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Do desiccation and storage of *Campomanesia adamantium* (Cambess.) O. Berg (Myrtaceae) seeds affect the formation and survival of seedlings?

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The objective of study was to verify if desiccation and storage of the seeds affect the morphoanatomy and histochemistry of the seedlings of *Campomanesia adamantium*. The fruits were processed and seeds were subjected to desiccation to 30, 20, 15, 10 and 5% water contents in laboratory conditions and subsequently subjected to the following storage conditions: In the laboratory (LAB) (25 ± 2°C, 35% RH), cold and dry chamber (CC) (16 ± 2°C, 40% RH), refrigerator (REF) (8 ± 2°C, 35% RH), and freezer (FZ) (-18 ± 1°C, 42% RH) for 0 (newly processed), 30, 60, 90, 120, 150, and 180 days. The evaluation of the seedling survival rate was performed for 42 days, calculated as the survival percentages for seedling shoot and primary root. Anatomical observations and histochemical tests were performed using fixed and non-fixed samples of the median region of xylopodium in normal and abnormal seedlings. In the morphoanatomy of normal seedlings, we observed cotyledons, hypocotyl, xylopodium, and well defined primary root and in abnormal seedlings, cotyledons, hypocotyl and xylopodium (with 20 and 15% water content), and hypocotyl and primary root (with 10% water content). The desiccation and storage of seeds affected the formation of seedlings by preventing the normal development of roots and shoots. The xylopodium of normal and abnormal seedlings showed positive reaction to starch and lipophilic substances. The presence of phenolic compounds and fructans were observed in parenchyma cells of the xylopodium in abnormal seedlings and absent in normal ones. The deleterious effects of desiccation in association with storage induce the production of phenolic compounds and fructans in abnormal *C. adamantium* seedlings.

Key words: Brazilian Savanna, xylopodium, water content, morphoanatomy, histochemistry.

INTRODUCTION

Seed storage is a safe and economical way to preserve genetic diversity of native plant species and represents a strategy to meet the continuous demand for seedlings for commercial purposes, reforestation, and recovery of

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degraded areas (Costa, 2009). However, the success of seed storage depends on understanding the behavior of these seeds during the storage process, which enables the use of appropriate conditions to maintain their viability (Hong and Ellis, 1996). The most important feature in seeds destined for long-term storage is longevity, which includes the seed survival time (Hay et al., 2010).

The storage capacity is expanded to many species when the reduction in seed water content is associated with decreases in environmental temperature (Walters et al., 1998). However, some species do not tolerate sharp decrease in temperature such as freezing due to the damage caused by negative that cause the formation of ice crystals inside the tissues, and consequently, the loss of seed viability (Chin et al., 1989; Fonseca and Freire, 2003).

Campomanesia adamantium (Cambess.) O. Berg (Myrtaceae) is a native non-cultivated fruit tree, abundant in the Cerrado area of Mato Grosso do Sul, and sometimes present beyond Brazil, reaching areas in Uruguay, Argentina, and Paraguay (Arantes and Monteiro, 2002; Lorenzi et al., 2006). The leaves and fruits have anti-inflammatory, antidiarrheal, and antiseptic properties in urinary tract infections (Vieira et al., 2011). Fruits collected at different ripening stages showed potential for "*in natura"* use, in food industry and as flavoring agent in beverage industry due to high levels of acidity (1.2 g in citric acid) and ascorbic acid (234 mg 100 g^{-1} vitamin C), minerals (K -1304 mg kg^{-1}, Ca, P, and Mg from 165 to 175 mg kg^{-1} concentration and the microelements Fe -11.3 mg kg^{-1} and Al -15.9 mg kg^{-1}) dietary fibers, and monoterpene hydrocarbons (a-pinene (10.6%), limonene (10.1%) and the b-(z)-ocimene (9.2%) present in greater amounts in the essential oil of the fruits and which give them the citrus scent (Vallilo et al., 2006).

A large number of fruit trees and forest species have seeds that are sensitive to desiccation (Villela and Peres, 2004); however, the information gathered about the storage of seeds that belong to the genus *Campomanesia* is still contradictory. Although *C. adamantium* is classified as recalcitrant, intolerant to storage and desiccation over a silica gel at water content of less than 15% (Dresch et al., 2014), it is suggested that desiccation and storage of its seeds change the formation of the seedlings.

Thus, an important tool to determine the changes in seedlings resulting from desiccation and storage are the morphoanatomical and histochemical studies that contribute to the identification of substances, present in storage organs and which may be part of adaptive mechanisms to adverse conditions. Therefore, this work aimed to verify if desiccation and storage of the seeds affect the morphoanatomy and histochemistry of the seedlings of *C. adamantium*.

MATERIALS AND METHODS

C. adamantium fruits were collected at the end of December 2011, from 30 tree samples in the Cerrado area (*sensu stricto*), in Ponta Porã- state of Mato Grosso do Sul (MS). After collection, the fruits were taken to the Laboratory of Plant Nutrition and Metabolism at the Federal University of Grande Dourados (UFGD) in Dourados-MS, where they were washed in running water and damaged fruits were discarded. Afterwards, the fruits were processed manually using sieves for separation of seeds. Then, the seeds were washed in running water and placed on Germitest® paper for 40 min at laboratory temperature (25 ± 2°C, 32% relative humidity - RH) to remove excess water (surface drying).

