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Reduced Sensitivity of *Campomanesia adamantium* (Cambess.) O. Berg Seeds to Desiccation: Effects of Polyethylene Glycol and Abscisic Acid

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Abstract

The *Campomanesia adamantium* is a threatened species from Brazil Savannah which seeds are desiccation-sensitive and do not withstand storage. This study aimed to reduce the sensitivity of *Campomanesia adamantium* seeds to desiccation using polyethylene glycol (PEG) and abscisic acid (ABA). Initially, seeds were subjected to PEG (0, −1.48, and −2.04 MPa) with or without ABA (100 μM) during 120 h, followed fast drying (silica gel) or slow drying (laboratory environment), at 20%, 15%, and 10% moisture content. In the second experiment, the seeds were PEG treated (−1.48 MPa) which provided the best results in the first experiment; the seeds were then subjected to different incubation times in PEG (30, 60, 90, or 120 h) and ABA (0, 10^{-3}, 10^{-4}, and 10^{-5} μM), following the seeds were fast dried at 15% moisture content. The slow drying should be avoided, even in seeds previously subjected to osmotic conditioning with or without ABA. Seeds submitted to PEG treatment (−1.48 MPa/120h) without ABA and PEG (−1.48 MPa) with 10^{-3} or 10^{-4} μM of ABA (90 h), followed by fast drying at 15% moisture content showed reduction of desiccation sensitivity and high germination and vigor when compared to the other treatments.

Keywords

Abscisic Acid, Osmotic Conditioning, Polyethylene Glycol, Water Stress

1. Introduction

There is a great consensus that for the continuous exploitation of tropical fruit

species in the future is necessary to increase the knowledge about the conservation of these seeds species. Many of these species produce seeds that are sensitive to desiccation and storage, and the lack of knowledge regarding longevity hinders their sustainable use and the maintenance of germplasm banks.

Campomanesia adamantium is popularly known in Brazil as "guavira" or "gabiroba" and it is native from Brazilian fields and savannahs from Distrito Federal, Goiás, Mato Grosso do Sul, Mato Grosso, Minas Gerais, São Paulo, Paraná and Santa Catarina [1] and is distributed from Venezuela, throughout the Amazon to Uruguay with its center of distribution in eastern Brazil [2]. Its fruits are appreciated by the local populations implying economical importance for the traditional farmers, especially between the October and December months when it occurs the fruits dispersion. Its propagation occurs via seeds, which show recalcitrant behavior and only tolerate 30 days storage if the water contents are reduced up to 15.3% [3].

The germination potential of *C. adamantium* seeds was directly correlated with the tolerable seeds moisture content associated with the drying method used; hence, fast drying (through silica gel) seeds beyond 21.1% moisture content and the slow drying (25˚C/35% UR) seeds beyond 17.2% moisture content injured the seeds vigor. The electrophoretic profile revealed that the RNA extracted from seeds was totally degraded following fast and slow drying at 4.5% and 5.4% moisture content, respectively [4].

According to the above mentioned, at the moment there is no technique to preserve the seeds viability or the genetic diversity of *C. adamantium* in seed banks. One possible approach for storing seeds that are intolerant to water content reductions is the osmotic way using polyethylene glycol (PEG) [5] with or without the addition of germination inhibitors, such as abscisic acid (ABA). During this, the germination process is induced by soaking seeds in water or in solutions containing exogenous molecules [6] controlling seed hydration up to a certain level.

ABA is directly or indirectly related to desiccation tolerance, and its synthesis is linked to seed maturation, as well as the stimulation of carbohydrate synthesis and gene expression that is related to desiccation tolerance [7] [8] [9] and evolved for cellular protection from water deficits [10]. Besides, exogenous ABA application in recalcitrant seeds results, for example in embryonic axes from the normally recalcitrant seeds of the silver maple can be made more tolerant to desiccation by pretreatment with ABA [11].

The effects of drying post-conditioned seeds depend on each species because they respond differently to dehydration. However, the success of conditioned seeds usually depends on the drying process because the water contents vary depending on the species and storage conditions. The slow-drying process induces viability loss at high water contents [12]. However, in desiccation-sensitive seeds, the faster drying occurs, the lower the water content is that they can tolerate because there is not enough time for the progress of the deleterious reaction effects that cause viability loss in the materials that are intolerant to desiccation [13].

Studies of the embryos of recalcitrant seeds of *Inga vera* Will. subsp. *affinis* (DC.) T.D. Pennington have shown that water mobilization control between the seed and the medium provides embryo germination rates that are greater than 80% after 90 days of storage at 10˚C when conditioned in solutions of PEG (−2.4 MPa) [14]. However, studies of *I. vera* embryos at different stages of maturation have reported that the drying of mature embryos at −4 MPa provides greater tolerance to temperature reductions up to water freezing levels (−2˚C), although no treatment results in tolerance to −18˚C [15].

