



Short Communication

Seedling production of bromeliad endemic to the semiarid region of Bahia, Brazil

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Abstract

Bromeliads are plants that, in addition to marked ecological importance, have increasing ornamental potential in the flower market, being commonly collected in an extractive way, which results in genetic loss of the exploited species, putting them in different degrees of threat. Culture of plant tissues has been shown to be a viable alternative for the propagation of many bromeliads. The aim of this study was to produce seedlings of *Neoregelia mucugensis* by micropropagation. *In vitro* multiplication of sprouts via direct organogenesis was obtained in static liquid medium containing 6-benzylaminopurine (BAP) cytokinin. The sprouts were rooted in activated charcoal medium and transferred to the *ex vitro* condition, and 100% plant survival was obtained. It is concluded, therefore, that the production of seedlings of *N. mucugensis* is viable by micropropagation.

Key words: activated charcoal, direct organogenesis, *in vitro* cultivation, *Neoregelia mucugensis*.

Resumo

As bromélias são plantas que além de acentuada importância ecológica, apresentam crescente potencial ornamental no mercado de flores, sendo comumente coletadas de forma extrativista, o que acarreta perda genética das espécies exploradas, pondo-as em diferentes graus de ameaça. A cultura de tecidos vegetais tem se mostrado uma alternativa viável para a propagação de muitas bromeliáceas. O objetivo deste estudo foi produzir mudas de *Neoregelia mucugensis* por micropropagação. A multiplicação de brotos via organogênese direta foi obtida em meio líquido estático contendo a citocinina 6-benzilaminopurina (BAP). Os brotos foram enraizados, em meio com carvão ativado, e transferidos para a condição *ex vitro*, sendo obtidos 100% de sobrevivência das plantas. Conclui-se, portanto que a produção de mudas de *N. mucugensis* é viável por micropropagação.

Palavras-chave: carvão ativado, organogênese direta, cultivo *in vitro*, *Neoregelia mucugensis*.

Bromeliads have been commercially exploited in Brazil since the 1970s, and the demand for these plants in the market is increasing (Anacleto *et al.* 2008) due to the exuberance of colors and shapes, being widely used as ornamental plants. However, several species are marketed in an extractive way (Anacleto & Negrelle 2009) and, consequently, there is a reduction in the natural population of these plants, with risk to the genetic material. As a result, several bromeliads are included in the red list of threatened species categorized at different levels.

Species of the genus *Neoregelia* are listed in the classifications of less worrying, vulnerable, almost threatened, endangered, and critically endangered (CNCFlora 2012).

Neoregelia mucugensis Leme is a rupicolous herb not yet evaluated for the risk of extinction, but it is native and endemic to Brazil, with confirmed occurrence only in the state of Bahia, in rupestrian grassland vegetation (Flora do Brasil 2020, continuously updated) and exclusively exploited in an extractive way. The predation of bromeliads

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can interfere with the dynamics of the ecosystem since there are species of invertebrate and vertebrate animals that use them in several ways: aquarium or water source, place of shelter or hunting territory, food and permanent habitat; in addition, other plant species grow in bromeliads (Frank & Lounibos 2009).

In order to preserve these plants and meet the flower market of Brazil, which according to data from Ibraflor (2021) is among the 15 largest flower producers in the world, it is necessary to study the application of sustainable propagation strategies such as the culture of plant tissues. *In vitro* cultivation can be influenced by luminosity, type and concentration of the culture medium that provide conditions for plant growth and multiplication (Rodrigues *et al.* 2019), type of explant, and concentrations of plant regulators (Lopes *et al.* 2017) and other compounds added to the medium, such as activated charcoal. For *N. mucugensis*, the existing publications address only the stages of *in vitro* establishment and *in vitro* multiplication (Bellintani *et al.* 2007a, 2008), but there are no reports for *in vitro* rooting and acclimatization. The objective of this study was to produce seedlings of *Neoregelia mucugensis*, aiming for a commercial scale.

