Light and ethephon overcoming seed dormancy in Melocactus zehntneri

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Abstract

Seed germination in Melocactus and other cactus species is hampered by the occurrence of dormancy. However, most studies failed to achieve high seed germination rates, suggesting a complex mechanism of dormancy in Cactaceae. Thus, the objective of this study was to demonstrate that dormancy in Melocactus seeds may be associated with factors such as light and phytoregulators. Two consecutive experimental sets were designed: one with seed germination paper under different wavelengths and Photosynthetically Photon Flux Densities (PPFDs); and one in vitro experiment using a culture medium to evaluate the influence of different plant growth regulators, both in the germination of seeds of Melocactus zehntneri. The results obtained showed that seeds of M. zehntneri are positive photoblastic. Red light and gradual increases in PPFD resulted in the highest germination rates and speed germination index. The experiment with phytoregulators showed a major effect of Ethephon to release the germination of dormant seeds of M. zenhtneri, totalizing 98% of seeds germinated under in vitro conditions, compared to 76% in control. The present study develops an efficient technique to break seed dormancy and the results can be useful to better understand cacti seed dormancy.

Original Articles

Light and ethephon overcoming seed dormancy in *Melocactus zehntneri*(Cactaceae)

Running head title: Overcoming dormancy of *Melocactus zehntneri* seeds

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Short running title : Overcome dormancy in *Melocactus zehntneri*

Key-words : cactus, dormancy breaking, *in vitro* germination, wavelength, light intensity, ethephon

Light and ethephon for overcoming seed dormancy in*Melocactus zehntneri* (Cactaceae)

Short running title : Dormancy breaking and germination in

Melocactus zehntneri

ABSTRACT

Seed germination in *Melocactus* and other cactus species is hampered by the occurrence of dormancy. However, most studies failed to achieve high seed germination rates, suggesting a complex mechanism of dormancy in Cactaceae. Thus, the objective of this study was to demonstrate that dormancy in *Melocactus* seeds may be associated with factors such as light and phytoregulators. Two consecutive experimental sets were designed: one with seed germination paper under different wavelengths and Photosynthetically Photon Flux Densities (PPFDs); and one *in vitro* experiment using a culture medium to evaluate the influence of different plant growth regulators, both in the germination of seeds of *Melocactus zehntneri* . The results obtained showed that seeds of*M. zehntneri* are positive photoblastic. Red light and gradual increases in PPFD resulted in the highest germination rates and speed germination index. The experiment with phytoregulators showed a major effect of Ethephon to release the germination of dormant seeds of *M. zenhtneri* , totalizing 98% of seeds germinated under in vitro conditions, compared to 76% in control. The present study develops an efficient technique to break seed dormancy and the results can be useful to better understand cacti seed dormancy.

Key-words : cactus, dormancy breaking, *in vitro*germination, wavelength, light intensity, ethephon

Introduction

The genus *Melocactus* comprises plants popularly known as 'chapéu-de-frade cactus and Brazil is the country with the greatest diversity, with 55% total number of *Melocactus* species in the world, occurring throughout the Northeast region, and in parts of the North and Southeast regions of Brazil (Machado, 2009).

The demand for *Melocactus* species in the commercial sector, especially for ornamentation and collecting purposes, has had remarkable growth in recent years, due to its peculiar aesthetics, ornamentation, and easy cultivation. However, it is difficult to be precise about the exact numbers of the trade of these plants, since the illegal trade is difficult to quantify (Cavalcante and Vasconcelos, 2016). The fact is that many *Melocactus* populations have been drastically reduced (Martinelli and Moraes, 2013).

Consequently, some *Melocactus* species are already under protection, as a way of controlling the high extraction for trade (Cites, 1994; Cites, 2019). The species *M. zehntneri* is classified as a least concern species, but its population has also shown a recent and constant decline, being recently placed under protection (Bravo Filho et al. 2018a; Machado, 2017). In part, its population reduction can be attributed to two main factors: the continuous deforestation of the Caatinga Biome and the replacement of wild species with agriculture and livestock activities and, the recent increase of commercial exploitation, resulting in the extraction of individuals for the production of food, traditional medicines, and animal fodder (Fabricante et al. 2010; Zappi et al. 2011), but especially for ornamental purposes (Correia et al. 2018a).