The use of osmotic techniques allows for reduction sensitivity in some recalcitrant seeds to desiccation and/or increases their longevity [5], but generally the major efforts limits the evaluations concerning the primary root protrusion and do not evaluate the completeness normal seedlings. However, to ascertain the effectiveness of these techniques in *C. adamantium* the hypothesis of this paper was that *C. adamantium* seeds can be desiccated through the fast or slow drying to less than 15% moisture content with PEG and ABA so that they maintain a high germination rate and vigor. Thus, the aim of this work was to reduce the sensitivity of *C. adamantium* seeds to desiccation with PEG and ABA.

2. Material and Methods

Campomanesia adamantium ripe fruits were harvested from thirty matrix in areas of the Cerrado (*stricto sensu*), in the city of Ponta Porã-MS, Brazil. After harvesting, the fruits were brought to the Laboratory of Plant Nutrition and Metabolism at the Federal University of Grande Dourados (UFGD), in Dourados-MS, where they were washed with tap water and the damaged fruits were discarded. The fruits were then manually processed using sieves to isolate seeds from the fruit waste.

In the first experiment, seeds from fruits that were collected in November 2014 underwent superficial drying (above filter paper) for 40 min at room temperature [25˚C ± 2˚C, 32% relative humidity (RH)]. After the drying, the seeds were incubated for 120 h in PEG 6000 at potentials of −1.48 and −2.04 MPa with or without ABA at a concentration of 100 μM and kept in B.O.D. (Biochemical Oxygen Demand) at 25˚C. The control treatment did not involve incubation with PEG or ABA, and the seeds were kept for 120 h in a plastic bag at laboratory environment (25˚C ± 2˚C and 35% RH). After seed removal from the osmotic conditioning, the seeds were washed in running water for 5 min to remove the PEG solution and surface dried on paper towels for 10 min at laboratory environment (25˚C ± 2˚C, 35% RH monitored with thermo-hygrometer). Then they were dried in activated silica gel (8% RH) (fast drying) or in the laboratory environment (slow drying).

For fast dehydration, the seeds were placed on a steel screen inside closed germination plastic boxes (“gerbox”) with silica gel at the bottom during hours. The silica gel was replaced as soon as it lost its indicative blue coloration. For slow dehydration, the seeds were placed inside open plastic containers at 25˚C ±

2˚C and 35% RH. The seeds were then weighed each hour until they achieved predetermined water contents such as 20%, 15% and 10%, respectively after 8 h, 13 h and 23 h through the fast drying; and after 12 h, 22 h and 30 h, respectively through the slow drying.

In the second experiment, the seeds were processed and subsequently submitted during 120 h to the best concentration of PEG (−1.48 MPa) that was obtained in the first experiment, and were kept in B.O.D. at 25˚C. After seed removal from the osmotic conditioning, we proceeded to wash them in running water for 5 min to remove the conditioning solution and surface-dried them on paper towels for 10 min at room temperature (25˚C ± 2˚C, 32% RH). Later, the seeds were dried in the best drying setup that was obtained in the first experiment (fast/15% water content).

In both experiments, after drying the seeds were pre-humidified at 100% RH and 25˚C under constant white light for 24 h in order to avoid damage by imbibition. The following characteristics were determined in order to assess physiological potential.

Water content: was determined at 105˚C ± 3˚C for 24 h [16] with three replicates of 5 g of seeds each, and the results were expressed on a wet basis.

Imbibition curve: the seeds were placed in 4-cm-tall plastic cups with a 5-cm diameter on a double layer of Germitest® moistened paper with 1 mL of the following solutions according to the treatment conditions: 1) distilled water; 2) PEG (−1.48 MPa); 3) PEG (−1.48 MPa) + ABA (100 μM); 4) PEG (−2.01 MPa); and 5) PEG (−2.01 MPa) + ABA. Two replicates with six seeds were used for each treatment. The imbibition was assessed hourly during the first eight hours and every 24 h thereafter up to 144 h. The seeds subjected to conditioning were washed in running water before being weighed in order to remove the PEG solution.

Primary root protrusion: was measured on paper rolls with four replications of 25 seeds each, germinated at B.O.D. 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 (%). *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 shoots and root system development as the criteria. The results were expressed in percentages (%).

Germination speed index (*GSI*): was calculated according to [17].

Seedling length: was obtained by measuring the lengths of the primary root and aerial parts using a millimeter ruler. The results were expressed in centimeters (cm).

In both experiments, the design was completely randomized. In the first experiment, the data were subjected to analysis of variance with mean comparisons by the Scott-Knott test with 5% probability. The second experiment was conducted in a factorial scheme (four imbibitions periods × four ABA concentra-

tions), and the data were subjected to a regression analysis with 5% probability. In both experiments, it was used the SISVAR software and Microsoft Office Excel. The drying curve and water content data were presented as mean ± standard deviation.

3. Results and Discussion

In the first experiment, the imbibition curve showed a gradual increase in the mass and water content values of *C. adamantium* seeds that were imbibed in distilled water (**Figure 1(a)** and **Figure 1(b)**). After 120 h of imbibition, the seeds showed primary root protrusion.

Osmotic conditioning with PEG treatments of −1.48 MPa (with and without ABA) and −2.01 MPa (with and without ABA) provided a reduction in the mass and water contents of the seeds due to the slow dehydration that was caused by the treatments (PEG) during the imbibition period (**Figure 1(b)**). The initial water content before immersion was 52%, and, after 144 h, these values decreased to 43%, 41%, 37%, and 38% in response to treatments of −1.48 MPa (with and without ABA) and −2.01 MPa (with and without ABA), respectively (**Figure 1(b)**).

