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In vitro conservation of mangaba (*Hancornia speciosa* Gomes): An important fruit tree of Brazilian Cerrado

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Abstract

This study aimed at evaluating the effect of osmotic agents, sucrose, and abscisic acid on the *in vitro* conservation of mangaba trees by slow growth. Two experiments were carried out. Nodal segments were inoculated in the MS medium with the addition of 1) osmotic agents (15, 20, and 25 g L^{-1} of mannitol and 10, 20, and 40 g L^{-1} of sorbitol) + control; 2) sucrose (0, 10, 20 and 30 g L^{-1}) and abscisic acid – ABA (0 and 0.5 mg L^{-1}). After carrying out the experiments, both were performed again, this time with the addition of 2 g L^{-1} activated charcoal to the medium for comparison. The survival rate and number of green leaves were evaluated after 45 and 90 days. For growth recovery, the explants were transferred to a specific growth medium, consisting of MS medium supplemented with 7 g L^{-1} agar, 1 mg L^{-1} 6-benzylaminopurine (BAP), 1 mg L^{-1} 1-naphthaleneacetic acid (NAA), 2 g L^{-1} activated charcoal. At 60 days, survival rate, number of green leaves, number of nodes, shoot length and fresh weight of the explants were assessed. The results showed that the use of 2 g L^{-1} of activated charcoal improves the growth and development of the explants. The tested doses of osmotic agents also did not influence the evaluated characteristics. However, there was significant effect of osmotic agent and time on survival rate and treatment *x* time interaction for the number of green leaves, having been influenced also by the use of osmotic agent at 45 days, in addition to significant differences between the control and the other treatments for this characteristic. The osmotic agents were not effective in plant conservation as well as the use of ABA. According to the conditions of this study, it is recommended the use of 30 g L^{-1} sucrose to the culture medium for up to 90 days in a row of *in vitro* conservation of mangaba plants.

Keywords: abscisic acid; *in vitro* preservation; osmotic agents; plant tissue culture.
Abbreviations: MS_Murashige and Skoog, ABA_abscisic acid, IAA_indole-3-acetic acid, BAP_6-benzylaminopurine, NAA_1-naphthaleneacetic acid.

Introduction

The Cerrado is the second largest Brazilian biome and is regarded as the richest savanna in the world. However, uncontrolled settlement and exploitation of Cerrado's resources have caused the extinction of native species, the loss of genetic variability, and the depletion of natural resources (Zuin, 2020). For that reason, for almost two decades, the Cerrado has been included in the world's biodiversity hotspot list, for it is an area of high endemism and it is at risk of permanent environmental damage (Myers et al., 2000; Zuin 2020).

In Brazil, the mangaba tree grows in sparse vegetation areas, mainly in the Cerrado and Caatinga biomes and in the Restingas, but there are records of it in the Amazon region (Lima and Scariot, 2010). Mangaba tree plays a key role in northeastern and central-west Brazil, for it provides thousands of families engaged in extractive activities with a living and food. However, mangaba naturally occurs in highly anthropized areas mainly due to real estate speculation and intense farming activities, which have radically changed the landscape and ended people's traditional way of life. Mangaba is currently included in the endangered species list (Silva et al., 2016).

The *in situ* conservation of mangaba germplasm has already been performed by research bodies (Almeida et al., 2019; Fajardo et al., 2018), but the accessions are highly vulnerable, mainly due to pest infestation, diseases, and bad weather, which stress the importance of developing complementary techniques of conservation, such as the *in vitro* conservation of germplasm (Lédo et al., 2011). The slow-growth *in vitro* conservation technique benefits include the pathogen-free plant material, the requirement of little space, material ready to be immediately propagated, and cost reduction (Shahzad et al., 2017). It aims to reduce plant growth and to increase the subculture interval by promoting physical changes to the environment (temperature and light) and/or chemical changes to the culture medium (growth regulators, organic and inorganic compounds) (Engelmann, 2011). The method is especially applied to the conservation of rare or endemic species and to those plants which are not easily propagated with traditional techniques (Coelho et al., 2020). For this reason, this study aims to enhance the

protocol for slow-growth *in vitro* conservation of mangaba and, thus, to contribute to species conservation.

Results and discussion

Effect of osmotic agents and the in vitro conservation of mangaba nodal segments

The individual analysis of the experiment showed that, in the absence of activated charcoal, the doses of osmotic agents did not affect the traits, and no difference between control and treatments was observed. However, there was a significant effect of the osmotic agent on the survival rate and the number of green leaves at 45 days of *in vitro* storage. Moreover, there was a significant effect of time on both traits assessed. Mangaba nodal segments were already in process of senescence on the 90th day of conservation, showing a 43.81% mean reduction in the survival rate and a 0.72% mean reduction in the number of green leaves (Table 1).

Concerning the osmotic agent's effect, it was found that the mannitol, when significant, provided a low number of green leaves and d low survival rate of nodal segments, compared to the sorbitol results, regardless of the time of *in vitro* conservation (Table 2).

