6-BENZILADENINE (BA) AND GIBERELIC ACID (GA_3) EFFECTS ON *in vitro* DEVELOPMENT OF *Selenicereus grandiflorus* (*L.*) BRITTON & ROSE (CACTACEAE)

EFEITOS DE 6-BENZILADENINA (BA) E ÁCIDO GIBERELICO (GA_3) NO DESENVOLVIMENTO *in vitro* DE *Selenicereus grandiflorus* (*L.*) BRITTON & ROSE (CACTACEAE)

PATRÍCIA FONTES ESTEVES[1]; ALICE SATO[2]; MARIA APPARECIDA ESQUIBEL[3]

[1] Mestre em Biotecnologia Vegetal - Universidade Federal do Rio de Janeiro (UFRJ) - Centro de Ciências e da Saúde – Avenida Carlos Chagas Filho, s/nº, Instituto de Biofísica Carlos Chagas Filho, Bloco G - 2º andar - Sala G2 050, Cidade Universitária, Ilha do Fundão - 21941-590 - Rio de Janeiro, RJ - patyfesteves@yahoo.com.br
[2] Doutora em Biotecnologia Vegetal - Universidade Federal do Estado do Rio de Janeiro (UNIRIO) – Avenida Pasteur, nº 458 – 4º andar – Sala 415, Urca - 22290-240 - Rio de Janeiro, RJ - alicesato@unirio.br
[3] Pós-Doutora em Bioeletrogênese - Universidade Federal do Rio de Janeiro (UFRJ) - Centro de Ciências e da Saúde – Avenida Carlos Chagas Filho, s/nº, Instituto de Biofísica Carlos Chagas Filho, Bloco G - 2º andar - Sala G2 050, Cidade Universitária, Ilha do Fundão - 21941-590 - Rio de Janeiro, RJ - maesquibel@gmail.com

ABSTRACT

Stem segments of *Selenicereus grandiflorus* (*L.*) Britton & Rose were cultured on MS medium supplemented with different concentrations of 6-benziladenine (BA) and gibberelic acid (GA_3) with the objective to investigate the effects on plant organogenesis and rhizogenesis. Cultures treated with BA in concentrations of 0.22 or 0.88 mM, induced mean production of 9 and 10 shoots/explant in 29.0 and 32.3% of explants, respectively in 30 days of culture; while in 75 days of culture, 58.1 and 48.4% of explants developmented 28 and 21 shoots/ explant. Regenerated shoots rooted within 30 days of culture in 100% of explants. The treatment with GA_3 at 0.29 or 0.88 mM, induced mean production of 9 and 5 shoots/explant with shoots percentage of 19.6 and 9.5, respectively, in 30 days of culture. However in 75 days of culture, the addition of GA_3 did not produce effect on shoots production. The application of GA_3 did not induce significant effect on roots development, besides inhibited its elongation.

Index terms: Cacti, Tissue culture, Growth regulators.

RESUMO

Segmentos caulinares de *Selenicereus grandiflorus* (*L.*) Britton & Rose foram cultivados em meio MS, suplementados com diferentes concentrações de 6-benziladenina (BA) e ácido giberélico (GA_3), a fim de verificar seus efeitos na organogênese de brotos e raízes. As culturas tratadas com BA nas concentrações de 0,22 ou 0,88 mM, em 30 dias de cultura, induziram produção média de 9 e 10 brotos/explante em 29,0 e 32,3% dos explantes, respectivamente, enquanto em 75 dias de cultura, 58,1% e 48,4% dos explantes desenvolveram 28 e 21 brotos/explante. A rizogênese foi de 100% em 30 dias de cultura. O tratamento com GA_3, 0,29 ou 0,88 mM promoveu, em 30 dias de cultura, produção média de 9 e 5 brotos/explante, com porcentagem de brotamento de 19,6 e 9,5%, respectivamente. Entretanto, em 75 dias de cultura, a adição de GA_3 não produziu efeito na produção de brotos. O ácido giberélico não promoveu desenvolvimento significativo das raízes, além de inibir o alongamento destas.

Termos para indexação: Cactus, Cultura de tecidos, Reguladores de crescimento.

