CONSERVATION OF *Campomanesia adamantium* (CAMB.) O. BERG SEEDS IN DIFFERENT PACKAGING AND AT VARIED TEMPERATURES[1]

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ABSTRACT –This article aims at evaluating the effects of different packaging and varied storage temperatures on the germination potential of seeds of *Campomanesia adamantium* Camb. O. Berg. The seeds were packaged in glass, aluminum foil and plastic containers, or maintained inside intact fruits at 5, 10 and 15 ºC during 0, 7, 14 and 21 days. After these periods the seeds were sown in Germitest® germination paper and maintained in incubation chambers at 25 ºC under constant white light for 42 days. Seed moisture contents were evaluated both before and after storage, as well as germination percentages, germination speed index, root and aerial portion of seedlings lengths, and total dry weights. All possible combinations of packing materials, temperatures and storage times were tested, with four repetitions of 25 seeds for each treatment. *C. adamantium* seeds showed initial water contents of 31.5%. Glass and aluminum packaging were efficient at maintaining the water content of the seeds, and provided greater germination speed index than the other packaging materials. Germination percentages, seedlings lengths and dry weights did not vary among the different temperatures tested. *C. adamantium* seeds can be stored for up to 21 days at temperatures between 5 and 15 ºC without altering their physiological quality. In terms of cost-benefit efficiencies, these seeds can be stored without significant damage for 21 days while still inside the fruits at temperatures of 5, 10 or 15 ºC.
Index terms: guavira; Brazilian savannah; native fruit; Myrtaceae; seed longevity.

CONSERVAÇÃO DE SEMENTES DE *Campomanesia adamantium* (CAMB.) O. BERG EM DIFERENTES EMBALAGENS E TEMPERATURAS

RESUMO – Objetivou-se com este trabalho avaliar o efeito de diferentes embalagens e temperaturas durante o armazenamento sobre o potencial de germinação das sementes de *Campomanesia adamantium* Camb. O. Berg. As sementes foram mantidas em embalagens de vidro, papel de alumínio, plástico e no interior do fruto, nas temperaturas de 5, 10 e 15 ºC, durante zero, sete, quatorze e 21 dias. Após esse período, as sementes foram semeadas em rolo de papel Germitest® e mantidas em B.O.D. a 25 ºC e luz branca constante durante 42 dias. Antes e após o armazenamento foram avaliados o teor de água das sementes, porcentagem e índice de velocidade de germinação, comprimento de raiz, parte aérea e total e a massa seca das plântulas. Todas as combinações de embalagens, temperaturas e tempos de armazenamento foram testadas com quatro repetições de 25 sementes para cada tratamento. Sementes de *C. adamantium* apresentam 31,5% de teor de água após o processamento. As embalagens de vidro e de alumínio foram eficientes para manter o teor de água das sementes, proporcionando maior velocidade de germinação em relação aos demais ambientes. A porcentagem de germinação, comprimento e massa seca de plântulas não variaram entre as temperaturas testadas. Sementes de *C. adamantium* podem ser armazenadas por até 21 dias nas temperaturas entre 5 e 15 ºC sem prejuízo para a qualidade fisiológica. Considerando-se o custo-benefício, as sementes podem ser armazenadas até 21 dias no interior do fruto em temperaturas de 5, 10 ou 15 ºC.
Termos para indexação: guavira, Cerrado, fruta nativa, Myrtaceae, longevidade de sementes.

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INTRODUCTION

The harvest periods of seeds rarely coincide with ideal planting times, and seed quality is influenced by storage during the period between harvesting and sowing. As such, considerable effort has been directed towards maintaining their physiological quality and prolonging their longevity (including monitoring their water content and controlling environmental temperatures and storage conditions) (NAGEL; BÖRNER, 2010).

Oliveira et al. (2011a) and Silva et al. (2011) reported that adequate storage can maintain higher seed viability and vigor than would normally be possible under natural conditions, and the types of packaging materials used, the temperatures and relative humidity of the storage environments play important roles in maintaining seed physiological quality.

Oliveira et al. (2011b) observed that some species of native Brazilian fruits, the genus *Campomanesia* (MYRTACEAE), have significant commercial potential due to their desirable agroeconomic characteristics such as high productivity and high brix values. These fruits can be consumed in the form of liquors, ice creams, jellies and jams, *in natura*, and used for flavor production – in addition to their medicinal value (as they are rich in vitamins).

