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Germination and acclimatization of *Melocactus sergipensis* Taylor & Meiado[1]

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ABSTRACT - *Melocactus sergipensis* is endemic to the state of Sergipe in northeastern Brazil, with an estimated population of 100 adult individuals. The present study evaluated the effect of imbibition on the *in vitro* and *ex vitro* germination of *M. sergipensis* seeds, and the influence of different substrates on acclimatization. The experimental design was completely randomized, and the data were evaluated using an Analysis of Variance with Tukey's test, considering a 5% significance level. Soaking the seeds for 6 h in 2 mg L^{-1} GA_3 increased *in vitro* germination significantly, from 10% to 38%, although no significant variation was found in germination rates among the different substrates. Acclimatization occurred in all substrates, with a survival rate of over 88%.

Keywords: Turk's cap cactus, *ex situ* condition, conservation, *in vitro* establishment

RESUMO - Germinação e aclimatização de *Melocactus sergipensis* Taylor & Meiado. *Melocactus sergipensis* é endêmica de Sergipe, no nordeste do Brasil, com população estimada em 100 indivíduos adultos. O presente estudo avaliou o efeito da embebição na germinação *in vitro* e *ex vitro* de sementes de *M. sergipensis* e a influência de diferentes substratos na aclimatização. O delineamento experimental foi inteiramente casualizado, e dados foram avaliados por meio de Análise de Variância com teste de Tukey, considerando-se um nível de significância de 5%. A imersão das sementes por 6 horas em 2 mg L^{-1} de GA_3 incrementou significativamente a germinação *in vitro*, de 10% para 38%, embora nenhuma variação significativa tenha sido encontrada nas taxas de germinação entre os diferentes substratos. A aclimatização ocorreu em todos os substratos, com uma taxa de sobrevida superior a 88%.

Palavras-chave: cabeça-de-frade, condição *ex situ*, conservação, estabelecimento *in vitro*

INTRODUCTION

The Turk's cap cacti of the genus *Melocactus* Link & Otto (Cactaceae) comprise 38 species distributed from Central America to northeastern Brazil, including the Caribbean, Andes, and the Amazon basin. Brazil is the principal center of diversity of this genus, with 23 native species, of which, 19 are endemic (Zappi *et al.* 2014). *Melocactus* is distinguished from all other cacti by its exclusively sexual propagation, the absence of shoots or branches, low survival rates during its initial development phase, and the presence of a terminal cephalium (Abreu 2008, Hughes 2009, Cruz 2011).

Five *Melocactus* species are found in the Brazilian state of Sergipe, where they occur in the coastal Restinga ecosystem and the semi-arid Caatinga biome. *Melocactus sergipensis* Taylor & Meiado, is the state's only endemic cactus, and is the only cactus species classified as Critically Endangered in the IUCN Red List (Taylor *et al.* 2014, Zappi *et al.* 2016, Bravo Filho *et al.* 2018,), based on criteria B1, B2, and C2 (IUCN 2001). In 2014, Bravo Filho *et al.* (2015) recorded a population of approximately 100 adult *M. sergipensis* growing on outcrops of granite and metamorphic rock within a small patch of Caatinga vegetation of approximately 1000 m^2 (Taylor *et al.* 2014, Herbário Alexandre Leal Costa 2016). This is the only known population of *M. sergipensis*. This reinforces the need for the development of propagation techniques that will increase the germination rate of *M. sergipensis* seeds and seedling survival.

Imbibing the soil in varying concentrations of gibberellin (GA_3) prior to sowing the seeds has been used to increase germination in a number of plant species, in different substrates and environments, and at varying temperatures (Bárbara *et al.* 2015, Moraes *et al.* 2018, Souza-Leal *et al.* 2019).

The germination process can be standardized by *in vitro* cultivation, which may increase both germination rates and seedling survival, producing a large number of seedlings, and potentially providing a viable alternative to the harvesting of plants from the wild for the commercial exploitation of *Melocactus* (Bravo Filho *et al.* 2015, Marchi 2016).

The present study evaluated the *in vitro* and *ex vitro* germination rates of *Melocactus sergipensis* seeds and the acclimatization of this species.