INTRODUCTION

Selenicereus grandiflorus (*L.*) Britton & Rose (Cactaceae), native of Mexico and West Indies, is a creeping and fleshy plant. In Brazil, it is known by the name of night-blooming cereus because its flowers expand at night and exhale a vanilla-like odor. The cladodes are therapeutically used in asthenia, bronchitis, chronic asthma, hemorrhoids cases and possess diuretic action, besides they have been utilized in cardiac diseases treatment (CORRÊA et al., 1998).

In general, succulent plants present slow growth and it can impose limitation in their reproductive capacity. The tissue culture is an efficient method for propagation of them, because it allows a fast and continuous production of new plants.

Cacti are commonly propagated by seeds or cuttings, but these methods show many disadvantages. Seeds present low germination rates and are difficult to be obtained due to self -incompatibility (BOYLE, 2003). The tissue culture allows fast multiplication from little plant

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material and provokes low impact on wild populations, and it is also a potential method for *ex situ* conservation of genetic diversity (GIUSTI et al., 2002).

This method has been showed adequate for several members of Cactaceae family (MAUSETH, 1979; ESCOBAR et al., 1986; HUBSTENBERGER et al., 1992; DEOLIVEIRA et al., 1995; MALDA et al., 1999; ESTRADA-LUNA et al., 2002; PELAH et al., 2002) using different explant sources: apex of plantlets (AULT & BAGAMON, 1985; SMITH et al., 1991; PELAH et al., 2002), pith excised from stem tissue of plants growing in nature (JOHNSON & EMINO, 1979), shoot tips of mature plants (CLAYTON et al., 1990), roots (BHAU, 1999) and explants with areoles (RAMIREZ-MALAGON et al., 2007).

Particularly for cactus, it has been observed that *in vitro* plants show fast growth and produce significant number of new shoots. Rodriguez & Rubluo (1993) showed that *Aztekium ritterii* Boed., a cactus of slow growth, produced 5-7 new shoots after 11 months of culture, while *ex vitro* specimens did not produce new shoots. *Mammillaria woodsii* Craig obtained from seeds needed at least one year to reach the same size than regenerated plants, which needed of few months of culture (VIZKOT & JARA, 1984). Comparable observations were showed for *Neomammillaria prolifera* (Mill.) Britton & Rose (MINOCHA & MEHRA, 1974) and *Cephalocereus senilis* (Haw.) Pfeiff (BONNES et al, 1993).

Many different media and hormones have been tested for cactus propagation, especially the interaction of auxins and cytokinins, but the response is different for each cactus species (GIUSTI et al., 2002). It has been suggested that each species might require a specific hormone combination (JOHNSON & EMINO, 1979; GIUSTI et al., 2002; RUBLUO et al., 2002). However, studies point out that an exogenous cytokinin is required to obtain *in vitro* proliferation, while auxins are not strictly necessary (RUBLUO et al., 2002). In this study we analyzed the morphogenetic responses in relation to the growth regulators influence on organogenesis and rhizogenesis of *in vitro* stem segments of *S. grandiflorus*.

MATERIAL AND METHODS

In vitro culture establishment.

Stem segments of *S. grandiflorus* were washed with water containing a few drops of 2% neutral commercial detergent (15 min), rinsed in sterile distilled water, followed by immersion in 50% commercial bleach (3% sodium hypochlorite, 15 min), rinsed in sterile distilled water, washed in 70% ethanol (10min) and finally washed three times (2min each) in sterile distilled water. This process was realized at flow chamber.

After sterilization process, the stem segments were isolated and transferred to solid medium (20 mL), consisting of full-strength MS salts and vitamins (MURASHIGE & SKOOG, 1962), 30 gL^{-1} sucrose, without growth regulators. This medium was named MS0 (control medium).The pH was adjusted for 5.8, solidified with 8 gL^{-1} of agar and sterilized in autoclave at 120°C for 15min.

The cultures were incubated in a growth room at 25±2°C with a 16-h photoperiod under irradiance of 23.0 mmoles of photon $m^{-2}\,s^{-1}$ for 90 days.

Growth regulators treatments

After three months of culture, stem segments (1cm) from MS0 were transferred to MS medium (50 mL) supplemented with 6-benziladenine (BA: 0.22, 0.44 or 0.88 M) and gibberelic acid (GA_3: 0.29 or 0.88 mM). Each treatment was replicated five times and 40 explants were used.

