In vitro germination of desert rose varieties[1]

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ABSTRACT

The drought stress resistance is a characteristic of the desert rose and its estimable beauty flowers, which gave it great relevance in the ornamental market. However, the desert rose production and germination is hampered by possible sterility of their male and female flowers and frequent problems in pollination, so the tissue culture is a promising alternative to the propagation of these plants. This study aimed to evaluate the effect of gibberellic acid on four commercial varieties of desert rose (*Adenium obesum*) cultivated *in vitro*. The seeds of the varieties 'Orange Pallet', 'Carnation violet', 'Diamond ring' and 'Vermiliont' were sterilized and inoculated on Water + Agar (T0), medium MS (T1), ½ MS (T2), MS + 0.25 mg L^{-1} GA_3 (T3), MS + 0.5 mg L^{-1} GA_3 (T4), ½ MS + 0.25 mg L^{-1} GA_3 (T5), ½ MS 0.5 mg L^{-1} GA_3 (T6). The seeds germination of *A. obesum* was initiated on the fourth day of cultivation and on the tenth day was possible to observe the expansion of the cotyledons and leaf expansion with subsequent development of early secondary root. The 'Orange pallet' variety germinated 100% of seeds on water + agar and MS ½ + 0.5 mg L^{-1} of GA_3. For 'Diamond Ring' and 'Carnation violet' the highest rate of germination occurred in treatments MS ½; 0.25 mg L^{-1} GA_3; MS + 0.5 mg L^{-1} GA_3 MS ½ + 0.5 mg L^{-1} GA_3 averaging 80% and 70%, respectively. For 'Vermiliont' the best response was in MS and MS ½ + 0.5 mg L^{-1} GA_3 ranging between 70-90% germinated embryos. It was registered different malformations in all treatments like absence of roots and apexes during seedling development. The concentrations of GA_3 did not affect significantly the seed germination.

Keywords: *Adenium obesum*, *In vitro* cultivation, ornamental plant, giberellic acid

RESUMO

Germinação *in vitro* de variedades de rosa-do-deserto

A rosa do deserto (*Adenium obseum*) conhecida por sua resistência ao estresse hídrico e por suas flores de estimável beleza, o que lhe atribui grande relevância no mercado ornamental. Contudo, a produção e germinação das sementes de rosa-do-deserto é dificultada pela possível esterilidade de suas flores masculinas e femininas e frequentes problemas na polinização, sendo a cultura de tecidos uma alternativa promissora para a propagação dessas plantas. O objetivo do trabalho foi avaliar a germinação e a influência do ácido giberélico em quatro variedades comerciais de rosa-do-deserto (*Adenium obesum*) cultivadas *in vitro*. As sementes das variedades 'Orange pallet', 'Carnation violet', 'Diamond ring' e 'Vermiliont' foram desinfestadas e inoculadas em Água + Ágar (T0), meio MS (T1), MS ½ (T2), MS + 0,25 mg L^{-1} de GA_3 (T3), MS + 0,5 mg L^{-1} de GA_3 (T4); MS ½ + 0,25 mg L^{-1} de GA_3 (T5) e MS ½ + 0,5 mg L^{-1} de GA_3 (T6). A germinação das sementes de *A. obesum* iniciou-se no quarto dia de cultivo e no décimo dia foi possível observar a expansão dos cotilédones com posterior expansão foliar e o desenvolvimento dos primórdios radiculares secundários. A variedade 'Orange pallet' teve 100% das sementes germinadas nos àgua+ ágar e MS ½ + 0,5 mg L^{-1} de GA_3. Para 'Diamond ring' e 'Carnation violet'o maior índice de germinação ocorreu nos tratamentos MS ½; 0,25 mg L^{-1} de GA_3; MS + 0,5 mg L^{-1} de GA_3 MS ½ + 0,5 mg L^{-1} de GA_3 com média de 80% e 70%, respectivamente. Já para 'Vermiliont' a melhor resposta foram nos tratamentos MS e MS ½ + 0,5 mg L^{-1} de GA_3 variando entre 70 a 90% de embriões germinados. Diferentes malformações como ausência de raízes e ápices foram observadas em todos os tratamentos no desenvolvimento das plântulas. As concentrações de GA_3 não influenciaram de forma significativa na germinação das sementes.