After, the seeds (fourteen thousand units) were dried under laboratory conditions on plastic trays and weighed every hour until they achieved the pre-established water content (30%, 20%, 15%, 10% and 5 ± 2°C) according to Sacandé et al. (2004) formula.

When the closest desired water content was achieved, a sample was homogenized, and divided into fractions packaged in clear plastic bags with a thickness of 0.20 mm (100 seeds) and subjected to the following storage conditions: Laboratory (LAB) (25 ± 2°C, 35% RH), cold and dry chamber (CC) (16 ± 2°C, 40% RH), refrigerator (REF) (8 ± 2°C, 35% RH), and freezer (FZ) (-18 ± 1°C, 42% RH). After 0 (newly processed), 30, 60, 90, 120, 150, and 180 days of storage, seeds were pre-humidified in 100% RH and at 25°C under continuous light for 24 h to avoid damage due to soaking, and the following, characteristics were determined to assess the physiological potential:

1. Water content: Determined at 105 ± 3°C for 24 h using the incubator method (Brasil, 2009), in three replicates each with 5 g of seed samples, and the results were expressed as percentage water content on a fresh weight basis.
2. Survival rate of shoot and primary root: A Germitest® paper roll was used for sowing four replicates, each of 25 seeds that were maintained in B.O.D. (Biochemical Oxygen Demand) incubator at 25°C under continuous light. Evaluations were performed for 42 days after sowing, calculating the percentages of survival of the shoot area and primary root of the seedlings and the results were expressed in percentage (%).

The design was a completely randomized factorial scheme (4 temperatures/environments × 7 storage periods). Differences in the temperature data designated as significant by analysis of variance were compared using Tukey's test and storage periods were adjusted by regression equations at 5% probability using the SISVAR software (Ferreira, 2011).

Morphoanatomical studies

Anatomical observations were made in the median region of the xylopodium of the *C. adamantium* seedlings. The cross sections obtained free hand were clarified with 20% sodium hypochlorite and, after being washed in 2% acetic alcohol and distilled water, were subjected to double staining using astra blue and safranin (Bukatsch, 1972) and mounted on glycerinated gelatin (Dop and Gautié, 1928).

Histochemical tests (qualitative)

Histochemical tests were performed using ten fixed and ten non-fixed samples of xylopodium of *C. adamantium* seedlings. The presence of lipophilic substances was visualized by using Sudan III (Sass, 1958), lugol for starch (Kraus and Arduin, 1997), and ferric chloride for phenolic compounds (Johansen, 1940). The slides were mounted using distilled water and observed posteriorly. For the fructans analysis, xylopodium samples were treated with sulfuric acid, and subsequently viewed under polarized light (Johansen, 1940).