The osmotic agents, in the absence of activated charcoal and regardless of the dose, did not differ from the control and did not inhibit the metabolism of the explants, although mannitol has helped to inhibit the development of nodal segments when compared to sorbitol. Thus, under these conditions, the osmotic agents are not suitable for the slow-growth *in vitro* conservation of the species.

Mannitol and sorbitol are sugar alcohols that are not usually metabolized by plants and therefore have been employed to reduce the water potential of a culture medium (George, 1993), interfering with the plant's water and nutrient intake and consequently with its development. The results, though, depend on many factors, such as osmotic agent content, culture medium, type of explant, nature of the study, and temperature. Lédo et al. (2013) reported that none of the doses up to 20 g L^{-1} mannitol added to the MS medium supplemented with 4.5 g L^{-1} Phytagel® was effective in the *in vitro* conservation of genip plantlets, for they could not inhibit growth. By contrast, mannitol or sorbitol employed in the *in vitro* conservation of Cape gooseberry (*Physalis peruviana*) helped to inhibit plant growth at 25 °C. At lower temperatures, sucrose alone provided better results (Rezende et al., 2018).

When using activated charcoal, the doses of osmotic agents did not affect the traits as well. However, there was a significant effect of the osmotic agent and time on the survival rate and of the treatment *x* time interaction on the number of green leaves, which was also affected by the osmotic agent on the 45th day, showing a significant difference between control and treatments.

Moreover, the explant survival rate was also low in a medium supplemented with 2 g L^{-1} activated charcoal on the 90th day of *in vitro* conservation, showing a 32.38 % decrease compared to the shorter period assessed. Regardless of the dose, mannitol resulted in nodal segments with a smaller number of green leaves compared to the control's, showing statistically equal values on the 45th and 90th days of *in vitro* conservation. The doses of sorbitol, on the other hand, did not show differences concerning the control, except for 10 g L^{-1}, which helped the development of explants with a smaller number of green leaves on the 90th day of *in vitro* conservation. However, on that dose, leaf senescence was higher over time (Table 1).

Comparing the effects of the osmotic agents, the results, when significant in the presence of activated charcoal, were similar to those without charcoal. Adding sorbitol to the culture medium provided a higher explant survival rate, whereas mannitol helped to reduce the number of green leaves in the nodal segments (Table 2).

By slow-growth *in vitro* conservation, one aims to find a methodology that helps to both reduce plant growth and increase survival rates. Mannitol in the presence of activated charcoal allowed mangaba nodal segments to retain the same number of green leaves over time, that is to say, it reduced explant metabolism. However, the survival rates were too low and the explants were already in process of senescence regardless of the use of osmotic agents on the 90th day of conservation, which showed that the treatments assessed were not effective for this purpose.

Likewise, mannitol had a better performance than sorbitol in reducing the shoot development of *Macrosyphonia velame* plantlets (Martins et al., 2011). Lédo et al. (2007a) reported that mannitol did not produce positive effects on the *in vitro* conservation of coconut palm, because it reduced the survival rates, despite lowering plantlet growth. Silva et al. (2019) observed that mannitol and sorbitol added to the culture medium for the conservation of *Poincianella pyramidalis* produced low plant survival rates, and for this reason, they were not effective for *in vitro* conservation, according to the authors.

Conversely, Santos et al. (2011), studying the *in vitro* conservation of mangaba nodal segments, found a significant difference among 10, 20, and 40 g L^{-1} sorbitol, when using a culture medium supplemented with 1 mg L^{-1} indole-3-acetic acid (IAA) and 1 mg L^{-1} 6-benzylaminopurine (BAP), which are auxin and cytokinin growth regulators, respectively. The authors concluded that 10 or 20 g L^{-1} osmoticum without sucrose in the culture medium is suitable for the conservation of mangaba explants for up to 120 days. Furthermore, Sá et al. (2011) also used a culture medium supplemented with the same growth regulators and reported a significant effect when testing 0, 10, 15, and 20 g L^{-1} mannitol doses, which helped to form shorter micro cuttings, but produced deleterious effects on the 90th day of *in vitro* conservation.

Auxins are substances that control cell growth and elongation, and cytokinins promote cell division and reduce apical dominance. The balance between these two regulators controls many aspects of cell differentiation and organogenesis in tissue cultures (Pasqual, 2001). The aforementioned studies indicate significant differences in the osmotic agent doses, probably because they employed a culture medium supplemented with those growth regulators, which promoted the explant's development and made them susceptible to the action of the osmotic agents. Moreover, one may infer that, because of the lack of such regulators in the culture medium used in this study, the mangaba nodal segments were less prone to develop and, therefore, were already in process of senescence on the 90th day of *in vitro* conservation.

Assessing the experiments together, there was a significant effect of the treatment *x* activated charcoal interaction on the number of green leaves and an isolated effect of this product on the survival rate. Nonetheless, there was no significant difference in the doses of activated charcoal on the survival rate. The 40 g L^{-1} sorbitol in the presence of

charcoal favored a higher development of green leaves on the 90th day, as well as the control, regardless of the *in vitro* conservation time (Table 3).