MATERIAL AND METHODS

Collection, storage, and disinfection of the seeds

The ripe fruits used in the germination experiment were collected in the municipality of Simão Dias, in Sergipe, Brazil, in the natural area of occurrence of the study species (10º46'16" S, 37º53'43" W). The seeds were washed and placed on filter paper to dry at ambient temperature for two days. They were then stored at 5±1°C prior to the experiment. In the laboratory, the seeds were washed with autoclaved water and neutral detergent, dried, and then immersed in 70% ethanol for one minute. The seeds were then immersed in a 1% (v/v) solution of sodium hypochlorite for 10 minutes and rinsed three times in autoclaved and distilled water.

In vitro germination

The *in vitro* germination experiment was based on three treatments: (i) treatment 0 (T0) – control (untreated seeds), (ii) treatment 1 (T1) – seeds soaked in distilled water for 6 hours, and (iii) treatment 2 (T2) – seeds soaked in a 2 mg L^{-1} solution of gibberellic acid (GA_3) for 6 hours. In a laminar flow cabinet, the seeds were inoculated into test tubes containing MS medium (Murashige & Skoog 1962), with 30 g L^{-1} of sucrose and 7 g L^{-1} of agar. The pH of the culture medium was adjusted to 5.8 prior to autoclaving at a pressure of 1.23 kgf/cm^2 and temperature of 121°C, for 15 minutes.

The test tubes were maintained in a growing chamber for 90 days, with a 12-hour photoperiod and light intensity of 45 µmol m^2 s^{-1}, provided by 40 W fluorescent lamps, at a mean temperature of 28±1°C.

Greenhouse germination

For greenhouse germination, the seeds were processed as for the *in vitro* experiment: (i) control (untreated seeds); (ii) seeds soaked in distilled water for 6 hours, and (iii) seeds soaked in a 2 mg L^{-1} solution of gibberellic acid (GA_3) for 6 hours. The seeds were planted in 100 mL transparent polypropylene pots containing 70 g of the substrate, maintained near the capacity of the pot. Five replicates were used per treatment, with 10 seeds per experimental unit.

Acclimatization

Acclimatization was initiated after 90 days of *in vitro* cultivation. Each of the four substrates was tested in five replicates, with five plants in each plot. The plants were transferred to 250-mL pots containing 170 g of the test substrate (Caatinga soil, Atlantic Forest soil, 'West Garden' humus, and 'West Garden' humus + washed sand at a proportion of 1:1 w/w) and 30 mL of water. The pots were covered with transparent plastic caps during the first 15 days and kept in the growing chamber for 30 days before being transferred to the greenhouse. The pots were kept covered for a further eight days, and then uncovered, when they were maintained under a 50% shading screen for 22 days and watered every two days. After this phase, the plants were grown unshaded during 30 days, when they were watered every eight days, prior to being transferred from the greenhouse.

Experimental design and analysis

The samples were evaluated in three phases: (i) at the end of the *in vitro* cultivation, (ii) at the beginning of the acclimatization trials, and (iii) at the end of the acclimatization trials. The following seven parameters were evaluated during the first phase (*in vitro* culture): (i) the index of germination velocity (IGV %), (ii) the germination rate (% G), (iii) percentage survival (%S), (iv) stem length (SL – cm), (v) stem diameter (SD – mm), (vi) radicle length (RL – cm), and (vii) fresh weight (FW – g). Three parameters were evaluated during the second and third phases: (i) percentage survival (% S), (ii) stem length (SL– cm), and (iii) stem diameter (SD – mm). These parameters were tested using an Analysis of Variance, with the results being analyzed using Tukey's *post hoc* test to identify significant differences between pairs of conditions. A 5% significance level was considered in all analyses.

The *in vitro* germination experiment had a completely randomized design, with three treatments, and 25 replicates of two seeds per experimental unit. The greenhouse germination experiments were also based on a random design, with three treatments and four replicates in both cases. In this case, the experimental unit was composed of 10 seeds. The acclimatization of the plants obtained by *in vitro* culture was tested in four substrates (Caatinga soil, Atlantic Forest soil, humus and humus + washed sand at a proportion of 1:1 w/w).

