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Storage of *Campomanesia adamantium* (Cambess.) O. Berg Seeds: Influence of Water Content and Environmental Temperature

Daiane Mugnol Dresch[1*], Silvana de Paula Quintão Scalon[2], Tathiana Elisa Masetto[2], Rosilda Mara Mussury[3]

[1]Pós-Doutoranda em Agronomia (PNPD/CAPES), Universidade Federal da Grande Dourados (UFGD), Dourados, Brasil
[2]Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados (UFGD), Dourados, Brasil
[3]Faculdade de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados (UFGD), Dourados, Brasil
Email: *daiamugnol@hotmail.com

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Abstract

The present work evaluated the conservation of *Campomanesia adamantium* seeds under different conditions. The fruits used in the study were collected from matrices located in the city of Ponta Porã-MS, Brazil. After processing, the seeds were slowly dried to different water content levels and subsequently exposed to various environmental conditions: 25˚C ± 2˚C, 35% relative humidity (laboratory); 16˚C ± 1˚C, 40% relative humidity (cold and dry); 8˚C ± 1˚C, 35% relative humidity (refrigeration); and −18˚C ± 1˚C, 42% relative humidity (freezing). Each treatment was conducted for 0 (recently processed seeds, with superficial drying of 40 minutes), 30, 60, 90, 120, 150, and 180 days. Seed potential was physiologically evaluated based on radicle protrusion, percentage of normal seedlings, seedling length (primary root, shoot and total), and dry mass of the seedlings. The experimental design was a completely randomized factorial scheme with split-split plots (4 temperatures/environments × 5 water content × 7 storage periods). *C. adamantium* seeds tolerated a reduction in the water content to 15.3% but did not tolerate the storage period, confirming the recalcitrant behavior of the seeds.

Keywords

Myrtaceae, Brazilian Savanna, Drying, Conservation

*Corresponding author.

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1. Introduction

Seed storage is a safe and economical way to preserve the genetic diversity of native plant species, and the method represents a strategy to meet the continued demand for seedlings for commercial purposes, reforestation, and reclamation [1]. However, the success of seed storage depends on knowledge regarding the behavior of seeds during the process, which enables the use of appropriate conditions that maintain their viability [2].

Seeds may be classified into two categories of behavior in storage: orthodox or recalcitrant [3]. Orthodox seeds remain viable after desiccation to water content levels of approximately 5% and may be stored at low temperatures for long periods. Recalcitrant seeds are sensitive to desiccation and do not survive with low levels of moisture, which prevents long-term storage. More recently, a third category has been proposed, in which seeds exhibit a storage behavior somewhere between orthodox and recalcitrant t [4]. Seeds that exhibit intermediate behavior tolerate dehydration to 7.0% - 10% humidity but do not tolerate low temperatures for extended periods [2].

Recalcitrant seeds have low viability in storage, which causes serious problems for the conservation of the germplasm of these species through ex situ conservation [5] and inclusion in plant restoration programs [6]. The retention of recalcitrant seeds could be favored by methods that limit the maximum possible growth of the embryonic axis, thereby maintaining the seeds at a sufficient water content [7].

The storage capacity of many species is increased when the reduction in water content of the seeds is associated with a reduction of the environmental temperature [8]. However, some species do not tolerate sharp reductions in temperature due to the damage caused by freezing (−18˚C) from the formation of ice crystals in the tissues, which leads to a loss of viability [9] [10].

The seeds of many fruit and forest species are sensitive to desiccation [11], but work concerning the storage of seeds of the genus *Campomanesia* has yielded contradictory results [12]-[14]. *C.adamantium* (Cambess.) O. Berg (Myrtaceae) is a native, uncultivated fruit that is abundant in the Cerrado of Mato Grosso do Sul [15]. Fruits collected at different ripening stages have the potential to be used "*in natura*", in the food industry and as a flavoring in the beverage industry due to their high acidity (1.2 g citric acid) and ascorbic acid (vitamin C) content (234 mg/100g), minerals (K = 1304 $mg \cdot kg^{-1}$; Ca, P, and Mg in concentrations between 165 and 175 $mg \cdot kg^{-1}$), dietary fiber, and oil monoterpenes, which are present in significant quantities in the volatile oil of the fruits and give them a citrus aroma and energetic value of 66.3 kcal/100g [16].