Melocactus present in its apex a peculiar structure to the genus called cephalium, which occurs only when the cactus reaches reproductive maturity, at approximately ten years of age, mainly due to very slow vegetative growth (0.04-4.7 cm per year) (Lafite and Salimon, 2020). The natural reproduction is exclusively by seeds due to limited vegetative propagation since they do not develop segmentations as observed in other cactus species (Bravo Filho et al. 2018a).

In addition, seed germination in different species of *Melocactus*is hampered by dormancy. Different studies have reported the use of chemical scarification to increase germination rates, and associated dormancy with the rigid seed coat. Also, phytoregulators, such as Gibberellic Acid (GA_3), have been used to increase the germination rate in *Melocactus* . In *M. azureus* , the imbibition of seeds in water or gibberellic acid (GA_3) for 2 hours resulted in an increase of 14 and 20% seeds germinated, compared to only 3% germinated seeds in the control (Bárbara et al. 2015).

Also, the use of *in vitro* germination has been proposed as an alternative to increase germination rates in *Melocactus* . Successful *in vitro* germination was reported for *M. glaucescens* (68.1%), *M. sergipensis* (64%), *M. zehntneri*(58.7%), and *M. violaceus* (59.3%) (Santos, 2019). Additionally, previous results obtained by our group have demonstrated similar percentages of seeds germinated (50% on average) for *M. zehntneri* using the chemical scarification of seeds (Magnani and Cardoso, 2022). Thus, the results obtained with different

species of*Melocactus* showed that a substantial part of the seeds (40-85%) continues with some unknown type of dormancy, with no germination.

Thus, the present study aimed to test and determine the main factors that can affect the germination of *M. zehntneri* seeds, such as the wavelength, light intensity, and phytoregulators. The experiments, from a practical point of view, can solve and discover the main causes of dormancy in *Melocactus* seeds that do not germinate, enable in vitro conservation and serve as a method for propagation of*Melocactus* species, and for further studies on seed dormancy breaking in Cactaceae.

Material and Methods

Plant material

For the experiment, *Melocactus zehntneri* was used, from the germplasm collection of the Center of Agrarian Sciences, UFSCar, catalog number ABBC280 (Fig. 1) by the *Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado*(SisGen/Brazil). Three adult plants in full fruiting were used as a source of seeds, and fruit were collected at the moment they were detached from the cephalium (Fig. 1).

2.2 Seed preparation and storage

Seeds were removed from the fruit and washed in a sieve under running water containing a few drops of neutral detergent to remove excess mucilage surrounding the seeds. After washing, seeds were placed in a grow room at 25-28°C to dry on filter paper for 24 hours. Seeds remained stored that way for another 14 days until the experiments. This procedure of removing mucilage and drying, demonstrated by previous experiments, is the best condition for the germination of seeds of this species.

2.3 Experiment under different light intensities and wavelengths for germination

In the implementation of the experiment, seeds of *M. zehntneri*were immersed in distilled water for 10 minutes, according to results previously obtained in our laboratory (Magnani and Cardoso, 2022). Later, using a laminar flow cabinet, seeds were subjected to asepsis, aiming at the reduction or even elimination of microorganisms. This was performed in 15 mL Falcon® tubes and seeds were immersed in 70% alcohol (v/v) for one minute and then in a solution containing 30% sodium hypochlorite (2.0-2.5% active chlorine) added with 5 drops of neutral detergent for every 100 mL solution, for 15 minutes under constant stirring, followed by three washes in previously autoclaved deionized water.

In the last rinse, approximately 2 mL deionized water with pH adjusted to 5.8 was kept as a vehicle for the inoculation of seeds on the plates. All seeds, 20 per plate, were sown in clear, smooth, and sterile polystyrene Petri dishes, previously filled with two layers of filter paper saturated with 5 mL sterile deionized water at pH 5.8 per dish.

