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Sucrose concentration alters the physiological potential of *in vitro* germinated gabiroba seeds

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ABSTRACT

The propagation of gabiroba occurs via sexual reproduction. However, their seeds are recalcitrant. The objective of this study was to evaluate the physiological quality of *Campomanesia adamantium* and *C. pubescens* seeds, sown in a growth medium with different sucrose concentrations, to understand the performance of these species on *in vitro* environment. An entirely randomized design was used in a 2x4 factorial scheme [2 species and sucrose concentrations (0, 20, 40 and 80 g L^{-1})]. The following were evaluated: germination, emergence, and growth parameters of seedlings. The presence of sucrose in the culture media did not improve the germination percentage. *C. adamantium* seeds germinated in supplemented culture media with up to 20 g L^{-1} showed higher emergence and vigor and considerable increase in the number and length of the roots. For *C. pubescens*, the culture media without the addition of sucrose provided better results.

Index terms: *Campomanesia adamantium; Campomanesia pubescens;* sexual reproduction; Myrtaceae.

INTRODUCTION

Campomanesia genus comprehends species known as gabirobeira or guabirobeira, which is distributed in the Midwest, North, Southeast, and South regions of Brazil, mostly in the Cerrado region. The genus includes 36 species (Govaerts et al., 2008; Sobral et al., 2016) with potential for the cosmetic and pharmaceutical industries, as well as for human food.

Despite the importance of these species, the harvest of the fruits occurs in an extractive way and live threatened due to intense deforestation for the introduction of agriculture and livestock, which can lead some species to near extinction. Therefore, germplasm conservation programs and the use of seeds for seedling production and vegetation recomposition require basic knowledge about the conditions necessary for storage, seed germination, and seedling establishment (Dresch et al., 2012). Studies show that the seeds of some *Campomanesia* species present recalcitrant behavior, not enduring desiccation and storage (Dousseau et al., 2011; Melchior et al., 2006). This makes seed analysis an essential tool in controlling the quality of these seeds, because the sum of genetic, physical, physiological, and sanitary attributes affects their performance.

The physiological quality of seeds has been routinely evaluated through germination tests that are conducted under optimal environmental conditions favoring the maximum germination potential (Marcos Filho, 2015). During the process of seed germination, using *in vitro* cultivation, the solutions of salts and sugars that compose the culture medium do not have a purely nutritive effect, but influence cell growth and morphogenesis through osmotic properties (George, 1996). The presence of sucrose in the extracellular environment, besides acting as a carbon source necessary for growth, can play a

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role in osmotic regulation of water stress causing a negative effect (Hilae; Te-Chato, 2005).

Some species require supplementation of the culture medium with sucrose, beneficially altering the physiological quality of the seedlings. Moreover, they can favor the maintenance of seedlings *in vitro* for a longer period of time, in order to be used in micropropagation or multiplication producing healthy seedlings, free of viruses, bacteria and fungi. However, according to Almeida (2013), depending on the species, there is no need for sucrose in the medium, especially in the early stages of the *in vitro* establishment of the plant.

This study aimed to evaluate the physiological quality of seeds of *Campomanesia adamantium* (Cambess.) O. Berg. and *Campomanesia pubescens* (DC.) O. Berg., sown in culture medium with different sucrose concentrations, in order to understand the performance of these species under *in vitro* conditions.

MATERIAL AND METHODS

Seeds of *C. adamantium* and *C. pubescens* were collected from thirty matrices of the germplasm bank of the Federal University of Jataí (17°56'13''S and 51°43'44''W, 620 m altitude). The climate of the region, according to Köeppen's classification (1948), is Cw, mesothermal, with dry and rainy seasons defined by the months of March to September and October to April, respectively. The exsiccates of the plant material are deposited in the Jataiense Herbarium, of that institution (5647, 5648, 5649, and 5650).

For both species, the fruits were harvested at full maturity stage and used right after harvest. The number of full and empty seeds per fruit was counted using 200 fruits of each species evaluated.