RESULTS AND DISCUSSION

Germination rates and *in vitro* development of the *M. sergipensis* seedlings

Significant differences (Tukey: $p < 0.05$) were found among the *in vitro* germination rates of the *M. sergipensis* seeds cultivated under different conditions (Tab. 1), but not for any of the other parameters, that is, germination

velocity, survival, stem length, radicle length or fresh weight. Soaking the seeds in 2 mg L^{-1} GA_3 for 6 hours resulted in a significant increase in the germination rate, from 10% in the control group to 38% in the GA_3 group (treatment 2). Gibberellic acid (GA_3) is a gibberellin plant hormone and growth regulator (Silva *et al.* 2013), and controls many developmental processes, including the seed germination.

Barbara *et al.* (2015) obtained similar results from *in vitro* germination tests on *Melocactus azureus*, with a two-hour pre-soak in 1000 mg L^{-1} of GA_3, resulting in an increase in the germination rate from 3% (control) to 20%. Marchi *et al.* (2013) also recorded an increase in the germination rates of *Discocactus zehntneri* seeds (from 7% to 24%) after they were pre-soaked for 2 hours in 1000 mg L^{-1} of GA_3.

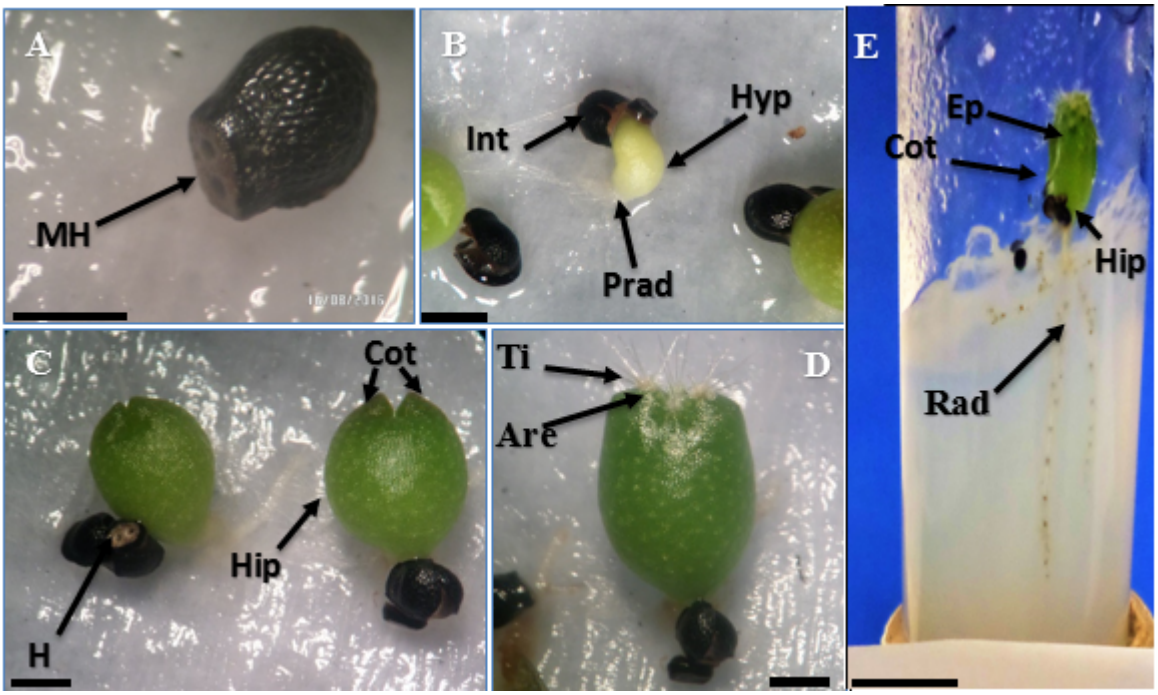
While the germination rate recorded for treatment 2 (soaked in GA_3) was also higher than that recorded for treatment 1 (T1: soaked in distilled water), the difference was not statistically significant. The germination rate recorded for T1 (30%) in this stuy was nevertheless equivalent to the best results obtained by Barbara *et al.* (2015) and Marchi *et al.* (2013), who recorded germination rates of only 20% and 24%, respectively, for the seeds soaked in GA_3. This indicates that soaking the seeds in water for 6 hours had a physiological effect similar to that of soaking in 2 mg L^{-1} of GA_3, and may have been at least as effective as the high concentrations of GA_3 used by Barbara *et al.* (2015) and Marchi *et al.* (2013). Reis *et al.* (2012) found that pre-soaking *Pilosocereus aurisetus* seeds in GA_3 for 24 hours did not improve the *in vitro* germination rate in comparison with the control (90%), whereas soaking the seeds 250 mg L^{-1} GA_3 reduced the germination rate to 18% and soaking in 500 mg L^{-1} GA_3 reduced it to 13%.