Neoregelia mucugensis seeds collected in Mucugê Municipal Park - Mucugê, Chapada Diamantina, BA, Brazil, were established *in vitro* (Bellintani *et al.* 2007a) and used as a source of explants. Whole 3-month-old plants were inserted into test tubes containing 5 mL of MS culture medium (Murashige & Skoog 1962) with half of the salt concentrations (MS 1/2) plus 30 g L⁻¹ sucrose (static liquid medium) and different 6-benzylaminopurine-BAP concentrations (0.0; 20; 25; 30; 35 µM). After 60 days, the percentage of responsive explants (%ReE) and number of sprouts per explant (NS) were evaluated. The experimental design was completely randomized (CRD) with 5 treatments. Each treatment consisted of 6 replicates with 5 samples.

Sprouts, 2 cm long, were inserted into test tubes containing 15 mL of MS1/2 culture medium plus 30 g L⁻¹ sucrose, 7 g L⁻¹ agar and 1 g L⁻¹ activated charcoal. The experimental design was CRD with two treatments: rooting for 30 and 45 days, with 30 replicates each. After the stipulated period, the percentage of rooted explants (%RoE), the number of roots (NR), and the length of the longest root (LLR) measured in centimeters (cm) were evaluated. Then, these microplants were

acclimatized. The microplants rooted *in vitro* were transplanted to a substrate composed of earth and vermiculite (1:1) (Bellintani *et al.* 2007b), in 50-mL transparent disposable plastic cups, and kept in a plastic tray with a film of water in a greenhouse with 70% shade net. At 30 days, the percentage of plant survival was evaluated.

The *in vitro* cultures were kept in a growth room at a temperature of 25 ± 3 °C, photoperiod of 16 h and active photosynthetic radiation of 60 µmol.m⁻².s⁻¹. The data were subjected to analysis of variance (ANOVA) and to regression for quantitative data, using the SISVAR 5.6 program (Ferreira 2019).

Regarding the *in vitro* multiplication of *N. mucugensis*, the results showed that the addition of BAP concentrations in the culture medium promoted a significant increase in %ReE and NS, which were formed by direct organogenesis pathway (Fig. 1). The results were described by an increasing linear regression model for both variables, with lower mean values in the control, being 11% of %ReE and 0.26 of NS, and incremented with the application of cytokinin, which led to maximum means of 70% and 3.27 for %ReE and NS, respectively (Fig. 1a-b). This attests to the importance of this plant regulator in the process of inducing sprouts, and indicates that

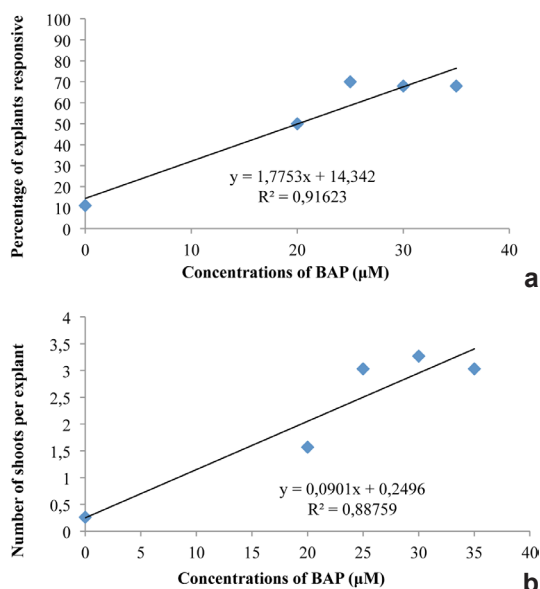


Figure 1 – a-b. Percentage of explants responsive to sprout formation (a) and number of sprouts per explant (b) as a function of different 6-benzylaminopurine (BAP) concentrations in *Neoregelia mucugensis* plants after 60 days of *in vitro* cultivation.

higher concentrations of BAP can be tested for this species.