C. adamantium seeds imbibed in distilled water showed initial germination stages (phases I, II, and III) (**Figure 1(a)**) that corresponded to the standard tri-phase [18]. However, this behavior was not observed in seeds imbibed in PEG with or without ABA, and these treatments produced a gradual and slow reduction of water content after 120 h of imbibitions (**Figure 1(b)**). Seed incubation in PEG regulated the amount of absorbed water by providing conditions for the development of the early germination stages (phases I and II), but without reaching stage III, which corresponded to radicle protrusion [18].

According to the fast drying, the highest values of the primary root protrusion were observed in seeds that were imbibed in PEG (−2.01 MPa) with and without ABA and subsequently dried to 20% water content (80% and 77%, respectively) and seeds that were imbibed in PEG (−1.48 MPa) without ABA and PEG (−2.01 MPa) with ABA and then subjected to drying, till the seeds reached a water content of 15% (85% and 82%, respectively) (**Table 1**). The osmotic conditioning in PEG (−1.48 and −2.01 MPa) and subsequent fast drying to 10% water content provided a marked reduction in primary root protrusion, which made normal seedling formation completely unviable.

Imbibition seeds in osmotic potential of −1.48 MPa without ABA and subsequent drying to 15% water content provided higher percentages of normal seedlings (84%) compared to the other treatments. It is important to note that seeds submitted to the osmotic stress with PEG (−1.48 MPa) in the absence of ABA and dried at 15% moisture content showed high root protrusion (85%) besides elevated appearance of normal seedling (84%) (**Table 1**); nevertheless the seeds previously submitted to the PEG treatment but with higher concentration (−2.01 MPA) added ABA and dried at 15% moisture content also showed high

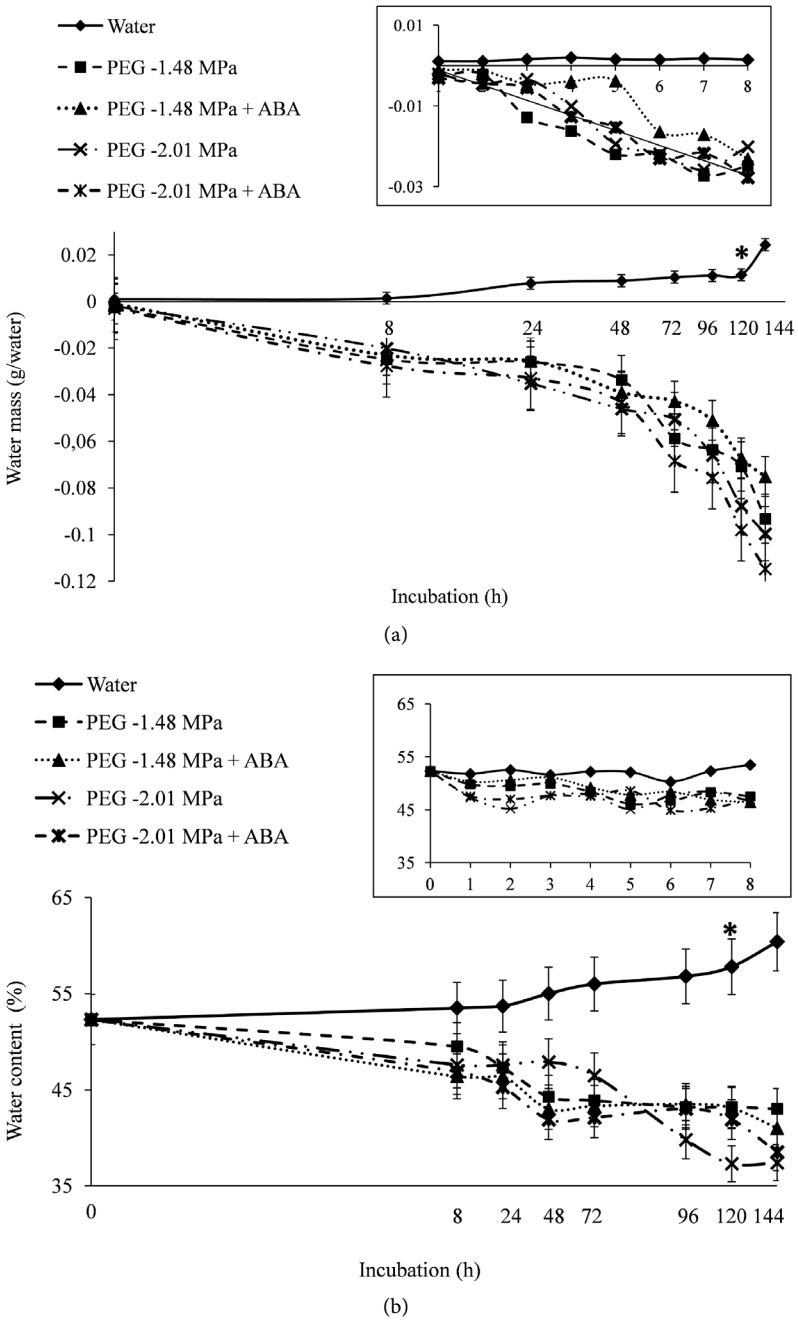

Figure 1. Imbibition curve (a) and water content (%) (b) of *Campomanesia adamantium* seeds submitted to treatments of polyethylene glycol (PEG) −1.48 and −2.01 MPa and associated (+) or not with abscisic acid (10^{-4} μM) (ABA) and control (water) during imbibitions. Bars indicate the standard deviation of the means. (*) Protrusion root primary.

