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Seed germination and substrates for seedlings cultivation of *Melocactus zehntneri*

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ABSTRACT

The populations of *Melocactus zehntneri* 'chapéu-de-frade' have shown an alarming decline due to the constant withdrawal of individuals from their natural areas. The low percentage of germination of *M. zehntneri* seeds, added to the slow growth, requires a system for commercial propagation of this species. This study aimed to test the influence of water, hydrochloric acid or acetic acid pre-treatments on seed germination and the influence of different substrates on the initial development of *M. zehntneri* seedlings. The seeds were immersed in deionized water, hydrochloric acid (5N) or glacial acetic acid (100%) for 10, 20, 30, 40 and 50 minutes and placed to germinate in a Petri dish containing filter paper moistened with 5 mL deionized water. The seedlings obtained from this germination (≡ 0.4 cm diameter) were cultivated on Carolina Soil® and vermiculite [2:1] substrates; Carolina Soil® and vermiculite [2:1] + drainage; medium-grained sand; medium-grained sand + drainage; medium-grained sand and organic compost [1:1]; medium-grained sand and organic compost [1:1] + drainage; and Carolina Soil® and medium-grained sand [1:1]. The highest percentage of germination was obtained with the pre-treatment by immersion in water, in the times of 10 and 30 minutes, totaling 55% of germinated seeds. Treatments with hydrochloric acid did not increase the percentage of germinated seeds, which were also close to 50%. In the test with the substrates, in Carolina Soil® there were the best rates of seedling survival after transplanting (87.5%) and seedling development performance. Treatments containing organic compost resulted in lower survival (69%) and seedling development. Seed germination and seedling development in Carolina Soil® substrate and sand proved to be an excellent alternative for the production of *M. zehntneri* seedlings.

Keywords: 'coroa-de-frade', globose cladode, seed dormancy, vegetative growth, pot cultivation.

RESUMO

Germinação de sementes e substratos para mudas de *Melocactus zehntneri*

As populações de *Melocactus zehntneri* (chapéu-de-frade) têm apresentado um declínio alarmante devido à constante retirada de indivíduos de suas áreas naturais. A baixa porcentagem de germinação das sementes, somada à lentidão de crescimento, exige um sistema de propagação comercial da *M. zehntneri*. Este trabalho teve como objetivos testar a influência dos pré-tratamentos com água, ácido clorídrico ou ácido acético na germinação de sementes e a influência de diferentes substratos no desenvolvimento inicial de plântulas de *M. zehntneri*. As sementes foram imersas em água deionizada ou ácido clorídrico (5N) ou ácido acético glacial (100%) por 10, 20, 30, 40 e 50 minutos e colocadas para germinar em placas de Petri contendo papel filtro umedecido com 5 mL de água deionizada. As plântulas obtidas (≡ 0,4 cm de diâmetro) foram cultivadas em substratos Carolina Soil® e vermiculita [2:1]; Carolina Soil® e vermiculita [2:1] + drenagem; areia média; areia média + drenagem; areia média e composto orgânico [1:1]; areia média e composto orgânico [1:1] + drenagem; e Carolina Soil® e areia média [1:1]. A maior porcentagem de germinação (55%) foi obtida com o pré-tratamento por imersão em água, nos tempos de 10 e 30 minutos. Os tratamentos com ácido clorídrico não aumentaram a porcentagem de sementes germinadas, que também ficaram próximas a 50%. No teste com os substratos, em Carolina Soil® houve as melhores taxas de sobrevivência de plântulas após o transplante (87,5%) e desempenho de desenvolvimento de plântulas. Os tratamentos contendo composto orgânico resultaram em menor sobrevivência (69%) e desenvolvimento das plântulas. A germinação de sementes e o desenvolvimento de mudas em substrato Carolina Soil® e areia mostraram-se uma excelente alternativa para a produção de mudas de *M. zehntneri*.

Palavras-chave: coroa-de-frade, cladódio globoso, dormência em sementes, crescimento vegetativo, cultivo em vaso.

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The genus *Melocactus* is made up of plants popularly known as coroa-de-frade or chapéu-de-frade and has a distribution in Central and South America, the Caribbean, the Andes and Mexico. In Brazil, the genus occurs in all northeastern states, except Maranhão, and in parts of the North and Southeast regions. Brazil is the country with the greatest diversity of *Melocactus* species in the world, presenting 21 endemic species, which represent about 55% of the total species already recognized (Zappi *et al*., 2015). *Melocactus* generally has a globose cladode and very slow vegetative growth. At its apex it is possible to locate the cephalium, a peculiar structure to

the genus, which occurs only when the cactus reaches reproductive maturity, with approximately ten years age.

