Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252021v34n101rc

IN VITRO SEED GERMINATION AND PLANT GROWTH OF "CABEÇA-DE-FRADE" (CACTACEAE)[1]

SHEILA VITÓRIA RESENDE[2]*, ALONE LIMA-BRITO[3], GABRIELA TORRES- SILVA[4], JOSÉ RANIERE FERREIRA DE SANTANA[3]

ABSTRACT – The genus *Melocactus* ("cabeça-de-frade") comprises 32 species in Brazil, of which *M. glaucescens* and *M. paucispinus* are threatened with extinction. The present work evaluated the effects of different concentrations of Murashige & Skoog (MS, MS/2 and MS/4) culture medium and sucrose (15 g L^{-1} and 30 g L^{-1}) on *in vitro* seed germination and plant growth of *M. glaucescens* and the efficiency of sterilization with sodium hypochlorite (NaOCl) on *in vitro* seed germination and plant growth of *M. glaucescens* when using seeds and *M. glaucescens* and *M. paucispinus* when using apical segment of cladode. In *M. glaucescens*, the final germination at the different MS and sucrose concentrations varied between 53.5 and 68.1% and the best results for *in vitro* growth were observed with the lowest mineral salt (MS/2 and MS/4) and sucrose (15 g L^{-1}) concentrations, with lengths of the aerial portion of 9.70 and 10.76 mm, respectively. There was no difference in seed germination and plant growth in chemical and autoclave medium. It is concluded that the use of chemical sterilization with NaOCl at low concentrations of salts (MS/2 and MS/4) and sucrose (15 g L^{-1}) are quite advantageous for producing ornamental plants germinated *in vitro* and/or apical segment of cladode of *M. glaucescens* and *M. paucispinus*, representing a reduction of costs for *in vitro* cultivation of this species.

Keywords: *Melocactus*. Culture medium. *In vitro* establishment. Chemical sterilization.

GERMINAÇÃO DE SEMENTES E CRESCIMENTO DE PLANTAS *IN VITRO* DE CABEÇA-DE-FRADE (CACTACEAE)

RESUMO – O gênero *Melocactus* (cabeça-de-frade) possui 32 espécies no Brasil, das quais *M. glaucescens* e *M. paucispinus* estão ameaçadas de extinção. O objetivo deste trabalho foi analisar o efeito de diferentes concentrações de sais do meio Murashige e Skoog (MS, MS/2 e MS/4) e sacarose (15 g L^{-1} e 30 g L^{-1}) na germinação de sementes e crescimento de plantas *in vitro* de *M. glaucescens* e a eficiência da esterilização do meio de cultura com hipoclorito de sódio (NaOCl) na germinação de sementes e crescimento de plantas *in vitro* de *M. glaucescens* ao utilizar sementes, *M. glaucescens* e *M. paucispinus* ao utilizar o segmento apical do cladódio. Para *M. glaucescens* a germinação final nas diferentes concentrações de MS e sacarose, variou de 53,5 a 68.1% e os melhores resultados para o crescimento *in vitro* foram observados nas menores concentrações de sais (MS/2 e MS/4) e de sacarose (15 g L^{-1}) com valores de comprimento da parte aérea de 9,70 e 10,76 mm, respectivamente. Não houve diferença na germinação das sementes e crescimento das plantas em meio químico e autoclavado. Conclui-se que, o uso da esterilização química com NaOCl em baixas concentrações de sais (MS/2 e MS/4) e sacarose (15 g L^{-1}) são as mais indicadas para produção de plantas ornamentais germinadas *in vitro* e /ou segmento apical do cladódio de *M. glaucescens* e *M. paucispinus*, o que representa uma redução de custo no cultivo *in vitro* dessas espécies.

Palavras-chave: *Melocactus*. Meio de cultura. Estabelecimento *in vitro*. Esterilização química.

*Corresponding author
[1]Received for publication in 06/04/2020; accepted in 09/02/2020.
Paper extracted from the doctoral thesis of the first author.
[2]Institute of Biology, Universidade Federal da Bahia, Salvador, BA, Brazil; sresende@ufba.br - ORCID: 0000-0003-1976-3924.
[3]Biological Science Department, Universidade Estadual de Feira de Santana, Feira de Santana, BA, Brazil; alone@uefs.br - ORCID: 0000-0003-4560-3182, raniere@uefs.br - ORCID: 0000-0003-0186-6888.
[4]Plant Biology Department, Universidade Federal de Viçosa, Viçosa, MG, Brazil; gabrielatorres.bio@gmail.com - ORCID: 0000-0002-9545-5053.