The water content in the seeds was determined using the oven method at 105 ± 3 ºC for 24 hours with four repetitions of 1 g. The result was expressed in percentage, based on the wet weight and dry weight of the seeds (Brasil, 2009).

The soaking curve was obtained using freshly harvested seeds with tegument isolated from the fruit, which were wrapped in a roll of paper moistened with 2.5 times the mass of the paper and placed in B.O.D. (Biochemical Oxygen Demand) at 27 ºC. Before the beginning of soaking and again at increasing every thirty minutes each intervals according to the water gain, the seeds were weighed (mg^{-1}) to determine the increment on the initial mass. The weighing was performed using a precision analytical balance (0.0001g). Four repetitions of ten seeds were made according to Justo et al. (2007).

To characterize the physiological quality of the seeds, the fruits were pulped, and the seeds remained in running water for 20 minutes and then were taken to the laminar flow chamber, where they were immersed in 70% alcohol for one minute and then transferred to sodium hypochlorite solution (1%) for seven minutes. After the process, they were washed three times with distilled and autoclaved water. The seeds were then inoculated in WPM culture medium (Lloyd; McCown, 1980) supplemented with 0, 20, 40, and 80 $g.L^{-1}$ sucrose and solidified with 0.7% (w/v) of agar. The pH of the culture environment was adjusted to 5.8 ± 0.1 before autoclaving at 120 °C for 20 minutes and 1 atm pressure. After inoculation, the seeds were maintained for 30 days in a growth room under irradiance of 36 µmol m^{-2} s^{-1}, photoperiod of 16 hours and temperature of 26 ± 1 ºC, and submitted to the following tests:

Radicle protrusion: percentage of *in vitro* seedlings that presented radicle protrusion, with approximately 2 mm in length;

Germination: for the percentage of normal seedlings, with all the essential structures, according to Brasil (2009);

Radicle protrusion speed index (RPSI) and germination speed index (GSI): calculated by the sum of the number of germinated and emerged seeds each day, divided by the number of days between sowing and germination or emergence, according to Maguire's formula (1962).

Number of leaves and number of roots: counting the number of leaves and roots presented in each seedling at 30 days of evaluation;

Seedling length: length of the primary root and the length of the primery aerial part of the seedlings, the results being expressed in millimeters (mm) by the use of a graduated measuring tape at the end of the thirty day period;

Total mass: obtained from the fresh weight of the seedlings and the dry weight in an incubator regulated at 105 ºC for 24 hours. The weighing was performed using a precision analytical balance (0.0001g) and the results expressed in mg $seedling^{-1}$ at the end of the thirty day period.

The data referring to the water content of the seeds were not submitted to statistical analysis. The seed soaking curve was adjusted to cubic equations. To evaluate the *in vitro* physiological quality of the seeds, the experiment was set up in an entirely randomized statistical design with five repetitions. Each repetition consisted of five test tubes with one seed. The treatments were arranged in a 2x4 factorial scheme: 2 species and 4 sucrose concentrations. The data related to the species were submitted to variance analysis using the F test, and the average values were compared using the Tukey test, at 5% probability level. The data related to sucrose concentrations were subjected to polynomial regression analysis, testing the linear and quadratic equations, being accepted the significant equations until 5% probability by the F test, with the highest determination coefficient (R2) and when there was interaction, the respective unfoldings were performed.

RESULTS AND DISCUSSION

The water content of the seeds of *C. adamantium* and *C. pubescens* without any type of storage were on average 62.3% and 58.74% respectively. These results are in agreement with Dresch et al. (2012) and Oliveira, Santana and Santos (2011), who found water contents of 57.00 and 54.98% for *C. adamantium*, respectively. For *C. pubescens*, similar values were also reported by Oliveira, Santana and Santos (2011). Recalcitrant seeds present high water content, as observed for the genus *Campomanesia*; moreover, they present intolerance to desiccation (Dousseau et al., 2011). Other Myrtaceae species also present sensitivity to desiccation, such as species of the *Eugenia* genus (Delgado; Barbedo, 2007).