The demand for *Melocactus* species in the commercial sector, especially for ornamental and collector's purposes, has had remarkable growth in recent years due to their peculiar aesthetics and easy maintenance. These plants present great attractive and economic power due to their ornamental attributes. However, it is difficult to estimate numbers regarding the trade of wild individuals (Cavalcante & Vasconcelos, 2016), being common the mass commercialization of adult plants removed from their habitat.

A concern about the extractivism of *Melocactus* is that the genus has more difficulty in recolonizing its habitats compared to other genera, besides the high degree of endemism it presents. As consequence, many *Melocactus* species are having their populations drastically reduced, so that the removal of individuals from its habitat is disproportionate to its growth time, making this practice non-sustainable. Consequently, some *Melocactus* species are already under protection, as a way to control the high flow of extraction for trade (Cites, 2019). Population of *M. zehntneri* has been showing recent and important decline in regions of the Northeast, where only small isolated populations remain (Machado *et al*., 2017; Bravo Filho *et al*., 2019; Bravo Filho & Ribeiro, 2018).

The natural reproduction of this genus was exclusively through seeds and has propagative limitations, since it do not develop segmentations as in other cactus species (Correia *et al*., 2018a). In addition, it also has a low survival rate in its initial development and few individuals reach the age of reproduction (Coelho *et al*., 2015). *Melocactus* seeds also didn't present a good germination rate soon after being extracted from the fruit due to the concentration of inhibitors, probably present in the forehead of the seeds (Correia *et al*., 2018b). The seeds are also lined with a mucilage similar to arilum, which has a great capacity to absorb water and serving as protection against the dehydration common in the semi-arid climate (Correia *et al*., 2018b). These features lead to believe that the seeds, in natural habitat, need to spend a period in humid conditions, or even pass through the digestive system of dispersers so that possible physical or chemical inhibitors of germination can be removed (Bárbara *et al*., 2015). In this way, the action of chemical agents, such as acids, could cause the tegument rupture, breaking the seed's dormancy and release the germination by chemical scarification.

After germination, the cultivation of seedlings can be realized using commercial substrates. The commercial production of cactus seedlings using seed germination followed by substrate cultivation under controlled environmental conditions is the unique technique that could replace the unsustainable extractivism of individuals in the commercial cultivation. However, until now, there are few information about the use and evaluation of substrates in the cultivation of *Melocactus* species (Bravo Filho & Santana, 2019).

Thus, the present study aimed to develop a method for seed germination and seedling production of *M. zehntneri*, as well as to analyze the influence of different substrates on the development of germinated seedlings.

MATERIAL AND METHODS

Description of the species and origin of seeds

We used the species *Melocactus zehntneri*, from the germplasm collection of the Centro de Ciências Agrárias at Federal University of São Carlos (CCA/UFSCar), registered as ABBC280 by the National System for Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) (Brasil, 2016). Three adult plants in full bloom and fructification were used as seed source, and the fruits were collected at the moment they were detached from the cephalium. The species *M. zehntneri* has a dark green or light green globular stem, generally opaque (Figure 1a). It has 8 to 13 spines per areolae, which can form a gradient of brown, yellow and opaque pink, covered by a grayish tone, except at the tips, which have a dark color and curvature. The cephalium presents all over its extension thin reddish or pinkish bristles (Figure 1a, 1b). The flowers at the cephalium are small and vary from pale to dark pink (Figure 1b, 1c). The fruits are small and juicy when ripe, being pink or pale lilac in color (Figure 1d), approximately 12 to 20 mm long, with black and round seeds of approximately 1.4 mm diameter (Figure 1e, 1f).

The seeds were removed from the fruits and, using a sieve, were washed in running water with a few drops of neutral detergent to remove the surplus of mucilage from the fruits and that lined them. After washing, they were placed in the shade and at room temperature to dry on filter paper for 24 hours. The seeds remained stored in these conditions for about 14 days until the experiments were performed.

Chemical scarification and germination of *M. zehntneri* seeds in Petri dishes

The seeds of *M. zehntneri* were exposed to treatments by chemical scarification using 3 treatments: 1) 100% glacial acetic acid (AA); 2) 5N hydrochloric acid (HCl) and; 3) deionized water (H_2O) all by immersion of seeds in these solutions during 10, 20, 30, 40 and 50 minutes.