Subsequently, dishes containing the seeds were arranged under different intensities and wavelengths given by LED lamps and cultivated in a grow room, as follows: blue LED (Phillips Greenpower LED Research Module Blue, 440 nm), red LED (Phillips Greenpower LED module HF deep red, 660 nm), blue(1)/red(1.5) LED (LabPar, with wavelength peaks at 447 nm, range 420-470 nm - blue and 667 nm, range 625-680 nm – red) and, as a white LED control (Ourolux(r), Brazil), with peaks at 440-450 nm (blue), 540-550 nm (green) and 610-620 nm (red).

The photosynthetically photon flux densities (PPFD) were measured using a PPFD Quantum meter, Apogee Instruments(r), Model SQ-520 (USA) of each light source and were obtained by placing the dishes in equidistant proportions from the LEDs (Table 1).

The experiment was a completely randomized design, in a 3 x 4 factorial (PPFD x wavelength) with four replications composed of individual Petri dishes containing 20 seeds each.

As a complementary experiment, darkness influence on the germination of*M. zehntneri* seeds was tested, with seeds kept protected from light, in a grow room, for three periods of 10, 20, and 30 days of darkness.

In this case, seeds under germination conditions were only exposed to a light source after remaining in the respective periods in the dark. For this, the same procedure of preparation and inoculation of seeds was carried out and the experimental design was completely randomized.

Petri dishes from both experiments were sealed with a transparent PVC film and kept in a grow room at 26 +- 1o C, and a photoperiod of 14 hours. Germination was evaluated twice a week; seeds were considered germinated when hypocotyl-radicle protrusion was equal to or greater than 0.1 cm. In the end, the Germination Percentage (G%), Average Germination Speed (AGS), and Germination Speed Index (GSI) were calculated.

2.4 In vitro germination of M. zehntneri under different concentrations of phytoregulators

The main objectives of this experiment were to evaluate the effect of different classes of plant growth regulators on the germination of*M. zehntneri* seeds and to develop a methodology for *in vitro* germination as an alternative to the method of seed germination in Petri dishes.

Procedures before sowing and aiming at seed asepsis were carried out in the same way as described in the previous experiment. In the last rinse, approximately 2 mL deionized water (pH 5.8) was maintained so that this solution containing the seeds could be used as a vehicle for inoculation in culture flasks containing 30 mL MS culture medium (Murashige and Skoog, 1962), containing sucrose (20 g L^{-1}), inositol (100 mg L^{-1}), activated charcoal (1 g L^{-1}), and the pH was adjusted to 5.8 before the addition of agar (6.4 g L^{-1}). Flasks containing the culture medium were autoclaved at 120oC for 25 minutes.

Phytoregulators tested for germination of *M. zehntneri* seeds and added to the MS culture medium were 6-benzylaminopurine (BAP) 1 mg L^{-1}; gibberellic acid (GA_3) 1 mg L^{-1}; and the combination of the two, BAP (1 mg L^{-1}) and GA_3 (1 mg L^{-1}) and a control without addition of phytoregulators.

Also, we evaluated the effect of pre-treatment of seeds in a solution containing 100 µL L^{-1} Ethrel® (240 g/L Ethephon - Bayer®, Brazil) for 24 hours before the experiment and later inoculated in culture media containing the same treatments described above. After inoculation, flasks containing the seeds were kept in a grow room under the same conditions as in the previous experiment, but using only the LED in the blue (1) and red (1.5) wavelengths.

The experiment was conducted in a Completely Randomized Design (CRD), in a 2 (pre-treatment with Ethrel®) x 4 (phytoregulators in the culture medium) factorial. In total, six replications were performed per treatment, each replication consisting of a flask containing 30 mL culture medium, with 10 seeds per flask.