The number of seeds per fruit averaged 3.25 for full seeds and 1.89 for empty, totaling an average of 5.14 seeds in *C. adamantium* fruits (Table 1). The minimum and maximum values of seeds per fruit, including full and empty, were two and eight. In this way, the number of full seeds (63.22%) represented most of the seeds in *C. adamantium* fruits. However, the fruits of *C. pubescens* presented a higher proportion of empty seeds (62.03%), being on average 1.93 and 3.15 the values found of full and empty seeds per fruit, respectively. The minimum and maximum number of seeds per fruit was three and eight seeds, with a total average of 5.08 (Table 1). As the flowers of the two species present similarities (Landrum, 1986), it was expected that a similar number of seeds would be maintained.

Landrum (1986) found *C. adamantium* fruits with one to four seeds. However, Oliveira, Santana and Santos (2011) reported that 48% of the fruits had only one seed. The average number of full seeds, reported by Melchior et al. (2006), in 42.8% of the fruits was 3.1 seeds, corroborating with the results observed in the present study. The number of full seeds described here also corroborates the results reported by Dresch et al. (2013), of 3.17 seeds in fruits of *C. adamantium*.

Table 1: Number of seeds per fruit of *Campomanesia adamantium* and *Campomanesia pubescens*.

Seed per fruit characteristic	Value				DP[1]	CV[2](%)
	Minimum	Avarage	Maximum	%		
Campomanesia adamantium						
Full seed	0	3.25	7	63.22	1.34	41.39
Empty seed	0	1.89	5	36.78	1.40	74.12
Seed Total	2	5.14	8	100	1.07	20.74
Campomanesia pubescens						
Full seed	1	1.93	4	37.97	0.93	48.03
Empty seed	0	3.15	6	62.03	1.35	42.73
Seed Total	3	5.08	8	100	1.08	21.27

[1] DP: Standard Deviation. [2] CV: Coefficient of variation.

The soaking curve of *C. adamantium* seeds (Figure 1a) had a constant and significant increase until 8 to 10 hours from the beginning of soaking (Phase I), reducing and stabilizing the increase in water content after 10 hours (Phase II). The seeds initiated Phase III of the germination process after 51 hours of soaking. This same three-phase model was also observed during *Eugenia pyriformis* germination (Justo et al., 2007), species from the same family. The soaking curve of *C. pubescens* (Figure 1b) followed the same triphasic pattern, with a substantial increase in weight gain (Phase I) in the first 9 hours, followed by a slow gain in mass (Phase II), and a slight acceleration in water gain after 46 hours, coinciding with the beginning of germination (Phase III), reaching a total mass increment of about 17%.

It is noteworthy that seeds of *C. adamantium* and *C. pubescens* present high initial germination percentage in the absence of sucrose (94%). Similar germination results were observed by Carmona, Rezende and Parente (1994), Melchior et al. (2006), Scalon et al. (2009), Oliveira, Santana and Santos (2011), Dresch et al. (2013) and Scalon et al. (2013), who obtained germination above 80% in an *ex vitro* environment, confirming the high germinability of *C. adamantium* and *C. pubescens* seeds, regardless the environment (*in vitro* or *ex vitro*) to which they are exposed.

The germination percentage decreased as seeds of *C. adamantium* and *C. pubescents* remained exposed to increasing sucrose concentrations in the culture medium, but for *C. adamantium* the decrease was more pronounced in concentrations higher than 20 g L^{-1} of sucrose, because the initial percentage found was 96% without sucrose (Figure 1c). Seeds of *C. pubescence* presented 64% of germination in this sucrose concentration at 20 g L^{-1}. Seeds inoculated in culture medium supplemented with 40 g L^{-1} of sucrose had a reduction in emergence of 44% and 65% for *C. adamantium* and *C. pubescens*, respectively. At the concentration of 80 g L^{-1} sucrose, the germination percentage was inferior to 10% for both species (Figure 1c). The presence of sucrose interfered in the osmotic regulation of the culture medium and, consequently, in the availability of water. The reduction in water content can generate a decrease in physiological quality of seeds, reducing vigor and percentage of emergence, as previously observed in *C. pubescens* (Dousseau et al., 2011).