Activated charcoal can modify the culture medium by adsorbing toxic substances, nutrients, vitamins, and growth regulators and, therefore, under certain circumstances, improve or regulate the growth of *in vitro* cultivated plants (George, 1993). Studies on mangaba tree (Lédo et al., 2007b) and barueiro (*Dipteryx alata* Vog.) (Silva et al., 2016) showed that adding activated charcoal to the culture medium improves the development of plantlet shoots.

During the growth recovery phase, the doses and the osmotic agents did not affect any of the traits assessed; however, there was a significant effect on the interaction between the control and treatments. Nonetheless, the fresh mass did not a show significant difference (Table 4).

Mangaba nodal segments in mannitol did not show survival rate after 60 days in culture medium, that is to say, the explant growth could not be recovered, affecting other traits, which were inferior to the controls. Moreover, nodal segments in sorbitol showed lower shoot length development compared to those in a medium without osmotic agents, except for the 20 g L^{-1} dose, which provided a similar number of nodes and shoot length to those of the control. It means that the explants had a better response to the stimuli to growth recovery in that dose, although the survival rate reduced 26.66 % since the assessment on the 90th day of *in vitro* conservation.

In the absence of osmotic agents (the control), the survival rate reduced only 6.67 % since the assessment on the 90th day, and the explants showed a good shoot development, after 60 days in culture medium. Genip explants in 15 and 20 g L^{-1} mannitol had a better morphogenetic response on the 120th day of growth recovery (Lédo et al., 2013), that is to say, mannitol produced an opposite effect, which the authors believe to be related to either the study species or the use of unsuitable doses. Santos et al. (2011) observed that mangaba nodal segments in a medium supplemented with growth regulators and 10 g L^{-1} sorbitol were more likely to recover growth on the 60th day compared to those in 20 and 40 g L^{-1} doses.

Based on the results, one may say that the explants in osmotic agents during the *in vitro* conservation period suffer from stress, which affects growth recovery, even after explants being transferred to a medium supplemented with growth regulators.

Effects of sucrose and abscisic acid in in vitro conservation of mangaba nodal segment

The individual analyses of the experiments showed that a culture medium without activated charcoal produced a significant effect of sucrose *x* time interaction on both variables studied, and of ABA *x* time interaction on the number of green leaves. However, in a medium supplemented with 2 g L^{-1} activated charcoal, there was a significant effect of the isolated action of sucrose and time on survival rate, and a triple action of such factors on the number of green leaves. In the combined analysis of the experiments, the treatment *x* activated charcoal interaction produced a significant effect on the survival rate, and the charcoal produced an isolated effect on the number of green leaves.

In the presence of activated charcoal, there was a low survival rate on the 90th day. The same result was reported in the absence of charcoal, except for the explants in a sucrose-free medium, whose survival rate reduced to zero on the 45th day of *in vitro* conservation (Table 5).

However, increasing the sucrose contents in the absence of activated charcoal provided a linear increase of survival rate: for each 1 g L^{-1} sucrose added to the culture medium, the rate increased 1.57 % and 2.90 % on the 45th and the 90th days, respectively. In the presence of charcoal, quadratic behavior was observed with a maximum survival rate of 75.67 % to 20.2 g L^{-1} sucrose (Figure 1).

According to Faria et al. (2006), sucrose in the culture medium is essential to maintaining the development of passion fruit explants. Carbohydrates are particularly important to plants, for they are a major structural component of plant cells and provide the process of respiration with an energy source, and the synthesis of biomolecules with carbon skeletons (Majerowicz, 2004).

Comparing both the experiments, it was found that adding activated charcoal to the culture medium provided a higher explant survival rate on the 45th day, when the explants were maintained in a sucrose-free medium or a 10 g L^{-1} sucrose medium; and in the middle sucrose doses on the 90th day, with no difference on the other treatments. The activated charcoal did not affect the number of green leaves (Table 6). The results are believed to be related to the fact that the activated charcoal can adsorb toxic substances and growth regulators from the medium.

The development of the number of green leaves in the absence of activated charcoal was higher as the sucrose content increased in the culture medium, growing at higher rates on the 45th day of *in vitro* conservation (Figure 2).

The explants showed higher leaf senescence in 20 and 30 g L^{-1} sucrose over time, but the small doses provided the same number of green leaves on the 45th and 90th days (Table 7).

Adding ABA to the culture medium without activated charcoal provided a low development of nodal segments because the number of green leaves recorded on both assessment intervals was statistically equal. Conversely, in the absence of such regulators, the explants showed a small number of green leaves on the 90th day of *in vitro* conservation.

ABA in the culture medium also helped to reduce the development of genip plantlets (Lédo et al., 2013), making it suitable for *in vitro* conservation. One of the most common effects of ABA is plant growth inhibition, when the hormone is transported to the buds, causing dormancy (Stacciarini-Seraphin, 2004).

Furthermore, it was found that the number of green leaves has an increasing linear behavior in the presence of charcoal, without ABA and regardless of time, that is to say, the higher the medium sucrose content, the higher the development of the leaves. In the presence of ABA, the variable showed a quadratic regression curve with a maximum of 3.37 leaves in 21.70 g L^{-1} sucrose. On the longest interval of assessment, the maximum was 3.70 leaves in 17.58 g L^{-1} sucrose (Figure 3).