root protrusion (82%), but this performance did not verified in the continuance of the seedlings development that was negatively affected (69%) (Table 1). ABA is a plant hormone found to regulate the acquisition of desiccation tolerance during seed maturation [9], but, possibly, under marked osmotic stress conditions, ABA exogenous may have acted as environmental response signal that

Table 1. Primary radicle protrusion (PRP) (%), percentage of normal seedlings (NS) (%), germination speed index (GSI), length of aerial parts (LAP) (cm), and length of roots (LR) (cm) of *Campomanesia adamantium* submitted to treatments of polyethylene glycol (PEG) −1.48 and −2.01 MPa and associated (+) or not with (−) abscisic acid (10^{-4} µM) (ABA) during imbibition and subsequent drying in silica gel (fast) at different water contents (WC).

Treatments			Variables				
WC	PEG	ABA	PRP	NS	GSI	LAP	LR
20%	0.00	(−)	66*± 6.9[1] b	63 ± 5.7 b	1.236 ± 1.23 a	3.50 ± 0.24 a	6.24 ± 0.56 a
	−1.48	(−)	48 ± 5.2 c	35 ± 3.4 c	0.580 ± 0.02 c	2.32 ± 0.10 d	4.58 ± 0.52 b
		(+)	62 ± 4.5 b	21 ± 2.5 d	1.124 ± 0.02 a	2.40 ± 0.09 d	1.76 ± 0.13 c
	−2.01	(−)	80 ± 2.8 a	72 ± 3.7 b	1.199 ± 0.03 a	3.79 ± 0.10 a	7.16 ± 0.21 a
		(+)	77 ± 5.0 a	70 ± 2.6 b	1.296 ± 0.08 a	3.70 ± 0.22 a	7.58 ± 0.29 a
15%	0.00	(−)	51 ± 3.0 c	16 ± 1.6 d	0.851 ± 0.06 b	2.52 ± 0.24 d	1.98 ± 0.25 c
	−1.48	(−)	85 ± 1.0 a	84 ± 3.3 a	1.383 ± 0.05 a	3.56 ± 0.16 a	8.29 ± 0.57 a
		(+)	37 ± 4.1 d	35 ± 3.4 c	0.470 ± 0.06 c	2.85 ± 0.09 d	4.14 ± 0.50 b
	−2.01	(−)	41 ± 3.0 d	34 ± 3.4 c	0.500 ± 0.04 c	3.34 ± 0.22 a	6.57 ± 1.37 a
		(+)	82 ± 2. 0 a	69 ± 3.4 b	1.313 ± 0.02 a	3.40 ± 0.05 a	5.59 ± 0.62 b
10%	0.00	(−)	50 ± 1.2 c	38 ± 1.2 c	0.856 ± 0.05 b	3.63 ± 0.18 a	5.59 ± 0.62 b
	−1.48	(−)	30 ± 4.8 e	20 ± 0.0 d	0.532 ± 0.08 c	1.99 ± 0.12 c	3.05 ±0.16 c
		(+)	23 ± 7.5 e	20 ± 0.0 e	0.435 ± 0.12 c	1.99 ± 0.12 c	3.05 ± 0.16 c
	−2.01	(−)	2 ± 1.2 f	0 ± 0.0 e	0.027 ± 0.02 d	0.00 ± 0.00 d	0.00 ± 0.00 d
		(+)	2 ± 1.2 f	0 ± 0.0 e	0.050 ± 0.03 d	0.00 ± 0.00 d	0.00 ± 0.00 d
CV[2]			16.0	14.7	15.3	11.5	22.7

*Means followed by the same letter in the columns do not differ significantly by the Scott-Knott test ($p \leq 0.05$), [(1)]Standard error and [(2)]coefficient of variation (CV).

relieves the stressful condition to a certain extent. Accordingly, for the *C. adamantium* seeds there are an association between the osmotic treatments techniques and the moisture content tolerate by the seeds that provides the resumption of vital functions of the seed and subsequent formation of the essential structures of the seedlings.

The germination speed index (GSI) exhibited better results in seeds imbibed in osmotic treatments of PEG (−1.48 MPa) with ABA (1.1242) and PEG (−2.01 MPa) with and without ABA (1.1995 and 1.2962, respectively) and subsequent drying to a 20% water content and PEG (−1.48 MPa) without ABA (1.3838) and PEG (−2.01 MPa) with ABA (1.3184) and further drying to a 15% water content (Table 1).