During *in vitro* germination, no significant interaction was observed between the species and sucrose concentrations for the parameters root protrusion and number of leaves. For both species there was no significant difference in the percentage of root protrusion and the value found for both species was 95% (Figure 1d). Independent of the sucrose concentrations, the process of root protrusion was recorded from the fourth day, extending until the eighteenth day for *C. adamantium*; for *C. pubescent*, it started on the third day and continued until the fourteenth day (Figure 1d). The onset of normal seedling formation (Brasil, 2009) for *C. adamantium* was recorded on the thirteenth day and for *C. pubescent* on the fifteenth day.

In the culture medium without the addition of sucrose, the seeds of *C. pubescens* showed significantly higher RPSI (2,1) than *C. adamantium* (2,0). However, for both species, the RPSI decreased as the sucrose concentration in the culture medium increased (Figure 1d). Similar behavior was observed in the GSI (Figure 1e), indicating a direct relationship between these parameters. The presence of sucrose in the extracellular environment, not only acts as a carbon source, but also plays a role of osmotic regulation (Hilae; Te-Chato, 2005), which may cause a decrease in the water potential of the culture medium. This was evidenced by the reduction of the RPSI and GSI, causing a decrease in the percentage of initial germination, by the protrusion of the radicle, and finally, formation of the normal seedling.

As for the number of roots, for *C. pubescens* the gradual increase of sucrose in the culture environment led to a decrease in the number of roots produced (Figure 2a). However, for *C. adamantium*, the presence of 20 g L^{-1} of sucrose provided an average number of roots 46.4% higher than seedlings without sucrose, reaching an average of 9.08 roots per seedling (Figure 2a).

The presence of sucrose promoted a reduction in the shoot length and primary roots of the species of gabiroba evaluated (Figure 2b, c). Similar results were observed for seeds of *Genipa americana*, for which sucrose in the external medium became dispensable for the initial growth of seedlings (Almeida et al., 2013), as well as for seeds of *Nopalea cochenillifera* (L.) Salm Dyck because there was no difference in the percentage of seed germination in the presence of sucrose, but there was a decrease in seedling growth (Castro et al., 2011).