These results are similar to those obtained by Lima *et al.* (2021) in a study with *Neoregelia mucugensis* in which, using BAP and NAA, a maximum rate of 70% for responsive explants and an average of 2.2 shoots in stem explants of plants cultivated *in vitro* for 365 days were achieved.

The maximum rate of explants that responded to sprout formation, 70%, was higher than that of 54.6% obtained by Bellintani *et al.* (2008) in stem segments of the same species, also in the presence of BAP, and the average number of sprouts was 4 to 6 per explant (on a measurement scale) in the combination of BAP and naphthalene acetic acid (NAA).

Although the results obtained by these authors for NS are more favorable, the stem segments came from 6-month-old mother plants and the variables were also evaluated at 6 months of inoculation, totaling a period of one year to obtain sprouts. In the present study, 3-month-old seedlings were used as explants and the final evaluation was performed at 60 days, totaling 5 months for the production of sprouts, which represents a reduction of time and costs in the production of seedlings.

The presence of BAP has also had a positive effect on the *in vitro* multiplication of other bromeliad species, such as the higher number of sprouts for *Sincoraea mucugensis* (Wand. & A.A. Conc.) & Wand. in a study conducted by Lima *et al.* (2020), and for *Aechmea miniata* Beer ex Baker and *Aechmea blanchetiana* (Baker) L.B.Sm in the study conducted by Garcia *et al.* (2021). For *Ananas comosus* L. Merrill, the use of BAP also generated an increase in sprouts compared to the absence of this plant regulator, in both liquid and semi-solid medium (Oliveira-Cauduro *et al.* 2016). Similar behavior was reported by Oliveira *et al.* (2007) in the *in vitro* multiplication of *Ananas lucidus* Miller, as this cytokinin promoted a higher response in the number of sprouts when compared to the control using liquid medium, while in solid medium there was no significant difference between the concentrations used.

BAP efficiency is associated with its ability to break the apical dominance of plants, which stimulates the development of lateral buds (Santa-Rosa *et al.* 2013), with control of cell division and induction and growth of sprouts (Nelson *et al.* 2015). On the other hand, when applied at high concentrations, it may cause phytotoxicity, depending on the species (Raposo *et al.* 2019).

Other bromeliad species have shown a positive effect of the liquid medium on *in vitro* cultivation, as can be seen in a study conducted by Mengarda *et al.* (2009), in which the bromeliads *Neoregelia cruenta* (R. Graham) L. B. Sm, *Tillandsia stricta* Sol., *Vriesea gigantea* Gaudich., *Vriesea guttata* Linden & André and *Vriesea incurvata* Gaudich showed higher average rate of sprout proliferation in *in vitro* multiplication in static liquid medium compared to semi-solid medium, static liquid medium with paper bridge and liquid medium under agitation. Moreover, the liquid culture medium was also used by Meneghetti *et al.* (2019) for *in vitro* multiplication of *Neoregelia johannis* (Carrière) L.B. Smith. The liquid medium can be beneficial in the *in vitro* cultivation of plants, as it increases the availability and absorption of nutrients by them (Oliveira *et al.* 2019), in addition to reducing the cost of production due to the non-use of gelling substance in the culture medium.