Aerial parts length had the largest growth outcomes in the seeds that were subjected to fast drying to levels of 20%, 15%, and 10% water contents, imbibed

in PEG (−2.01 MPa) with and without ABA, desiccated to levels of 20% and 15%, imbibed in PEG (−1.48 MPa) without ABA, and desiccated to a 15% water content (Table 1). This oscillation in the results was also observed in the primary root length, whereas seedlings from seeds that were imbibed in PEG (−1.48 MPa) without ABA and subjected to fast drying to 15% provided the largest increases; nevertheless, they did not differ significantly from untreated seeds and were dried to a 20% water content. The results showed that even seeds that were previously treated with PEG with or without ABA and subjected to fast drying at 10% had the lowest growth rates of aerial parts and roots, indicating the seeds sensitivity to decrease of the water content.

The percentage of primary root protrusion, normal seedlings, and the GSI had higher values in seeds that were not previously treated with PEG and subsequently dried (slow drying) at 20% water content (83%, 71%, and 1.547%, respectively) (Table 2). The same behavior was observed for aerial parts length and

Table 2. Primary radicle protrusion (PRP) (%), percentage of normal seedlings (NS) germination speed index (GSI), length of aerial parts (LAP) (cm), and length of roots (LR) (cm) of *Campomanesia adamantium* submitted to treatments of polyethylene glycol (PEG) −1.48 and −2.01 MPa and associated (+) or not with (−) abscisic acid (10^{-4} μM) (ABA) during imbibition and subsequent drying in the laboratory environment (slow) at different water contents (WC).

Treatments			Variables				
WC	PEG	ABA	PRP	NS	GSI	LAP	LR
20%	0.00	(−)	83* ± 1.0[1] a	71±3.0 a	1.547 ± 0.03 a	3.69 ± 0.09 a	7.58 ± 1.07 a
	−1.48	(−)	58 ± 1.2 b	44 ± 4.3 b	0.820 ± 0.02 b	2.81 ± 0.10 b	5.04 ± 0.63 b
		(+)	46 ± 2.0 c	34 ± 5.2 d	0.909 ± 0.06 b	2.56 ± 0.03 b	1.95 ± 0.40 c
	−2.01	(−)	57 ± 3.0 b	12 ± 3.3 e	0.787 ± 0.06 b	2.19 ± 0.13 c	1.19 ± 0.04 d
		(+)	60 ± 0.0 b	45 ± 2.5 b	0.887 ± 0.01 b	2.82 ± 0.16 b	4.17 ± 0.29 b
15%	0.00	(−)	61 ± 3.0 b	43 ± 7.0 b	0.906 ± 0.03 b	2.84 ± 0.16 b	4.22 ± 0.44 b
	−1.48	(−)	46 ± 5.0 c	33 ± 3.8 c	0.813 ± 0.04 b	2.52 ± 0.10 b	2.80 ± 0.28 c
		(+)	26 ± 1.2 e	20 ± 0.0 e	0.306 ± 0.01 d	2.27 ± 0.03 c	2.81 ± 0.04 c
	−2.01	(−)	36 ± 0.0 d	28 ± 0.0 d	0.618 ± 0.04 c	2.62 ± 0.20 b	2.62 ± 0.31 c
		(+)	16 ± 4.0 f	0 ± 0.0 f	0.291 ± 0.06 d	0.00 ± 0.00 d	0.00 ± 0.00 d
10%	0.00	(−)	38 ± 4.2 d	17 ± 1.9 e	0.562 ± 0.07 c	2.37 ± 0.03 c	1.95 ± 0.05 d
	−1.48	(−)	24 ± 4.9 e	15 ± 1.0 e	0.390 ± 0.06 d	2.55 ± 0.19 b	3.24 ± 0.66 c
		(+)	32 ± 4.6 d	18 ± 3.5 e	0.584 ± 0.07 c	2.45 ± 0.14 c	1.94 ± 0.03 c
	−2.01	(−)	33 ± 3.8 d	34 ± 0.8 d	0.476 ± 0.04 c	2.54 ± 0.04 b	3.72 ± 0.31 b
		(+)	12 ± 1.6 f	15 ± 0.9 e	0.159 ± 0.03 e	2.08 ± 0.10 c	3.79± 0.66 b
CV[2]			14.9	22.3	14.0	9.6	29.0

*Means followed by the same letter in the columns do not differ significantly by the Scott-Knott test ($p \leq 0.05$), [(1)]Standard error and [(2)]coefficient of variation (CV).

primary roots in seedlings from seeds that were not subjected to osmotic treatments and slowly desiccated to 20% water content which showed the highest growth (3.69 cm and 7.58 cm, respectively).

The seeds that were subjected to osmotic conditioning initially showed 40.5% water content. After different imbibitions times, reductions were observed in the water levels, with the most expressive slow dehydration occurring after 120 h of imbibition in different concentrations of ABA (**Figure 2(a)**). There was a significant interaction between the ABA concentration and the imbibition time with root protrusion, the percentage of normal seedlings, the GSI, and the aerial parts length (**Figures 2(b)-(d)** and **Figure 3(a)**).