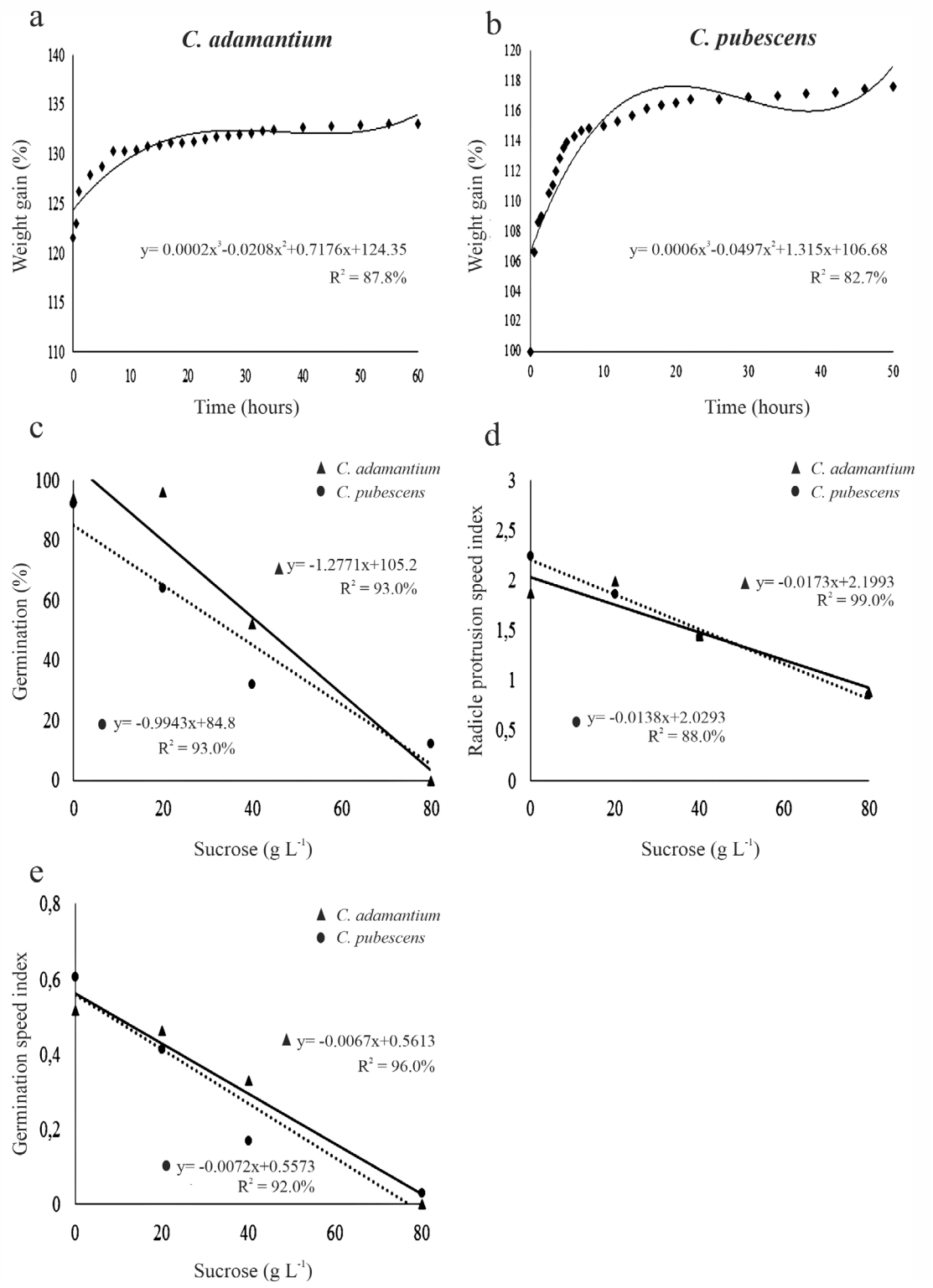

Figure 1: *In vitro* germination of *Campomanesia adamantium* and *C. pubescens* in different sucrose concentration. (a, b). Soaking curve of seeds of *Campomanesia adamantium* (a) and *Campomanesia pubescens* (b) by the increment of initial mass. (c) Germination percentage. (d) Root protrusion velocity index. (e) Germination velocity index.