The experimental design was entirely randomized, in factorial 3 x 5 (solutions x immersion time), distributed in five repetitions, consisting of individual Petri plates containing 20 seeds each. The treatments were performed using 5 mL of each solution in a Falcon® tube containing 100 seeds each. As control, seeds were taken directly from the fruit without any treatment, including the mucilage around seeds.

After the treatment times, the seeds received triple rinsing with 10 mL deionized water for three minutes each under agitation, also performed in a Falcon® tube aiming to stop the reaction of acids, which can cause damages in the embryos.

All seeds were sown in Petri plates containing filter paper saturated with 5 mL deionized water, obtained from a solution containing 2 mL/L

of DioxiPlus® (7% active chlorine) containing stabilized chlorine dioxide and used to reduce the microbial load on the filter paper during the cultivation period. There was no need to replace the volume of water throughout the germination time. The Petri dishes were then closed and sealed with transparent PVC film and maintained in a growth room at 26±1ºC, photoperiod of 14 hours, light intensity of 30 µmol/m²/s obtained by cold white fluorescent lamps.

Germination was checked daily and been considered germinated seeds when a visible protrusion of the hypocotyl root axis equal to or greater than 0.1 cm appeared. At the end, the germination percentage and germination speed index (GSI) were calculated.

Acclimatization of Petri-dish seedlings and cultivation in substrate

After germination and initial seedling development of *M. zehntneri,* the individuals obtained went through the acclimatization stage. Overall, 533 germinated individuals were acclimatized. The used substrate was Carolina Soil® (peat, carbonized rice husk and vermiculite), placed in a plastic container containing approximately 500 mL of substrate. Small surface cavities were made in the substrate and the seedlings were planted. The seedlings remained under illumination of cool white fluorescent lamps and at room temperature, with minimum of 16°C and maximum of 28°C for a period of three months.

Irrigation was performed once a week, together with fertilizer application, using Plant Prod® 20-20-20 fertilizer plus micronutrients (1.2 g/L) and the salts $Ca(NO_3)_2$ (0.3 g/L) and $MgSO_4$ (0.25 g/L). Before fertigation, the nutritive solution had its pH adjusted to 6.0-6.5.

Experiment with seedling cultivation and development in different substrates

After the acclimatization period of three months, individuals of approximately 0.5 cm diameter of cladodes were obtained (Figure 2a). The used substrates consisted of different proportions in volume as follows: Carolina Soil® number 25 (peat, carbonized rice husk and vermiculite), medium grained sand and organic compost from emerald grass waste and bovine manure obtained according the methodology of Zanello & Cardoso (2016). The proportions and combinations of elements are described in Table 1. Besides the four treatments, three others were used with the same elements, but with the difference that gravel type stone (small pieces) was incorporated to test the interaction between substrate and drainage, since most of these cacti occur in rocky habitat with high drainage (Correia *et al*., 2018b), thus totaling seven treatments.

The *Melocactus* seedlings were individually arranged in different substrates for evaluation of characteristics that meet the most adequate and rapid development for the species. The experiment was done

Figure 1. Plant material of *Melocactus zehntneri* used for the study: a) Morphology and general characteristics of the species; b) Characteristics of the cephalium showing the flower's development; c) Morphology representation: Flower (c.a); flower crosssection (c.b); fruit (c.c); fruit crosssection (c.d); d) Fruits from *M. zehntneri* species; e) Germinated seeds (red arrows) showing the green hypocotyl radicular axis; f) Non-germinated seeds cultivated directly from the fruit (control) (Original figure from MCFM). Araras, UFSCar, 2020.

in an entirely randomized design, with four types of substrates without drainage and three with drainage layer sourced by gravel small stones. Each treatment was composed of 24 repetitions (seedlings), totaling 168 individuals, so that each cell had only one individual of the plant species. The substrates were placed in polyethylene trays containing 128 cells. The treatments with the use of gravel had the stones placed at the bottom of the cell forming a single layer.

All substrates were evaluated at the beginning of the experiment about the pH and electrical conductivity (EC). For this analysis, 250 mL of substrate, obtained from the same source used for the experiments were diluted in 250 mL distilled water and put under agitation for 30 minutes. Thus, the solutions were filtrated using cellulose filter. Therefore, the pH using pHmeter Model Seven Compact (Mettler, Switzerland) and electrical conductivity (EC) using condutivimeter (Hannah Instruments, Portugal) were evaluated in filtrated aqueous solutions (Table 1).