C. adamantium produces seeds sensitive to desiccation and storage, and the lack of knowledge regarding their longevity complicates the use of the fruits, the optimization of the cultivation of this species, and the maintenance of germplasm banks. Several studies have suggested that the species can be classified as recalcitrant based on its germination and storage behavior for short periods, which leads us to believe that a critical limit to the loss of water exists for storage, and this limit is dependent on desiccation, temperature, and storage time. The hypothesis put forth in this paper is that the seeds of *C. adamantium* can be stored for a period exceeding 30 days at low temperatures, provided their water content is maintained at approximately 25%. To test this hypothesis, this study evaluated the effects of variations in water content, environmental conditions, and storage periods on the conservation of *C. adamantium* seeds.

2. Material and Methods

Campomanesia adamantium fruits were collected at the end of December 2011 from 30 arrays located in an region of the Cerrado (sensu stricto) in Ponta Porã-MS. After collection, the fruits were brought to the Laboratory of Nutrition and Metabolism of Plants, Universidade Federal da Grande Dourados (UFGD) in Dourados-MS, where they were washed in running water and any damaged fruits were discarded. Subsequently, the fruits were processed manually and on sieves to separate the seeds. The seeds were then washed with tap water and placed on Germitest® paper for 40 minutes at room temperature (25˚C ± 2˚C, 32% relative humidity) to remove excess moisture.

After water was removed from the seed surfaces, the seeds were dried under laboratory (25˚C) conditions on plastic trays and weighed until they reached the pre-established water content (30%, 20%, 15%, 10% and 5%), as calculated according to the formula of [17].

When the water content levels of the seeds were found to be near those desired, samples were taken, homogenized, and divided into fractions packaged in clear plastic bags with a thickness of 0.20 mm. These samples were subjected to the following storage conditions: 25˚C ± 2˚C, 35% relative humidity (laboratory); 16˚C ± 1˚C, 40% relative humidity (cold and dry); 8˚C ± 1˚C, 35% relative humidity (refrigeration); and −18˚C ± 1˚C, 42% relative humidity (freezing). After 0 (newly processed), 30, 60, 90, 120, 150, and 180 days of storage, the seeds were rehumidified to 100% relative humidity and 25˚C under continuous white light for 24 hours, thereby preventing damage from soaking. The following physiological characteristics were then determined.

The water content was determined at 105˚C ± 3˚C for 24 h using the greenhouse method [18] with three replicates of 5 g of seeds each, and the results were expressed on a wet basis.

Protrusion of the primary root was measured on Germitest® paper rolls with four replications of 25 seeds each, germinated with B.O.D. (Biochemical Oxygen Demand) at 25˚C under continuous white light. Assessments were conducted daily, and the root was considered protruded when it reached a length of 5 mm. The results were expressed in percentages (%).

The percentage of normal seedlings was determined in Germitest® paper rolls with four replications of 25 seeds each, which were germinated with BOD at 25˚C under continuous white light. Evaluations were performed forty-two days after sowing by computing the percentages of normal seedlings, using the issuance of shoot and root system development as the criteria [13]. The results were expressed in percentages (%).

Seedling length was obtained by measuring the lengths of the primary root, shoot and total plant using a millimeter ruler. The results were expressed in centimeters (cm).

The total dry mass was obtained from seedlings that had been dried in an oven at 60˚C for 48 hours using an analytical balance (0.0001 g), with the results expressed in grams (g).

The design was a completely randomized factorial scheme with split-split plots (4 temperatures/environments × 5 water content × 7 storage periods). Differences in the temperature data designated as significant by analysis of variance were compared using Tukey's test and water content and storage periods were adjusted by regression equations at 5% probability using the SISVAR software [19].

3. Results

Slow (ambient) drying required 18 hours for the initial water content of newly processed seeds (42.1%) to be reduced to 5.5% (**Figure 1**). The seeds placed in the cold/dry chamber (16˚C), refrigerator (8˚C), and freezer (−18˚C) all showed small variations in water content during storage. However, the seeds kept at room temperature in the present study showed reductions in the water content after storage for 30 days; these reductions were more pronounced in seeds with an initial water content of 31.2%; the water content reached 17.2% at 30 days and 8.0% after 180 days (**Figure 2**).

For radicle protrusion, the seeds with a water content of 21.8% showed an average maximum protrusion of 53.8% after storage in the cold/dry chamber (16˚C) (**Figure 3(a)**). The seeds showed a radicle protrusion rate of over 50% at 30 days only under cold (16˚C) and dry storage (52.3%) and a water content of 21.5% (51.2%); other conditions yielded rates below 50% (**Figure 3(b)**, **Figure 3(c)**). The dehydration of seeds under different storage conditions intensified the process of deterioration over time, causing primary root protrusion rates lower than 50% (**Figure 3(b)**, **Figure 3(c)**).