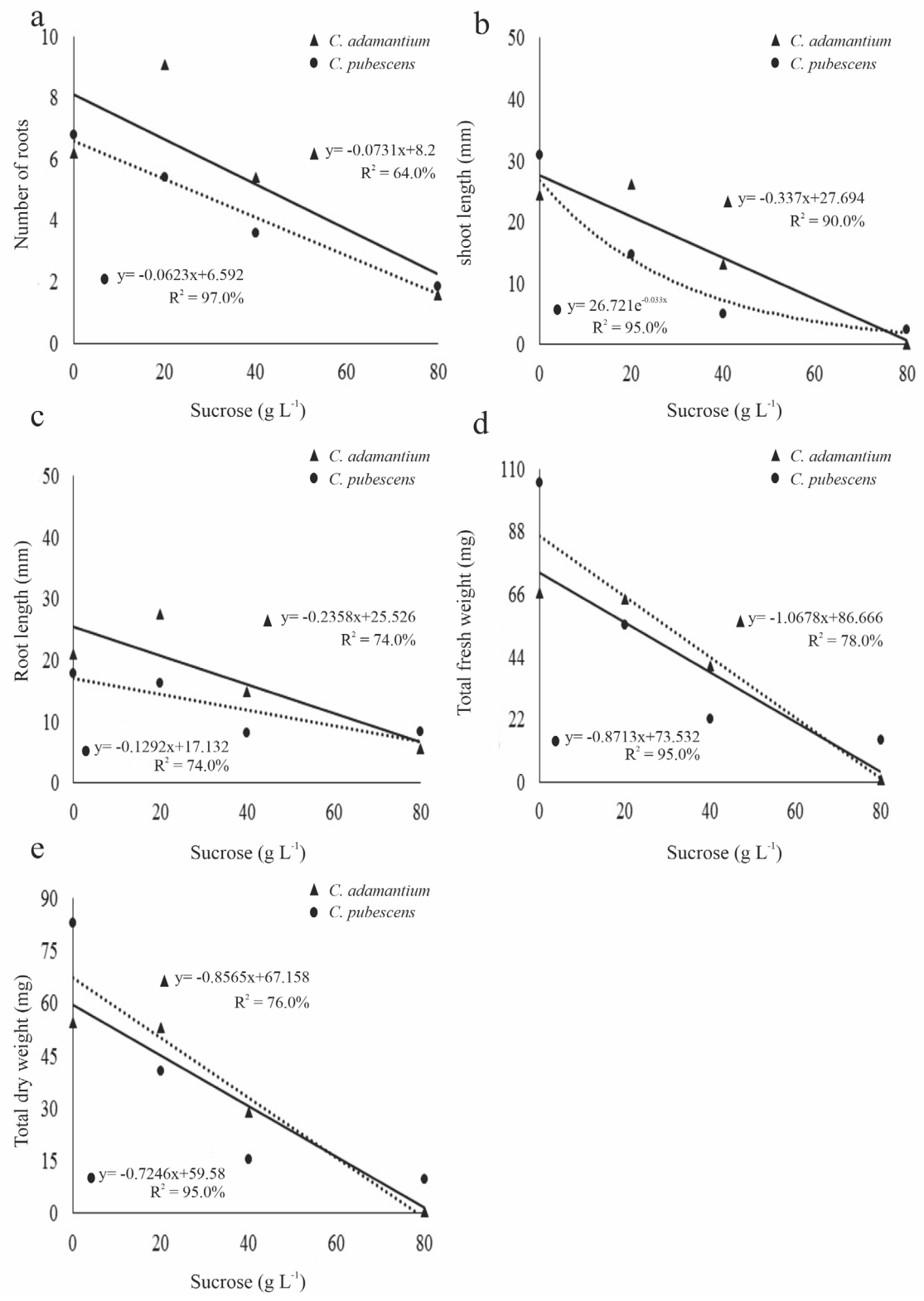

Figure 2: Establishment of *in vitro* seedlings of *Campomanesia adamantium* and *C. pubescens* in different sucrose concentration. (a) Number of seedling roots. (b) Shoot length. (c) Primary root length. (d) Fresh weight. (e) Dry weight.

Evaluating the total fresh and dry weight (Figure 2d, e) it was observed that, for *C. pubescens*, the values found in the absence of sucrose were notably higher. The total fresh and dry weight of *C. adamantium* seedlings did not vary up to 20 g L^{-1} of sucrose but reduced drastically at 40 g L^{-1}. Studies performed by Poothong et al. (2020) showed that reducing sucrose in MS medium increased the number and length of raspberry buds in culture medium without or with low sucrose concentration. The excess of sucrose in the culture medium can inhibit chlorophyll synthesis and reduce the photosynthetic capacity, which may result in lower carbon gain and dry weight (Ayub et al. 2019), as observed in the present study.

In summary, we report here that the absence of sucrose favored *in vitro* germination and initial establishment of gabiroba seedlings. This result will aid in the determination of micropropagation and biotechnology protocols for *Campomanesia spp.* contributing to the process of conservation and domestication of this important fruit tree of the Brazilian Cerrado.

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