The trays were kept in a greenhouse at 28.2±6.8ºC and relative air humidity of 71.3±21.2%, measured by Incoterm® thermo-hygrometer. The cooling was controlled by the Pad-Fan system and the shading used was 50%, obtained by the use of Aluminet® shade net. The environmental conditions used in the greenhouse are considered standard, and can reach >90% survival of seedlings of other species (Iiyama & Cardoso, 2021). *M. zehntneri* was irrigated with tap

Table 1. Composition of substrates used in the cultivation of *M. zehntneri* and relative proportions used as treatments for the initial development of seedlings. Araras, UFSCar, 2020.

Substrates	Proportion (v/v)	pH	Electrical conductivity (mS/cm)
Carolina Soil® + vermiculite	2:1	5.47	1.08
Carolina Soil® + vermiculite + gravel drainage	2:1	5.47	1.08
Medium grained sand	1	6.31	0.08
Medium grained sand + gravel drainage	1	6.31	0.08
Medium grained sand + organic compost	1:1	5.88	6.0
Medium grained sand + organic compost + gravel drainage	1:1	5.88	6.0
Carolina Soil® + medium grained sand	1:1	6.17	0.50

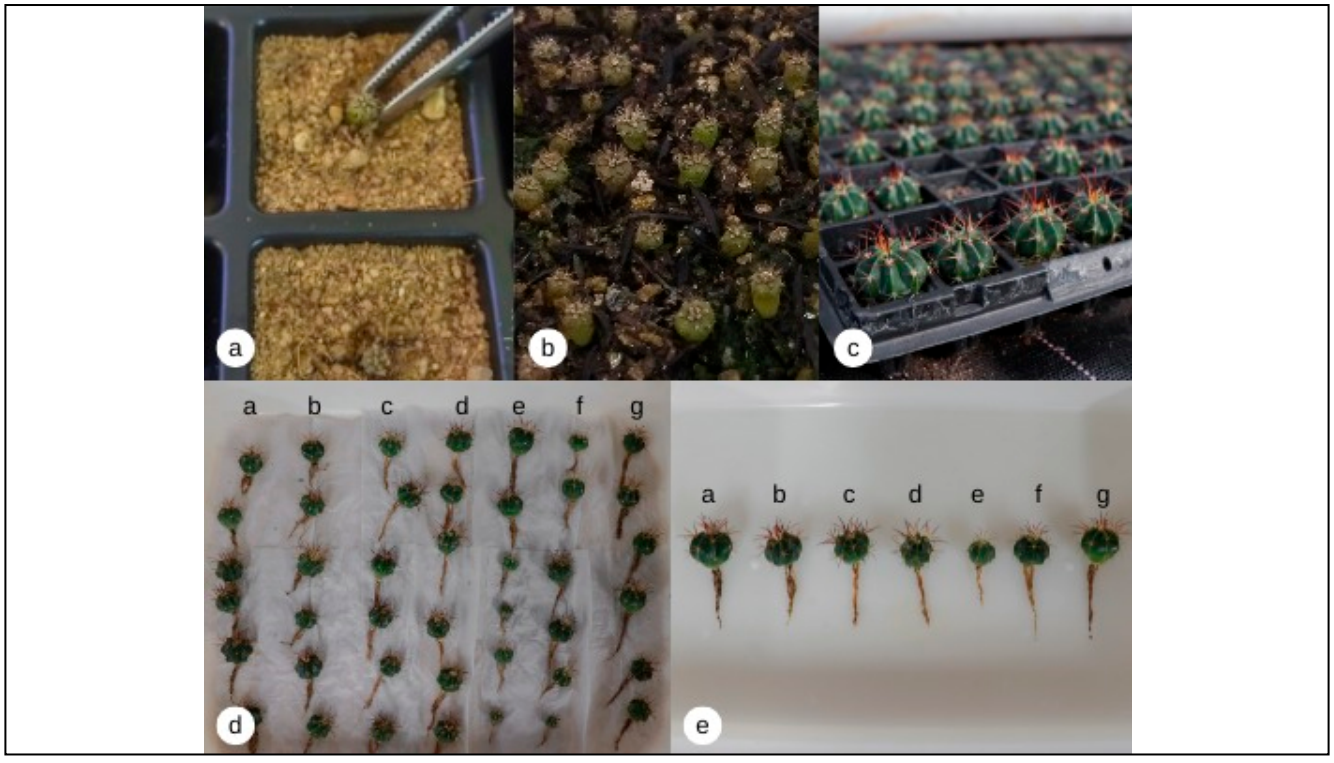

Figure 2. Different substrates in the cultivation of *Melocactus zehntneri* seedlings: a) *M. zehntneri* seedlings in sand substrate; b) Seedlings of *M. zehntneri* 74 days after acclimatization used for experiments with different substrates; c) General view of the seedlings in the experiment with different substrates; d) and e) Individuals cultivated under different growing substrates and greenhouse conditions. Araras, UFSCar, 2020.