INTRODUCTION

The genus *Melocactus* (L.) Link & Otto (Cactaceae), popularly known as melon cactus, or in Brazil as "cabeça-de-frade", comprises 38 species, of these 32 species and 10 subspecies occur in Brazil, of which 22 and 9, respectively, are endemic to this country (ZAPPI; TAYLOR, 2020). The high degree of endemism of these plants, associated with factors such as habitat degradation and the harvesting for commercial purposes as ornamental, has included some species in the main lists of species threatened with extinction, such as *M. glaucescens* Buining & Brederoo and *M. paucispinus* G. Heimen & R. Paul (IUCN, 2020). These species are restricted to highlands of the Chapada Diamantina and adjacent mountain systems in Bahia and Minas Gerais on substrates derived from quartzitic material and sandstone (TAYLOR et al., 2014).

Plant tissue culture techniques represent a viable alternative to the conventional propagation of "cabeça-de-frade" species, which grow slowly, require about ten years to reach its reproductive stage, and reproduce exclusively from seeds in their natural habitat, because these species do not ramify or produce lateral shoots unless the plant suffers some injury to both meet commercial demands and help preserve these plants in their natural habitats (MACHADO, 2009; LEMA-RUMIŃSKA; KULUS, 2014; PÉREZ-MOLPHE-BALCH et al., 2015).

In vitro establishment is the first step of micropropagation and its success depends on diverse factors of tissue culture techniques, such as the culture medium (PHILLIPS; GARDA, 2019). The medium formulated by Murashige and Skoog (1962) is typically used for *in vitro* cultivation of species of the Cactaceae family (LEMA-RUMIŃSKA; KULUS, 2014). Sucrose is an immediately available source of carbohydrate and has an important role in *in vitro* culture as source of metabolic energy and carbon skeletons, and it also can function as osmotic agent (SANTOS et al., 2011; HUANG et al., 2014).

Sterilization of the culture medium by autoclaving is the method most commonly used in plant tissue culture and adds significant costs to *in vitro* cultivation due to energy consumption and the high initial investments in equipment (TEIXEIRA et al., 2006; 2008; RIBEIRO; TEIXEIRA; BASTOS, 2011). Additionally, autoclaving can cause the decomposition of organic components of the culture medium such as sucrose (PHILLIPS; GARDA 2019). According to Brondani et al. (2013), treatment with NaOCl would avoid the degradation of carbohydrates at high temperatures and pressures, causing the formation of furfural form, which is toxic to the tissues. These factors argue for the substitution of the autoclaving of the culture medium with other less expensive forms (e.g. chemical sterilization) that will not otherwise compromise the integrity of the nutritive medium and eradicate pathogens such as fungi and bacteria (BRONDANI et al., 2013).

The use of sodium hypochlorite (NaOCl) in culture media can greatly reduce costs and has actually been shown to be more efficient at inhibiting contamination than autoclaving, being commonly used for asepsis of plant tissues (TEIXEIRA et al., 2006; 2008; BRONDANI et al., 2013).

The present work evaluated the effects of different concentrations of Murashige & Skoog (MS, MS/2 and MS/4) culture medium and different concentrations of sucrose (15 g L^{-1} and 30 g L^{-1}), on *in vitro* seed germination and plant growth of *M. glaucescens* and the efficiency of sterilization with sodium hypochlorite (NaOCl) on *in vitro* seed germination and plant growth of *M. glaucescens* when using seeds and *M. glaucescens* and *M. paucispinus* when using apical segment of cladode.

MATERIAL AND METHODS

Effects of mineral salts and sucrose concentrations on *in vitro* germination and growth

Ripe fruits of *Melocactus glaucescens* were collected from natural populations located in Morro do Chapéu, Bahia State, eastern Brazil (11°29′38.4″ S; 41°20′22.5″W) on March 10th, 2007 and stored in micro-centrifuge tubes (2.0 mL) at ambient temperature until used on March 4th, 2008. The pulp was removed with the aid of filter paper and the seeds were dried on filter paper at ambient temperature for 48 h. The seeds were soaked in a solution of 1000 mg L^{-1} gibberellin (GA_3) for 2 h and then disinfected with ethanol 96% for 1 min, posteriorly in a 2% solution of sodium hypochlorite (NaOCl), commercial bleach (QBoa®), for 10 min, and subsequently washed thrice in sterile water under aseptic conditions. Seeds were germinated in 250 mL glass flasks containing 50 mL of Murashige & Skoog (MS) culture medium (MURASHIGE; SKOOG, 1962) with the following salt concentrations: full-strength (MS); half-strength (MS/2); and a quarter-strength (MS/4) combined with two concentrations of sucrose (15 g L^{-1} and 30 g L^{-1}), solidified with 6.5 g L^{-1} of agar (Merck). The glass flasks were covered with two layers of polyvinyl chloride (PVC). The pH of the medium was adjusted to 5.7 before chemical sterilization, based on the protocol developed by Teixeira et al. (2006).