Campomanesia adamantium, also known as "guavira", can be found in the states of São Paulo and Mato Grosso do Sul in Brazil and is recommended for revegetation projects in the *Cerrado areas* (Brazilian savannah). While the available data are still qualitative, there is evidence that the pleasant taste and aroma of these fruits and their high levels of vitamin C make them popular in the state of Mato Grosso do Sul, and that a tea made from their leaves is used in traditional folk medicines to treat intestinal problems and urinary tract infections (UTI) (COUTINHO et al., 2010).

Germination studies with *C. adamantium* seeds were undertaken by Carmona et al. (1994), Melchior et al. (2006), and Scalon et al. (2009) although their results, especially in terms of their ideal storage times, seed water contents, and the environmental conditions and packaging materials necessary to conserve their physiological quality are somewhat contradictory suggesting that the seeds are sensitive to desiccation and to storage procedures (ZAIDAN; CARREIRA, 2008).

As such, investigations on the ideal conditions for storing *C. adamantium* seeds will allow proceeding on improving techniques for seedling production that can provide healthy plantings with high production rates. Therefore, the present study evaluated the physiological quality of *C. adamantium* seeds stored under different environmental conditions in different packaging materials.

MATERIALS AND METHODS

The fruits of *C. adamantium* were collected in 2010 from 12 spontaneous plants growing on the Santa Madalena Farm (22º08'25" S x 55º08'17" W) in the municipality of Dourados, State of Mato Grosso do Sul (MS), Brazil.

The harvested fruits were taken to the Plant Nutrition and Metabolism Laboratory at the *Faculdade de Ciências Agrárias of the Universidade Federal da Grande Dourados* (UFGD), Dourados, MS, where the seeds were manually extracted by macerating the fruits in a fine mesh sieve under running water. The loose seeds were then placed in a single layer over paper towels at room temperature (26 ºC ± 1 °C and 60% RH) to drain off the water excess. The water contents of the seeds were determined by drying them at 105 ± 3 ºC for 24 hours (with four repetitions of 10 seeds each); the results were expressed as percentages.

Tests of storage conditions used seeds that were allowed to remain inside intact fruits without any packaging (10 fruits) as well as processed seeds that were divided into three subsamples and stored in the following packaging materials: 1) hermetically sealed screw-top glass containers; 2) aluminum foil; and 3) transparent plastic bags (0.25 mm thickness). The fruits and seeds were then placed in B.O.D. (Biochemical Oxygen Demand) chambers with 85% RH at 5 º, 10 º and 15 ºC for periods of 0, 7, 14 and 21 days.

The water contents of the seeds after the different storage periods were determined as described above, while other seeds from the same groups were sown onto Germitest® germination paper (three layers of paper humidified with distilled water in quantities equivalent to 2.5 times the weight of the dry paper). The paper rolls were then placed in plastic bags and maintained in B.O.D at 25 ºC under constant white light. The paper humidity was accomplished and it was re-established when necessary.

The effects of the different types of packaging, storage temperatures and storage periods on the physiological potential of the seeds were evaluated by examining their seedlings 42 days after sowing in terms of the following characteristics: **Germination** - evaluating the formation of normal seedlings exhibiting all of the anatomical structures essential for development. **Germination speed index (GSI)** - calculated as cited by Ranal and Santana (2006). **Seedling growth** - 10 seedlings were chosen at random to evaluate their total lengths and the lengths of their primary roots and aerial portions using a millimeter graduated ruler; the results are presented as cm/seedling. To determine seedlings dry weight, the seedlings were dried in a forced-air oven at 65 ºC to a constant weight (as measured using an analytical balance); the results are presented as mg/seedling.

All possible combinations of packing material, temperatures, and storage times were tested, using four repetitions with 25 seeds for each treatment a randomized casualized delineament. The data were analyzed for inconsistency; and as it was pertinent, the averages related to packing type and temperatures were compared using Tukey Test; the averages related to storage times were adjusted using regression equations. Both types of calculation were made using SANEST® statistical software at a 5% level of significance.

RESULTS AND DISCUSSION

No significant interactions were observed between temperatures, packaging types, and storage times for any of the characters evaluated. *Campomanesia adamantium* seeds had 31.5% water contents immediately after extraction from the fruits. Similar results (30%) were reported by Melchior et al. (2006) after fermentation removal of the mucilage covering these seeds. Dousseau et al. (2011) reported a 35% water content of *C. pubescens* seeds after extraction from the fruits and superficial drying.