Seeds that were imbibed in PEG with ABA at a concentration of 10^{-3} µM showed a maximum value of primary root protrusion of 99% (77 h); however, for concentrations of 10^{-4} and 10^{-5} µM of ABA, there were no significant results in the mean values of root protrusion of 96% and 95%, respectively, between the imbibitions times (**Figure 2(b)**). The percentage of normal seedlings and the GSI exhibited the highest values in response to the concentrations of ABA of

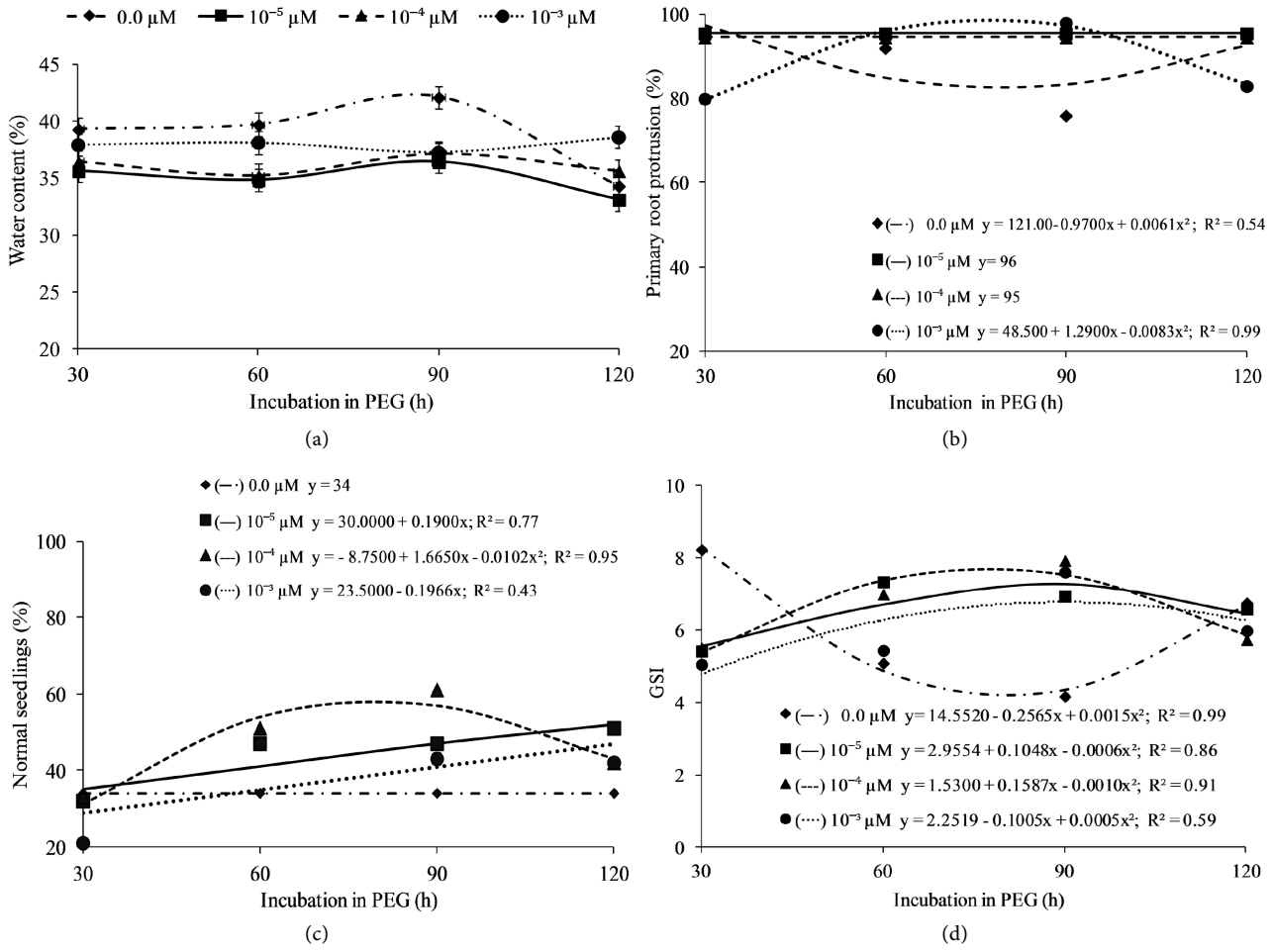

Figure 2. Water content (%) (a), primary root protrusion (%) (b), percentage of normal seedlings (%) (c), and germination speed index (GSI) (d) of *Campomanesia adamantium* from seeds conditioned with polyethylene glycol (PEG −1.48 MPa) during incubation times (30, 60, 90, and 120 h) and ABA concentrations (0, 10^{-3}, 10^{-4}, and 10^{-5} µM).