Germination was checked twice a week, and seeds were only considered germinated when embryo protrusion was equal to or greater than 0.1 cm. After 42 days, the germination percentage (%G), the AGS, and GSI were also evaluated.

2.5 Statistical analysis

Data obtained were tested by analysis of variance (ANOVA). For the comparison of means, Tukey's test was applied at 5% significance, using the software AgroEstat (Barbosa and Maldonado Júnior, 2009) and RStudio (Solanki et al. 2017).

Germination Percentage (G%) was determined by $G\% = \frac{\sum_{ni}}{N}.100$, where $\sum$ni is the total number of germinated seeds and N is the total number of seeds tested (Labouriau, 1983). The Average Germination Speed was determined according to the expression $AGS = \frac{ni}{ti}$, in which "ni" is the number of seeds germinated at time "i" and "ti" is the time after implementation of the test (Carvalho and Carvalho, 2009). The Germination Speed Index (GSI) was determined with the expression $GSI = \frac{G_1}{N_1} + \frac{G_2}{N_2} \ldots + \frac{G_n}{N_n}$,where G_1, G_2, $\ldots G_n$refer to the number of germinated seeds and N_1, N_2, N_n, to the number of days after sowing (Maguire, 1962).

Results

The use of the filter paper saturated with deionized water in Petri dishes or the *in vitro* germination using culture media containing phytorregulators resulted in a successful and rapid cultivation system for observing

and quantifying the germination of *Melocactus*seeds. Germination of *M. zenhtneri* seeds began with the protrusion of the hypocotyl-radicle axis, as the result of the growth and enlargement of the embryo, which leads to the seed coat disruption (Fig. 2).

3.1 Effects of light intensity and wavelength on germination

Variations in the PPFD of different LEDs wavelengths were reported. The highest PPFD values were observed in the Red > White > Blue > Red and Blue LEDs (Table 1).

The percentage of seeds germinated was affected by wavelength and PPFD, but there was no interaction between these two factors. The highest percentage of germination range from 60.8% to 61.7% and was reported in the white, red, and red/blue LEDs. The use of only blue LEDs resulted in a drastic reduction in seed germination to 37.5% (Fig. 3A) and the lowest GSI values (Fig. 3B).

About the PPFD, the gradual increases of light PPFDs resulted in a positive and significant correlation with the percentage of germinated seeds of *M. zenhtneri* (Fig. 3C). The GSIs values were also increased in the higher PPFDs (II and III), compared to the lowest PPFD used (Fig.3B).

The germination of the first seeds was reported four days after seeding (DAS) in the red/blue and white LEDs and at seven DAS in the other wavelengths. The blue light resulted in the late beginning of germination, at nine DAS, with the lowest average germination speed (AGS) (Fig. 3D). For the red, white, and red/blue LEDs, the maximum AGSs occurred between DAS 9 and 14 (Fig. 3D). Increases in PPFD were those resulting in the highest values of AGS.

The different wavelengths also affected the color of embryos after germination. In the red light, 100% germinated embryos had a light green color, related to chlorophyll biosynthesis, while in the blue LED and white LED, the red-purple color was predominant in the embryos. Intermediately, the presence of green and purple embryos in the red/blue LED was also observed (Fig. 2).

The use of darkness for the germination of *M. zehntneri* seeds drastically reduced the germination rate from 63.75% (control using light) to 11.3, 2.5, and 3.8% when seeds were cultivated in darkness for 10, 20, and 30 days, respectively (Table 2). Interestingly, seeds subjected to darkness for these short-period treatments, followed by exposure to light conditions were not able to germinate, for up to 12 months, suggesting the acquisition of secondary and light-irreversible dormancy.

3.3 Sowing M. zehntneri seeds in vitro under different concentrations of plant growth regulators

The germination percentage of *M. zehntneri* seeds using *in vitro* conditions in a culture medium was 74% (Fig. 4), higher than observed using the same light conditions in the previous experiment with Petri dishes and filter paper (61.3%).

