
5 MEDIUM MS AT DIFFERENT CONCENTRATIONS IN THE INITIAL DEVELOPMENT *IN VITRO* OF MELOCACTUS CONOIDEUS BUINING & BREDEROO

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ABSTRACT

The *Melocactus conoideus* Buining & Brederoo is an ornamental plant popularly known as "crown-of-friar" or "head-of-friar", considered endemic to Vitória da Conquista, Bahia and is critically endangered. It belongs to the Cactaceae family, with representatives that are characterized by being succulent plants, which have developed adaptations to reduce water loss. The main form of reproduction of naturally propagated cacti is sexed, and it can also occur asexually, through *in vitro* propagation, the seed being more commonly used as an explant, this is a good alternative for the multiplication of cacti of the genus *Melocactus*. It is necessary for the development the *in vitro* propagation of this species that will enable the production of seedlings in the scientific environment. In order to have a better development in the germinative process, it was proposed to analyze the medium MS and MS ½ in *in vitro* propagation, in order to obtain seedlings of the species. The experimental design was completely randomized, with 5 repetitions of 10 tubes for each treatment, totaling 100 tubes. After 60 days, the germination rate of the plant and variables such as diameter, length of the largest root, number of roots and length of the plant were analyzed in both medium.

The experiments were compared using the Hotelling test, in which two multivariate samples were analyzed with the same number of variables. Better *in vitro* development rates were observed in the MS½ medium. Evidencing, then, that the concentrations of salts of the MS medium are reduced, for the germination of *Melocactus conoideus*. After the initial seedling development, there was acclimatization, which was done after their natural rooting.

Keywords: *In vitro* propagation. *Melocactus conoideus*. Medium ms and ms ½. Germination.

RESUMO

O *Melocactus conoideus* Buining & Brederoo é uma planta ornamental popularmente conhecida como “coroa-de-frade” ou “cabeça-de-frade”, considerada endêmica de Vitória da Conquista, Bahia e se encontra criticamente ameaçada de extinção. Pertence à família Cactaceae, com representantes que caracterizam-se por serem plantas suculentas, que desenvolveram adaptações para reduzir perda de água. A principal forma de reprodução das cactáceas de propagação natural é a sexuada, e também pode acontecer de forma assexuada, através da propagação *in vitro*, sendo mais comumente utilizada a semente como explante, esta é uma boa alternativa para a multiplicação de cactos do gênero *Melocactus*. Torna-se necessário para o desenvolvimento a propagação *in vitro* desta espécie que irá viabilizar a produção de mudas no meio científico. Com o intuito de ter um melhor desenvolvimento no processo germinativo, foi proposto analisar os meios MS e MS ½ na propagação *in vitro*, afim de obter mudas da espécie. O delineamento experimental foi inteiramente casualizado, com 5 repetições de 10 tubos para cada tratamento, totalizando 100 tubos.

Após 60 dias, foi analisado a taxa de germinação da planta e variáveis como o diâmetro, comprimento da maior raiz, número de raízes e comprimento da planta, em ambos os meios. Os experimentos foram comparados pelo teste de Hotelling, em que foi analisado duas amostras multivariadas com o mesmo número de variáveis. Foi observado melhores taxas de desenvolvimento *in vitro* no meio MS½. Evidenciando então, que sejam reduzidas concentrações de sais do meio MS, para a germinação de *Melocactus conoideus*. Após o desenvolvimento inicial das plântulas, houve aclimatização, que foi feita após enraizamento natural das mesmas.

Palavras-chave: Propagação *in vitro*. *Melocactus conoideus*. meio ms e ms ½. Germinação.