Number of germinated seeds was evaluated weekly for 11 consecutive weeks, considering germination as the emergence of root. After 120 days, we evaluated the final germination (%), considering the number of plants formed, length of the aerial portion (LAP) and fresh weight of the aerial portion (FWAP), length of the largest root

(LLR), and total dry weight (TDW). Dry weight was determined after drying in a forced ventilation oven at 60 ºC for 72 hours.

The experiment was conducted in a completely randomized design in 3 X 2 factorial scheme (salt concentrations X sucrose concentrations). Germination tests were performed using ten repetitions of 20 seeds each and analyses of growth involved five repetitions of ten randomly selected plants each.

The efficiency of chemical sterilization of the culture medium

Two types of plant material were used to evaluate the efficiency of chemical sterilization: seeds of *M. glaucescens* and the apical segment of cladode of *M. glaucescens* and *Melocactus paucispinus*.

Ripe fruits of *M. glaucescens* were collected from natural populations located in Morro do Chapéu, Bahia State, eastern Brazil (11°29′38.4″S; 41°20′22.5″W) on April 2nd, 2008. The pulp was removed with the aid of filter paper and the seeds were dried on filter paper at ambient temperature for 48 h and then stored in micro-centrifuge tubes (2.0 mL) at ambient temperature until used on May 22nd, 2009. The seeds were soaked in a solution of 1000 mg L^{-1} gibberellin (GA_3) for 24 h, disinfected as in the previous experiment, and then inoculated into 250 mL glass flasks containing 50 mL of MS/2 culture medium supplemented with 15 g L^{-1} of sucrose and solidified with 6.5 g L^{-1} of agar (Merk).

The chemical sterilization of the culture flasks was done based on Teixeira et al. (2006) methodology. The culture flasks were washed with detergent, rinsed firstly with tap water and secondly in a solution containing 10 drops of 2% of QBoa® for each liter of distilled water. Subsequently, they were dried in a forced ventilation oven at 100 ºC and kept in bags until the date of use. Before preparing the medium, the flasks were rinsed again in a solution containing 0.0003% of QBoa® for each liter of distilled water and dried in a forced ventilation oven at 100 ºC. The glassware used to prepare the medium was washed with detergent, rinsed firstly with tap water and secondly in a solution containing 0.0003% of QBoa® for each liter of distilled water.

The culture medium was chemically sterilized with 0.0003% of QBoa® (T1) following the protocol of Teixeira et al. (2006) or sterilized by autoclaving (121 ºC for 15 min) (T2). For the chemical sterilization of the culture medium, three drops of 2% NaOCl were added to each liter of distilled water at least one hour before adding the other components. The salts and sucrose were added to water and then 0.0003% of QBoa® (0.15 mL/per liter) was added. After 15 minutes the volume of water was completed and the pH was adjusted to 5.7. The culture medium was prepared in a non-sterile environment and its distribution in the culture flasks and the explant inoculation occurred in a laminar flow chamber. The glass flasks were covered with two layers of PVC.

At 150 days after inoculation without change of culture medium, the final germination (%), considering the number of plants, diameter of the aerial portion (DAP), LAP, LLR, and TDW were evaluated. The experiment was conducted in a completely randomized design. Germination tests were performed with ten repetitions of 20 seeds each. The analysis of growth employed seven repetitions with three randomly selected plants each.

Plants of *M. glaucescens* and *M. paucispinus* germinated *in vitro* with 340 days of culture had their apical segments of cladode separated, and these segments were inoculated into 250 mL glass flasks containing 50 mL of MS/2 medium supplemented with 15 g L^{-1} of sucrose and solidified with 6.5 g L^{-1} of agar (Merk) (TORRES-SILVA et al., 2018). The culture medium was chemically sterilized with 0.0003% of QBoa® (T1) (TEIXEIRA et al., 2006) or sterilized by autoclaving (121 ºC for 15 minutes) (T2). The plants generated from the apical segments of cladode were evaluated 120 days after inoculation regarding the following parameters: DAP, LAP, LLR, and TDW.