The five stages of the *in vitro* post-seminal development of *M. sergipensis* seedlings from inoculation to 90 days are shown in Figures 1A-E. The *M. sergipensis* seed has a mean diameter of 1 mm, and is characterized by a wire-micropylar region with two depressions (Fig. 1A). Germination is epithelial and *in vitro*, it began on day 6 and extended to day 13, occurring synchronously throughout the period. The process begins with the release of the hypocotyl radicular

Table 1. *In vitro* germination and development of *M. sergipensis* seedlings 90 days after inoculation. Values in the same column followed by different letters (A or B) are significantly different ($p < 0.05$) from each other, based on Tukey's *post hoc* test.

Treatment	Germination rate (%)	Index of germination velocity (IGV)	Survival (%)	Stem length (cm)	Stem diameter (mm)	Radicle length (cm)	Fresh weight (g)
T0	10B	0.04 A	100 A	1.05 A	4.24 A	1.41 A	0.14 A
T1	30 AB	0.16 A	100 A	0.97 A	4.92 A	1.20 A	0.15 A
T2	38A	0.09 A	100 A	0.98 A	4.76 A	1.26 A	0.13 A

T0 = untreated seeds (control); T1 = seeds soaked in distilled water for 6 hours; T2 = seeds soaked in 2 mgL^{-1} GA_3 for 6 hours.



Figs. 1A-E. Development of the morphology of *Melocactus sergipensis* seedlings. **A.** Micropillary hilum region (MH); **B.** Hypocotyl (Hyp), Integument (Int) and Primordial radicle (Prad); **C.** Hilum (H), Cotyledon (Cot) and hypocotyl (Hyp); **D.** Areola (Are) and Trichoma (Ti); **E.** Cotyledon (Cot), Epicotyl (Ep), Hypocotyl (Hyp), Root (Rad). Source: Bravo Filho (2016). Bars: **Figs. 1A-D** = 1mm; **Fig. 1E** = 1 cm.

axis through the opening of the operculum (Fig. 1B). At this stage the coloration of the seedling varied from white to cream. Complete tegument release was observed eight days after sowing, when the cotyledons are exposed, although trichomes and areolas are absent at this stage, whereas the hypocotyl is fully erect, and the seedling is green in color and globular in shape (Fig. 1C). By 15 days after inoculation, setae began to appear on the apical part of the seedling, and the stem was elongated (Fig. 1D). At 90 days after inoculation, the plants had a mean length of 1.05 cm, a well-developed root, with a mean length of 1.41 cm, and a large number of trichomes, well-developed epicotyl, while the cotyledons are absorbed (Fig. 1E).

Similar results were obtained by Reis *et al.* (2012) in a study of the *in vitro* germination and post-seminal development of *Pilosocereus aurisetus* (kappa-la-lapa) seedlings, in which the development of the seedling followed a sequence similar to that observed in *M. sergipensis*. Germination began on the seventh day after inoculation, after which a translucent seedling emerged (Fig. 1B), which subsequently became hlorophyllous (Fig. 1C). The stem axis initially protruded without the emission of the radicle (Fig. 1B), with the seedling elongating 10 after inoculation.

Germination of the *Melocactus sergipensis* seeds and development of the seedlings under greenhouse conditions

The results of the greenhouse germination and growth experiment are shown in Table 2. All the plants survived, and the only significant variation (Tukey test) found between treatments was in the fresh weight, which was significantly greater in treatment 2 (seeds soaked in GA_3) in comparison with both the control and treatment 1 (seeds soaked in water). Germination rates were 82% in treatment 1 and 92% in treatment 2 (seeds soaked in GA_3), which were similar to that of the control (86%), which indicates that the pre-soaking of the seeds, whether in water or GA_3, did not affect germination rates under the experimental conditions adopted in the present study. Reis *et al.* (2012) obtained similar results in a study of *Pilosocereus aurisetus*, although germination rates did not exceed 80% in either the control group or the seeds soaked for 24 hours. In a study of the germination of *Melocactus bahiensis* in different substrates and at distinct temperatures, by contrast, Lone *et al.* (2009) obtained low germination rates of between 45% and 48%, values approximately half those recorded in the present study.