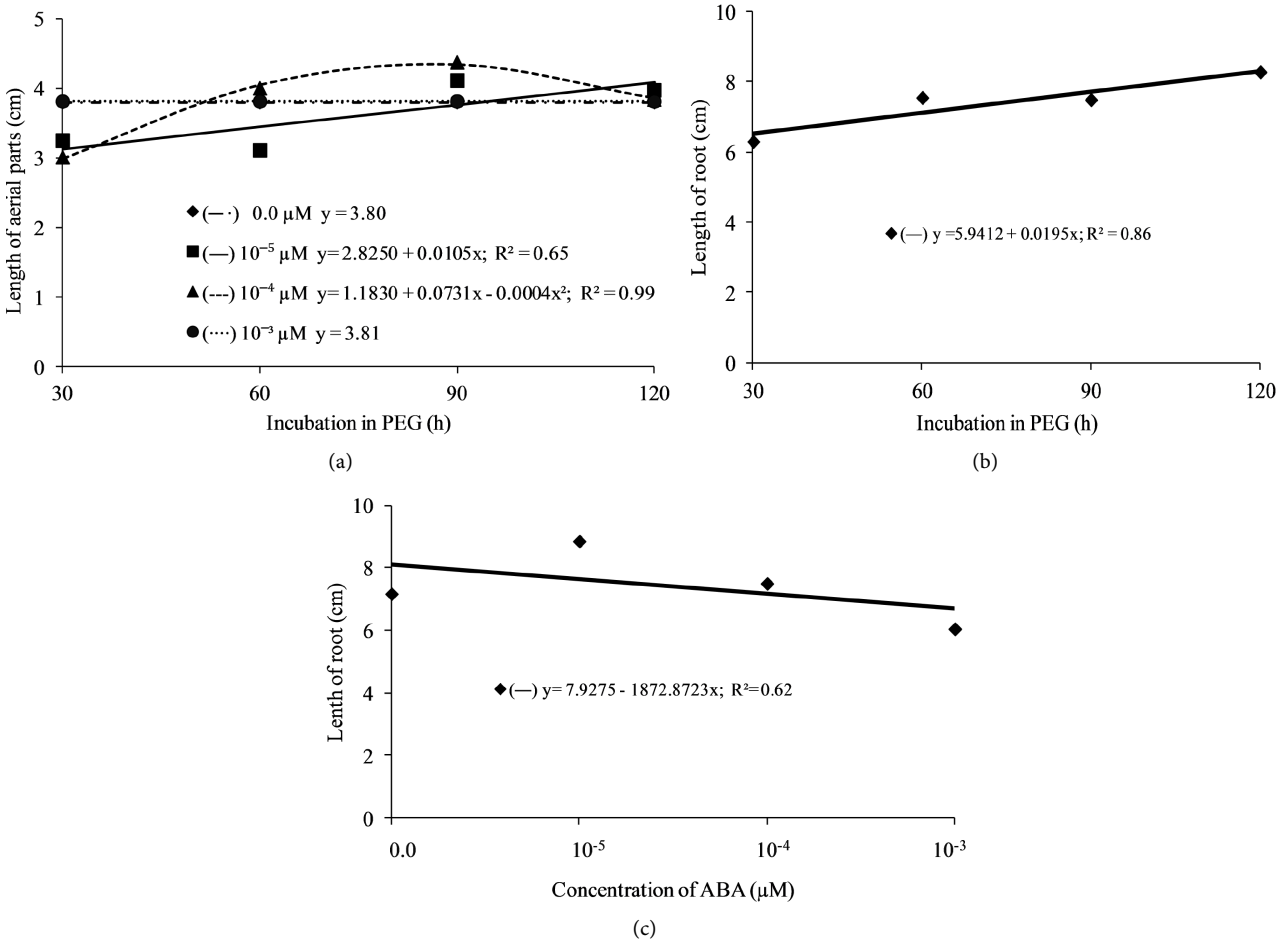

Figure 3. Length of aerial parts (cm) (a) and length of root (b) and (c) of *Campomanesia adamantium* seeds incubated in polyethylene glycol (PEG −1.48 MPa) during different incubation times (30, 60, 90, and 120 h) and ABA concentrations (0, 10^{-3}, 10^{-4}, and 10^{-5} µM).

10^{-4} µM (59% and 7.823% in response to imbibition periods of 82 and 79 h, respectively) and 10^{-5} µM (51% and 7.459% in response to imbibition periods of 120 and 83 h, respectively) (**Figure 2(c)** and **Figure 2(d)**).

The maximum growth in aerial parts length was observed at a concentration of ABA of 10^{-4} µM (4.53 cm) with 91 h of imbibition time, which was followed by 10^{-5} µM of ABA that resulted in linear growth over 120 h of imbibition (4.08 cm) (**Figure 3(a)**). For the primary root length the interactions between the concentrations of ABA and the imbibition time were not significant, and the factors were presented in isolation (**Figure 3(b)** and **Figure 3(c)**). The imbibition of seeds in increasing concentrations of ABA negatively affected root growth, with the lowest values observed at a concentration of 10^{-3} µM of ABA (6.06 cm) (**Figure 3(b)**). However, seeds that were imbibed up to 120 h resulted in greater shoot length (8.28 cm) (**Figure 3(c)**).