For the interaction of water content x ambient conditions, the maximum percentage of normal seedlings was observed in seeds stored under cold (16˚C) and dry conditions with a water content of 21.5% (32.5%) (**Figure 3(d)**). After 30 days of storage, gradual and significant reductions in the survival of normal seedlings (greater than 50%) were observed for all environments, storage periods, and water content, indicating that the seeds did not tolerate desiccation and storage (**Figure 3(e)**, **Figure 3(f)**). Seeds stored under the cold/dry chamber (16˚C) conditions presented a minimal percentage of normal seedlings (4.7%) after 143 days of storage. Under the laboratory (25˚C) and refrigerator conditions and for water content of 10.2% and 5.5%, storage for 90 days or longer made the seeds completely unviable the seeds conservation (**Figure 3(e)**, **Figure 3(f)**).

The shoot length was negatively influenced by dehydration over different periods of storage under the various environmental conditions (**Figures 4(a)-(c)**). Regarding the interaction of water content and environmental conditions, the longest shoot lengths were observed in cold (16˚C) storage at a water content of 25.1% and under refrigeration (8˚C) at a water content of 18.9% (4.36 and 1.86 cm, respectively) (**Figure 4(a)**). The refrigerator (8˚C) and laboratory (25˚C) storage conditions prevented the growth of shoots after 60 days of storage. For the cold and dry chamber (16˚C) condition, the shoot length decreased over the 180 days of the experiment, with the minimum value (2.01 cm) observed at 162 days (**Figure 4(b)**). The shoot length decreased after 30 days of storage at all water content levels, and no growth was observed after 60 days of storage for water content levels of

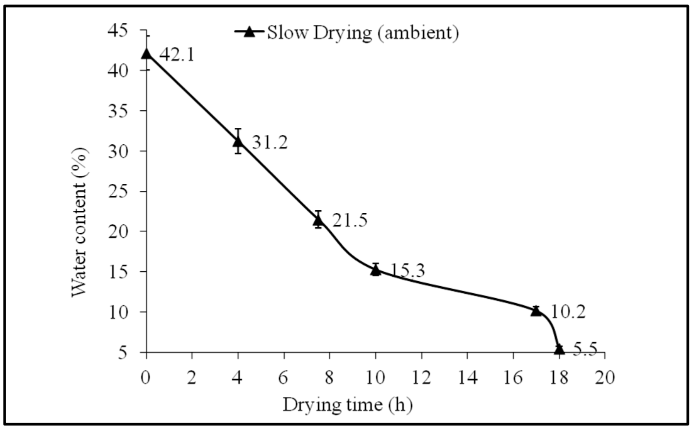

Figure 1. Curve slow drying *Campomanesia adamantium* seeds.

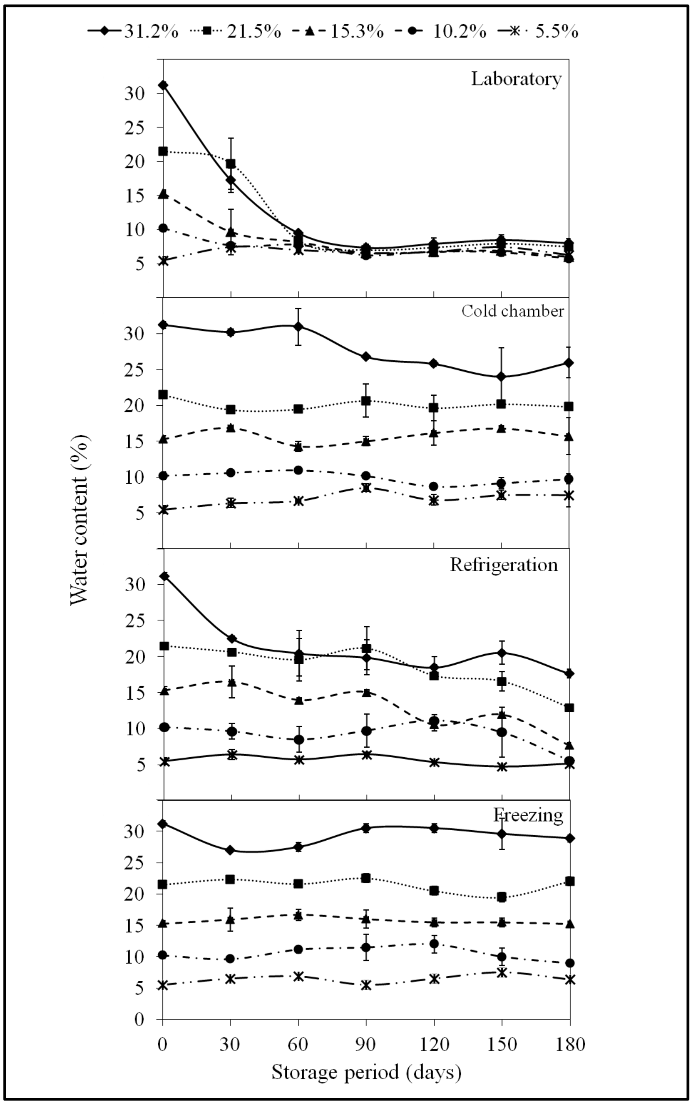

Figure 2. Water content (%) *Campomanesia adamantium* seeds packed with different water contents (%), environmental temperature and stored for different periods.