The experiment was conducted in a completely randomized design in 2 X 2 factorial scheme (species X sterilization techniques), with five repetitions and seven plants per repetition.

The culture medium was also visually evaluated in both experiments for contamination, considering those cultures with no perceptible contamination on or in the culture medium to be uncontaminated (TEIXEIRA et al., 2008).

Experimental conditions and Statistical analyses

The cultures were maintained at 25±3 ºC under two fluorescent lamps (Philips 100W) with photosynthetically active radiation levels of 60 $mmol.m^{-2}.s^{-1}$ and photoperiod of 16/8 h light/dark.

The data were submitted to analysis of variance using the Sisvar 5.3 statistical program (FERREIRA, 2011). The averages were compared using the Tukey test at 5% probability level. The final germination results, expressed as percentages, were arc-sine transformed $(\%G\ /100)^{0.5}$ to normalize their distribution.

RESULTS AND DISCUSSION

The numbers of seeds of *Melocactus glaucescens* germinating during the first week after inoculation varied among the different treatments, with more than 90 seeds germinating in the culture media having the lowest mineral salt concentrations: MS/4 + 30 g L^{-1} (91) and MS/4 + 15 g L^{-1} (98)

(Figure 1). The lowest levels of germination were observed in culture media with the greatest mineral salt concentrations: MS + 30 g L^{-1} (51) and MS +15 g L^{-1} (62). Similar results were observed for *Lophohora williamsii* and *Astrophytum asterias*, whose early germination may be enhanced by the half-strength of MS formulation (LEMA-RUMIŃSKA; KULUS, 2012; CORTÉS-OLMOS et al., 2018). In the following weeks, there was a reduction in germination (Figure 1) followed by cladode growth (Figure 2).