The morphoanatomical results were analyzed and illustrated by

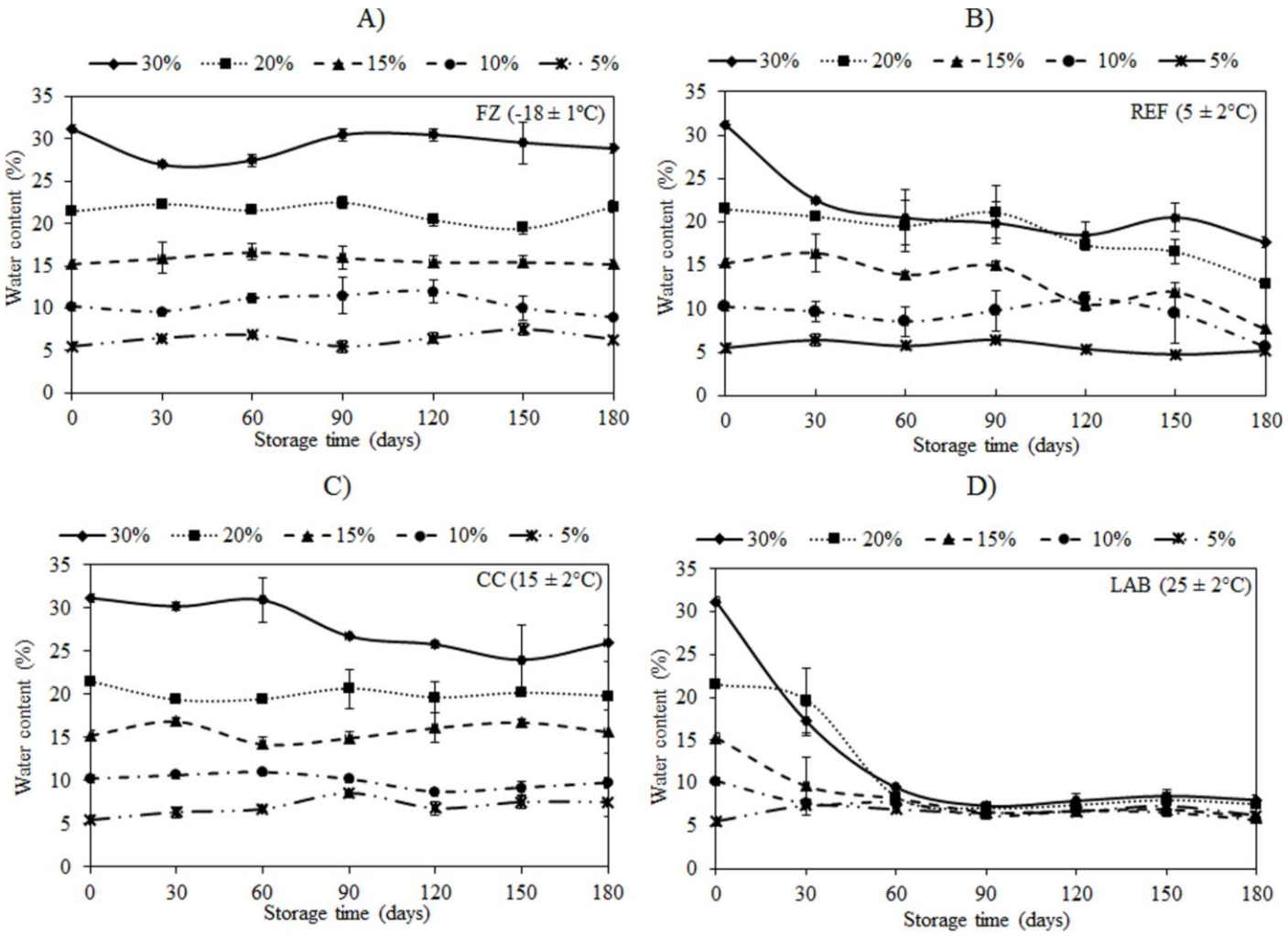

Figure 1. Water content (%) of *C. adamantium* seeds packed with different water contents (30, 20, 15, 10 and 5%), environmental conditions LAB - laboratory (A), CC - cold and dry chamber (B), REF- refrigerator (C), and FZ - freezer (D), and storage times (0, 30, 60, 90, 120, 150 and 180 days).

means of photographic equipment Sony Cyber-shot (Sony Electronics Inc., Japan) mounted on microscope Nikon Eclipse E 200 (Nikon Co., Tokyo, Japan). In all cases, scales were added according to the optical conditions used. The data for water content were presented as the average results and standard deviation.

RESULTS

The temperature of cold and dry chamber (16 ± 2°C), refrigerator (8 ± 2°C) and freezer (-18 ± 1°C) provided small variations in the water content levels during storage (Figure 1a to c). The seeds stored in laboratory temperature (25 ± 2°C) showed reductions in water levels over time, and these values were more pronounced in seeds with high water content (30 and 20%), so that at the end of 180 days showed water content of 8.0 and 7.5%, respectively (Figure 1d).

The survival rate of shoot and primary root was influenced by desiccation, environmental conditions, and seed storage time (Figures 2 and 3). Initially, the survival rate of shoot and primary root primary root was not affected by the environmental conditions (temperatures and storage times).

The survival of the shoot of the seedlings with reduced storage time in all water contents, the levels of 30 in 15% survival was greatest when seeds were stored in cold and dry chamber (Figure 2a to c). When the seeds were stored with water content below 15% survival percentage was close to zero from the 30 days of storage (Figure 2d and e). The seeds stored for up to 60 days in cold storage and water content of 15% showed high survival percentage of the shoot (over 50%).

For the survival of the primary root, the results were similar to the shoot, however, the seeds stored in cold and dry chamber with 15% water content the survival was less than 50% at 30 days (Figure 3a to e).