Seed imbibition in Ethephon solution as a pre-treatment resulted in a significant and striking increase in the percentage of *in vitro*germinated seeds of *M. zehntneri* . The best treatment and response of seeds to Ethephon was reported in the culture medium without phytoregulators, which resulted in 98% seeds germinated (Fig. 4). In the control culture medium without the pre-treatment with Ethephon, the germination rate was only 76% (Fig. 4). The pre-treatment of seeds with Ethephon also promoted faster seed germination, with the greater number of seeds germinated within 11-14 days, compared to the control (18 days) (Fig. 5) and the highest GSI (2.93) compared to untreated (2.14) and the other plant growth regulators (BAP, GA_3 or BAP+GA_3) added to the culture media (Fig. 6).

Discussion

4.1 ***Seed dormancy in Cactaceae***

Different authors reported dormancy in many Cactaceae species, related to the xeric environment, with highly limited natural resources to support seed germination, seedling development, and survival. Some of these studies associate physical dormancy with mechanical resistance (Rojas-Aréchiga et al. 2011; Pérez-Molphe-Balch et al. 2015) caused by the rigid coat surrounding the embryo, which could limit the water uptake into

the seeds. Seeds of different species of Cactaceae have rigid and hard coats (Archibald, 1939; Orozco-Segovia et al. 2007). However, a study on the anatomy of seeds of different Cactaceae species, including *Opuntia tomentosa* , demonstrated that in these seeds there are no characteristics that explain the occurrence of physical dormancy or any limitation to the water uptake (Orozco-Segovia et al. 2007). At the same time, treatments to reduce the mechanical resistance of seeds using chemical or mechanical scarification have been one of the most studied in different species of Cactaceae, but failed to achieve high rates (>90%) of seed germination (Delgado-Sánchez et al. 2010; Barrios et al. 2020; Magnani and Cardoso, 2022).

In addition, recent studies increased the evidence that only physiological dormancy exists in Cactaceae seeds (Barrios et al. 2020; Rojas-Aréchiga and Garcia-Morales, 2022).

In *Melocactus* , the presence of seed dormancy was widely reported, with percentages of germinated seeds between 8 and 65%, depending on the species, genotype, harvest time, germination temperature, cultivation conditions (*in vitro* , Petri dish or *in vivo*), and pre-treatments or treatments applied to the seeds (Zamith et al. 2013; Bravo Filho et al. 2019; Chaves et al. 2021). However, the main biological evidence and reasons why 35-90% seeds did not germinate, as well as, the treatments required to further increase the germination rates of dormant seeds in Cactaceae, and more specifically in*Melocactus* , are not yet fully elucidated.

4.2 Light strongly influenced seed germination in Melocactus zehntneri

Regarding the wavelengths, except for monochromatic blue light that reduced the germination percentage of *M. zehntneri* seeds (38%), all other wavelengths resulted in germination percentages between 61.0% and 62.0%. Except for monochromatic blue, all other LEDs tested in this study contained in their spectral composition, emission peaks in the red wavelength range, such as the monochromatic red LEDs, the red/blue LEDs, and the white LEDs. Red light seems to be the most important wavelength that accelerates, promotes, and increases the germination of seeds of*M. zehntneri* seeds.

Red light is an important wavelength mediating seed germination. The photomorphogenic responses, which involve light receptors like phytochromes, control the plant development through the presence or absence of light, and also the information and interpretation of different wavelengths in the environment, which guides the most appropriate development, including seed germination (Kami et al. 2010; Neff et al. 2009).

Cho et al. (2012) also demonstrated that germination of*Arabidopsis* seeds is promoted by red light-enriched environments by the activation of Phytochrome B, which results in a gradual increase in the levels of gibberellins that trigger germination. Interestingly, different studies in Cactaceae species sought to correlate the presence of light with increasing GA_3 in seeds due to germination gains with these treatments (Ortega-Baes and Rojas-Aréchiga, 2007). However, there was no strong evidence and no effectiveness of the application of external GA_3 which leads to the release of a large number of seeds from dormancy in this plant family (Barrios et al. 2020). The percentage of germinated seeds under different treatments rarely exceeds the average values observed in the present study with *M. zehntneri* .