After obtaining the sprouts, *in vitro* rooting was performed, which did not indicate the influence of the tested periods on %RoE, NR and LLR (Tab. 1). However, it is worth noting that high rates of rooted sprouts were generated, 76 to 93%, and that NR and LLR (cm) ranged from 1.86 to 2.03 and from 2.13 to 2.38, respectively (Tab. 1). These results indicate that the 30-day period is sufficient to induce the *in vitro* formation of *N. mucugensis* roots and corroborate the efficiency of activated charcoal in this process. The same was observed by Lima *et al.* (2020) in a study with *S. mucugensis* in which it was found that the 30-day period is indicated for rooting with activated charcoal. Activated charcoal is commonly added to the culture medium in order to improve morphogenic responses because it reduces or suppresses undesirable compounds due to its adsorption capacity (Sáenz *et al.* 2010), such as plant exudates, toxic metabolites and phenolic compounds (Rondon *et al.* 2019; North *et al.* 2012). The use of activated charcoal in the culture medium to the detriment of the addition of auxins for the *in vitro* formation of roots is advantageous due to the low cost of this substance compared to the acquisition of plant regulators.

The efficiency of the rooting step is of great importance for acclimatization, and the strategies to be applied vary according to the species. For *N. mucugensis*, there are no records of previous studies for these steps, and in this study the results indicate that the survival rate after 30 days of

Table 1 – Percentage of rooted explants (%RoE), the number of roots (NR), and the length of the longest root (LLR) as a function *in vitro* rooting treatments of *Neoregelia mucugensis*.

Treatments (1g L ⁻¹ de activated charcoal)	%RoE	NR	LLR (cm)
rooting for 30 days	93 A	2,03 A	2,13 A
rooting for 45 days	76 A	1,86 A	2,38 A

Means followed by the same uppercase letters in the columns do not differ from each other by Tukey test at 5% probability level.

acclimatization was 100%, which confirms the success of micropropagation of this species, whose steps are shown in Figure 2. Likewise, for the bromeliad *S. mucugensis* the study conducted by Lima *et al.* (2020) concluded that no type of strategy to control water loss needs to be applied and that microplants of this species can be directly exposed to the environment during acclimatization without compromising their survival, whose rate was 95%. Similarly, the transfer to the *ex vitro* environment of the *Nidularium minutum* Mez microplants was performed without control of water loss in a greenhouse, and a 100% survival was recorded (Kurita *et al.* 2013).

The study makes it possible to conclude that BAP stimulation in liquid medium induces the formation of sprouts by direct organogenesis in *N. mucugensis* seedlings, the 30-day period

is sufficient to induce the *in vitro* formation of roots with activated charcoal, and microplants show a high survival rate after acclimatization; consequently, the production of seedlings by micropropagation is viable for the studied species.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that there is no data sharing of this manuscript.