Palavras-chaves: *Adenium obesum*, cultivo *in vitro*, planta ornamental, ácido giberélico

1. INTRODUCTION

Adenium obesum (Apocynaceae) commonly known as desert rose is a succulent plant native from Senegal, Ethiopia, Somalia and Tanzania, also found in Saudi Arabia, Oman and Yemen as wild plant. (DIMMIT e HANSON, 1991; OYEN, 2008). Its leaves are simple and spirally arranged at the ends of the branches; the flowers are variable in shape and color, but without fragrance. The fruit is termed follicle, and when mature splits along one side to release seeds with hairy tuffs (pappus) attached for dispersion by the wind (McLAUGHLIN e GAROFALO, 2002).

The desert rose keeps its flowers for 2-4 months every year and it is widely used for indoor and outdoor

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decoration (McLAUGHLIN and GAROFALO, 2002; WANNAKRAIROJ, 2008). Currently, it is one of the main ornamental plants cultivated in Brazil, especially in arid regions, with great relevance in the ornamental market due to its sculptural aspect, resistance to drought stress, and very showy flowers (McLAUGHLIN and GAROFALO, 2002; CHUHAIRY and SITANGGANG, 2004; WANNAKRAIROJ, 2008; HASTUTI et al., 2009; VERSIANI et al., 2014).

The propagation of desert rose can be done via seeds or cuttings. The first option is not reliable due to the low seed production, a result of pollination problems, and possible sterile male and female flowers (McLAUGHLIN and GAROFALO, 2002). The easiest method of propagation is cuttings, however the plants obtained through this method are not well accepted in the ornamental market, since they produce underground caudex and do not show the same exuberance of the plants propagated via seeds (KANCHANAPOOM et al., 2010).

Studies reporting the *in vitro* cultivation of plants of the genus *Adenium* are scarce in the literature. KANCHANAPOOM et al. (2010) cultivated shoot tips of *A. obesum* using a combination of the growth regulators BA and NAA to induce indirect organogenesis. These authors obtained a high frequency of 5.20±1.10 shoots per explant using 22.2 µM BA. Flow cytometry analysis indicated differences in the DNA content between the plants obtained by *in vitro* culture and those obtained *in vivo*. Using juvenile leaf explants of *Adenium multiflorum*, TALUKDAR (2012) established a protocol, based on indirect regeneration, for *in vitro* production with conservation perspectives, considering the low index of seed production and the germination potential of this species. CARVALHO E TOMBOLATO (2004) highlighted the necessity of optimizing the conditions of different stages of the *in vitro* production of ornamental seedlings, since tissue culture is a promising alternative for the propagation of these plants. This tool allows for a fast development, high multiplication rates and the production of pathogen-free plants (KANCHANAPOOM et al., 2010).

The addition of plant growth regulators in the culture media compensate for any possible deficiencies of the hormonal endogenous levels in the explants (VIDAL et al., 2013). Among the plant growth regulators, gibberellins show physiological activities in the induction of seeds germination and in breaking dormancy. Gibberellins promote the expression of genes that control the synthesis of enzymes involved in the degradation of the endosperm cell walls, inducing the embryo growth and stimulating the germination process (CARDOSO, 2004).

Considering the importance of the desert rose commercialization for the market of ornamental plants and the growing cultivation of the species in the Brazilian floriculture, this work aimed to evaluate the germination of four commercial varieties of desert rose *Adenium obesum* and the influence of the gibberellic acid in the *in vitro* germination and culture.

2. MATERIAL AND METHODS

Seeds of 4 commercial varieties of desert rose (60 seeds per each variety), 'Orange pallet', 'Carnation violet', 'Diamond ring' and 'Vermiliont', were disinfected under aseptic conditions in a laminar flow cabinet, by immersion in 70% ethanol for 1 minute, followed by immersion in a solution of 2.5% (w/v) sodium hypochlorite (NaClO) containing two drops of Tween-20 for 6 minutes. Seeds were then rinsed four times in sterile distilled water.