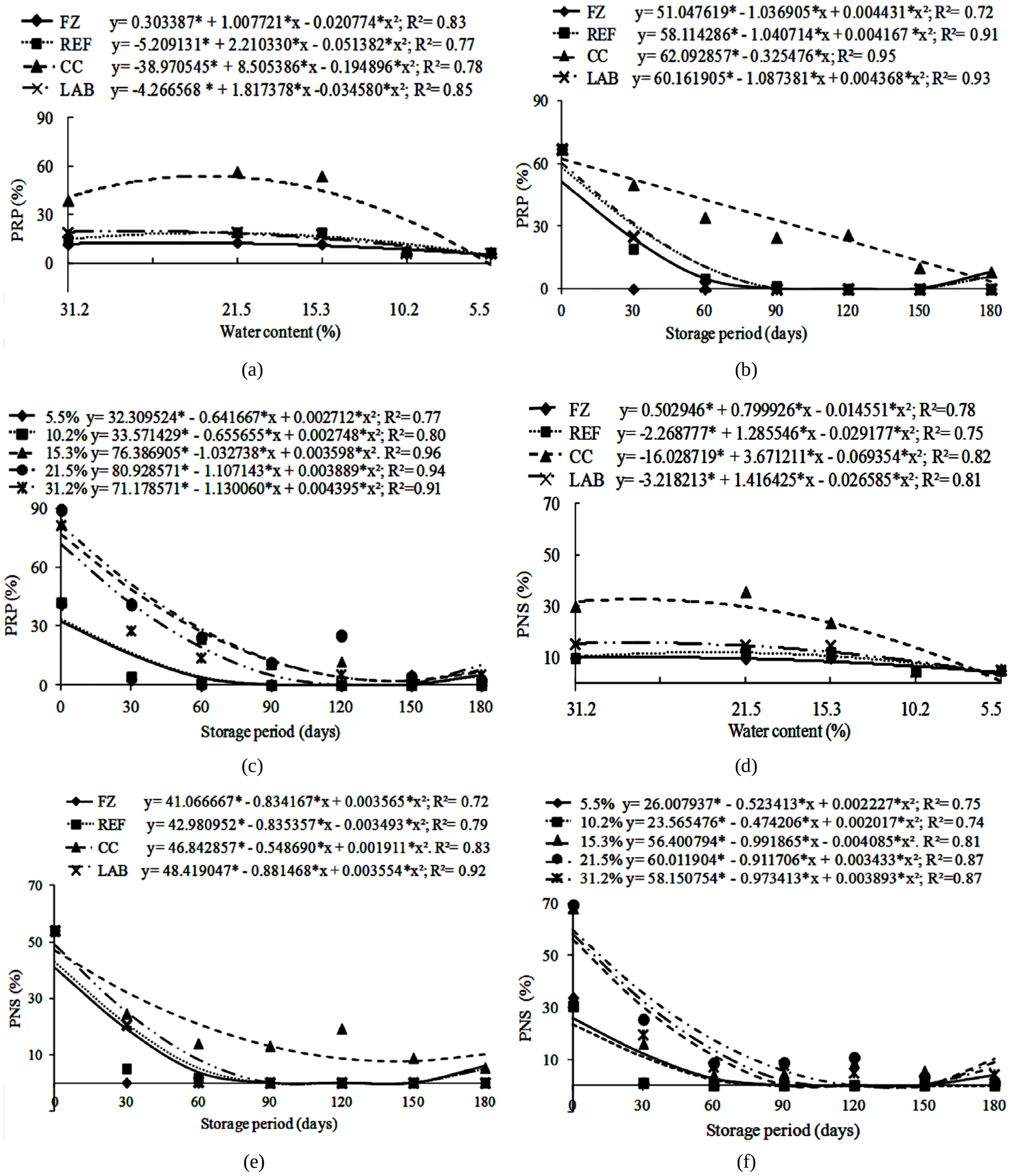

Figure 3. Primary root protrusion (PRP) (%) (a), (b), (c) and percentage of normal seedlings (PNP) (%) (d), (e), (f) *Campomanesia adamantium* seeds due to interactions of water content x environmental conditions (a), (d), storage periods x environmental conditions (b), (e) and storage periods x water content (c), (f).