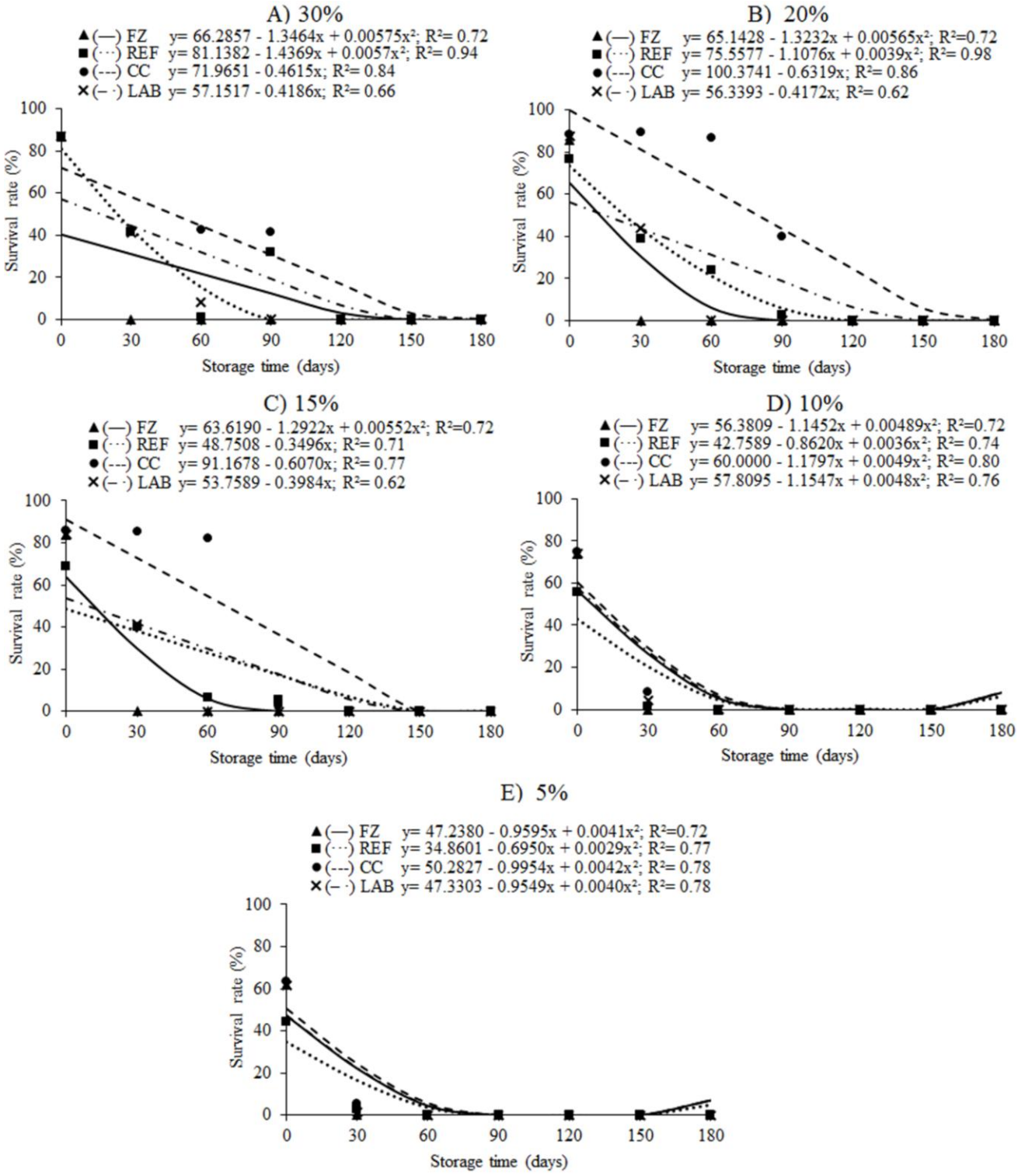

Figure 2. Survival rate of shoot of *C. adamantium* seedlings stored at different water contents of 30 (A), 20 (B), 15 (C), 10 (D) and 5% (E), environmental conditions (LAB - laboratory (25°C), CC - cold and dry chamber (16°C), REF- refrigerator (8°C), and FZ - freezer (-18°C), and storage times (0, 30, 60, 90, 120, 150 and 180 days).

Starting at 30 days of storage, we found that the seeds stored under freezer conditions showed no germination and consequently no formation of seedlings over the 180 days of storage (Figures 2 and 3).