Nonetheless, there was no significant difference between the presence or absence of ABA in the medium with up to 20 g L^{-1} sucrose, but there was higher leaf senescence in explants maintained in ABA with 30 g L^{-1} sucrose (Table 8).

Increasing the sucrose content promoted the development of mangaba nodal segment shoot maintained in growth regulator medium (Santos et al., 2011) and of physic nut embryos (Nunes et al., 2008). The results show that increasing the availability of sucrose in the culture medium

Table 1. Survival rate and number of green leaves of mangaba nodal segments grown in medium added of mannitol or sorbitol, with and without activated charcoal, on the 45 and 90th days of *in vitro* conservation.

Absence of activated charcoal					
Osmotic agent	Dose ($g\ L^{-1}$)	Survival rate (%)		Number of green leaves	
		45 days	90 days	45 days	90 days
Control	0*	40.00	0.00	0.53	0.00
Mannitol	15	53.33	0.00	0.40	0.00
	20	46.67	6.67	0.93	0.27
	25	20.00	0.00	0.20	0.00
Sorbitol	10	66.67	26.67	1.53	1.00
	20	73.33	13.33	1.93	0.27
	40	73.33	20.00	1.40	0.33
------ Mean ------		53.33a	9.52b	0.99a	0.27b
Presence of activated charcoal					
Osmotic agent	Dose ($g\ L^{-1}$)	Survival rate (%)		Number of green leaves	
		45 days	90 days	45 days	90 days
Control	0*	80.00	40.00	3.07a	2.40a
Mannitol	15	26.67	0.00	0.13a*	0.00a*
	20	53.33	26.67	0.67a*	0.53a*
	25	46.67	20.00	0.73a*	0.27a*
Sorbitol	10	86.67	33.33	2.13a	0.13b*
	20	60.00	33.33	2.87a	0.93b
	40	80.00	53.33	1.87a	1.60a
------ Mean ------		61.90a	29.52b	1.64	0.84

Means within each row followed by a different letter differ from one another at 5 % probability according to Tukey's test; *different on Dunnett's test at 5 % probability.

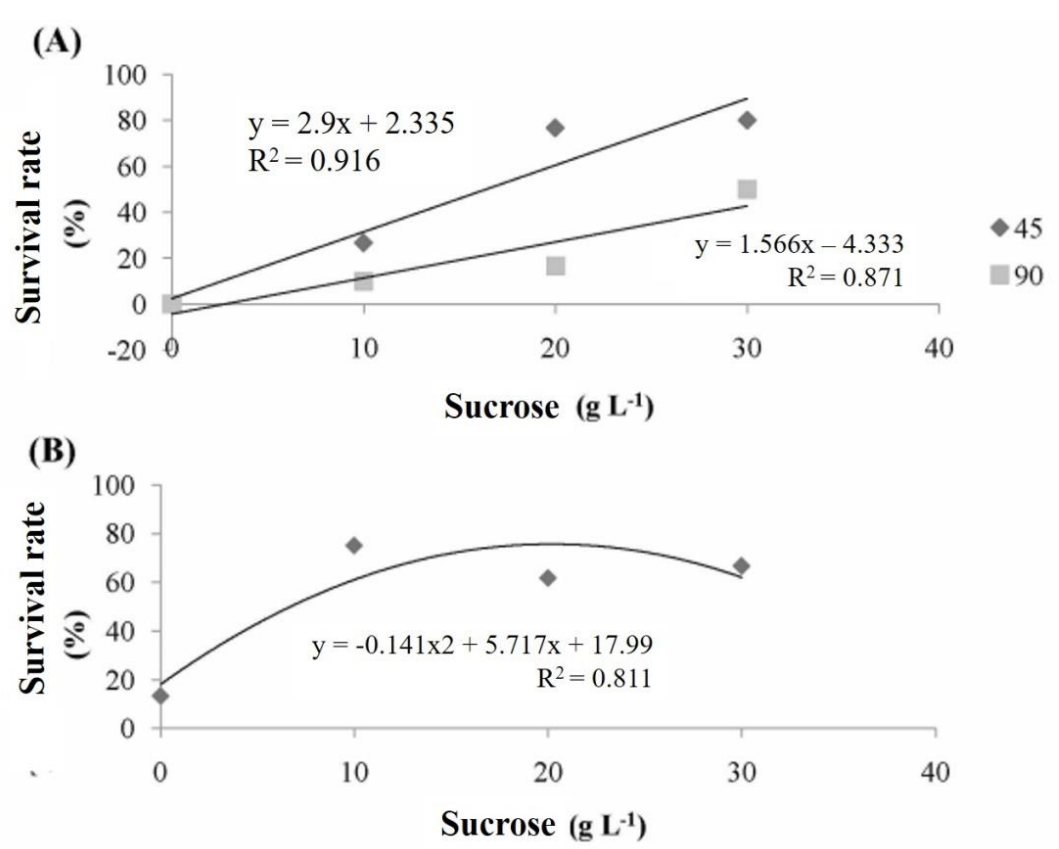

Figure 1. Survival rate of mangaba nodal segments related to sucrose content, *in vitro* conservation time and absence of activated charcoal (A); and related to sucrose content and presence of activated charcoal (B).