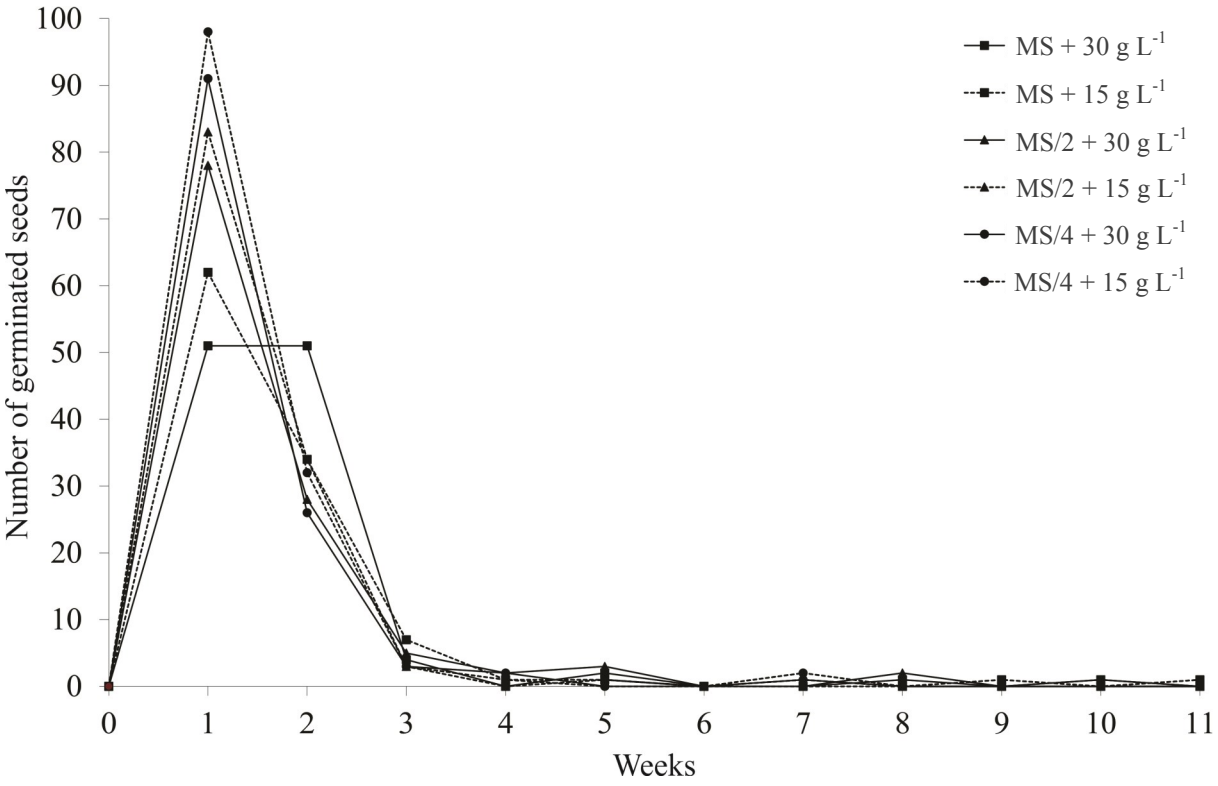

Figure 1. Number of germinated seeds of *Melocactus glaucescens* during 11 weeks, at different concentrations of salts from the MS medium and sucrose.

Figure 2. Germination of *Melocactus glaucescens* seeds at 9 days in MS/2 and 15 g L^{-1} of sucrose (A), at 50 days in MS and 30 g L^{-1} of sucrose (B) and at 92 days in MS/4 and 15 g L^{-1} of sucrose (C) bar = 1 cm.

Higher concentrations of salts and sucrose in the culture medium tend to diminish its water potential, thus reducing the rate of seed hydration and delaying the activation of the enzymes that will hydrolyze storage reserves during phase I, consequently delaying growth of the embryonic axis (BEWLEY et al., 2013). This situation can explain the decrease of germinating seeds during the first week after inoculation into MS + 30 g L^{-1} and MS + 15 g L^{-1} media when compared to the others treatments. In spite of this initial delay, the final germination values for *M. glaucescens* in the different treatments did not show any significant differences 11 weeks (120 days) after inoculation, varying between 53.5 and 68.1% (Table 1).

Table 1. Effects of different mineral salt and sucrose concentrations in Murashige & Skoog (MS) medium on the *in vitro* seed germination and plant growth of *Melocactus glaucescens* 120 days after inoculation.

MS mineral salt concentrations	Sucrose (g L^{-1})		Overall average
	15	30	
		Final germination (%)	
MS/4	68.10Aa	61.50Aa	64.75A
MS/2	61.00Aa	59.50Aa	58.81AB
MS	53.50Aa	55.00Aa	55.53B
Overall average	60.83a	58.67a	-
		Length of the aerial portion (mm)	
MS/4	10.76Aa	8.74ABb	9.75A
MS/2	9.70ABa	9.71Aa	9.71A
MS	9.36Ba	8.13Bb	8.74B
Overall average	9.94a	8.86b	-
		Length of the largest root (mm)	
MS/4	22.02Aa	21.10Aa	21.56A
MS/2	18.42Ba	20.26Aa	19.34B
MS	18.50Ba	17.28Ba	17.89B
Overall average	19.65a	19.55a	-
		Fresh weight of the aerial portion (mg)	
MS/4	114.28Aa	81.63Bb	97.95B
MS/2	121.41Aa	117.89Aa	119.65A
MS	101.50 Aa	83.28Ba	92.41B
Overall average	112.41a	94.26b	-
		Total dry weight (mg)	
MS/4	3.53Ab	5.47Aa	4.50A
MS/2	3.98Ab	5.27Aa	4.66A
MS	3.49Ab	4.64Aa	4.06A
Overall average	3.67b	5.14a	-

Averages followed by the same upper-case letters in the columns or the same lower-case letter in the rows do not differ significantly by the Tukey test ($p<0.05$).

The germination values observed for *M. glaucescens* are relatively high when compared to the *in vitro* seed germination of other species in the Cactaceae family such as *Notocactus magnificus* (13%) (MEDEIROS et al., 2006). As observed for *M. glaucescens*, high values for *in vitro* germination were found in *Melocactus sergipensis* (82-92%) (BRAVO FILHO et al., 2019), *Melocactus zehntneri* (85.5%), *M. sergipensis* (79.7%), *Melocactus violaceus* (58.2%) (SANTOS et al., 2020), *A. asterias* (67.5%-77.5%) (CORTÉS-OLMOS et al., 2018) and *Obregonia denegrii* (72.7%) (CARDARELLI; BORGOGNONE; COLLA, 2010).