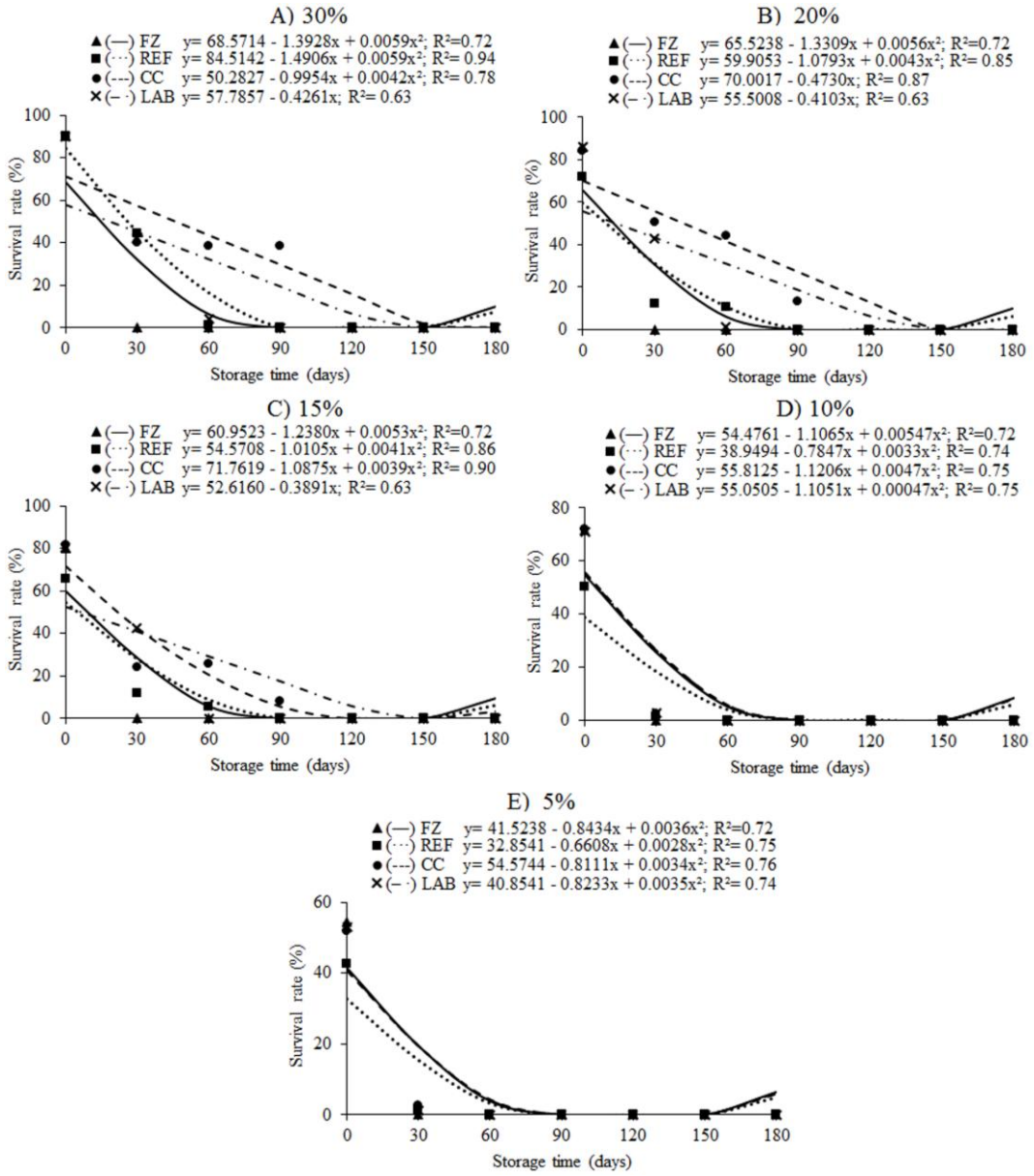

Figure 3. Survival rate of primary root of *C. adamantium* seedlings stored at water contents of 30 (A), 20 (B), 15 (C), 10 (D) and 5% (E), environmental conditions (LAB - laboratory (25°C), CC - cold and dry chamber (16°C), REF- refrigerator (8°C), and FZ - freezer (-18°C), and storage times (0, 30, 60, 90, 120, 150 and 180 days).

Desiccation of seeds to different water levels associated with storage environment and refrigerator conditions prevented the formation of shoots and primary roots after 60 days of storage (Figures 2 and 3). The desiccation of the seeds to water levels of 10 and 5% compromised seedling formation after 30 days of storage, regardless of storage conditions.

In relation to non stored seeds (storage time zero), we can emphasize that the relationship between shoot and primary root was proportional, reducing the value rate with seed desiccation. These high values in survival rates are related to high incidence of normal seedlings, which are characterized by the presence of expanded cotyledons, hypocotyl, xylopodium, and well-defined primary root (Figure 4).

The reduction in the survival rate of primary roots was due to the high incidence of abnormal seedlings, which are characterized by the presence of expanded cotyledons, hypocotyl, xylopodium, and nonexistent or stunted primary root (Figure 4).

The anatomical characterization of the xylopodium in normal and abnormal seedlings show a normal pattern in

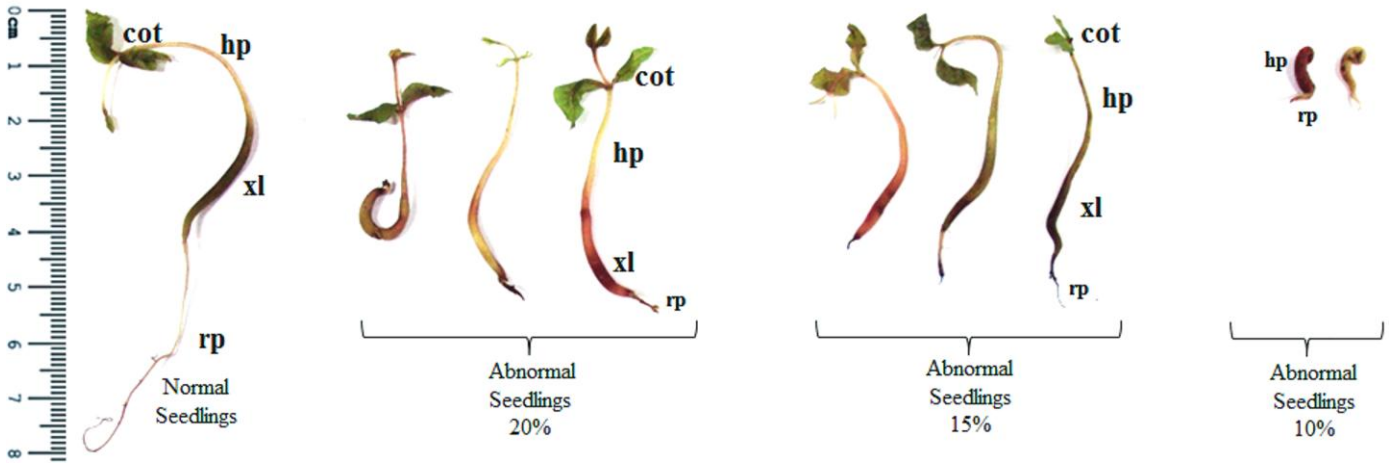

Figure 4. Overview of normal and abnormal *C. adamantium* seedlings (grown from seeds with water content of 20, 15 and 10%). cot, expanded cotyledons; hp, hypocotyl; xl, xylopodium; pr, primary root.