Table 2. Effect of osmotic agent on survival rate and number of green leaves of mangaba nodal segments in the absence of activated charcoal on the 45th and 90th days of *in vitro* conservation.

Absence of activated charcoal				
Osmotic agent	Survival rate (%)		Number of green leaves	
	Time (days)		Time (days)	
	45	90	45	90
Mannitol	40.00B	2.22B	0.51B	0.75A
Sorbitol	71.11A	20.00A	1.62A	0.97A
Presence of activated charcoal				
Osmotic agent	Survival rate (%)		Number of green leaves	
	Time (days)		Time (days)	
	45	90	45	90
Mannitol	42.22B	15.55B	0.51B	0.27A
Sorbitol	75.55A	40.00A	2.29A	0.89A

Means within each column followed by a different letter differ from one another at 5 % probability according to Tukey's test.

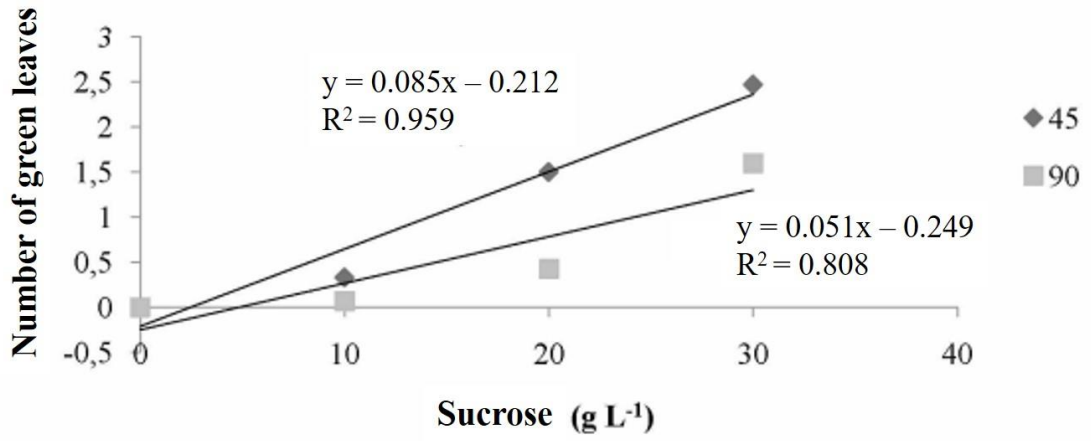

Figure 2. The number of green leaves of mangaba nodal segments related to the sucrose content and *in vitro* conservation time in the absence of activated charcoal.

Table 3. Effect of activated charcoal on the survival rate and number of green leaves of mangaba nodal segments in mannitol and sorbitol doses on the 45th and 90th days of *in vitro* conservation.

Time (days)	*Osmotic agent*	*Dose (g L^{-1})*	*Survival rate (%)*		*Number of green leaves*	
			Activated charcoal (g L^{-1})		*Activated charcoal (g L^{-1})*	
			0	*2*	*0*	*2*
45	*Control*	*0*	*40.00*	*80.00*	*0.53b*	*3.07a*
	Mannitol	*15*	*53.33*	*26.67*	*0.40a*	*0.13a*
		20	*46.67*	*53.33*	*0.93a*	*0.67a*
		25	*20.00*	*46.67*	*0.20a*	*0.73a*
	Sorbitol	*10*	*66,67*	*86.67*	*1.53a*	*2.13a*
		20	*73.33*	*60.00*	*1.93a*	*2.87a*
		40	*73.33*	*80.00*	*1.40a*	*1.87a*
90	*Control*	*0*	*0.00*	*40.00*	*0.00b*	*2.40a*
	Mannitol	*15*	*0.00*	*0.00*	*0.00a*	*0.00a*
		20	*6.67*	*26.67*	*0.27a*	*0.53a*
		25	*0.00*	*20.00*	*0.00a*	*0.27a*
	Sorbitol	*10*	*26.67*	*33.33*	*1.00a*	*0.13a*
		20	*13.33*	*33.33*	*0.27a*	*0.93a*
		40	*20.00*	*53.33*	*0.33b*	*1.60a*
		Means	*31.43a*	*45.71a*	*0.63*	*1.24*

Means within each row followed by a different letter differ from one another at 5% probability according to Tukey's test.