5.5% to 10.2% (**Figure 4(c)**). Comparatively, the minimum shoot lengths were observed at 137 days for the 31.2% water content level, at 155 days for 21.5%, and at 136 days for 15.3% (0.56, 0.75, and 0.68 cm, respectively) (**Figure 4(c)**).

Storing seeds in different environments and at various water content levels intensified the process of deterioration, affecting protrusion and primary root growth the most (**Figures 4(d)-(f)**). The cold/dry chamber (16˚C) condition was less damaging to the primary root length of seeds with a water content of 30.5% (4.18 cm), but the shortest roots (1.73 cm) were observed after 130 days of storage in this condition (**Figure 4(d)**, **Figure 4(e)**). The seeds at the water content of 15.3% and 21.5% had minimal primary root lengths at 135 days (0.38 cm) and 125 days (0.63 cm) of storage, respectively (**Figure 4(f)**).

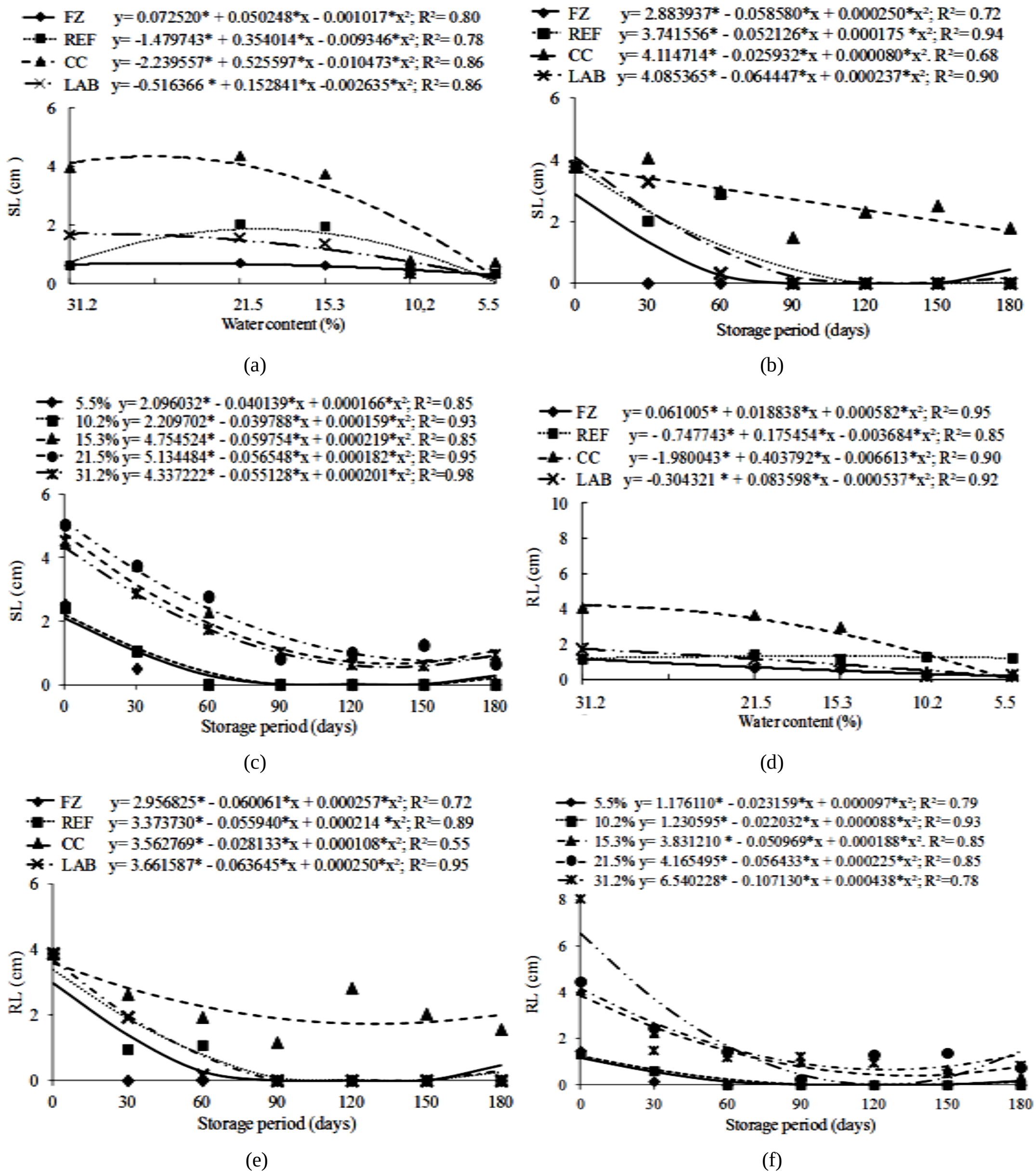

Figure 4. Shoot of length (SL) (cm) (a), (b), (c) and root length (RL) (cm) (d), (e), (f) *Campomanesia adamantium* seedlings due to interactions of water content x environmental conditions (a), (d), storage periods x environmental conditions (b), (e) and storage periods x water content (c), (f).

The storage of seeds at different water content levels, environmental conditions, and storage periods negatively influenced total seedling growth (**Figures 5(a)-(c)**). The highest seedling growth was observed in seedlings from seeds stored in cold chamber (16˚C) storage at a water content of 27.9% (8.38 cm) and in the refrigerator with a water content of 20.3% (3.15 cm) (**Figure 5(a)**).

The cold and dry chamber (16˚C) condition allowed for seedling growth over the 180 days of storage; the lowest growth value was observed at 140 days (3.78 cm) (**Figure 5(b)**). The water content levels of 15.3% and 21.5% yielded the lowest growth values at 134 days (1.03 cm) and 139 days (1.46 cm), respectively (**Figure 5(b)**).

The reduction in total seedling growth due to seed desiccation and storage was also accompanied by the decreased accumulation of total dry mass (**Figures 5(d)-(f)**). The seeds kept under refrigeration (8˚C) with a water content of 20.8% (0.0061 g) and those kept in the cold chamber (16˚C) with a water content of 30.9% (0.0280 g)