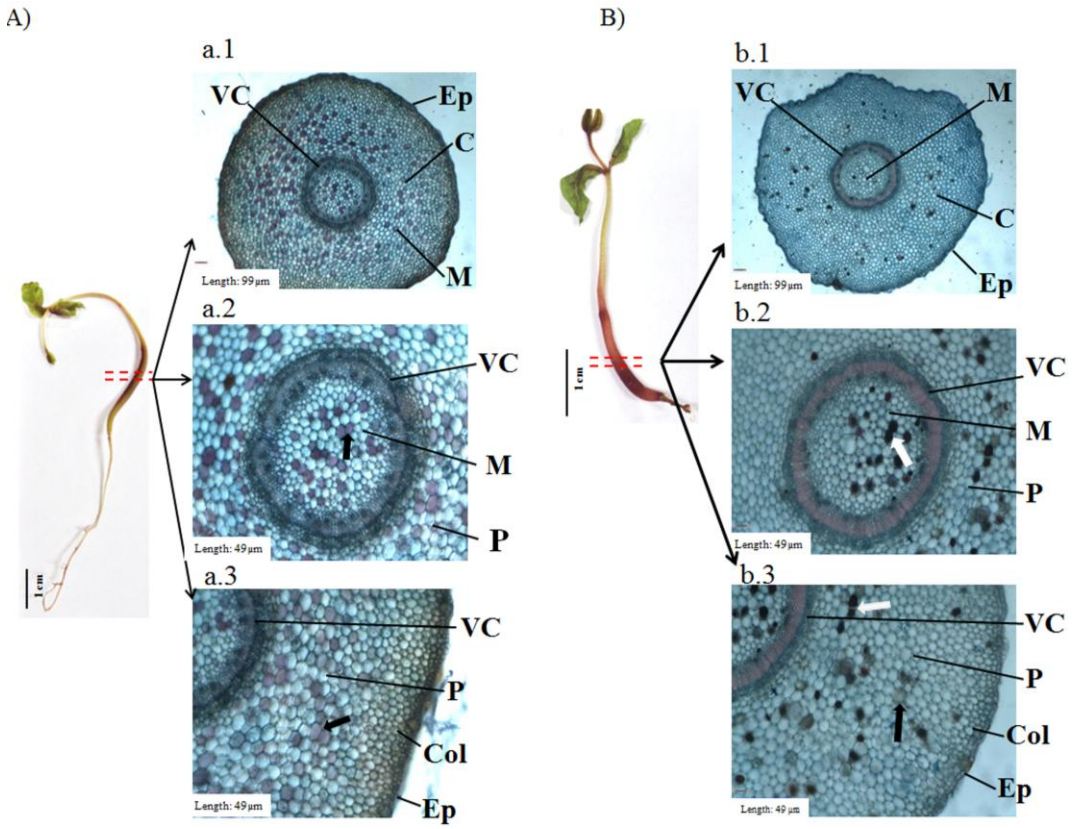

Figure 5. Cross section of xylopodium in primary stage of normal (a) and abnormal (b) seedling growth (M = medulla, C = cortex, P = parenchyma, Col = collenchyma, VC = vascular cylinder, Ep = epidermis, black arrow = lipids detected with Sudan III, and white arrow = phenolic compounds detected with ferrous chloride).

the early phase of development with well-defined regions such as the epidermis, cortical, and medullary regions. The innermost layer of the cortex is composed of lignified cells. The xylopodium in normal and abnormal seedlings shows lipids present in the cortex and medulla (Figures 5a and b). However, the phenolic compounds at this

Table 1. Histochemical tests of xylopodium in normal and abnormal seedlings *C. adamantium* from desiccated seeds with 20, 15 and 10% water contents.

Seedlings/water content	Classes of compounds			
	Lugol (starch)	Ferrous chloride (phenolic compounds)	Sulfuric acid (fructans)	Sudan III (lipophilic substance)
Normal	+	-	-	+
Abnormal / 20%	+	+	+	+
Abnormal /15%	+	+	+	+
Abnormal / 10%	+	+	+	+

(+) Positive and (-) negative.

stage of development were detected only in the cortex and medulla of abnormal seedlings.

In the histochemical test results, lugol used for identification of starch grains and Sudan III for lipids in general, showed positive reactions in the xylopodium of normal and abnormal seedlings (Table 1). Ferrous chloride used for identification of phenolic compounds and sulfuric acid (fructans) showed a strong positive reaction, especially in parenchyma cells of xylopodium in abnormal seedlings and a negative reaction in normal seedlings.

DISCUSSION

The formation of *C. adamantium* seedlings was influenced by desiccation, environmental conditions, and seed storage time. The dehydration of the seeds at different water contents associated with the storage conditions intensified the process of deterioration over time decreasing the rate in development of shoot and primary root to below 50% after 30 days of storage, except under the freezer condition.

The seeds packaged in semi-permeable plastic packaging in laboratory temperature allowed the exchange of water vapor between the seeds with high water content and the external environment, changing the level of hydration of seeds. These changes in water levels associated with the storage time influenced negatively the survival of the shoot and primary roots, confirming the recalcitrant behavior due to the sensitivity to drying and storing (Melchior et al., 2006; Scalon et al., 2013; Dresch et al., 2012, 2014).

The negative effects of desiccation, mainly at water contents below 15% associated with the storage times, resulted in the formation of abnormal seedlings, which had a missing or stunted primary root. Possibly, desiccation of these seeds associated with storage favored deterioration, which has as main cause lipids peroxidation (McDonald, 1999). Therefore, lipid peroxidation, occurring in the mitochondria of cells at the radicle end caused reduction in seedling growth in the most deteriorated seeds (Marcos Filho, 2005). The occurrence of seedling abnormalities observed in the final stages of decay is determined by the death of important tissues in different regions of the seeds that cause severe damage to cellular metabolism and consequently disruption in seedling growth (Matthews, 1985; Marcos Filho, 2005).