5.1 INTRODUCTION

The *Melocactus conoideus* Buin. & Bred. is an ornamental plant, endemic to Vitória da Conquista-BA, which is characterized by being xerophilic and stores in its tissues large amounts of water and nutrients (ZAPPI e TAYLOR, 2016). They stand out, for having a conical globose body with plump buds, white marrow, angular rib, 9 or more straight spines and central spines over 1cm, dark green epidermis and the fruit is magenta in color. After a decade of their vegetative development, they reach a typical stature, evidenced by the cephalium located at the apex of adult individuals, which protects flowers and fruits in development (CERQUEIRA-SILVA and SANTOS, 2008; MACHADO, 2009; ZAPPI *et al.*, 2019).

The number of species in the Cactaceae family is still uncertain, there are differences in many studies. According to Cerqueira-Silva (2007) the number of species found in this genus is limited to 38, but there are researchers who say it is more than 50. According to Cruz. (2011), the genus *Melocactus* is composed of 38 species, most of which are endangered and are distributed in Central American countries, the Caribbean, the Andes and Brazil, especially in the Northeast, which covers an endemic quantity of *Melocactus*. Brazil has the largest diversity center of the genus, with 23 species, according to Taylor *et al.* (2015), 21 are endemic to the country. According to Batista *et al.* (2018), more current, the genus has 35 species, 23 of which are Brazilian endemic species.

Known as “crown-of-friar” or “head-of-friar”, according to Menezes *et al.* (2011; 2013) in the state of Bahia, its diversity center, has 18 species, 10 of which are endemic to the state. *M. conoideus* is endemic to Vitória da Conquista-BA and is threatened with extinction. This species is classified as critically endangered by IUCN (International Union for the Conservation of Nature) and is present in Appendix I of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora since 1992 (CITES, 2013).

Dubbed as “Bahian Switzerland”, Vitória da Conquista is located in the southwest of Bahia, according to Rocha (2013), since 1750, when the first Portuguese expeditions took place, the territory was inhabited by indigenous tribes, who lived in the surroundings of Serra do Periperi, a place that presents a population of individuals with noticeable phases in the same or different biological stages of development of *M. conoideus*.

Vitória da Conquista is privileged with a tropical climate, mitigated by being a relatively high place and composed of vegetation distributed in ranges. According to Medeiros (1996), Range A composes the Caatinga, Range B carrasco vegetation, Strip C Vine Forest, Range D Mata de Larga, Strip E Cold Forest and F Wet River Forest, being the Serra do Periperi,

according to Vitória da Conquista Municipal (2013), composed mostly of Range C, a region of Vine Forest.

Serra do Periperi is an area where many anthropic activities occur due to disorderly urbanization, according to Benedictis (2007), the Serra is close to neighborhoods in the city such as Guarani and Cruzeiro, with many environmental impacts on flora and fauna, such as fires, pollution, among other illegal actions often carried out intentionally. According to Zappi et al. (2011), these attitudes interfere with the size of natural populations and puts some species of cacti at risk of extinction.

In this Serra, there is an important Conservation Unit (CU), in which is the Municipal Park of Periperi, aiming to conserve and protect the fauna and flora. The Park was created by the Vitória da Conquista Municipal Government, decree nº 9.480 / 99, to meet the need of the Participatory Government to adopt preservation measures in the Serra, such as preventing disorderly occupation, mining and deforestation activities, this Serra it has the springs of the main rivers of the city like Rio Verruga and Rio Pardo.

According to Taylor and Zappi (2004), the country moves slowly in relation to the studies of cacti compared to other countries in relation to morphology, germination and physiology, and for having great wealth and diversity of plants of the Cactaceae family, with several endemic species. The propagation of the species is done exclusively by seeds. According to Nascimento (2015), the success of the perpetuation of many species of cacti is intrinsically related to the germination of their seeds dispersed by animals, in the genus *M. conoideus* are mainly ants, lizards and birds.