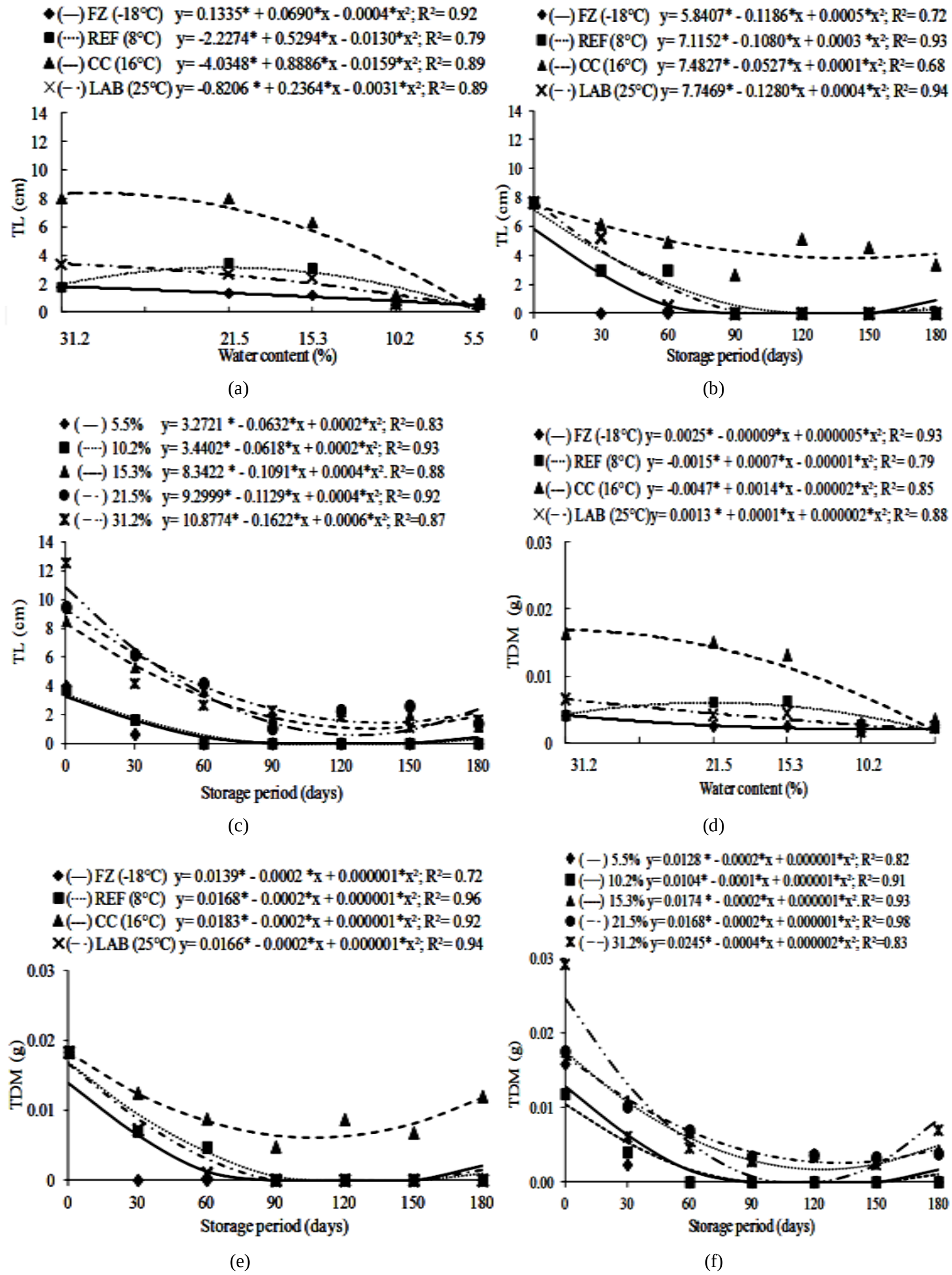

Figure 5. Shoot of length (SL) (cm) (a), (b), (c) and root length (RL) (cm) (d), (e), (f) *Campomanesia adamantium* seedlings due to interactions of water content x environmental conditions (a), (d), storage periods x environmental conditions (b), (e) and storage periods x water content (c), (f).

exhibited higher accumulations of total dry mass than seeds stored in other environments (**Figure 5(d)**). The seeds kept in the cold chamber (16˚C) accumulated mass over the entire 180 days, with the lowest value observed at 113 days (0.0054 g) (**Figure 5(e)**). Seeds with water content levels of 15.3% and 21.5% showed the lowest accumulations at 126 days (0.0014 g) and 108 days (0.0053 g), respectively (**Figure 5(f)**).

4. Discussion

Seed drying to obtain the desired water content before storage was performed slowly at room temperature (25˚C) to minimize damaging the integrity of the cell membranes in the seed, which can occur during rapid water removal. The seeds placed in the cold/dry chamber, refrigerator, and freezer all showed small variations in water content during storage, indicating that low and controlled temperatures are efficient for maintaining the water content of seeds, even in semipermeable containers such as plastic bags. The same behavior was observed in seeds of *Eugenia brasiliensis* Lam.; when seeds of this species were stored in perforated plastic bags and kept in a cold chamber, they presented small variations in the water content levels of 23.6% (1.8 percentage points) and 35.1% (3.5 percentage points) that were achieved after drying [20].

Although the packages are semipermeable, they allow for the exchange of water vapor between seeds with a high water content and the external environment, thereby reducing the moisture level of the seeds. This alteration in the hydration level of the seeds was more pronounced at 25˚C (laboratory), and at the end of the 180 days of storage, the higher water content levels (31.2%, 21.5%, and 15.3%) had been drastically reduced to values between 10% and 5%. The changes in temperature and relative humidity cause constant adjustments in the water content of seeds during storage [21].