Statistical analysis

Growth regulators effects were determined according to shoots number, the higher length of shoot and root, shoots production and rhizogenesis percentage. Each fifteen days were measured the shoot and root production, during 75 days. The obtained results were analyzed by ANOVA and the means were compared by Tukey test at the 0.05 level of significance. The difference between percentage test (p_1 and p_2) was applied in the comparative study of shoots production and rhizogenesis percentage, at the 0.05 level of significance, using Statistic for Windows ™, version 5.0.

RESULTS AND DISCUSSION

The sterilization protocol utilized was efficient and none contamination was observed after 30 days of culture (Figure 1A).

Due to the culture multiplication capacity in different concentrations of BA, the shoots number developed in 30, 45, 60 and 75 days of culture was determined. After 30 days of culture, the explants cultivated in 0.22 and 0.88 mM of BA showed significantly higher shoots production (9 and 10 shoots/explant, respectively) when compared to MS0 (3 shoots/explant) (Table 1). After 75 days of culture, the media supplemented with BA (0.22; 0.44 and 0.88 mM) induced production of 28, 19 and 21 shoots/explant, respectively, but these results did not show statistic difference in relation to MS0.

Similar results were obtained by Ruvalcaba et al. (1999), studying *Agave parrasana* Berger cultures. When BA was not added to the medium, shoots production was imperceptible (0.1 shoot/explant), however when BA (13.3 to 53.2 mM) was added to the medium, the shoots production increased (25 shoots/explant).

The efficiency of cytokinins on shoots proliferation was also observed in *Escobaria minima* (Baird) D. Hunt, *Mammillaria pectinifera* (Ruempler) F.A.C.Weber and *Pelecyphora aselliformis* Ehrenberg cultures. The higher BA concentration (22.2 mM) induced mean of 3 shoots / explant, while the lower concentration (0.44 mM) induced mean of 1.3 shoots/explant only in *E. minima*. Kinetin (KIN) at high concentration (23.23 mM) with low concentration of NAA (0.05 mM) induced mean of 10.2 shoots/explant in *P. aselliformis*; low concentrations of KIN induced less or none axillary shoots formation (GIUSTI et al., 2002). In disagreement with these studies, Bhau (1999) showed that KIN and BA alone did not induce morphogenetic response in culture of *Coryphantha elephantidens* (Lem.) Lem.. The maximum shoots production (1.6 shoots/explant) was obtained on medium with 2.2mM 2.4-D and 4.6 mM KIN. Nikam (1997) also demonstrated that high concentrations of KIN reduce the number of shoots/explant, and BA was also inefficient on *Agave sisalana* Per. shoots production. These results were similar for *A. cantala* Roxb. and *A. fourcroydes Lem.* (BINH et al., 1990).

In the Cactaceae family, interaction of auxins and cytokinins for *in vitro* regeneration is well documented (HUBSTENBERGER et al., 1992) however the authors showed that an exogenous cytokinin is necessary to obtain shoot proliferation moreover auxins are not required. On the other hand, Rubluo et al. (2002) demonstrated a linear positive correlation between auxin concentration and shoots proliferation. The same results were obtained for *Echinocereous* spp. and *Opuntia polycantha Haw.* (MAUSETH & HALPERIN 1975; HUTABARAT, 1986), which demonstrate that morphogenetic responses are