water once a week and fertigation was done every two weeks using PlantProd® 20-20-20 fertilizer plus micronutrients (1.2 g/L), $Ca(NO_3)_2$ (0.3 g/L) and $MgSO_4$ (0.25 g/L) salts with pH adjusted to 6.0-6.5 before the fertigation.

The cacti remained under the treatment substrates for a period of 14 months. To determine the effects of substrate on the cultivation and development of *M. zehntneri* seedlings, the following variables were analyzed: percentage of seedling survival (%S); polar and equatorial diameter (PD) of the cladodes; equatorial diameter (ED); root length (RL); number of roots (NR); root fresh mass (RFM); root dry mass (RDM); aerial fresh mass (AFM) and aerial dry mass (ADM). For the characteristics %S, PD and ED, measurements were made in all 24 individuals of each treatment (Figure 2b). For the other characteristics, six plants per treatment were used, totaling 42 individuals. These were analyzed with the aid of a millimeter ruler, pachymeter and precision analytical balance Mettler ML201.

To establish the weight of fresh and dry mass of the roots and globose part of the cactus, sections of the parts were made, separating the root system from the aerial part. After weighing the fresh masses, longitudinal cuts were made in the cladodes to facilitate the total loss of water in the oven. The materials were packed in kraft paper bags separately and placed in an oven at 65°C for 48 h until reaching constant mass. Then, using an analytical balance, the weight of dry mass in grams was determined.

Data analysis

The obtained data were submitted to analysis of variance (ANOVA). The Tukey test at 5% significance was made to compare means using the software AgroEstat (Barbosa & Maldonado Júnior, 2009) and RStudio (Patel *et al*., 2017).

RESULTS AND DISCUSSION

Chemical scarification and germination of *M. zehntneri* seeds in Petri dishes

The results showed differences among the treatments, but no significant interactions were observed among the studied factors (product used x immersion time). The seeds of *M. zehntneri* remained in Petri dishes for a total of 23 days. The onset of germination occurred with the protrusion of the hypocotyl-radicular axis, resulting from embryo expansion and tegument rupture.

Table 2. Percentage of germinated seeds (%G) and mean values of germination index (GSI) of *Melocactus zehntneri* seeds submitted to different treatments. Araras, UFSCar, 2020.

Times (min)	Treatments	Germination (%)	GSI
10	Glacial acetic acid (AA)	34 b	1.76 b
	Hydrochloric acid (HCl)	50 a	3.44 a
	Deionized water (H_2O)	55 a	3.16 a
20	AA	28 b	1.32 b
	HCl	35 a	1.81 a
	H_2O	35 a	2.27 a
30	AA	25 b	0.80 b
	HCl	48 a	2.96 a
	H_2O	55 a	3.22 a
40	AA	20 b	0.75 b
	HCl	51 a	2.94 a
	H_2O	47 a	2.80 a
50	AA	4 b	0.15 b
	HCl	26 a	1.68 a
	H_2O	47 a	2.94 a
CV (%)		37.61	34.07
F		2.0585^{NS}	1.665^{NS}

Means followed by same letters in the columns do not differ significantly according to Tukey test (5%). NS = not significant.

Germination of pre-treated seeds with hydrochloric acid (HCl) or water (H_2O) started on the third day of seeding. Germination percentages were higher in seeds pre-treated with the hydrochloric acid (HCl) and deionized water (H_2O), not differing significantly from each other. Using HCl, the highest seed germination was reported with 10, 30 and 40 minutes of immersion, which resulted in 50%, 48% and 51% of germinated seeds, respectively. Interestingly, the immersion of seeds of *M. zehntneri* for 10 and 30 minutes in H_2O also presented higher seed germination (55%) (Table 2).

Using glacial acetic acid (AA), the germination of seeds started from the sixth day only in the times of 10, 20 and 30 minutes, however, in lower percentages compared to the seeds immersed in HCl and H_2O. The low germination rate of seeds under the AA may be related to the concentration used, or even due to the chemical structure of this organic acid, which may have caused toxicity to the embryo. The phytotoxic effect of the majority of organic acids occurs in the initial phases of development and may directly harm germination (Lemes *et al*., 2014).