The monochromatic blue light substantially reduced the seed germination of *M. zenhtnerii* seeds. The reduction of seed germination in response to blue light is not exclusive to the family Cactaceae and it is frequently reported to inhibit the germination of dormant seeds in cultivated grasses (Hoang et al. 2013; Jacobsen et al. 2013). Some studies suggest that the interaction of blue light with cryptochrome 1 results in an increased concentration of Abscisic Acid (ABA) that inhibit germination (Barrero et al. 2014; Hofmann 2014).

The majority, if not all, Cactaceae species are positive photoblastic (Rojas-Aréchiga and Garcia-Morales, 2022). Flores et al. (2007) reported that from a total of 28 cactus species, all were considered positive photoblastic, and these authors also described the occurrence of secondary dormancy as a consequence of seed exposure to a period of darkness during germination. The same result was observed with *M. zehntneri* in the present study, in which light was necessary for seed germination and exposure of seeds to dark conditions, even for short periods (10, 20, and 30 DAS), significantly reduced the percentage of seed germination (Table 2). Interestingly, even after a short period of darkness (10 days), seeds are unable to germinate even after up to 12 months under the same light conditions that promote germination, with the acquisition of irreversible

secondary dormancy.

Under light conditions, the percentage of germinated seeds of *M. zehntneri* was, on average, 40-60% (Magnani and Cardoso, 2022), showing their photoblastic positive response. Otherwise, 40-60% of seeds are not able to germinate under light conditions, suggesting another type of dormancy not directly associated with light. However, the studies carried out so far, even under light conditions and when applying other types of treatments to break seed dormancy, fail to indicate the main cause(s) of non-germination of seeds that remains dormant.

4.3 Ethephon releases the germination of dormant seeds of M. zehntneri

The treatments with phytoregulators have been used as a way to break dormancy in seeds of different Cactaceae species. The most important effects observed in the present study with *M. zehntneri* occurred when seeds were pre-treated with Ethephon (2-chloroethylphosphonic acid). The treatment using Ethephon promoted a germination percentage of 98% seeds in the culture medium free of other phytoregulators, compared to the 76% observed in the same culture medium without pre-treatment, and promoted the highest values of AGS and SGI.

Different commercial products containing Ethephon have been used as a direct substitute for the ethylene gas hormone, as it is easy to apply and effective in releasing ethylene in an alkaline solution (Zhang et al., 2010). In this way, Ethephon was the unique treatment promoting the germination of dormant seeds in *M. zehntneri* , but the knowledge about how this plant growth regulator affects cactus development is still limited. Among the few reports, ethylene is responsible for the closing, wilting, and pollination of flowers (Doorn 2002) and fruit ripening (Esparza et al. 2006) in some cactus species. However, the effects of ethylene on releasing seed dormancy in Cactaceae have not yet been reported.

Although little explored and used in Cactaceae, ethylene is considered a key hormone that regulates dormancy and seed germination, as well as the establishment of seedlings after germination in many plant species. This hormone is effective, at concentrations from 0.1 to 200 μL L^{-1}, in releasing seeds from dormancy (Corbineaeu et al. 2014). In the present study, the pre-treatment with ethephon was effective to release the germination of *M. zenhtineri* seeds when applied by immersing seeds in a solution at 100 μL $L^{-1}$for 24-h using the commercial product Ethrel®(Bayer®, Brasil), which contains 240 g L^{-1} Ethephon, thus, at a concentration of 24 μL L^{-1}.

Ethylene acts in the release of seeds from dormancy, especially involving a complex interaction with other hormonal groups in seeds, such as ABA, Gibberellins, Nitrous Oxide (NO), and reactive oxygen species (ROS) (Arc et al. 2013). Different studies have demonstrated that, through the use of ethylene biosynthesis and action inhibitors, ET insensitive mutants, and the use of Ethylene biosynthesis precursors, such as 1-aminocyclopropane 1-carboxylic acid (ACC), ethylene is involved in overcoming the dormancy and promoting germination (Corbineau et al. 2014). In addition, this hormone is also capable of neutralizing the effects of ABA on seed dormancy.