The germination rates recorded in the greenhouse trials were significantly different from those recorded in the *in vitro* experiment for all treatments. The greatest difference was found in the control, in which the germination increased from 10% (*in vitro*) to 86% (greenhouse), followed by T2, in which it increased from 38% (*in vitro*) to 92% (greenhouse), and T2, in which it increased from 30% to 82%.

Acclimatization

At the end of the acclimatization period, no significant variation was found among treatments in the survival of the *M. sergipensis* seedlings, or in the length of the stem or radicle (Tab. 3), although significant differences were found in stem diameter and fresh weight. Survival rates ranged from 88 to 100% in the different treatments, rates considered satisfactory, given that 80% survival is considered to be a good survival rate, considering the difficulty of transferring plants from *in vitro* to *ex vitro* conditions.

In general, the Atlantic Forest soil (T2) provided the best substrate for the development of the seedlings, with the highest values being recorded for all the variables analyzed (except for survival, which was slightly lower than that recorded for the Caatinga soil). Even so, significant differences were only found in stem diameter and fresh weight, with T2 returning significantly higher values than those recorded for treatment 4 (humus + sand).

Table 2. *In vitro* germination and growth of the *M. sergipensis* seedlings 90 days after planting in a greenhouse. Values in the same column followed by different letters (A or B) are significantly different ($p < 0.05$) from each other according to Tukey's *post hoc* test.

Treatment	Germinatin rate (%)	Index germination velocity (IGV)	Survival (%)	Stem length (cm)	Stem Diameter (mm)	Radicle Length (cm)	Fresh weight (g)
T0	86 A	0.59 A	100 A	0.77 A	5.10 A	1.18 A	0.12 B
T1	82 A	0.41 A	100 A	0.65 A	5.0 A	1.11 A	0.11 B
T2	92 A	0.46 A	100 A	0.71 A	5.0 A	1.48 A	0.16 A

T0 = untreated seeds (control), T1 = seeds soaked in distilled water for 6 hours; T2 = seeds soaked in 2 mg L^{-1} GA_3 for 6 hours.

Table 3. *Ex situ* growth of *M. sergipensis* seedlings in distinct substrates after 60 days of acclimatization. Values in the same column followed by different letters (A or B) are significantly different ($p < 0.05$) from each other according to Tukey's *post hoc* test.

Treatment	Survival (%)	Stem Length (cm)	Stem Diameter (mm)	Radicle Length (cm)	Fresh weight (g)
T1	100 A	1.63 A	6.5 AB	0.80 A	0.30 AB
T2	96 A	1.71 A	7.2 A	0.96 A	0.41 A
T3	88 A	1.64 A	6.5 AB	0.51 A	0.34 AB
T4	92 A	1.50 A	5.8 B	0.74 A	0.29 B

T1 = Caatinga soil; T2 = Atlantic Forest soil; T3 = humus; T4 = humus + washed sand (1:1 w/w).

These findings contrast with those of Marchi (2016) and Resende *et al.* (2010), who evaluated the effects of different substrates on root growth during the acclimatization of *Melocactus glaucescens*. In these studies, the humus/washed sand mixture was the most adequate substrate for the development of the roots, whereas in the present study, it performed worst in most parameters (Tab. 3).

By the end of the acclimatization period, the seedlings had grown significantly in comparison with the period of *in vitro* culture, with stem length increasing from 0.98 cm to 1.71 cm, stem diameter from 4.92 mm to 7.20 mm, and fresh weight from 0.15 g to 0.41 g. However, the length of the root decreased from 1.41 cm to 0.96 cm.

Soaking the *Melocactus sergipensis* seeds in either distilled water or GA_3 resulted in a significant increase in the germination rate, both *in vitro* and in the greenhouse. During the acclimatization experiment, the soil from the Atlantic Forest was the most adequate substrate for the development of the seedlings.

The *M. sergipensis* seeds that were not imbibed presented allow rate of germination, especially in comparison with the seeds imbibed in water for six hours (with or without the application of GA_3 at a concentration of 2 mg L^{-1}).

In the greenhouse, high germination rates (82–92%) were recorded in all treatments, and were considerably higher than the *in vitro* rates (which reached a maximum of 38% in T2). During acclimatization, the Atlantic Forest soil was the most suitable substrate for the development of the seedlings.

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