Santos *et al*. (2020), in their study about the influence of light and different growing environments on the germination of *Melocactus* species (*M. sergipensis*, *M. violaceus*, *and M. zehntneri*), concluded that seeds of these species are photoblastic positive. These authors also demonstrated higher rates of seed germination on the seventh day after sowing, and showed very similar results to the actual study.

The fact that germination did not exceed 55% may be explained by the unviability of the seeds (absence or unviable embryo) or the presence of

Table 3. Mean values of survival percentage (%S), polar diameter (PD), equatorial diameter (ED), root length (RL) and number of roots (NR), aerial fresh mass (AFM), aerial dry mass (ADM), root fresh mass (RFM), root dry mass (RDM) of *Melocactus zehntneri* individuals on different substrates evaluated at 14 months of cultivation in each substrate. Araras, UFSCar, 2020.

Treatment	%S	PD (cm)	ED (cm)	RL (cm)	NR	AFM (g)	ADM (g)	RFM (g)	RDM (g)
a	91.7 a	1.76 a	2.05a	4.23 ab	17.50ab	5.52a	0.28a	0.18a	0.02ab
b	83.3 a	1.62 a	1.88a	4.35 ab	21.00a	4.82a	0.31a	0.12ab	0.02ab
c	79.2 a	1.37 ab	1.71ab	5.30a	11.33bc	4.57a	0.27a	0.10bc	0.02ab
d	79.2 a	1.33 ab	1.71ab	5.07a	8.83c	4.88a	0.37a	0.12ab	0.02a
e	70.8 a	0.85 b	0.98c	3.32b	8.83c	3.15a	0.07c	0.06c	0.01ab
f	66.7 a	1.02 b	1.23bc	3.97ab	11.50bc	3.14a	0.11bc	0.07bc	0.01b
g	95.8 a	1.70 a	2.11a	4.48ab	17.00ab	4.92a	0.21ab	0.09bc	0.02ab
F value	NS	6.6792**	8.0855**	3.7215**	8.1437**	NS	8.9318**	8.0762**	3.8905**
Average	80.96	1.38	1.67	4.39	13.71	4.43	0.23	0.11	0.02
CV (%)	17.66	45.84	50.37	11.43	24.02	43.82	26.73	6.66	4.65

Means followed by same letters in the columns do not differ significantly (NS) according to Tukey test at 5% (*) or 1% (**) significance level. a= Carolina Soil® + vermiculite; b=Carolina Soil® + vermiculite + gravel drainage; c= Medium grained sand; d= Medium grained sand + gravel drainage; e= Medium grained sand + organic matter; f= Medium grained sand + organic matter + gravel drainage; g= Carolina Soil® + medium grained sand.

another type of dormancy unresolved by the treatments used in our study, what we consider the most likely. It has already been observed in other species of *Melocactus* that its seeds present low to medium germination rates (Arias & Lemus, 1984). In a study of the *Melocactus azureus* species, also around 50% of the seeds germinated (Bárbara *et al*., 2015*)*. These results are consistent with the data obtained in our study using HCl and H_2O treatments for *M. zehntneri*.

In addition, studies of Arias & Lemus (1984) about germination of *Melocactus caesius* seeds, demonstrated that washing of seeds before sowing is required to obtain good germination rates. All seeds used in our study with *M. zehntneri* were also previously washed and the treatments with immersion in water resulted in increased germination (Table 1). Previous study with *M. zehntneri* seeds from the same batch, in the same conditions of Petri dishes, showed no germination with unwashed seeds (data not presented). This leads to the conclusion that the contact of seeds with mucilage inhibits its germination in this genus and could be related to the presence of germination inhibitors in the mucilage. These data and results indicate the necessity of further studies about the inhibitory compounds contained in the tegument, mucilage or aril of this cactus seeds, which can be removed by water and release the germination process.

The germination speed index (GSI) was also improved using HCl and H_2O, similarly to that observed with germination rates. The highest GSI where obtained with HCl and H_2O, with no significant differences between them (Table 2). The use of AA resulted in reduced GSI values. In addition, the highest germination rates were observed between 9 to 11 days after sowing using HCl or water pre-treatments. Bravo Filho & Santana (2019) observed that germination of *M. zehntneri* and *M. violaceus*, reached the highest germination rates after the sixth day of culture.

Acclimatization of germinated seedlings

The cacti that went through acclimatization presented adequate development, within the parameters of growth time of the genus, which is considered slow. There was no effect of chemical scarification of seeds on the efficiency of seedlings acclimatization.