Among the plant growth regulators used to study germination, one of the most used was Gibberellic Acid or GA_3 (Mascot-Gómez et al. 2019). This plant growth regulator has shown divergent results in releasing dormant seeds of Cactaceae, in some cases even reducing the percentage of germinated seeds, as observed in *Ferocactus* species (Amador-Alférez et al. 2013). In the present study, seeds of *M. zenhtneri* did not respond to the presence of GA_3 at 1.0 mg L^{-1} in the culture medium (72% germinated seeds) and did not differ from the control without phytoregulators (76% germinated seeds). These results differ from the *in vitro*germination of *M. sergipensis* , in which the addition of GA_3 at 2 mg L^{-1} for 6 hours increased the percentage of seeds germinated in this species. However, the maximum percentage of germinated seeds observed for this species and this treatment was 38% (Bravo Filho et al. 2019), much lower than observed in our study with *M. zenhtnerii* .

The main justification for the wide use of GA_3 for overcoming seed dormancy is the fact that germination release in dormant seeds is associated with a balance between Abscisic Acid (ABA), which maintains seeds dormant, and some gibberellins such as GA_3, which promotes seed germination (Yazaki and Kikuchi 2005;

Rodríguez-Gacio et al. 2009; Yang et al. 2020). The external application of GA_3 in seeds results in increased gibberellin contents in the embryos, which leads to dormancy overcoming and germination release in some species. Nevertheless, the limited positive results to increase germination by the use of GA_3 in several cacti species may be related to the differential sensitivity of seed tissues to GA_3 (Kucera et al., 2005). In addition, recent studies reported that the differential sensitivity of tissues to GA_3 and ABA is also caused by the epigenetic control of the metabolism of GA_3 and ABA (Sano and Marion-Poll, 2021; Smolikova et al. 2021).

Cytokinins and ethylene have been identified as hormonal groups that upregulate themselves and help to overcome seed dormancy (Zdarska et al. 2015). However, in the present study, the addition of BAP, a synthetic cytokinin, or the BAP + GA_3 combination in the culture medium reduced the effects associated with germination promoted by the pre-treatment containing Ethephon. For example, in treatments containing this cytokinin in the culture medium, there were no differences in the percentage of germinated seeds pre-treated or not with Ethephon. The effects of cytokinins on the germination of Cactaceae seeds are practically non-existent, although positive effects of cytokinins both in increasing the germination percentage and GSI have been demonstrated in species from other plant families (Nikolić et al. 2006; Wang et al. 2011). In our study on *Melocactus* , BAP, and GA_3reduced the GSP compared to the Ethephon pre-treatment. Cytokinins also have other positive effects, such as kinetin (KIN) and BAP that were successfully used to promote organogenesis in *in vitro* cladodes segments of *Melocactus glauscecens* , allowing the *in vitro*clonal multiplication of this species (Torres-Silva et al. 2018).

Other groups of phytoregulators, such as 3-indoleacetic acid (AIA), caused no effects on the germination of different Cactaceae species (Mascot-Gómez et al. 2019) or even inhibited the germination of these seeds (Amador-Alférez et al. 2013), such as the putrescine used for germination of *Turbinicarpus lophophoroides* and *T. pseudopectinatus* (Flores et al. 2007). Salicylic acid and Acetylsalicylic acid also considered phytoregulators, completely inhibited the germination of several cactus species (Mihalte et al. 2011).