Imbibition seeds in an osmotic potential of −1.48 MPa without ABA and further drying them over silica gel (fast) to a 15% water content significantly increased normal seedlings compared to seeds that were directly submitted to

drying at 15% water content (**Table 1**). These results showed that the incubation period of 120 h in PEG without the exogenous ABA provided a slow and gradual dehydration-triggering protective mechanism to desiccation in the seeds. It has been reported that slow water loss can allow for protective changes not only in germinated seeds but also in the development of orthodox seeds [19], thus allowing subsequent endurance of the severe dehydration [20] that is provided by seed quick drying (in silica gel) at low moisture contents, such as 15%.

Studies have suggested that the stress that is caused by drought and decreased cell volume during desiccation induces the accumulation of ABA [21] [22]. In this way, many of the physiological and biochemical changes caused by ABA in developing embryos can be induced by low osmotic potential [18]. Otherwise osmotic treatment with PEG (−1.48 MPa) without ABA promoted the highest germination results (84%) and vigor of the *C. adamantium* seeds submitted to fast drying at 15% moisture content, indicating the positive effects of the osmotic treatment on reducing the seeds sensitivity to desiccation. The beneficial effects of PEG has been also observed in other sensitive desiccation organisms as germinated seeds of *Medicago truncatula*, confirming that osmotic stress led to slow (and limited) water loss by incubation in the PEG solution (−1.8 MPa), which resulted in the restoration of the desiccation tolerance and high rates of seedling root lengths up to 2 mm [20]. The incubation of seedlings of *Tabebuia impetiginosa* in PEG and ABA significantly increased the re-induction of DT, indicating the important role of ABA in this process [23]. In the germinated seeds of *Cedrela fissilis* treated with PEG (−2.04 MPa) and ABA (100 μM) for 72 h before dehydration was efficient in restoring desiccation tolerance in seeds with 1-mm-long radicles (100% survival) [24]; in germinated seeds of *Sesbania virgata* PEG treated was able to re-establish DT, at least partially, with 2, 3 and 4 mm but not in 5 mm radicle lengths [25].

Thus, the positive effects of PEG were evident in seeds dried to 15% moisture content through the fast drying; otherwise the seeds without osmotic treatment and subjected to the same water content showed only 16% germination (**Table 1**). With slow drying, it was not possible to reduce the seed sensitivity to desiccation, which was viable only up to 20% water content without being subjected to osmotic conditioning and ABA (**Table 2**). Possibly, slow drying after osmotic treatment resulted in an acceleration of the seed deterioration process, since slow dehydration and the beginning of metabolic activities occur during PEG incubation. These processes are induced with slow drying that occurs naturally for a long time, which consequently favors the occurrence of catabolic reactions. In addition, slow drying can promote increased protein maturation (heat resistant). However, these proteins are not capable by themselves of promoting desiccation tolerance and, hence, maintaining seed viability [26].

Based on these results, treatment of seeds with PEG (−1.48 MPa) without ABA and later dried in silica gel (fast) to a 15% water content was effective in reducing sensitivity to desiccation, which was not observed in seeds exposed to

slow drying (Table 2). A possible explanation for the different responses in the drying rates observed in the present study was that the fast drying provided a reduction in the time in which the seeds were exposed to deleterious processes during the drying period [27]. Nevertheless, it was evident that the possibility of reduced seed sensitivity of *C. adamantium* to desiccation was subject to osmotic treatment with PEG (−1.48 MPa), which was followed by fast drying up to a 15% water content.

Osmotic conditioning with PEG and 10^{-4} and 10^{-5} μM of ABA provided reduced sensitivity to desiccation as seen through superior normal seedlings compared to the treatments without the addition of ABA (Figure 2(c)). According to the data, we observed the best results with different imbibition times. However, based on the regression analysis of the different characteristics, the average incubation period of 90 h and further drying over silica gel to 15% water content was the imbibition time that was satisfactory to stimulate protective mechanisms against damage caused by desiccation (Figure 2(c)).

C. adamantium seeds submitted to slow dry at 15% moisture content showed 23% of normal seedlings after storage for a period of over 30 days [3]; and showed 30% of normal seedlings after fast dry at 15% moisture content [4] both without any osmotic treatments. Our results demonstrated that the use of PEG treatment (−1.48 MPa for 120 h) and PEG (−1.48 MPa + 10^{-3} or 10^{-4} μM ABA for a period of 90 h), followed by fast drying at 15% moisture content was effective for reducing the sensitivity of *C. adamantium* seeds to desiccation; it was evidenced not only by the high root protrusion, but mainly through the high production of normal seedlings that implies the complete resumption of the vital functions of the seed. However, slow drying should not be used, even in previously osmotic-conditioned seeds that were or not subjected to ABA.

4. Conclusions

The seeds of *C. adamantium* are desiccation-sensitive and it hinders the species germplasm maintenance in seed banks as strategies for the *ex situ* conservation. The present study found an alternative method to prolong the vital functions of the seeds through the treatment of the seeds with PEG (−1.48 MPa) without ABA and later dried in silica gel (fast) to a 15% water content, which was effective in reducing sensitivity to desiccation, since the seeds without osmotic treatment and subjected to the same water content did not withstand dehydration state or showed low germination percentage.

Besides, the results highlight the positive effects of fast drying after the seeds osmotic treatment to minimize the eventual damages caused by dehydration and reducing the desiccation sensitivity of *C. adamatium* seeds.

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