Subsequently, the seeds were placed on Murashige and Skoog (1962) semi-solid medium (MS) supplemented with MS vitamin solution, 0.01% (w/v) myo-inositol, 0.3% (w/v) sucrose and 0.11% (w/v) agar, following the treatments: (T0) Water + Agar, (T1) MS (T2) MS ½, (T3) MS + 0.25 mg L^{-1} GA_3, (T4) MS + 0.5 mg L^{-1} GA_3; (T5) MS ½ + 0.25 mg L^{-1} GA_3, (T6) MS ½ + 0.5 mg L^{-1} GA_3.

The pH of the medium was adjusted to 5.7 ± 0.1, prior to sterilization at 121° C and 1 atm for 15 minutes in a vertical autoclave. Then the medium was poured into test tubes (10 mL in each tube) under laminar flow cabinet. One seed was inoculated per tube, and each treatment had 10 replicates performing a total of 240 tubes.

After 30 days of culture the number of germinated seeds (after the radicle protrusion and cotyledon expansion), abnormalities of the embryos and seedlings, number of leaves and plant height were evaluated.

Following the evaluation, seedlings measuring ± 5 cm were transferred into plastic cups containing washed sand + black soil + crushed charcoal pieces (1:1:1) and acclimated to greenhouse conditions. The plants were watered daily.

The experiment was laid out as a completely randomized design (CRD) with 10 replicates per treatment and one seed in each replicate. The data were submitted to analysis of variance (ANOVA) and treatment means were compared by Tukey test at 5%, using the software SISVAR 5.3 (FERREIRA, 2003).

3. RESULTS AND DISCUSSION

The seeds of all desert rose varieties showed low rates of contamination, as indicated: 'Orange pallet' 6,66%, 'Diamond ring' 5%, 'Carnation violet' 6.66% and Vermiliont 1.66%. For all the treatments, the seeds started to germinate after 4 days in culture, with radicle protrusion (Figures 1A-B). In the 10th day of culture, it was already possible to observe the cotyledon and leaf expansion (Figure 1C), and the secondary root primordia development (Figure 1D).