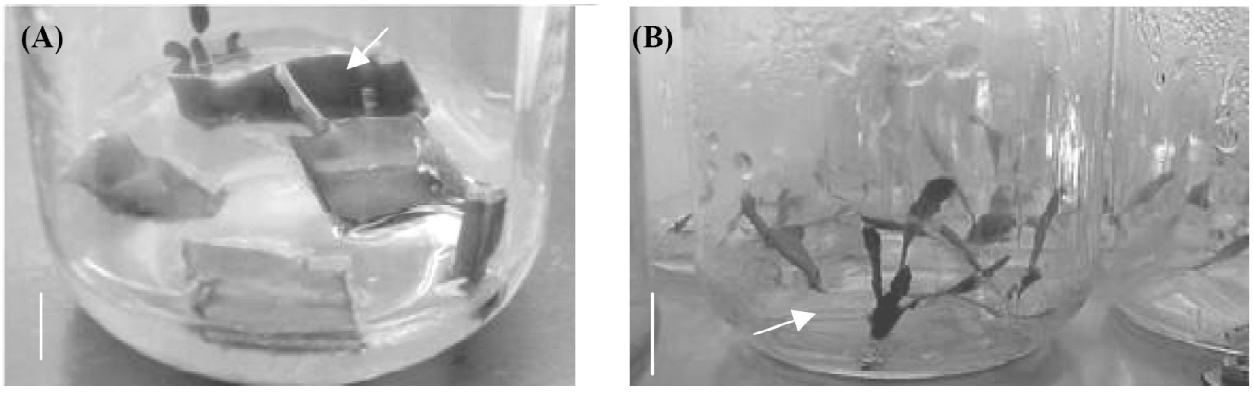

FIGURE 1 – Micropropagation of *S. grandiflorus* from stem segments. (A) Axillary shoots development on MS0 medium (arrow). (B) Proliferation of shoots obtained with 0.22μM BA (bar = 1 cm).

singular for each cactus specie (JOHNSON & EMINO, 1979; CLAYTON et al., 1990).

In relation to shoots production percentage, after 30 days of culture, the media containing BA (0.22 and 0.88 mM) induced greater shoots production percentage (29.0% and 32.3%, respectively) while MS0 showed 5.7% of shoots production percentage (Figure 1B). However, after 75 days of culture, BA addition (58.1; 42.9 and 48.4%) did not induce better results than MS0 (38.5%) (Table 1).

The positive effect of BA on shoots production confirms the BA efficiency on multiplication of many species and it may be the perfect cytokinin for aerial parts multiplication and axillary shoots induction. It is important to jut out the influence of concentration on success *in vitro* multiplication (TORRES & CALDAS, 1990).

Other authors also observed positive effect of BA (0.44 – 44 mM) in *Opuntia polyacantha Haw.*, *O. amyclaea* Ten. and *Mammillaria* spp. cultures (MAUSETH & HALPERIN, 1975; MAUSETH, 1979; ESCOBAR et al., 1986).

In regeneration of *Cereus peruvianus* (*L.*) Mill. from callus culture, lateral explant in medium with auxins (IAA and NAA) and cytokinins (BA and Kinetin) showed positive results on shoots regeneration, while apical explants did not showed response for *in vitro* multiplication (MACHADO & PRIOLI, 1996).

In 30 days of culture, the addition of GA_3 induced mean production of 9 and 5 shoots/explant, while MS0 induced 3 shoots/explant (Table1). However after 75 days of culture, MS0 induced higher shoots production than the media with GA_3 and it can also be observed on shoots production percentage, where the medium supplemented with 0.29 and 0.88 mM GA_3 showed 20.0 % and 14.6 %, respectively (Table 1).

Shoot elongation

The shoots elongation was significantly greater (0.8 and 0.9 cm, respectively) in the media supplemented with 0.22 and 0.88 mM BA than MS+0.44 mM BA (0.3 cm), after 30 days of culture. In 75 days of culture, plants cultivated in MS0 did not show difference to plants submitted to BA (Table 2). Exogenous GA_3 did not promote effects on shoots elongation (Table 2) but rather inhibited it. Although gibberellins have been reported to promoted growth of shoot and root, cell division and cell elongation were not distinguished (SPONSEL &HEDDEN, 2004). This suggested that the stem segments contains enough gibberellins to support elongation growth and that exogenous addition of GA3 results in a supra–optimal concentration of this growth regulator in the segment.

TABLE 1 – Effect of 6-benziladenine (BA) and giberelic acid (GA_3) on the morphogenetic response of stem segments from *Selenicereus grandiflorus* for 30 and 75 days of culture. The values represent mean ± standard deviation and percentage (n=40/treatment).