The seedlings characterized as abnormal showed accumulation of phenolic compounds and fructans in parenchyma cells of the xylopodium, while the same does not occur in normal seedlings. The phenolic compounds result from lipid peroxidation due to seed deterioration (Marcos Filho, 2005). The damage caused by the deterioration may have contributed to the accumulation of phenolic compounds that triggered the malformation and growth of primary roots in seedlings. However, fructans accumulation may be associated with loss of membrane integrity during seed desiccation and storage. Fructans play an important role in osmotic regulation and in preventing membrane damage, maintaining the integrity and cell function, allowing not only the survival but also the growth even under conditions of low water availability, which occur due to either low temperature or lack of water in the environment (Brocklebank and Hendry, 1989; Demel et al., 1998; Vereyken et al., 2001).

Furthermore, the fructans are a source of energy or carbon reserve and therefore, like the phenolic compounds, are related to a species' tolerance to environmental stresses during growth and development, especially in the Cerrado area, a place where *C. adamantium* occurs naturally and where long droughts and fires can happen (Melo-de-Pinna and Menezes, 2003; Detmann et al., 2008). Several studies suggest that fructans provide the plants with resistance to drought and/or tolerance to cold (Livingston and Henson, 1998; Pilon-Smits et al., 1995, Van Den Ende et al., 2000). In studies of anatomy of the underground system in *Vernonia grandiflora* Less. and *V. brevifolia* Less. (Vernonieae, Asteraceae), was observed that the occurrence of these bud-forming underground systems, which stored reserve compounds, enabled these plants to survive throughout unfavorable environmental conditions in the Cerrado, such as dry season and

frequent fires in the winter (Hayashi and Appezzato-da-Glória, 2007).

The presence of fructans and phenolic compounds demonstrates the adaptive mechanism of the species in response to seed desiccation and storage temperature. In surveys conducted in the Cerrado area, it has been found that many species have underground storage organs that accumulate large amounts of fructans (Figueiredo-Ribeiro et al., 1986; Tertuliano and Figueiredo-Ribeiro, 1993; Hayashi and Appezzato-da-Glória, 2007; Appezzato-da-Glória and Cury, 2011).

The results obtained in this study are in agreement with our initial hypothesis that desiccation and storage does not affect the morphoanatomy and histochemistry of *C. adamantium* seedlings. It is worth noting that after the evaluation period of seedling survival, the emergence of secondary roots (data not shown) in abnormal seedlings was observed, reinforcing the information from the literature that fructans confer tolerance under stress conditions, such as desiccation and seed storage temperatures, thereby ensuring the survival of seedlings.

Future work should be conducted to assess the development of the root system and its implication in seedling production from seeds that are desiccated and stored at tolerable water levels in germplasm banks.

Thus, we conclude that the desiccation and storage of seeds affects seedlings formation by preventing normal development of the primary root and shoot structures. The deleterious effects of desiccation associated with storage triggers the onset of phenolic compounds and fructans in abnormal *C. adamantium* seedlings.

Conflict of Interest

The authors declare they have no conflict of interest.

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REFERENCES

Appezzato-Da-Glória B, Cury G (2011). Morpho-anatomical features of underground systems in six Asteraceae species from the Brazilian Cerrado. An. Acad. Bras. Cienc. 83(3):981-991.

Arantes AA, Monteiro R (2002). A família Myrtaceae na estação ecológica de Panga, Uberlândia, MG. Lundiana 3(2):111-127.

Brasil (2009). Ministério da Agricultura, Pecuária e Abastecimento - Regras para análises de sementes**.** Ministério da Agricultura, Pecuária e Abastecimento. Secretaria da Defesa Agropecuária. Brasília, DF: Mapa/ACS, P. 399.

Brocklebank KJ, Hendry GAF (1989). Characteristics of plant species which store different types of reserve carbohydrates. New Phytol. 112:255-260.

Bukatsch F (1972). Bemerkungen zur Doppelfärbung Astrablau - Safranin. Mikrokosmos 61(8):225.

Chin HF, Krishnapillay B, Stanwood PC (1989). Seed moisture: recalcitrant vs. orthodox seeds. In: Stanwood PC, Mcdonald MB. [ed.]. Seed moisture. Madison: Crop Sci. Soc. of Am. pp.15-22.

Costa CJ (2009). Armazenamento e conservação de sementes de espécies do Cerrado. Embrapa Cerrados (Documentos/Embrapa Cerrados), Planaltina, DF, P. 30.

Demel RA, Dorrepall E, Ebskamp MJM, Smeekens JCM, Kruijff B (1998). Fructans interact strongly with model membranes. Biochim Biophys Acta Rev. Biomembranes 1375:36-42.

Detmann KDSC, Delgado MN, Rebello VPA, Leite TDS, Azevedo AA, Kasuya MCM, Almeida AMD (2008). Comparação de métodos para a observação de fungos micorrízicos arbusculares e endofíticos do tipo *dark septate* em espécies nativas de Cerrado. Rev. Bras. Ciênc. Solo 32(5):1883-1890.

Dop P, Gautié A (1928). Manuel de technique botanique. [2 ed.] Paris: J. Lamare, P. 594.

Dresch DM, Scalon SPQ, Masetto TE, Vieira MC (2012). Germinação de sementes de *Campomanesia adamantium* (Camb.) O. Berg em diferentes temperaturas e umidades do subtrato. Scientia Forestalis 40:223-229.

Dresch DM, Scalon SPQ, Masetto TE, Mussury RM (2014). Storage of *Campomanesia adamantium* (Cambess.) O. Berg seeds: influence of water content and environmental temperature. Am. J. Plant. Sci. 5:2555-2565.