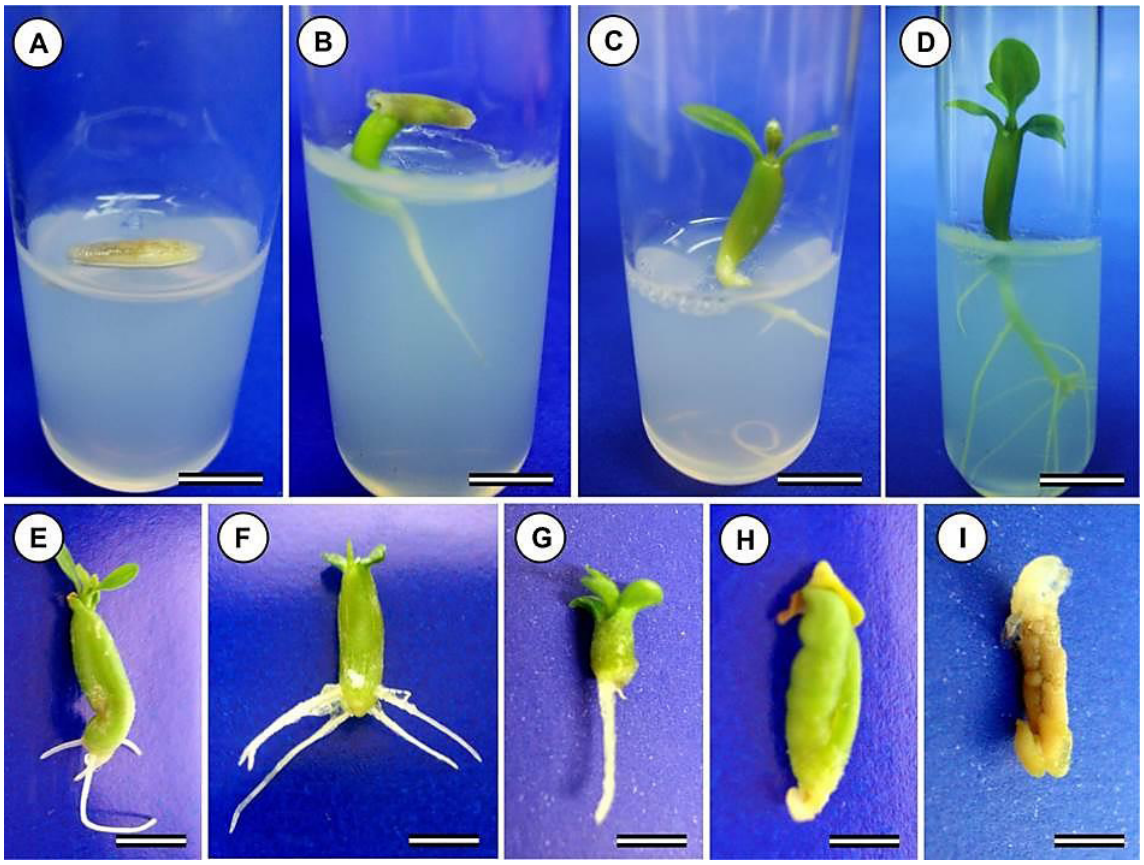

Figure 1. Aspects of *in vitro* germination of seeds and seedlings development of *A. obesum* in MS medium and abnormal in seedlings grown in medium in the absence of salts and sucrose. (A) Seed pink-the-desert cultivated *in vitro*; (B) Top of germination radicle emission; (C) Expansion of cotyledons after 15 days of cultivation leaf primordia and developing leaves; (D) Issuance of secondary roots after 30 days of cultivation; (E) Seedling with stem malformation in the region and stunting of shoots; (F) Seedling with malformation in the shoot; (G) A reduction of seedling stem region and shoots; (H) Embryo in the process of conversion into seedlings with malformations of the basal and apical regions; (I) Embryo with loss of regenerative capacity. Bars.: A-D: (1,4,5,7cm, respectively); E-I: (3,2,1,3,2 cm, respectively).
Figura 1. *Aspectos da germinação in vitro de sementes e desenvolvimento das plântulas de Adenium obesum em meio de cultura MS e malformações em plântulas cultivadas em meio de cultura na ausência de sais e sacarose. (A) Semente de rosa do deserto cultivada in vitro; (B) Início da germinação com a emissão da radícula; (C) Expansão das folhas cotiledonares aos 15 dias de cultivo e primórdios foliares em desenvolvimento; (D) Emissão das raízes secundárias aos 30 dias de cultivo. (E) Plântula com malformação na região caulinar e atrofiamento da parte aérea; (F) Plântula com malformação na parte aérea; (G) Plântula apresentando redução da região caulinar e parte aérea; (H) Embrião em processo de conversão em plântula com malformações das regiões basal e apical; (I) Embrião com perda da capacidade regenerativa. (Barras: A-D: 1, 4, 5 e 7 cm, respectivamente; E-I: 3, 2, 1, 3, 2 cm, respectivamente).*

The seedlings did not show hyperhydricity symptoms during the *in vitro* cultivation. Probably, the concentration of agar used in the medium provided a more solid surface with balanced osmotic potential. KANCHANAPOOM et al. (2010) also reported that *A. obseum* plants obtained via indirect organogenesis did not show hyperhydricity, when using MS medium solidified with agar.

Particularly in desert rose, the drought stress causes swollen basal caudex and the conical apical region divides in irregular branches (McLAUGHLIN and GAROFALO, 2002), which facilitates its commercialization. The typical swollen caudex and conical apical part were also observed in the *in vitro* germinated plants, after the radicle protrusion and the expansion of the leaf primordia.