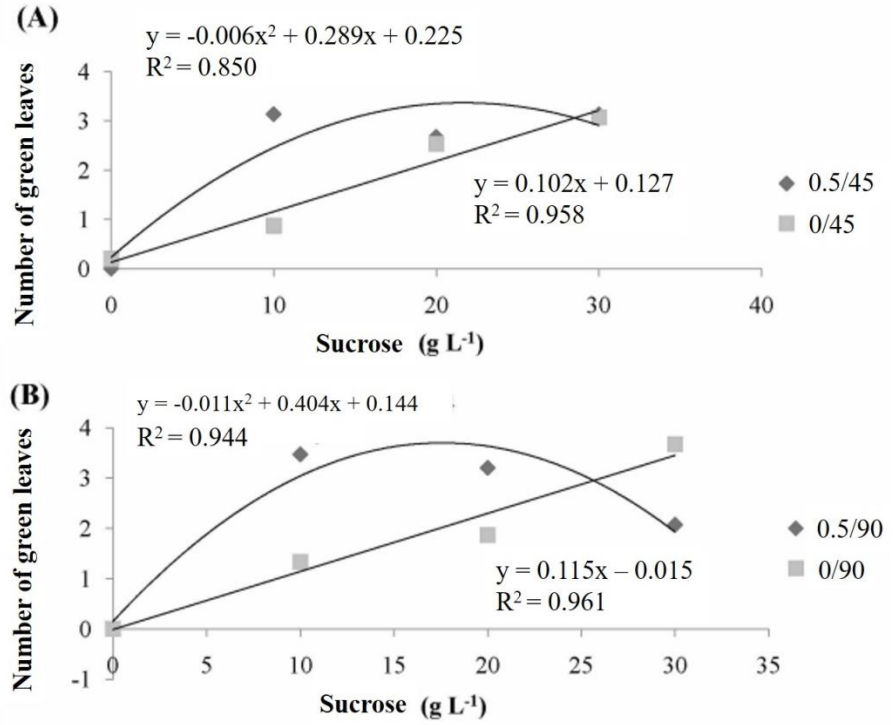

Figure 3. Number of green leaves of mangaba nodal segments related to sucrose and ABA doses with activated charcoal on the 45th (A) and 90th (B) days of *in vitro* conservation.

Table 4. Means of survival rate (SR), number of green leaves (NGL), number of nodes (NN), shoot length (SL), and fresh weight (FW) of mangaba nodal segments in medium supplemented with 2 g L^{-1} activated charcoal and different doses of mannitol and sorbitol on the 60th day of growth recovery.

Osmotic agent	*Dose (g L^{-1})*	*SR(%)*	*NGL*	*NN*	*SL (cm)*	*FW (g)*
Control	*0**	*33.33*	*2.07*	*1.20*	*1.10*	*0.072*
Mannitol	*15*	*0.00**	*0.00**	*0.00**	*0.00**	*0.000*
	20	*0.00**	*0.00**	*0.00**	*0.00**	*0.000*
	25	*0.00**	*0.00**	*0.00**	*0.10**	*0.016*
Sorbitol	*10*	*6.67**	*0.40**	*0.07**	*0.03**	*0.015*
	20	*6.67**	*0.40**	*0.80*	*0.63*	*0.047*
	40	*0.00**	*0.00**	*0.27**	*0.07**	*0.009*

*Different at 5% probability according to Dunnett's test.

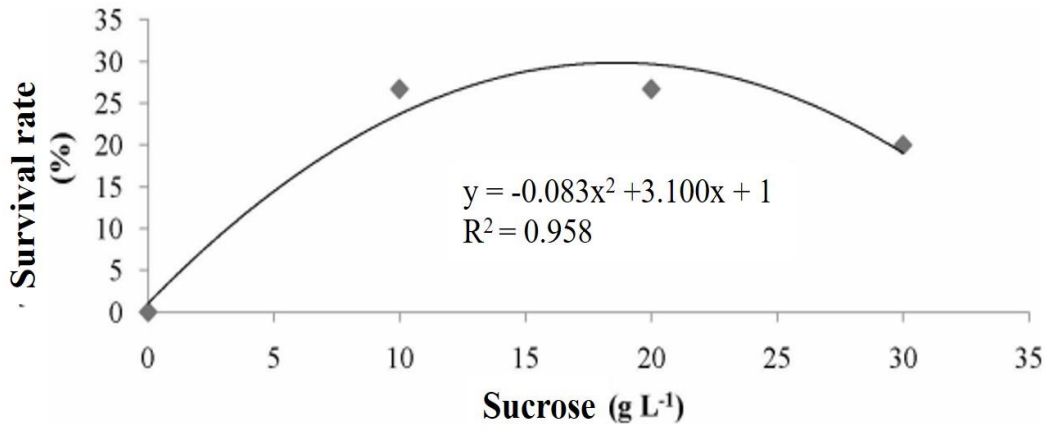

Figure 4. Survival rate of *in vitro* mangaba nodal segments related to sucrose content on the 60th day of growth recovery.

Table 5. Means of survival rate (%) of mangaba nodal segments in sucrose doses with and without activated charcoal on the 45th and 90th days of *in vitro* conservation.

Sucrose (g L^{-1})	Activated charcoal (g L^{-1})			
	0		2	
	Time (days)		Time (days)	
	45	90	45	90
0	0.00a	0.00a	26.67	0.00
10	26.67a	10.00b	83.33	66.67
20	76.67a	16.67b	73.33	50.00
30	80.00a	50.00b	80.00	53.33
Means	45.83	19.17	65.83a	42.50b

Means within each row followed by a different letter differ from one another at 5% probability according to Tukey's test.