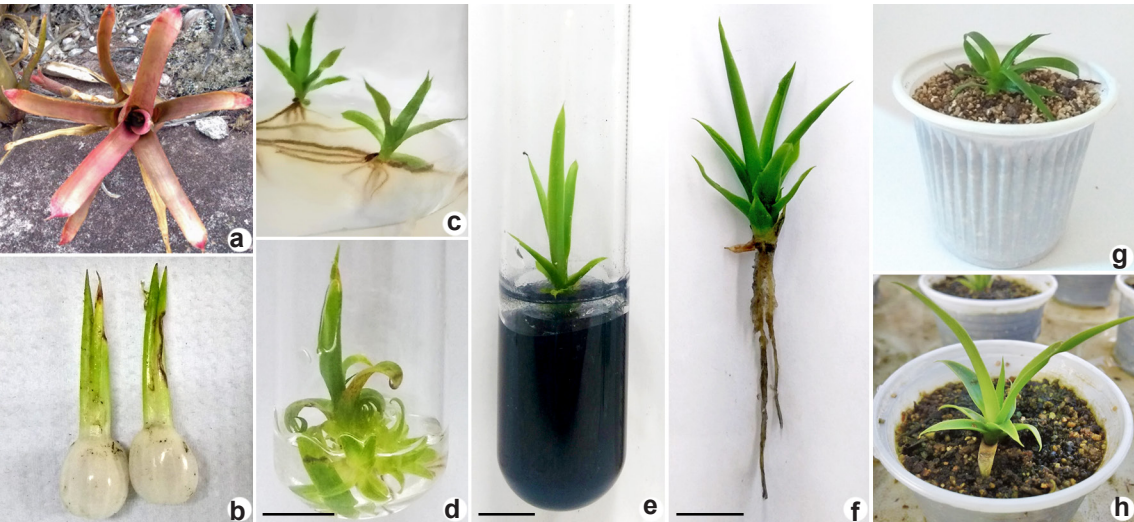


Figure 2 – a-h. Micropropagation of *Neoregelia mucugensis* – a. plant in natural environment; b. capsules containing seeds; c. plants germinated *in vitro*; d. sprouts obtained after 60 days of *in vitro* cultivation in liquid medium under the effect of 6-benzylaminopurine (BAP); e. sprout under rooting with activated charcoal; f. rooted microplant; g. plant transferred to substrate, initial time; h. plant after 30 days of acclimatization. Scale bar = 1 cm.