Differences regarding the germination rate were observed among the treatments for two varieties. For 'Orange pallet' (Figure 2A), the maximum germination rate was 100% for seeds germinated in water + agar and MS ½ + 0.5 mg L^{-1} GA_3 and the minimum was 60% for seeds germinated in MS + 0.5 mg L^{-1} GA_3. For 'Diamond ring' (Figure 2B) the highest germination rate (80%) occurred in the treatments MS ½, MS + 0.25 mg L^{-1} GA_3, MS + 0.5 mg L^{-1} GA_3 and MS ½ + 0.5 mg L^{-1} GA_3, which were statistically different from the remaining treatments showing 20 to 50% of germination.

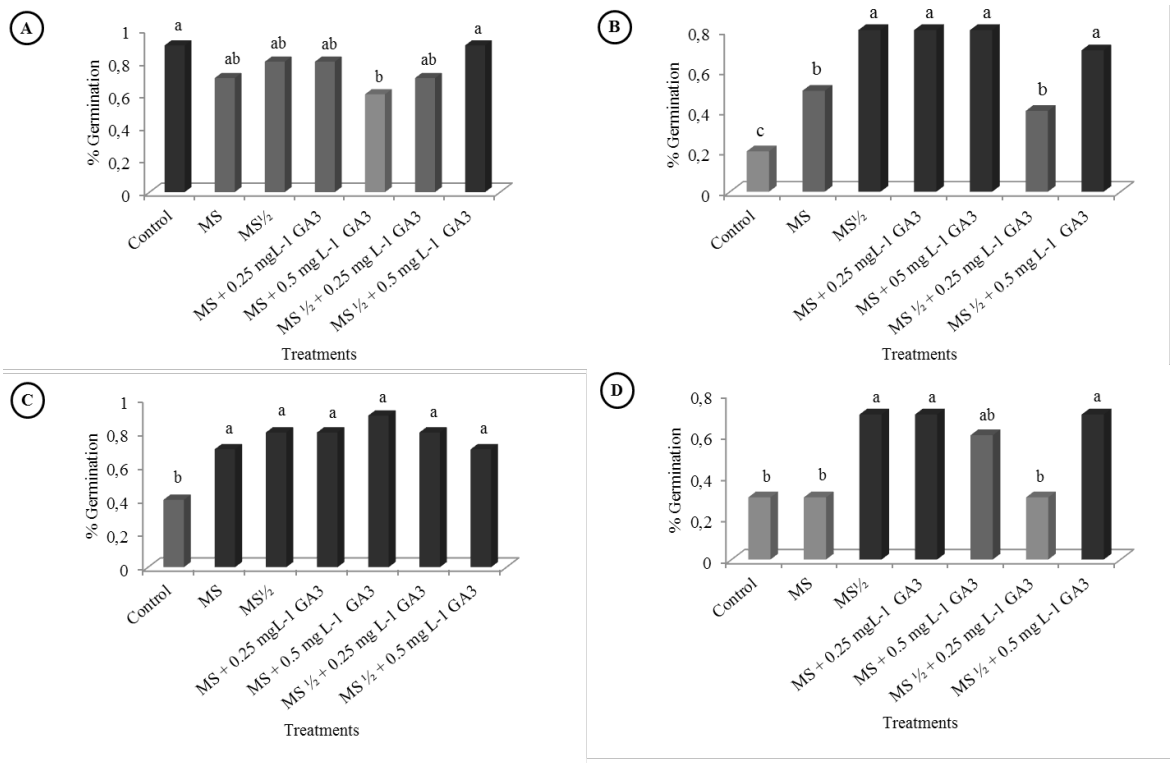

Figure 2. *In vitro* germination of seeds of *A. obesum*. (A) Variety Orange pallet; (B) Diamond ring; (C) Vermiliont; (D) Carnation violet. (T0) Control water and agar in the medium; (T1) MS; (T2) MS ½; (T3) MS + 0.25 mg L^{-1} GA_3; (T4) MS + 0.5 mg L^{-1} GA_3; (T5) MS ½ + 0.25 mg L^{-1} GA_3; (T6) MS ½ + 0.5 mg L^{-1} GA_3. Means followed by the same letter do not differ statistically by the Tukey test ($p < 0.05$) in each treatment.