Conclusions

Red light effectively contributed to the germination of *M. zehntneri* seeds, while blue light significantly decreased the percentage of germinated seeds. The exposure of seeds to darkness during germination, even for short periods, leads seeds to secondary dormancy. However, the greatest effect observed in this study on dormant seeds of*Melocactus* was found with the use of ethephon, which induced 98% seed germination. The present study also can serve as a reference for breaking dormancy and promoting seed germination in *Melocactus* .*In vitro* cultivation can still serve as a tool for studying the germination and propagation of cacti species, aiming at their large-scale production or even conservation. The present study also pointed to a possible new mechanism that explains seed dormancy in Cactaceae seeds: the differential biosynthesis or sensitivity of cacti embryos to ABA.

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Table 1 . Photosynthetically Photon Flux Density (PPFD) sourced by LEDs wavelengths used for seed germination in *Melocactus zehntneri.*

	Photosynthetically Photon Flux Density (PPFD ± SD (μμολ $\mu^{-2}\varsigma^{-1}$)	**Photosynthetical**
LED wavelength	**I**	**II**
Blue	37.83 ±0.50	67.19±0.42
Deep Red	53.59 ±0.64	97.58±0.73
Blue/Red LED	41.28 ±0.33	64.02±0.75
White LED	56.83±0.59	96.19±0.21
Mean	47.38	81.25

Table 2 . Effects of different periods of darkness on the percentage of seed germination of *Melocactus zehntneri* .

	Germination (%)
Control under light	63.75 a
10 days	11.25 b
20 days	2.5 b
30 days	3.8 b
F	31.386*
CV(%)	84.223

*Means followed by different letters were significantly different by Tukey's Test at 5% probability

Fig. 1 . Adult plant of *M. zehntneri* with cephalium (left) and details of cephalium containing mature fruit (right) used to collect seeds for the experimental procedures. Original photos of JCC and MFCM

Fig. 2 . Germination of seeds and color of seedlings of*Melocactus zehntneri* under different wavelengths: Red (V); Blue (A); Red and Blue (V+A) and; White (B).

Fig. 3. Germination (%) of seeds of *Melocactus zehntneri* cultivated under different wavelengths and PPFDs source by LEDs: Percentage of seed germination (A) (Different lowercase letters in the same column indicate the effects of the wavelength and uppercase letters indicate the effects of PPFDs on germination. Shapiro-Wilk Normality Test = 0.36, Levene Test for Homoscedasticity = 0.71. Coefficient of Variation = 13.2%. F-values for: wavelengths (10.81**), PPFDs (4.86*), and Interaction (1.67 ns). Significant at 5%(*) and 1%(**) probability. ns, non-significant); speed germination index (GSI) (Shapiro-Wilk Normality Test = 0.50, Levene Test for Homoscedasticity = 0.68. Coefficient of Variation = 24.95%. F-values for: wavelengths (12.87**), PPFDs (4.42*), and Interaction (2.05 ns). Significant at 5%(*) and 1%(**) probability. Ns, non-significant); correlation between percentage of seed germination and light PPFD (C) and speed germination average under different LEDs wavelengths (D).

Fig. 4 . Germination percentage of seeds of *Melocactus zenhtnerii* subjected to pre-treatment with Ethephon and to different phytoregulators in the culture medium. Shapiro-Wilk Normality Test = 0.857; Levene Test of Homoscedasticity = 0.7278. Coefficient of Variation = 32.7%. F-values for phytoregulators (1.58 ns), ethephon (11.37**), and Interaction (0.82 ns). Significant at 5%(*) and 1%(**) probability. Ns, non-significant.

Fig. 5 . Average germination speed (AGS) of *Melocactus zehntneri* seeds cultivated under different wavelengths (Blue, Deep red, Red and Blue, White) sourced by LEDs and photosynthetically photon flux densities (PPFDs)

Fig. 6 – Germination Speed Index (GSI) of *M. zenhtineri*seeds subjected to different phytoregulators added to the culture media and pre-treatment with Ethephon. Shapiro-Wilk Normality Test = 0.63; Levene Test of Homoscedasticity = 0.0975. Coefficient of Variation = 28.3%. F-values for phytoregulators (2.75 ns), Ethephon (9.98**), and Interaction (0.49 ns). Significant at 5%(*) and 1%(**) probability. ns, non-significant.

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