It should be noted that during storage under laboratory conditions (25˚C), several of the seeds with a water content of 31.2% exhibited primary root protrusion while still inside the plastic bag, indicating that the high water content prevented seed storage. Seed germination inside the storage bag has previously been observed in seeds of *Euterpe edulis* Mart. [22] under storage at 15˚C with a 44% water content level and in seeds of *Euterpe oleracea* Mart. [23] under storage at 20˚C with water content levels of 43.4% and 37.4%.

A high water content, in conjunction with high temperatures, allowed for the root protrusion of seeds in storage and for the development of fungi such as *Aspergillus* sp. and *Penicillium* sp. Understanding the presence of microorganisms and their effects on decay due to changes in the water content of seeds requires rigorous observations because microflora represent an important variable determining the performance of recalcitrant seeds under various environmental conditions [24].

However, the seed dehydration under different storage conditions intensified the process of deterioration over time, causing primary root protrusion rates lower than 50%. The loss of structural water during the drying of recalcitrant seeds can cause severe alterations in their metabolic systems and membranes, initiating the process of deterioration [25].

Similar results were observed in seeds of *Artocarpus integrifolia* L.: after 30 days of storage under controlled conditions (10˚C and 40% RH), the germination of seeds with a moisture content of 56% decreased from 51% to 27% and reached zero after 60 days [26]. Although the minimum temperature tolerated in storage varies among species, recalcitrant seeds cannot be stored at temperatures below 15˚C [27], similar to *Bactris gasipaes* (Kunth) [28], *Mangifera indica* L. [29], *Myrciaria dubia* (Kunth) McVaugh [30] and *Artocarpus integrifolia* L. [26].