Figura 2. *Germinação in vitro das sementes de Adenium obesum. (A) Variedade Orange pallet; (B) Diamond ring; (C) Vermiliont; (D) Carnation violet. (T0) Controle no meio de água e ágar; (T1) MS; (T2) MS ½; (T3) MS + 0,25 mgL^{-1} de GA_3; (T4) MS + 0,5 mg L^{-1} de GA_3; (T5) MS ½ + 0,25 mg L^{-1} de GA_3; (T6) MS ½ + 0,5 mg L^{-1} de GA_3. As médias seguidas pelas mesmas letras não diferem estatisticamente pelo teste de Tukey (p < 0,05), em cada tratamento.*

Differences in the conditions of *in vitro* culture between these two varieties were observed, wherein 'Orange pallet' seeds germinated even in the absence of nutrients in the medium (water + agar). Other factors may have influenced the germination, such as the osmotic potential of the medium, luminosity conditions, and the seed coats supply the nutrient requirements for the development of seedlings. On the other hand, for the 'Diamond ring' variety we observed the necessity of nutrients from the MS medium, to obtain a higher rate of germinated seeds.

There was no statistical difference among the treatments for the 'Vermiliont' variety (Figure 2C) and the germination rates ranged from 70% to 90%, thus the use of GA_3 for this *A. obesum* variety is unnecessary. The seeds of Vermiliont do not show dormancy and the *in vitro* conditions induce the germination and normal development of the seedlings. The GA_3 can be added to the medium to elongate the shoots, however, it is not frequently used because its endogenous content is enough (GRATTAPAGLIA and MACHADO, 1998) to induce the seeds germination.

Also for the 'Carnation violet' variety (Figure 2D) we did not observe statistical differences among the treatments MS ½, MS + 0.25 mg L^{-1} GA_3, MS + 0.5 mg L^{-1} GA_3 and MS ½ + 0.5 mg L^{-1} GA_3, that showed around 70% of germinated seeds. We could observe that the *A. obesum* varieties do not have strict requirements of nutrients and growth regulators to break the dormancy of their seeds. Although the *A. obesum* seeds do not show dormancy, the results demonstrated that the medium favored de germination and fast development of the seedlings cultivated *in vitro*. The use of GA_3 at different concentrations did not produce significantly different responses in the *in vitro* germination of seeds of *A. obesum* varieties.

Different abnormalities were observed during the seedlings development (Figures 1E-I) in all treatments, though the seeds cultivated in tubes containing water + agar (T0) were the most affected, with an average of 60% of plants showing abnormalities. Therefore we can infer that the absence of nutrients combined with genetic factors may cause higher rates of abnormal plants. When cultivating *Arabidopsis thaliana* using different concentrations of sugar, ECKSTEIN et al. (2012) observed that plants cultivated without this carbohydrate were slower to develop and did not reach the generative phase. Some species do not depend on nutritive medium, for example the genipap, which showed a higher rate of germination and formation of normal seedlings when cultivated without salts and sucrose (ALMEIDA et al., 2013). Some malformations observed,

such as the lack of primary and secondary roots and stem apex (Figures 3H e I), cause irreversible damage in the *A. obesum* seedlings. On the other hand, plants with other malformations like reduction of the stem and apical region are able to develop the whole vegetative body. Thus, deeper studies are still required to investigate the cause of these malformations, since from the mechanism of gametophytic cells formation onwards. The incidence of *Adenium* plants with severe damage in their development undermines their commercial production.