Significant variations were observed in seeds water content among the three storage temperatures and different packaging materials tested (Table 1). Seeds that remained within the fresh fruits at 15 ºC had the lowest water contents (25.6%), indicating that this higher temperature did not conserve the fruits very well nor maintain the water contents of the seeds. Seeds maintained within the fruits showed reduced moisture contents at 10 and 15 ºC, while those stored in plastic bags showed reduced moisture contents at 5 ºC.

Cisneiros et al. (2003) observed that packaging materials and the temperatures and relative humidities of the storage facility will directly influence the seeds water contents. The results of the present study indicated that glass and aluminum recipients were efficient in maintaining the water contents of *C. adamantium* seeds at all three temperatures evaluated – acting as impermeable barriers to gas exchange with the environment and thus minimizing water losses (Figure 1A). Similar results were reported by Melchior et al. (2006), who recommended storing *C. adamantium* seeds at humidity levels less than 30% in glass recipients at 25ºC. According to Martins et al. (2009), the efficiency of packaging in maintaining the original seed moisture content supports the treatments identity related with seeds moisture content and allows for reliable comparisons between different storage treatments.

The storage of intact fruits in plastic bags, however, resulted in marked oscillations in seed water contents during the different storage periods (Figure 1A). It was observed that after 21 days of storage at 5 and 15 ºC seed moisture contents were reduced, probably due to their adjustment to environment conditions, while seed stored at 10 ºC demonstrated high water contents at the end of these same storage periods (Figure 1B). It is important to note that storage conditions that can maintain seed physiological quality depend on intrinsic seed longevity and the humidity and temperature of the storage facilities (which must be able to reduce the biological activities of the seeds). Thus, stored seeds with water contents of 20 to 45% (as seen with *C. adamantium* seeds) tend to continue their biological processes, resulting in the warming of the seed lot due to respiratory activity.

No significant interactions were observed between the packaging materials and storage temperatures or any direct effects of these factors on seed germination. Maximum germination (93.2%) was observed after nine days of storage (Figure 2A). It is worth noting that the lowest recorded seed water content was 25.6%, but without any alteration in percent germination suggesting that all of the methods used for storing *C. adamantium* seeds were equally efficient within the time periods considered. Tershikh et al. (2008) reported that seeds will gradually deteriorate even under optimal storage conditions, thus reducing the quality of the stored lots. One of the major causes of seed deterioration during prolonged storage is lipid peroxidation, as free radicals attack the non-saturated fatty acids composing the phospholipid membranes, resulting in excessive losses of solutes when the seeds rehydrate. Additionally, these free radicals can attack other sub-cellular structures, including organelles, proteins and DNA.

Walters (2007) reported that low temperatures help maintain seed water contents and low metabolic levels – while environments with more elevated temperatures allow more rapid losses of seed viability by accelerating their metabolic reactions (and often by increasing their water contents). This author suggested that the association of low temperatures and impermeable packaging diminished cell metabolism, thus favoring seed longevity. Although the different packaging materials used here showed differential seed humidity values, these changes were not sufficient to alter their germinative potential, quite possibly because the storage temperatures used (between 5 and 15 ºC) were within the tolerance range of *C. adamantium* seeds for the storage periods evaluated.

There is considerable controversy in the literature concerning the storage tolerance of *C. adamantium* seeds. Melchior et al. (2006) reported a germination rate of 60% after storing seeds with water contents near 30% for 30 days in hermetically sealed glass containers at 25ºC. Scalon et al. (2009) reported that *C. adamantium* seeds had high germination levels three days after removal from the fruits, and that neither the cleaning processes nor the incubation temperatures used influenced germination – although they observed that seeds sown nine days after removal from the fruits showed both reduced germination percentages and germination velocities; these authors did not, however, indicate the initial water contents of the seeds. Dresch et al. (2012) demonstrated that *C. adamantium* showed low longevity and high sensibilities to desiccation (27%) and to storage at low temperatures.

Similar results were observed in the present study in relation to the germination speed index of the seeds stored in different packaging materials. The water contents (Figure 1A) and GSI (Figure 2B) of seeds stored in glass containers or in aluminum indicated that there was a direct relationship between these parameters, in that the packaging materials that maintained the highest seed water contents also had the greatest GSI (after storage for 21 days). Seeds that were maintained within intact fruits in plastic bags demonstrated greater water losses during storage and had lower GSI.