Of the 533 acclimatized seedlings, 416 individuals showed vigorous development totaling 78% survival of seedlings after three months of cultivation under substrate. The rate of individuals that survived to the acclimatization in the current study was similar to that described by Bravo Filho *et al*. (2019), who observed 80% of seedlings survival rate, since seedlings were transplanted from Petri dishes in laboratory conditions to substrate and under greenhouse conditions. Also, we noted a good growth of *M. zehntneri* seedlings regarding cultivation in substrate, based on the height and diameter of the cladodes, presenting on average 0.8 and 0.5 cm, respectively. In the upper axis of the cladodes, the areolas containing the spines were visible (Figure 1f).

Seed germination using Petri dishes followed by acclimatization in substrate can also be a method of propagation with commercial advantages, since the cost is low and the ease of storage and transport of large quantities of seeds (Bravo Filho & Santana, 2019). Furthermore, the species under study has a good annual production of fruits and seeds per adult plant, observed from the three donor plants of fruits and seeds of *M. zehntneri* used in this experiment. A study on the viability of *Melocactus guitartii* seeds stored for up to twelve months showed the maintenance of the viability of the seeds along storage, demonstrating that this is another advantage in the use of seeds for seedling production, and may

also consider the possibility of forming seed banks of *Melocactus* species, also contributing to the conservation of the species (Correia *et al*., 2018a).

Propagation from seeds has biological relevance, since it allows the genetic diversity between progenies to be maintained, besides being a more economical alternative compared to other propagation techniques, such as vegetative propagation. Studies on sexual propagation can contribute to the conservation of cacti, since the commercial production of seedlings is associated with a decrease in the demand for individuals from natural populations in their natural areas of occurrence (Coelho *et al*., 2015).

In conclusion, seed propagation is an excellent alternative both for species conservation and aiming the commercial propagation of the *M. zehntneri*.

Seedling cultivation and development in different substrates

The development of *Melocactus zehntneri* seedlings germinated in Petri dishes and acclimatized on substrates was satisfactory, and demonstrated its good development (Figure 2c). The values observed for the percentage of survival (%S) of *M. zehntneri* individuals did not show significant difference between the substrates applied to the seedlings (Table 3). Furthermore, the survival rate, considering all treatments, was close to 81%. The high percentage of survival of *Melocactus* individuals may be related to its physiology and morphology adapted to hot and dry climatic environments, such as the presence of the Crassulacean Acid Metabolism (CAM), fascicular and superficial root system, aquiferous parenchyma, presence of spines and wax, which reduce water loss (Cavalcante *et al*., 2013) and maintain the functioning of the physiological system temporarily when under stress.

It was possible to observe better results of the variables polar diameter (PD) and equatorial diameter (ED) in treatments 'a', 'b' and 'g', differing significantly from the other treatments (Table 3; Figure 2d, 2e).

On the other hand, the treatments with low performance in the variables PD and ED, in comparison to the other substrates, were treatments 'e' and 'f', in which treatment 'e' presented 0.85 cm for PD and 0.98 cm for ED; and treatment 'f' presented 1.02 cm for PD and 1.23 cm for ED. For the variable ED, both treatments (e and f) showed significantly lower results (Table 3). Treatments 'e' (medium grained sand + organic matter) and 'f' (medium grained sand + organic matter + drainage) were the only ones in which we could observe individuals with no development, being visually contrasting with the other treatments. The individuals under this treatment presented 0.9 cm height (polar diameter) and 1.0 cm equatorial diameter of the cladodes (Table 3). 37.3% of individuals showed no development in treatment 'e' and 37.5% in treatment 'f'.

Comparing the results of variables PD and ED with the results obtained from Cavalcanti & Resende (2007) on the effect of different substrates on the development of mandacaru (*Cereus jamacaru*), facheiro (*Pilosocereus pachycladus*), xique-xique (*Pilosocereus gounellei*) and coroa-de-frade (*Melocactus bahiensis*), more specifically the species of *Melocactus bahiensis,* the height and diameter were about five times greater than the results found in the actual study, even occurring within the same period of time and under similar substrates. These higher differences can be explained by multiple factors such as the genotypic differences in growth rate of *M. bahiensis* and *M. zehntneri*; the environmental conditions of cultivation of actual and that conducted with *M. bahiensis*; the volume of the cultivation container in which the individuals of *Melocactus bahiensis* developed (22 cm height and 15 cm diameter).