According to Simão *et al.* (2007), the main form of reproduction of naturally propagated Cactaceae is sexual, according to Abreu (2008), of every 10,000 seeds that germinate naturally, only one plant reaches its reproductive stage. Resende *et al.* (2010) worked with in vitro propagation with species of the same genus and demonstrated that tissue culture is a good alternative for the propagation of cacti. Many species of cacti show slow growth and low seed germination. Thus, in order to increase the production of these plants, in vitro propagation becomes an essential tool (Medeiros *et al.*, 2006).

Multiplication is fast through tissue culture in vitro, they are used as explants for seed, stem, leaf and root inoculation, and it is a good alternative for conservation, according to Zappi and Taylor (2011), with this technique, one can reduce the impact on populations, especially endemic ones, by increasing the number of individuals.

Because it is a plant with high ornamental potential, endemic and critically endangered, according to IUCN, it has an occurrence span of less than 100 km² and the population is in

continuous decline, further increasing the decrease in the occurrence of this species. Souza and Lorenzi (2012) argue that micropropagation can be a viable technique for Cactaceae, thus obtaining *M. conoideus* seedlings through seed propagation in a short period of time.

According to Grattapaglia and Machado (1998), all plant species grown *in vitro* are capable of regenerating new individuals, due to cellular totipotency, as long as they are in adequate aseptic conditions. Considering the urgency in relation to the rapid development of *Melocactus conoideus* Buin. & Bred with the *in vitro* propagation technique, it is necessary and urgent, the development of actions that enable the production of seedlings of this species in the scientific environment, since it is an endemic species, in which there is an interest to conserve because it is seriously threatened by urban sprawl, fires and quartz mining.

Consequently, with these anthropic actions, there are also several socio-environmental problems such as flooding throughout the city, such as the erosion of the mountains, contributing to the decrease of a large part of the population in nature. In addition, the population has been attacked by a fungus not yet identified, so there is great concern about its spread.

5.2 MATERIALS AND METHODS

The ripe fruits were collected in the Serra do Periperi, located in the municipality of Vitória da Conquista - BA. The material was sent to the Botany Laboratory of the Federal University of Bahia, Anísio Teixeira campus of IMS (Multidisciplinary Institute in Health). This work was authorized by the National System for the Management of Genetic Heritage and Associated Traditional Knowledge - SisGen (ADB361C) and Chico Mendes Institute for Biodiversity Conservation - ICMBIO (authorization for collection n° 69333-1).

The explant used for the *in vitro* establishment was the seed. This was washed with neutral detergent, disinfected with immersion in 70% alcohol for 3 minutes, followed by sodium hypochlorite (2.5% active chlorine) for 15 minutes, washed in distilled water 3 times in a laminar flow chamber and inoculated in test tubes containing 10mL of MS medium (MURASHIGE and SKOOG, 1962) and MS $\frac{1}{2}$, plus 30g.L⁻¹ of sucrose and 7g.L⁻¹ of agar.

A completely randomized design with 5 replications was used, in which each plot consisted of 10 test tubes for each treatment. The tubes were kept in a growth room, under a 16 h photoperiod at 25°C \pm 2 of temperature. The parameters of the best seed germination averages were analyzed in the MS (control) and MS $\frac{1}{2}$ medium. The germinated seedlings were used to evaluate two types of MS culture medium: MS (control) and MS $\frac{1}{2}$. Biometric analyzes of the

germinated seedlings were performed for statistical comparison of the variables between treatments.

The following attributes for in vitro development were measured and analyzed: Plant length, diameter, number of roots and length of the largest root. The data were analyzed using the Hotelling test, in which there were two multivariate samples (treatments) with the same number of variables and a probability of 0.05 to verify the statistical difference between the groups.

5.3 RESULTS AND DISCUSSION

The seeds only germinated after 30 days in both media, in the MS medium, germination occurred in 34% of the inoculated seeds, and in the MS ½ medium, 28%. It is observed in table 1, that the analysis of the germination of *Melocactus conoideus* obtained by the Hotelling Test, proved to be better in the MS medium (treatment 2), where there was germination of 17 explants in a total of 50 test tubes.