Table 6. Effect of activated charcoal on survival rate and number of green leaves of mangaba nodal segments in sucrose and ABA doses on the 45th and 90th days of *in vitro* conservation.

Time (days)	Sucrose (g L^{-1})	ABA (mg L^{-1})	Survival rate (%)		Number of green leaves	
			Activated charcoal (g L^{-1})		Activated charcoal (g L^{-1})	
			0	2	0	2
45	0	0	0.00a	20.00a	0.00	0.20
		0.5	0.00b	33.33a	0.00	0.00
	10	0	26.67b	73.33a	0.60	0.87
		0.5	26.67b	93.33a	0.07	3.13
	20	0	73.33a	73.33a	2.27	2.53
		0.5	80.00a	73.33a	0.73	2.67
	30	0	86.67a	73.33a	3.07	3.07
		0.5	73.33a	86.67a	1.87	3.13
90	0	0	0.00a	0.00a	0.00	0.00
		0.5	0.00a	0.00a	0.00	0.00
	10	0	0.00b	60.00a	0.00	1.33
		0.5	20.00b	73.33a	0.13	3.47
	20	0	20.00 b	53.33a	0.67	1.87
		0.5	13.33b	46.67a	0.20	3.20
	30	0	53.33a	53.33a	1.53	3.67
		0.5	46.67a	53.33a	1.67	2.07
		Means	32.50	54.17	0.80a	1.95a

Means within each row followed by a different letter differ from one another at 5% probability according to Tukey's test.

Table 7. Means of number of green leaves of mangaba nodal segments in sucrose and ABA doses in the absence of activated charcoal on the 45th and 90th days of *in vitro* conservation.

Sucrose (g L^{-1})	Time (days)	
	45	90
0	0.00a	0.00a
10	0.33a	0.07a
20	1.50a	0.43b
30	2.47a	1.60b
ABA (mg L^{-1})		
0	1.48aA	0.55bA
0.5	0.67aB	0.50aA

Means within each row and column (lower case and upper case, respectively) followed by a different letter, differ from one another at 5% probability according to Tukey's test.

Table 8. Means of number of green leaves of mangaba nodal segments related to sucrose and ABA doses with activated charcoal on the 45th and 90th days of *in vitro* conservation.

Sucrose (g L^{-1})	ABA (mg L^{-1})	Time (days)	
		45	90
0	0	0.20aA	0.00 aA
	0.5	0.00 aA	0.00 aA
10	0	0.87 aA	1.33 aA
	0.5	3.13 aA	3.47 aA
20	0	2.53 aA	1.87 aA
	0.5	2.67 aA	3.20 aA
30	0	3.07 aA	3.67 aA
	0.5	3.13 aA	2.07bA

Means within each row and column (lower case and upper case, respectively) followed by a different letter, differ from one another at 5% probability according to Tukey's test.

Table 9. Survival rate (SR), number of green leaves (NGL), number of nodes (NN), shoot length (SL), and fresh weight (FW) of mangaba segments in medium supplemented with 2 g L^{-1} activated charcoal and different doses of sucrose and ABA on the 60th day of growth recovery.

Sucrose (g L^{-1})	ABA (mg L^{-1})	SR (%)	NGL	NN	SL (cm)	FW (g)
0	0	0.00a	0.00a	0.00a	0.00a	0.000a
	0.5	0.00a	0.00a	0.00a	0.00a	0.000a
10	0	20.00a	0.80a	0.33a	0.47a	0.020a
	0.5	33.33a	2.80a	1.20a	1.50a	0.093a
20	0	20.00a	1.60a	0.93a	0.93a	0.050a
	0.5	33.33a	2.33a	1.47a	1.53a	0.087a
30	0	20.00a	4.00a	2.07a	2.33a	0.157a
	0.5	20.00a	1.73a	0.93a	0.80a	0.050a
CV (%)		62.98	119.29	115.29	111.35	114.54

Means within each column followed by a different letter differ from one another at 5% probability according to Tukey's test.

promoted the explant metabolism and, consequently, its development.

Chagas et al. (2005) observed that higher content of gibberellic acid is required in an activated charcoal culture medium to produce an effect on citrus explants. Thus, one can infer that the adsorption of ABA was smaller in 30 g L^{-1} sucrose due to a larger quantity of medium components, reflecting on the explant development over time.

On the growth recovery phase, there was a significant difference in sucrose doses for survival rate only. There was no significance in the use of ABA and the interaction of those factors for any of the traits assessed (Table 9). This indicates that the mangaba nodal segments maintained in an ABA medium show the same performance as those in a medium without that regulator during the growth recovery phase. Although no significant difference was reported for the other traits, the mangaba nodal segments showed better shoot development on 30 g L^{-1} sucrose without ABA.

The increase of sucrose content produced a quadratic behavior on the regression curve for the survival rate, reaching up to 29.83% on 18.60 g L^{-1} (Figure 4), with a considerable reduction since the assessment on the 90th day of *in vitro* conservation.