References

- Anacleto A, Negrelle RRB & Koehler HS (2008) Germinação de *Aechmea nudicaulis* (L.) Griseb. (Bromeliaceae) em diferentes substratos alternativos ao pó de xaxim. *Acta Scientiarum Agronomy* 30: 73-79.
- Anacleto A & Negrelle RRB (2009) Extrativismo de rametes e propagação vegetativa de *Aechmea nudicaulis* (L.) Griseb. (Bromeliaceae). *Scientia agraria* 10: 85-88.
- Bellintani MC, Lima CC, Lima-Brito A & Santana JRF (2007a) Estabelecimento *in vitro* de *Orthophytum mucugense* e *Neoregelia mucugensis*, bromélias endêmicas da Chapada Diamantina, Bahia - Brasil. *Revista Brasileira de Biociências* 5: 1101-1103.
- Bellintani MC, Lima CC, Lima-Brito A & Santana JRF (2007b) Efeito da ventilação *in vitro* na aclimatização de plantas micropropagadas de *Orthophytum mucugense* Wand e Conceição. *Revista Brasileira de Biociências* 5: 1098-1100.
- Bellintani MC, Lima-Brito A, Santana JRF & Dornelles A (2008) Resposta regenerativa *in vitro* de explantes caulinares de bromélias endêmicas da Chapada Diamantina, Bahia. *Magistra* 20: 328-337.
- CNCFlora (2012) *Neoregelia* in Lista Vermelha da flora brasileira versão 2012.2. Centro Nacional de Conservação da Flora. Available at <<http://cncflora.jbrj.gov.br/portal/pt-br/profile/Neoregelia>>. Access on 18 February 2020.
- Ferreira DF (2019) SISVAR: a computer analysis system to fixed effects split plot type designs. *Revista Brasileira de Biometria* 37: 529-535.
- Flora do Brasil 2020 (continuously updated) *Neoregelia* in Flora do Brasil 2020 (continuously updated) Jardim Botânico do Rio de Janeiro. Available at <<http://reflora.jbrj.gov.br/reflora/floradobrasil/FB16614>>. Access on 28 January 2020.
- Frank JH & Lounibos LP (2009) Insects and allies associated with bromeliads: a review. *Terrestrial Arthropod Reviews* 1: 125-153.
- Garcia FR, Nepomuceno CF, Rocha MACD, Lima-Brito A & Santana JFR (2021) Micropropagação de *Aechmea miniata* e *Aechmea blanchetiana*. *Rodriguésia* 72: 1-10.
- IBRAFLO - Instituto Brasileiro de Floricultura (2021) Available at <<https://www.ibraflor.com.br/numeros-setor>>. Access on 04 October 2021.
- Kurita FMK, Machado BDM, Texeira NB, César CDA, Nievola CC & Tamaki V (2013) Fenologia, cultivo *in vitro* e aclimatização da bromélia ameaçada de extinção *Nidularium minutum* Mez. *Biotemas* 27: 59-69.
- Lima APPS, Lima-Brito A & Santana JRF (2020) Micropropagation of Chapada Diamantina ornamental bromeliad. *Ciência Rural* 50: 1-6.
- Lima APPS, Bastos FDJO, Lima-Brito A, Fernandes GB & Santana JRFD (2021) Modulation of culture medium on the *ex situ* conservation of *Neoregelia mucugensis* Leme (Bromeliaceae). *Revista Caatinga* 34: 763-771.
- Lopes CA, Dias GDMG, Silveira FA, Rodrigues FA, Pio LAS & Pasqual M (2017) Propagação *in vitro* de pitaia vermelha. *Plant Cell Culture & Micropropagation* 13: 21-27.
- Meneghetti EC, Oliveira LS & Almeida M (2019) Multiplicação *in vitro* de *Neoregelia johannis* (Carrière) LB Smith em meio líquido sob diferentes concentrações de nutrientes. *Iheringia Série Botânica* 74: 1-8.
- Mengarda LHG, Povoas L, Debiassi C & Pescador R (2009) Estado físico do meio de cultura na propagação *in vitro* de Bromeliaceae. *Scientia Agraria* 10: 469-474.
- Murashige T & Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiology Plantarum* 15: 473-497.
- Nelson BJ, Asare PA & Arthur Junior R (2015) *In vitro* growth and multiplication of pineapple under different duration of sterilization and different concentrations of benzylaminopurine and sucrose. *Biotechnology* 14: 35-40.
- North JJ, Ndakidemi PA & Laubscher CP (2012) Effects of antioxidants, plant growth regulators and wounding on phenolic compound excretion during micropropagation of *Strelitzia reginae*. *International Journal of the Physical Sciences* 7: 638-646.
- Oliveira BC, Oliveira MEBSD & Cardoso JC (2019) Feasibility of the new method for orchid *in vitro* rooting using liquid and chemical sterilized culture medium under different sucrose concentration. *Ornamental Horticulture* 25: 263-269.
- Oliveira MKT, Bezerra Neto F, Câmara FAA, Nunes GHS & Oliveira FDA (2007) Propagação “*in vitro*” da cultura do abacaxizeiro ornamental (*Ananas lucidus* Miller). *Revista Caatinga* 20: 167-171.
- Oliveira-Cauduro Y, Lopes VR, Bona CM, Alcantra GB & Biasi LA (2016) Micropropagação de abacaxizeiro com enraizamento *in vitro* e *ex vitro*. *Plant Cell Culture & Micropropagation* 12: 53-60.
- Raposo A, Yamura RBT, Silva DA, Vasconcelos JM & Manfio CE (2019) Cultivo *in vitro* de *Piper aduncum* espécie com potencial econômico da Amazônia Sul-Occidental. *Evidência* 19: 167-184.
- Rodrigues MA, Bertolucci SKV, Alvarenga ICA, Silva ST, Carvalho AAD & Pinto JEBP (2019) Cytokinins in the *in vitro* multiplication and analysis of the volatile fraction of *Hyptis arruboides*. *Revista Ciência Agronômica* 50: 90-99.
- Rondon MJP, Sousa TI, Araujo DAA, Araujo IS & Fernandes DÁ (2019) Benefícios do carvão ativado no meio de cultura para os explantes de banana prata, nanica e terra. *Connection line-revista eletrônica do Univag* 21: 71-81.
- Sáenz L, Herrera-Herrera G, Uicab-Ballote F, Chan JL & Oropeza C (2010) Influence of form of activated

charcoal on embryogenic callus formation in coconut (*Cocos nucifera*). Plant Cell, Tissue and Organ Culture (PCTOC) 100: 301-308.
Santa-Rosa S, Souza FV, Vidal AM, Ledo CADS &

Santana JRF (2013) Micropropagação das bromélias ornamentais vulneráveis *Aechmea blanchetiana* e *Aechmea distichantha*. Horticultura Brasileira 31: 112-118.

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