There was a gradual increase in the speed of seed germination until the 14th day after sowing, followed by decreasing speed until the end of the evaluation period, with little differences compared to seed groups stored under different conditions (Figure 2C). It should be noted that all of the experimental conditions, independent of the type of packaging used or the storage environments, stimulated increases in the GSI of the seeds, apparently by slowing physiological and/or biochemical alterations typical of deterioration processes that would otherwise compromise seed vigor and survival.

Root length and total seedling length were stimulated by storage for up to 14 days (Figure 3A), with total length reaching its maximum value between the seventh and the 14th day of storage (maximum 13.02 cm after 12 days of storage).

The average length of the seedling radicle was 8.46 cm after approximately 13 days of storage. Growth of the aerial portion of the seedling did not significantly vary as a function of storage time, maintaining an average length of 4.60 cm for 21 days. Hypocotyl lengths demonstrated variations during the different storage periods, with a decrease of 6.0×10^{-4} cm near the third day of storage (Figure 3A).

Seedlings dry weights increased independent of the packaging material used up until the 14th day of storage (Figure 3B). It was noted that although seeds stored within the intact fruits had the lowest water contents, the dry weights of their seedlings increased until the 21st day of storage (0.062 mg). It is possible that the reduced metabolic activity of these seeds due to the reductions in their water contents during storage resulted in less metabolic reserve consumption and greater subsequent translocation of these nutrients to the growing embryonic axis. Seedlings provided from seeds maintained in all of the other packaging materials until the end of the 21-day storage period had average dry weights of 0.047 mg $seedling^{-1}$ (Figure 3B).

The results of the present study demonstrated that the successful storage of *C. adamantium* seeds was possible under the experimental conditions evaluated, and that the low costs involved indicated these techniques as efficient strategies for seed conservation. It is suggested that future research be focused on testing packaging materials and storage conditions over longer periods of time to determine the behavior of *C. adamantium* seeds during prolonged storage.

TABLE 1 – Water content of Campomanesia adamantium seeds after storage in different packaging materials and at different temperatures for 21 days. UFGD, 2012.

	5 °C	10 °C	15 °C
Fruit	32,8 aA	29,4 bB	25,6 bC
Glass	32,5 aA	32,9 aA	31,6 aAB
Aluminun	32,2 aA	32,2 aA	32,6 aA
Plastic bag	28,8 aB	30,0 aA	29,3 aB
C V = 4,98%			

Means within same column (minuscule letter) and within same row (capital letter) do not differ significantly at P<0,05 (Tukey Test).