There were no interactions between the concentrations of salts in the MS medium and the concentrations of sucrose for germination ($p<0.05$). These parameters were individually influenced by the different concentrations of sucrose tested. The germination values of MS/4 (64.75%) and MS/2 (58.81%) do not differ statistically, while the most concentrated medium (MS) had the lowest germination (55.53%) (Table 1). These results can be related to the greater water potential of the MS/4 and MS/2 culture media, which could favor seed germination.

The use of seeds for *in vitro* establishment culture of cactus represents a viable alternative for obtaining aseptic explants, because spines and/or hairs refuge a variety of microorganisms, which can make it difficult to disinfect the explants (MEDEIROS et al., 2006). In addition, the establishment of *in vitro* cultures from seeds is important for the formation of germplasm banks. Besides that, *in vitro* culture could contribute to the *ex situ* conservation of plants, with the aim to reintroducing them into their habitat to restore extinct or critically endangered natural populations (CORTÉS-OLMOS et al., 2018).

Although the *in vitro* germination of *M. glaucescens* was not influenced by the treatments tested, the plant growth parameters varied according to the factors analyzed. Significant interaction was observed between the concentrations of salts in the MS medium and sucrose concentrations for LAP

($p<0.05$). The highest value for LAP was obtained in the treatment with MS/4 + 15 g L^{-1} of sucrose (10.76 mm) (Table 1).

The other plant growth parameters analyzed were individually influenced by the different salt concentrations of MS medium and sucrose tested. LLR and FWAP were influenced by the concentrations of the MS medium and, in general, the reduction in the salt concentration of the MS medium was beneficial to the growth of *M. glaucescens* plants *in vitro*. The high values for LLR and FWAP were observed in the MS/4 and MS/2 media, respectively (Table 1).

As the primary objective of the present work was to obtain viable explants, we determined that the MS/4 medium supplemented with 15 g L^{-1} of sucrose promoted the greatest LAP, thus providing the greatest numbers of explants derived from the cladode. The greater availability of water in the medium with 15 g L^{-1} may have favored growth in length, since the turgor is important for the processes of cellular elongation. According to the results, it was observed that lowest salt concentration of MS medium is efficient to obtain plants of *M. glaucescens* germinated *in vitro*, and can be extended to other species of this genus as it was noted with the results for *Melocactus paucispinus.*

At 120 days after inoculation, LAP of *M. glaucescens* was 8-10 mm, making it possible to obtain 2 explants from each plant. Besides that, one apical cladode segment can be grown *in vitro* in PGR-free medium and can be used as source of explants (TORRES-SILVA et al., 2018). *In vitro* germination is a useful method for obtaining aseptic plants. This technique is very common to species of Cactaceae family, mainly when the goals are micropropagation and *in vitro* conservation (MEDEIROS et al., 2006; LEMA-RUMIŃSKA; KULUS, 2012; SANTOS et al., 2020).

The lowest values of LAP were observed at high concentration of sucrose and full-strength MS, which indicates that the use of these osmotic agents could be an efficient alternative to *in vitro* slow-growth strategies, a technique that promotes the success of *in vitro* conservation of *Melocactus* species.

The efficiency of chemical sterilization of the culture medium

No contamination of the culture medium was observed during the entire evaluation period in both experiments. The efficiency of the chemical method using NaOCl to disinfect both the seeds and the culture medium was confirmed by the absence of any contamination during the entire experiments.

The maintenance of succulent plant collections can be problematic, as many of these species are very susceptible to rots by bacteria and fungi (CASTRO et al., 2011). The presence of chlorine reduces the risk of contamination by endogenous microorganisms, which can be highly advantageous when using explants derived from field plants (TEIXEIRA et al., 2008). Additionally, NaOCl will inhibit contamination caused by handling of the medium or the culture flasks or introduced during the inoculation of the explants (TEIXEIRA et al., 2008).

The values for the variables analyzed as final germination and plant growth parameters (DAP, LAP, LLR, and TDW) did not differ statistically between the two sterilization techniques. This result demonstrates that media sterilized with NaOCl, at the concentrations used in this work, can be readily used to obtain plants from seed of *M. glaucescens* and were not toxic to the growth of the explants used (Table 2). Brondani et al. (2013) warn that active chlorine added to a culture medium can both cause damage to tissues and be beneficial depending on the concentration. Oliveira et al. (2015) observed that sterilization with NaOCl allowed similar or superior results compared to autoclaving for the analyzed variables for *Ananas comosus* var. *comomus*. In *Sequoia sempervirens*, the medium sterilized with NaOCl above 0.003% showed greater efficiency in the formation and average length of the branches, when compared to the autoclaved medium (RIBEIRO; TEIXEIRA; BASTOS, 2011).