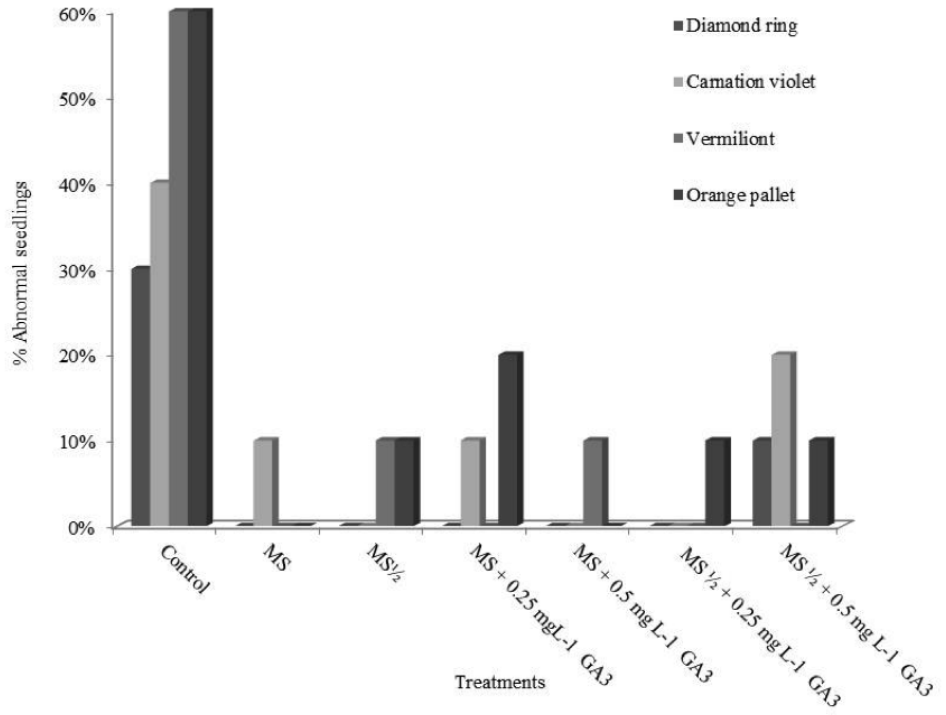

Figure 3. Percentage of abnormal seedlings of *A. obesum* cultured *in vitro*. Means followed by the same letter do not differ statistically by the Tukey test ($p < 0.05$) in each treatment.

Figure 3. *Percentagem de malformações (%) das variedades de A. obesum cultivadas in vitro. Médias seguidas pelas mesmas letras não diferem estatisticamente pelo teste de Tukey ($p < 0,05$), em cada tratamento.*

Regarding the height of the cultivated seedlings, we did not find significant differences among the treatments of the 'Orange pallet' variety. For this variety, the highest average of plant height (3.15 cm) was observed in the treatment MS + 0.25 mg L^{-1} (Table 1). The GA_3 acts mainly elongating axillary organs such as stems, flowers and inflorescences, and in the development of trichomes and seeds. This elongation probably occurs due to the increase of soluble carbohydrates, product of GA_3 metabolic activity (DeMASON, 2005; BIALECKA and KĘPCZNSKI, 2007).

The 'Diamond ring' variety also showed the best results when cultivated in MS + 0.25 mg L^{-1} GA_3 with average of plant height of 4.03 cm. For this variety, significant differences were detected among the treatments, and the lower rates (average of 0.7 cm) were found in T0 (control) (Table 1). As for the 'Vermiliont' variety, the best results were found using MS, which produced an average of plant height of 4.56 cm, while the lowest average (0.80 cm) was noticed for the treatment of water + agar (Table 1). The 'Carnation violet' variety also had the lowest average of plant height (0.35 cm) in the water + agar treatment but the highest average of plant height (4.57 cm) was observed for the T6, meaning that the use of half of the concentration of macro and micro MS nutrients plus 0.5 mg L^{-1} GA_3 is more efficient to stimulate the growth and elongation of this variety (Table 1).