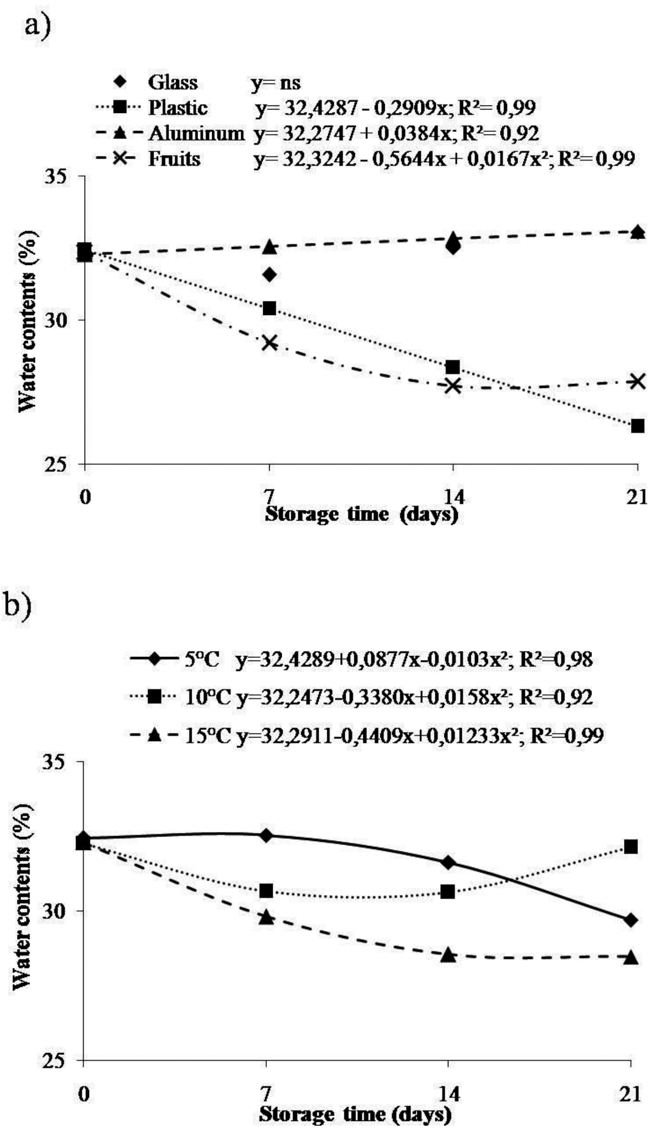

FIGURE 1 – Water content of *Campomanesia adamantium* seeds stored in different packaging materials (a) and at different temperatures (b).

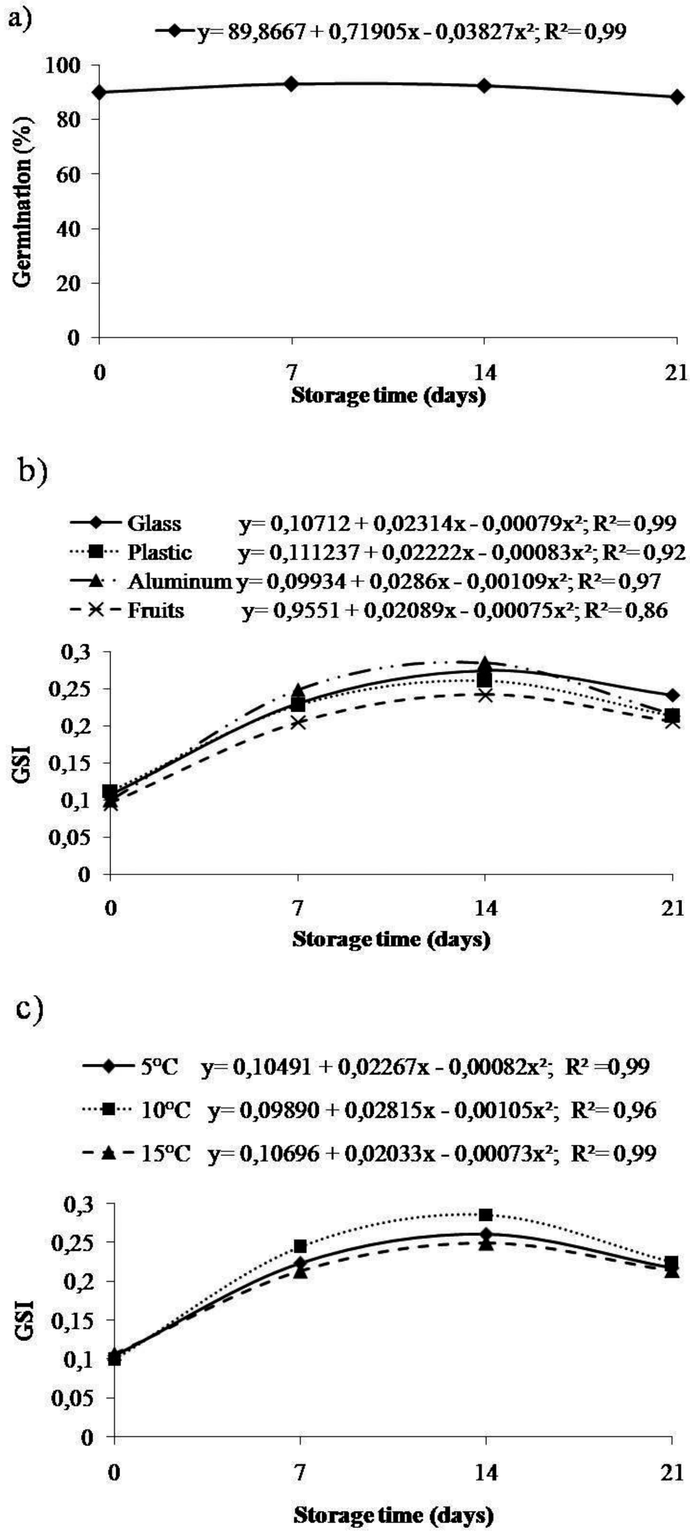

FIGURE 2 - Germination percentages of *Campomanesia adamantium* seeds (a); germination speed index of *C. adamantium* seeds stored in different packaging materials (b) and at different temperatures (c).