Table 2. Final germination (%), diameter of the aerial portion (DAP), length of the aerial portion (LAP), length of the largest root (LLR), and total dry weight (TDW) of *Melocactus glaucescens* 150 days after inoculation into culture medium that was either autoclaved or sterilized with sodium hypochlorite.

Sterilization	Final germination	DAP (mm)	LAP (mm)	LLR (mm)	TDW (mg)
Chemical	13.5a	5.08a	6.36a	14.09a	5.10a
Autoclave	18.5a	4.59a	6.26a	14.86a	4.83a

Averages followed by the same letter in the columns do not differ significantly by the Tukey test ($p<0.05$).

The final germination values for *M. glaucescens* were low but did not significantly differ between the two sterilization treatments used (13.5 and 18.5%) (Table 2). This relatively low germination can be attributed to the viability of the seed lots used, since the experimental conditions

were the same as the previous germination and the seeds were soaked in GA_3 solution for more time (24 h).

The analyses of the plant growth regenerated from apical segments of cladode in culture medium that was either autoclaved or chemically sterilized indicated no interactions between species and sterilization techniques for any of the variables ($p<0.05$). The DAP, LAP, LLR and TDW values demonstrated no significant differences between the types of sterilization for the two species studied, while for the LAP variable, significant differences were observed between species. The highest averages of LAP were obtained by the species *M. glaucescens* (10.77 mm) (Table 3).

Table 3. Growth of plants regenerated from the apical segments of cladode of *Melocactus glaucescens* and *Melocactus paucispinus* 120 days after inoculation into culture medium that was either autoclaved or chemically sterilized with sodium hypochlorite.

Type of sterilization	Species		Overall Average
	M. glaucescens	*M. paucispinus*	
	Diameter of the aerial portion (mm) – DAP		
Chemical	7.39Aa	7.23Aa	7.31A
Autoclave	7.58Aa	6.93Ab	7.26A
Overall Average	7.49a	7.08a	-
	Length of the aerial portion (mm) – LAP		
Chemical	10.79Aa	9.76Ab	10.27A
Autoclave	10.75Aa	9.59Ab	10.17A
Overall Average	10.77a	9.68b	-
	Length of the largest root (mm) – LLR		
Chemical	21.85Aa	19.66Aa	20.76A
Autoclave	20.82Aa	17.94Aa	19.39A
Overall Average	21.34a	18.80a	-
	Total dry weight (mg) – TDW		
Chemical	18.08Aa	18.10Aa	18.09A
Autoclave	18.84Aa	15.20Aa	17.02A
Overall Average	18.46a	16.65a	-

Averages followed by the same upper-case letters in the columns or the same lower-case letters in the rows do not differ significantly by the Tukey test ($p<0.05$).

Teixeira et al. (2008) observed that the culture medium sterilization protocol using NaOCl differed from the traditional protocol developed by Murashige and Skoog (1962), as it employs a chlorine solution to sterilize the utensils used to prepare and hold the culture medium, and adds NaOCl directly to the culture medium. This fact can be advantageous for the production of plants with ornamental use, such as *M. glaucescens* and *M. paucispinus*, because chemical sterilization allows reduction of costs by dispensing with autoclave.

CONCLUSION

The use of chemical sterilization with NaOCl at the lowest concentrations of salts (MS/2 and MS/4) and sucrose (15 g L^{-1}) are quite advantageous for producing ornamental plants germinated *in vitro* and/or apical segment of cladode of *Melocactus glaucescens* and *Melocactus paucispinus*, representing reduction of costs for *in vitro* cultivation of this species.

ACKNOWLEDGEMENTS

We thank Delmar Lopes Alvim for the help during fieldworks.

REFERENCES

BEWLEY, J. D. et al. **Seeds: physiology of development, germination and dormancy**. 3 ed. NewYork: Springer, 2013. 392 p.

BRAVO FILHO, E. S. et al. Germination and acclimatization of *Melocactus sergipensis* Taylor & Meiado. **Iheringia**, Série Botânica, 74: e2019009, 2019.

BRONDANI, G. E. et al. Chemical sterilization of culture medium: a low cost alternative to *in vitro* establishment of plants. **Scientia Florestalis**, 41: 257 -264, 2013.