Table 1. Ratings for height (cm) of plants for varieties of *A. obesum* after 30 days of *in vitro* culture.
Tabela 1. *Médias da altura (cm) das plantas para as variedades de Adenium obesum, após 30 dias de cultivo in vitro.*

	Treatments						
Varieties	Control	MS	MS½	MS+ 0.25 mg L^{-1} GA_3	MS+ 0.5 mg L^{-1} GA_3	MS½+ 0.25 mg L^{-1} GA_3	MS½+ 0.5 mg L^{-1} GA_3
Orange palett	2.52 a	1.57 a	2.97 a	3.15 a	2.99 a	2.85 a	2.69 a
Diamond ring	0.7 b	2.7 ab	3.6 a	4.03 a	2.2 ab	3.02 ab	3.54 a
Vermiliont	0.80 b	3.82 a	4.56 a	2.55 ab	3.30 a	4.19 a	3.50 a
Carnation violet	0.35 c	1.40 bc	3.6 ab	2.65 abc	2.78 abc	2.25 abc	4.57 a

Means followed by the same letter do not differ statistically by the Tukey test ($p < 0.05$) in each treatment.
As médias seguidas pelas mesmas letras não diferem estatisticamente pelo teste de Tukey ($p < 0,05$), em cada tratamento.

The presence or lack of GA_3 did not affect the number of leaves for the varieties studied. The 'Vermiliont' and 'Carnation violet' varieties showed higher number of leaves in the plants cultivated in MS ½ (Table 2). The MS + 0.25 mg L^{-1} GA_3 treatment produced the best results in terms of leaf production, for the 'Diamond ring' variety (Table 2). Differently, we did not observe statistical difference among the leaf production treatments for the 'Orange pallet' variety (Table 2). Gibberellins like GA_3 activate the cotyledons growth and the expansion of young leaves delaying leaf senescence (LENY et al., 2005; TAIZ and ZEIGER, 2010). The difference found in the number of leaves of the *A. obseum* varieties can be due to the plant genotypes demonstrating variability for this trait.

Table 2. Ratings of the number of leaves for the varieties of *A. obesum* cultivated *in vitro.*
Tabela 2*. Médias do número de folhas para as variedades de A. obesum cultivadas in vitro.*

Varieties	Treatments						
	Control	MS	MS½	MS+ 0.25 mg L^{-1} GA_3	MS+ 0.5 mg L^{-1} GA_3	MS½+ 0.25 mg L^{-1} GA_3	MS½+ 0.5 mg L^{-1} GA_3
Orange palett	2.6 a	2.7 a	2.3 a	1.8 a	1.8 a	1.9 a	2.3 a
Diamond ring	0.5 b	2.1 ab	2.4 ab	3.4 a	1 ab	2 ab	2.4 ab
Vermiliont	0.90 b	3.40 a	4.30 a	2.50 ab	2.40 ab	2.50 ab	3.40 a
Carnation violet	0.2 b	1.20 ab	3.0 a	2.30 a	2.40 a	1.40 ab	2.90 a

Means followed by the same letter do not differ statistically by the Tukey test ($p < 0.05$) in each treatment.
Médias seguidas pelas mesmas letras não diferem estatisticamente pelo teste de Tukey ($p < 0,05$), em cada tratamento.

Plants with normal development were acclimated. The substrate used, containing washed sand + black soil + crushed charcoal pieces and the greenhouse conditions favored the efficiency of acclimation and the survival percentage was 95% for seedlings of all the varieties. After 60 days, using the MS macro and micronutrients as fertilizer, we obtained, for all varieties, plants around 20 cm tall, with radial and prominent expansion of the stem, which is the typical morphology of the species. According to ROCHA (2009) the acclimation is the most critical phase of the micropropagation process. This difficulty is due to the stress caused by temperature change, relative humidity of the air and substrate as well as luminosity.

4. CONCLUSIONS

- The *in vitro* culture of the desert rose can be considered a viable propagation method, since on the 10th day the

seedlings had already developed leaf primordia and roots. However malformations can occur during the seedlings development and these malformations may be due to pollination problems.

- The use of GA_3 in different concentrations did not affect significantly the *in vitro* germination of seeds of *A. obesum* varieties.

- Obtaining health and vigorous plants of desert rose *in vitro* is a promising step to the establishment of regeneration pathways such as somatic embryogenesis.

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