Although there was no great difference between the treatment variables, the test showed that there was a statistically significant difference, assuming a p of 0.05.

For the variables plant length, diameter, number of roots obtained lower averages in the complete MS medium, only in the variable length of the largest root there was a higher average.

The MS ½ medium demonstrated, according to the test, the highest growth averages, that is, a better development. In in vitro germination works of the genus *Melocactus* and other plants of the same family, the reduction of ions has been more favorable to germination (PASQUAL *et al.*, 2001).

Table 1 - Seed germination within the treatments MS ½ and treatment MS, considering 50 the total of seeds in each

Half MS	Half MS
14 * sprouted seeds	17 * sprouted seeds

* Statistically significant difference for both treatments (P> 0.05).

The solutions of salts and sugars (carbon sources) that make up the culture media do not only have a purely nutritive effect, but can also influence morphogenesis, cell growth and development, through their osmotic properties (GEORGE *et al.*, 2008).

According to Santos (2018), for *Melocactus conoideus*, lower concentrations of salts and sucrose in the MS medium, favor an increase in the germination rate, as was demonstrated in this study in which higher germination rates were observed in relation to most of the averages of the variables.

As shown in table 2, the medium MS $\frac{1}{2}$ promoted a greater number of roots. Dilutions of the MS medium, for $\frac{1}{2}$, $\frac{1}{3}$ and even $\frac{1}{4}$ of salts, have enabled better results for many plant species. Less concentrated basic media such as WP, White, Knop, Heller can be equally favorable (ALOUFA, 2003).

Table 2 - Average values of the variables analyzed from the MS and MS $\frac{1}{2}$ media, limit value of the variables $p = 0.05$. Value found $p = 0.0081$

	Plant diameter	Length of largest plant	Length of largest root	Root number
Half MS $\frac{1}{2}$	0.4571	12.786	24.286	10.929
Half MS	0.4471	0.5647	95.956	0.3276

According to Retes RETES-PRUNEDA *et al.* (2007), the species of the genus *Melocactus*, do not branch or emit lateral shoots, thus needing inducers (plant regulators) such as auxins (NAA - naphthalene acetic acid) and cytokinins (BAP-6-benzylaminopurine), however this work proved that the species *Melocactus conoideus* showed branching of roots according to the root growth observed in the media, even without adding regulators to the medium.

Roots were emitted in both media demonstrating that this plant has enough endogenous hormones for this response. According to Resende *et al.*, (2010) endogenous hormones can promote explant development via direct organogenesis, which is a process rarely observed in *Melocactus* plants under natural conditions, but according to Peres (2002), conditions such as explant source and the mineral composition of the culture medium directly interfere in this sense.

After rooting *in vitro*, pre-acclimatization was performed, in which the tubes were uncapped with holes, remaining for 7 days in the growth room. After 7 days, the seedlings were transferred to the nursery to acclimate.

Although a larger number of seeds germinated in the MS medium, better rates of *in vitro* development are observed in the MS $\frac{1}{2}$ medium. Evidencing then, that concentrations of salts

of the MS medium are reduced, for the germination of *Melocactus conoideus*, also having as base some species of the same genus.

According to Correia *et al.* (2018), it is observed that when adding the gibberellin plant regulator in concentrations below 28.87 μM in culture media, there is favoring the germination of *Melocactus conoideus*, contributing to the process in general. Studies should then be carried out using MS $\frac{1}{2}$ medium or further reducing the salts (1/3 and up to 1/4) and adding gibberellic acid in low concentrations in order to obtain satisfactory germinations in the *in vitro* process of *Melocactus conoideus*.

5.4 FINAL CONSIDERATIONS

For the germination of *Melocactus conoideus*, it is recommended that the concentrations of mineral salts in the MS medium be decreased. The species *Melocactus conoideus*, even without plant inducers, has root branching in the medium MS and medium MS $\frac{1}{2}$.

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