Ferreira DF (2011). Sisvar: A computer statistical analysis system. Ciênc. agrotec. 35:1039-1042.

Figueiredo-Ribeiro RCL, Dietrich SMS, Chu EP, Carvalho MAM, Vieira CCJ, Graziano TT (1986). Reserve carbohydrates in underground organs of native Brazilian plants. Rev. Bras. Bot. 9:159-166.

Fonseca SCL, Freire HB (2003). Sementes recalcitrantes: problemas na pós-colheita. Bragantia 62(2):297-303.

Hay FR, Smith RD, Ellis RH, Butler LH (2010). Developmental changes in the germinability, desiccation tolerance, hardseededness, and longevity of individual seeds of *Trifolium ambiguum*. Ann. Bot. 105:1035–1052.

Hayashi AH, Appezzato-Da-Glória B (2007). Anatomy of the underground system in *Vernonia grandiflora* Less. and *V. brevifolia* Less. (Asteraceae). Braz. Arch. Biol. Technol. 50(6):979-988.

Hong TD, Ellis RH (1996). A protocol to determine seed storage behavior. In: Engels JMM, Toll J. Rome: IPGRI, P. 62. (IPGRI Technical Bulletin n.1)

Johansen DA (1940). Plant microtechnique. McGraw-Hill Book Company, New York. P. 790.

Kraus JE, Arduin M (1997). Manual básico de métodos em morfologia vegetal. Rio de Janeiro: EDUR, P. 198.

Livingston DP, Henson CA (1998). Apoplastic sugars, fructans, fructan exohidrolase, and invertase in winter oat: responses to second-phase cold hardening. Plant Physiol. 116:403-408.

Lorenzi H, Cacher L, Lacerda M, Sartori S (2006). Frutas brasileiras e exóticas cultivadas (de consumo *in natura*). São Paulo, Instituto Plantarum. P. 640.

Matthews S (1985). Physiology of seed ageing. Outlook Agric. 14(2):89-94.

Marcos Filho J (2005). Fisiologia de sementes de plantas cultivadas. Piracicaba: FEALQ, P. 495.

Melchior SJ, Custódio CC, Marques TA, Machado Neto NB. (2006). Colheita e armazenamento de sementes de gabiroba (*Campomanesia adamantium* Camb. – Myrtaceae) e implicações na germinação. Rev. Bras. Sementes 28:141-150.

Mcdonald MD (1999). Seed deterioration, physiology, repair and assessment. Seed Sci. Technol. 22(3):531-539.

Melo-De-Pinna GFA, Menezes NL (2003). Meristematic endodermis and secretory structures in adventitious roots of *Richterago* Kuntze (Mutisieae-Asteraceae). Rev. Bras. Bot. 26:1-10.

Pilon-Smits EAH, Ebskamp MJM, Paul MJ, Jeuken MJW, Weisbeek PJ, Smeekens SCM (1995). Improved performance of transgenic fructan-accumulating tobacco under drought stress. Plant Physiol. 107:125-130.

Sacandé M, Joker D, Dulloo M, Thomsen KA (2004). [ed] Comparative storage biology of tropical tree seeds. Roma: International Plant Genetic Resources Institute, P. 363.

Sass JE (1958). Botanical microtechnique. Iowa State University, Ames. P. 228.
Scalon SPQ, Oshiro AM, Masetto TE, Dresch DM. (2013) Conservation of *Campomanesia adamantium* (Camb.) O. Berg seeds in different packaging and at varied temperatures. Rev. Bras. Fruticultura 35:262-269.
Tertuliano MF, Figueiredo-Ribeiro RCL (1993). Distribution of fructose polymers in herbaceous species of Asteraceae from the cerrado. New Phytol. 123:741-749.
Vallilo MI, Lamardo LCA, Gaberlotti ML, Oliveira E, Moreno PRH (2006). Composição química dos frutos de *Campomanesia adamantium* (Cambessédes) O. Berg. Ciênc. Tecnol. Aliment. 26(4):805-810.
Van Den Ende W, Michiels A, Roover J, Verhaert P, Van Laere A (2000). Cloning and functional analysis of chicory root fructan 1-exohydrolase I (1-FEH I): A vacuolar enzyme derived from a cell-wall invertase ancestor? Mass fingerprint of the 1-FEH I enzyme. Plant J. 24:447-456.
Vereyken IJ, Chupin V, Demel RA, Smeekens SCM, Kruijiff B (2001). Fructans insert between the headgroups of phospholipids. Biochim. Biophys Acta Re. Biomembranes 1510:307-320.
Vieira MC, Perez VB, Heredia ZN, Santos MC, Pelloso I, Pessoa SM (2011). Nitrogênio e fósforo no desenvolvimento inicial da guavira (*Campomanesia adamantium* (Cambess.) O. Berg) cultivada em vasos. Rev. Bras. Plantas Med. 13:542-549.
Villela FA, Peres WB (2004). Coleta, secagem e beneficiamento de sementes. In. Ferreira, AG, Borguetti R. (Ogs). Germinação: do básico ao aplicado. Porto Alegre: Artmed, pp. 265-281.
Walters C (1998). Understanding the mechanisms and kinetics of seed aging. Seed Sci. Res. 8:223-244.