CASTRO, J. P. et al. *In vitro* germination and

disinfestation of sweet cactus (*Nopalea cochenillifera* (L.) Salm Dyck). **Acta Scientiarum Agronomy**, 33: 509-512, 2011.

CARDARELLI, M.; BORGOGNONE, D.; COLLA, G. *In vitro* propagation of *Obregonia denegrii* Frič. (Cactaceae). **Propagation of Ornamental Plants**, 10: 29-36, 2010.

CORTÉS-OLMOS C. et al. *In vitro* germination and growth protocols of the ornamental *Lophophora williamsii* (Lem.) Coult. as a tool for protecting endangered wild populations. **Scientia Horticulturae**, 237: 120-127, 2018.

FERREIRA, D. F. Sisvar: um sistema computacional de análise estatística. **Ciência e Agrotecnologia**, 35: 1039-1042, 2011.

HUANG, H. P. et al. Germplasm preservation *in vitro* of *Polygonum multiflorum* Thunb **Pharmacognosy Magazine**, 10: 179-184, 2014.

IUCN. **IUCN Red List of Threatened Species**. Version 2020.1. Available at: <https:// www.iucnredlist.org/>. Accessed date: 22 apr. 2020.

LEMA-RUMIŃSKA, J.; KULUS, D. Induction of somatic embryogenesis in *Astrophytum asterias* (Zucc.) Lem. in the aspect of light conditions and auxin 2,4-d concentrations. **Acta Scientiarum Polonorum - Hortorum Cultus**, 11: 77-87, 2012.

LEMA-RUMIŃSKA, J.; KULUS, D., Micropropagation of cacti – a review. **Haseltonia**, 18: 46-63, 2014.

MACHADO, M. C. The genus *Melocactus* in eastern Brazil: part I - an introduction to *Melocactus*. **British Cactus & Succulent Journal**, 27: 1-16, 2009.

MEDEIROS, L. A. et al. *In vitro* propagation of *Notocactus magnificus*. **Plant Cell Tissue and Organ Culture**, 84: 165-169, 2006.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum**, 15: 473-497, 1962.

OLIVEIRA, R. S. et al. Esterilização química e agentes geleificantes alternativos na propagação vegetativa *in vitro* do abacaxizeiro 'Vitória'. **Revista Brasileira de Fruticultura**, 37: 819-826, 2015.

PÉREZ-MOLPHE-BALCH, E. et al. Tissue culture of ornamental cacti. **Science in Agriculture**, 72: 540 -561, 2015.

PHILLIPS, G. C.; GARDA, M. Plant tissue culture media and practices: an overview. ***In Vitro* Cell Developmental Biology - Plant**, 55: 242-257, 2019.

RIBEIRO, J. M.; TEIXEIRA, S. L.; BASTOS, D. C. Cultivo *in vitro* de *Sequoia sempervirens* em meio de nutritivo esterilizado com hipoclorito de sódio. **Ciência Florestal**, 21: 77-82, 2011.

SANTOS, M. C. et al. Efeito da sacarose e do sorbitol na conservação *in vitro* de segmentos nodais de mangabeira**. Revista Ciência Agronômica**, 42: 735-741, 2011.

SANTOS, P. A. A. et al. Do diferente cultivation environments influence the germination and initial growth of three threatened Brazilian cacti species? **South African Journal of Botany**, 132: 363-370, 2020.

TAYLOR, N. P. et al. A new *Melocactus* from the Brazilian state of Sergipe. **Bradleya**, 32: 99-104, 2014.

TEIXEIRA, S. L. et al. Influence of NaClO on nutrient medium sterilization and on pineapple (*Ananas comosus* cv Smooth cayenne) behavior. **Plant Cell Tissue and Organ Culture**, 86: 375-378, 2006.

TEIXEIRA, S. L. et al. Utilização de hipoclorito de sódio na esterilização de meio de cultura para a multiplicação *in vitro Eucalyptus pellita* L. **Ciência Florestal**, 18: 185-191, 2008.

TORRES-SILVA, G. et al. *In vitro* shoot production, morphological alterations and genetic instability of *Melocactus glaucescens* (Cactaceae), an endangered species endemic to eastern Brazil. **South African Journal of Botany**, 115: 100-110, 2018.

ZAPPI, D.; TAYLOR, N. 2020. *Cactaceae in* **Flora do Brasil 2020 em construção**. Jardim Botânico do Rio de Janeiro. Available at: <http:// reflora.jbrj.gov.br/reflora/floradobrasil/FB1558 Accessed date: 23 aug. 2020.