Material and methods

Plant material

The experiments used mangaba fruits (identified as var. gardneri) which were picked from native trees between Uberlândia and Campo Florido cities, in Minas Gerais State, Brazil. The seeds were cut from the fruits and macerated under running water with the aid of a sieve to take the excess flesh. Then, they passed by standard laboratory sterilization comprising 70 % ethanol immersion for 1 minute, followed by 2.5 % sodium hypochlorite immersion for 30 minutes, and finally washing with autoclaved, distilled water inside a laminar flow chamber. The seeds were inoculated into 40 mL MS medium (Murashige and Skoog, 1962) supplemented with 2 g L^{-1} activated charcoal, 7 g L^{-1} agar, and pH adjusted to 5.7, inside clear glass flasks previously autoclaved at 121 °C for 20 minutes and capped with a rubber stopper. The flasks were stored in a growth room at 25 ± 2 °C and for a 16-hour photoperiod under 20 W white fluorescent lights. After 120 days, nodal segments measuring about 1 cm long were cut from the growing plants and used as explants in the *in vitro* conservation phase.

Two experiments were conducted. In the laminar flow chamber, the nodal segments were inoculated into 40 mL MS medium supplemented with 7 g L^{-1} agar inside flasks, according to the different treatments. The medium pH was adjusted to 5.7 and the flasks were autoclaved at 121°C for 20 minutes the day before the inoculation. The survival rate and number of green leaves were evaluated on the 45th and 90th days of conservation. Shapiro-Wilk and Levene tests were run to test the data for the normality assumptions of residuals and the homogeneity of variances, respectively, at 1 % probability with the aid of SPSS software (IBM, 2013).

Experiment 1: Osmotic agents

The experiment was arranged in a 7x2 time split-plot design, where the plot comprised seven treatments hierarchically

arranged, and the subplot comprised two-time intervals (45 and 90 days). The treatments comprised the osmotic agents and their doses (15, 20, and 25 g L^{-1} mannitol and 10, 20, and 40 g L^{-1} sorbitol), as well as an additional treatment corresponding to the dose zero (control). The assessments were conducted on the 45th and 90th days of *in vitro* conservation. The experiment was arranged in a completely randomized design and three replicates, each one comprising five flasks with one explant. After receiving the mangaba nodal segments, the flasks were stored in a growth room at 25 ± 2 °C and for a 16-hour photoperiod under 20 W white fluorescent lights. The experiment was later repeated adding 2 g L^{-1} activated charcoal to the medium and the results were compared to those without charcoal.

The survival rate and the number of green leaves data became arcsinev(x/100) and √(x+0.5), respectively. Data were fed into Sisvar (Ferreira, 2014) for the analysis of variance. Tukey's test was used to compare the qualitative means, and the curve fitting polynomial regression was used to compare the quantitative ones. Dunnett's test was used to compare the treatments with the control (additional treatment) using the statistical software Assistat (Silva and Azevedo, 2016). To check the effects of the activated charcoal on the medium, the experiments were compared in a combined analysis using Genes software (Cruz, 2013) ($P<0.05$).

Experiment 2: Sucrose and abscisic acid

The experiment was arranged in an 8x2 time split-plot design, where the plot comprised a 4x2 factorial corresponding to four sucrose contents (0, 10, 20, and 30 g L^{-1}) and two ABA contents (0 and 0.5 mg L^{-1}), and the subplot comprised two intervals (on the 45th and 90th days). The experiment was arranged in a completely randomized design and three replicates, each one comprising five flasks with one explant. The flasks were stored in a growth room at 25 ± 2 °C and for a 16-hour photoperiod under 20 W white fluorescent lights. The experiment was later repeated adding 2 g L^{-1} activated charcoal to the medium and the results were compared to those without charcoal. The variables were fed into Sisvar (Ferreira, 2014) for the analysis of variance. Tukey's test was used to compare the qualitative means, and the curve fitting polynomial regression was used to compare the quantitative ones. To check the effects of the activated charcoal on the medium, the experiments were compared in a combined analysis using the software Genes (Cruz, 2013). The tests were performed at 5% probability.

Explant growth recovery after conservation period

Ninety days after the *in vitro* conservation, the explants were inoculated into 40 mL MS medium supplemented with 7 g L^{-1} agar, 1 mg L^{-1} 6-benzylaminopurine (BAP), 1 mg L^{-1} 1-naphthaleneacetic acid (NAA), 2 g L^{-1} activated charcoal, and pH adjusted to 5.7, inside flasks previously autoclaved at 121 °C for 20 minutes. The flasks were stored in a growth room at 25 ± 2 °C and for a 16-hour photoperiod under 20 W white fluorescent lights. On the 60th day, nodal segment survival rate, number of green leaves, number of nodes, shoot length, and fresh mass were evaluated. Statistical analysis procedures were similar to those of the *in vitro* phase.

Conclusion

Activated charcoal in the culture medium favors both the survival rate and the development of mangaba segments. However, the osmotic agents, i.e. mannitol and sorbitol, do not prove to be effective in conserving the explants. The best result for plant conservation is achieved by adding 30 g L^{-1} sucrose to the culture medium for up to 90 days in a row of *in vitro* conservation. Adding ABA does not produce a positive effect on inhibiting growth.

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