Seeds stored in the freezer (−18˚C) did not present radicle protrusion nor seedling development after 30 days of storage, suggesting that they were unable to tolerate freezing temperatures. The main consequence of the formation of ice crystals is the mechanical disruption of both the structure and the cytoplasmic membrane of the cell due to the expansion of freezing water, resulting in cellular breakdown [31]. Similar results were observed in seeds of *Eugenia pyriformis* Cambess., for which 30 days of storage under freezer conditions (−18˚C ± 1˚C) prevented the germination of seeds (water content of 25%) [32]. The same behavior was observed in embryos of *Inga vera* Willd. subsp. affinis (DC.) T. D. Penn. (Inga), which showed sensitivity to dehydration at −18˚C [33].

The reductions in seed hydration of 10.2% and 5.5% in newly processed seeds and those stored within the first 30 days under different environmental conditions, respectively, intensified the process of deterioration, verifying the results for the primary root protrusion and the survival of normal seedlings and confirming the recalcitrant behavior of the species. The maintaining the viability of the seeds with this storage behavior is directly related to the reduction in the level of tolerable water in the seed. However, for *C. adamantium*, it was not possible to determine the level of tolerable water combined with the precise period of storage that would minimize the deterioration reactions. According to the results, reduction of the water content to 15.3% without subjecting the seed to storage facilitates germination of more than 50% normal seedlings. Seeds recalcitrant do not tolerate storage at low temperatures and are sensitive to desiccation, which complicates their conservation for prolonged periods [2]. Seeds that are intolerant of desiccation are often also considered to be intolerant to temperatures below 15˚C [34] [35].

Seeds of *C. adamantium* (Cambess.) O. Berg can be classified as recalcitrant due to their inability to be stored at a low temperature (8˚C) and their intolerance to desiccation (water content of 28%) [12]. Similar results were observed by reducing the water content of seeds from 57% to 27%, followed by storage under lab conditions; this procedure negatively affected germination, indicating the sensitivity of *C. adamantium* seeds to desiccation [13].

However, seeds of *C. adamantium* stored in different packages at temperatures of 5˚C, 10˚C, and 15ºC with an initial moisture content of 31.5% for seven, 14 and 21 days showed a germination percentage of 88.1% up to 21 days of storage and showed no significant difference between the temperatures and packages tested, but the water content was reduced to 26.3% [14]. It is emphasized that these high germination values observed at 21 days are due to seeds that were not initially subjected to reduced water content levels, suggesting that the loss of seed viability observed in the present study is related to a reduction in initial water content (less than 21.5%) associated with the storage, which may have triggered damage at the cellular level in the embryo.

In addition to the percentage of seeds that exhibited primary root protrusion and the survival of normal seedlings, other features were indicative of the reduction of seed vigor due to the damage caused by the deterioration of the seeds during desiccation and storage, including seedling length and total dry mass.

The storage of seeds in different environments and water content levels intensified the process of deterioration, affecting seedling growth (length of primary roots and shoots). Similar results were observed with the seeds of *Euterpe espiritosantensis* Fernandes. The storage of seeds for 60 days at 10˚C, 15˚C, and 20˚C - 30˚C, with or without pulp, was detrimental to the length of the shoot and primary root [36]. Seeds of *Albizia hassleri* (Chod.) Burkart. are stored at room temperature (23.6˚C and 72.7% RH) or slightly cooler (17˚C and 69% RH) gradually decreases in the average lengths of shoots and roots were observed, indicating the loss of seed viability during storage (270 days) [37]. A reduction in root length values was also observed for seeds of *Tabebuia serratifolia* (Vahl.) Nich stored in polyethylene bags under laboratory conditions (27˚C ± 3˚C and 62% ± 2% RH) [38]. However, the storage of seeds of *C. adamantium* in different containers and at temperatures did not harm the final germination percentage; consequently, the lengths of shoots and roots may be associated with the elevated initial water content of the seeds [14].

The reduction in total seedling growth due to seed desiccation and storage was also accompanied by the decreased accumulation of the total dry mass. These results provide evidence that the damage caused by deterioration is not restricted to the onset of germination; the final stages of formation can also be affected, as indicated by similar data observed for *Myrciaria dubia* (HBK) McVaugh seedlings stored at 10˚C [30].

Additional studies should be conducted to reduce the sensitivity of the seed to desiccation, making it possible to preserve seed viability and maintain germplasm banks.

5. Conclusion

C. adamantium seeds tolerated a reduction in the water content to 15.3% but did not tolerate the storage period, confirming the recalcitrant behavior of the seeds.

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