According to Zaccheo *et al*. (2013), the volume of the cultivation container can interfere with the development of the roots and the aerial part of the plant, so that when a plant is in a container too tight and disproportionate to its size it can alter the physiological responses of the plant, reflecting on the quality of the seedling. However, other factors such as climatic conditions of cultivation and differences in growth rates between *M. zehntneri* and *M. bahiensis* species also have high importance in these comparisons. During the removal of *M. zehntneri* individuals for evaluation, it was observed that the roots and aerial part occupied a large part of the substrate contained in the cell container. Therefore, it is possible that *Melocactus* seedlings require larger containers or require reduced period of development of the cactus in the cell-trays before the transplantation to a new container with greater volume.

Similar to that observed in the cladode development, the use of organic matter in substrates 'e' and 'f' significantly reduced the RL (root length), the number of roots and root fresh mass of *M. zehntneri* (Table 3). In addition, the higher values for root analysis were obtained using sand (substrate 'c') and the commercial substrate Carolina Soil® ('a'). Better results for root fresh mass were also reported in the substrates 'a' (0.175 g); 'b' (0.122 g) and 'd' (0.118 g) and the lowest values in substrates 'e' (0.068 g) and 'f' (0.0617 g). These results demonstrated that the best substrates for *M. zehntneri* are those with greater capacity for water drainage, similar to that observed for other species of *Melocactus* (Cavalcanti & Resende, 2006; Resende *et al*., 2010; Bravo Filho & Santana, 2019), such as sand and Carolina Soil®, which also promote large number, length and fresh mass of roots. A large number of roots is generally associated with good root development, because it allows a greater absorption of water and minerals and, consequently, better plant development (Xavier *et al*., 2019). In addition, other factor that contributes with better development of roots is the low to medium values of electrical conductivity (EC) of the substrates sand (0.08 mS/cm) and Carolina Soil (1.08 mS/cm), while the addition of organic compost to sand resulted in highest values of EC (6.0 mS/cm) and limited development of the seedlings.

The highest weights for CDM (cladode dry mass) of seedlings of *M.*

zehntneri were obtained in the substrates 'a' (0.28 g), 'b' (0.31 g), 'c' (0.27 g) and 'd' (0.37 g), the same with best root development. The highest weights of RDM (root dry mass) of seedlings of *M. zehntneri* were only obtained using the treatment 'd' (0.021 g), differing significantly from the others.

An analysis of the pH and electrical conductivity (EC) of the treatments (Table 1) assists to better understand the relationship of substrates with the plant development of the *Melocactus*. According to the results found in Table 3, it is possible to consider that the treatments that had the best results were those with lower to medium electrical conductivity in the substrate (EC between 0.08 and 1.08). The pH of the substrates with the best development for *M. zehntneri* cultivation were 5.5 (Carolina Soil®) and 6.3 (sand) and the worst was 5.9. These results suppose that the pH between 5.5 and 6.3 is adequate for cultivation of *M. zehntneri* seedlings and the predominant factor that affects the seedling's growth and development was the electrical conductivity in the actual study.

As already mentioned, *Melocactus* populations are found in semiarid regions and the soil of this type of region is very poor in organic matter and minerals, and, according to Souza *et al*. (2015), with chemical characteristic of acidic tendency to neutral. Knowing this and from the results found, it is also possible to conclude that the substrates that present in their composition organic matter (OM) aren't indicated for the cultivation of *Melocactus,* since the OM appears to retain enough moisture to interfere in the good general performance of the cactus, besides having a high EC. As already mentioned, only in substrates with OM in its composition ('e' and 'f') there were relevant numbers of *M. zehntneri* individuals with impaired development.

In conclusion, it was possible to establish a methodology for seed germination in Petri dishes, followed by seedling production of *M. zehntneri* using commercial substrate and cultivation in a greenhouse. The maximum value of germination obtained was 55%. According to our results, there is no physical or mechanical dormancy in *Melocactus zehntneri* seeds, since the use of previous immersion in water for 30 min was positive for the germination of this species, with similar germination percentage obtained with HCl scarification.

The acclimatization of the seedlings from the Petri dish to the substrate showed high survival of seedlings. The best survival and development were observed using sand and Carolina Soil® as substrate. Maybe, the use of deeper cells in plastic trays could represents a benefit for this cactus cultivation, since in other species of *Melocactus* different authors observed higher growth rates than the ones observed in our experiment with *M. zehntneri* (Lone *et al*., 2007; Resende *et al*., 2010).

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