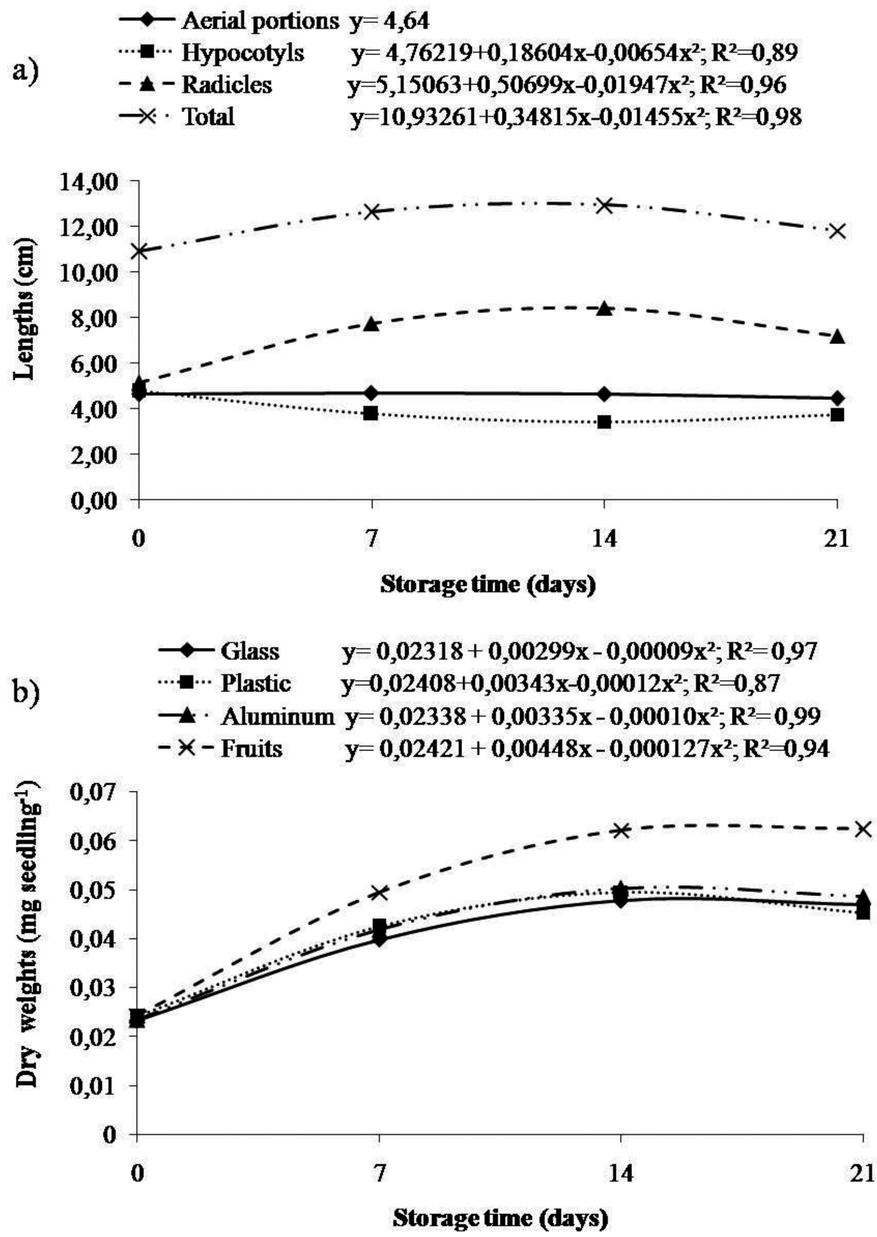

FIGURE 3 - Lengths of the aerial portions (PA), hypocotyls (Hyp), radicles (R), and total lengths (Total) of *Campomanesia adamantium* seedlings as a function of seed storage time (a) and dry weight of *C. adamantium* seedlings derived from seeds stored in different packaging materials (b). UFGD, 2012.

CONCLUSION

1-Glass and aluminum foil packaging maintained high seed water contents for up to 21 days.

2-Temperatures between 5 ºC and 15 ºC were efficient for seed storage and did not influence seed germination or seedling growth.

3-Storing *C. adamantium* seeds still within intact fruits in glass containers or aluminum foil packaging or within plastic bags maintained high physiological quality for at least 14 days.

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