Growth regulators	Concentration (μM)	Shoots production (%)		Shoots/explant Mean ± standard deviation		Roots formation (%)	
		30 days	75 days	30 days	75 days	30 days	75 days
BA	0.22	29.0 [b]	58.1 [a]	9 ± 0.23 [*]	28 ± 0.96	100 [b]	100 [b]
BA	0.44	11.4 [a]	42.9 [a]	4 ± 0.46	19 ± 0.90	100 [b]	100 [b]
BA	0.88	32.3 [b]	48.4 [a]	10 ± 0.32 [**]	21 ± 0.70	100 [b]	100 [b]
GA3	0.29	19.6 [a]	20.0 [b]	9 ± 0.39 [*]	9 ± 0.42	84.8 [b]	88.9 [b]
GA3	0.88	9.5 [a]	14.6 [b]	5 ± 0.40 [*]	6 ± 0.52	64.3 [a]	82.9 [b]
Control		5.7 [a]	38.5 [a]	3 ± 0.47	24 ± 0.79 [**]	40.4 [a]	66.7 [a]

* $P < 0.05$. ** $P < 0.01$ (Tukey's test). Values with different letters are significantly different from each other at a 5% level by difference between percentage tests.

TABLE 2 – Effect of 6-benziladenine (BA) and giberelic acid (GA_3) on shoots and roots length of *S. grandiflorus* in 30 and 75 days of culture. The values represent mean ± standard deviation. (n=40/treatment).

Growth regulators	Concentration (μM)	Shoot length (cm) Mean ± standard deviation		Roots length (cm) Mean ± standard deviation	
		30 days	75 days	30 days	75 days
BA	0.22	0.8±0.2*	1.6±0.94	3.1±1.24**	7.6±0.97**
BA	0.44	0.3± 0.11	1.7±0.81	1.3±0.65	4.7±0.86
BA	0.88	0.9±0.3*	2.4±0.97	4.0±1.22 **	11.5±1.02**
Control		0.5±0.15	2.1± 0.72	1.6±0.79	3.7±0.81
GA3	0.29	0.1±0.04	1.5±0.15	1.7±0.30	2.2±0.21
GA3	0.88	0.1±0.04	1.0±0.11	1.5±0.30	1.7±0.19
Control		0.1±0.04	1.6± 0.13	3.1±1.1**	4.8±0.95**

* $P < 0.05$. ** $P < 0.01$ (Tukey's test).

Like the results of our study, KIN was responsible for the fast growth of *Mammillaria pectinifera* F.A.C. Weber and *Pelecyphora aselliformis* Ehrenberg; shoots reached height of 1.8 to 2.5 cm (GIUSTI et al., 2002). Nikam (1997) showed the same result in *Agave sisalana* culture, where shoots cultivated with 0.93 to 6.98 mM KIN attained height of 1 cm in 35 days of culture. Differently, Bhau (1999) showed that shoots in medium supplemented with 2.2 μM 2.4-D and 4.6 mM KIN attained height of 3.4 cm in 28 days of culture; the shoots production was not observed in medium supplemented with kinetin or BA alone. To reach this production, the balance between cytokinins and auxins was necessary.

Rooting

To promote rooting in cactus, MS without growth regulators has usually been used (NIKAM, 1997; RUVALCABA et al., 1999; GIUSTI et al., 2002; RUBLUO et al., 2002). However, to *S. grandiflorus* stem explants exogenous BA concentrations utilized were more efficient on rooting induction when compared to control medium. They induced rooting on 100% of explants in 30 days, while the plants cultivated in MS0 attained the greater rooting perceptual (66.7) only after 75 days of culture (Table 1). Since the beginning of culture the medium MS+ 0.88 M BA induced roots with higher length reaching an average of 11.5 cm after 75 days (Table 2).

The medium supplemented with 0.29 mM GA_3, induced the greater rooting in 30 and 75 (84.8 and 88.9 %, respectively) days of culture (Table 1). Meanwhile, addition of GA_3 inhibited the roots length (Table 2).

For rooting stage it is also commonly used a dilutions of salt concentrations of culture medium. Therefore *Cereus jamacaru* presented higher root number and root elongation on MS 25% additioned of 2.0 mg.L-1 IBA (OLIVEIRA et al., 2008).

CONCLUSION

The BA addition on culture medium showed significant effect on roots development as well as on shoots production and elongation of shoots and roots in 30 days of culture.

Media supplemented with GA_3 stimulated shoots production but did not induce significative elongation. BA was also efficient on roots development, and medium enriched with 0.88mM BA induced greater elongation while GA_3 inhibited roots elongation.

The present results suggest that cytokinins may be involved in *in vitro* morphogenetic response of cactus

and confirm that each cactus species show specific response to different growth regulators.

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