Ciência Rural, Santa Maria, v.48:07, e20170817, 2018
http://dx.doi.org/10.1590/0103-8478cr20170817
ISSNe 1678-4596
BIOLOGY

Micropropagation of two species of foliage anthurium by direct organogenesis

Arlene Santisteban Campos[1] **Ana Cristina Portugal Pinto de Carvalho**[2*]
Ana Cecília Ribeiro de Castro[2] **Cândida Hermínia Campos de Magalhães Bertini**[3]

[1]Programa de Pós-graduação em Ciência do Solo (PPG Solos), Universidade Federal do Ceará (UFC), Fortaleza, CE, Brasil.
[2]Embrapa Agroindústria Tropical, CE, 60511-110, Fortaleza, CE, Brasil. E-mail:cristina.carvalho@embrapa.br. *Corresponding Author.
[3]Programa de Pós-graduação em Agronomia/Fitotecnia (PPGAF), Universidade Federal do Ceará (UFC), Fortaleza, CE, Brasil.

ABSTRACT: *The objective of the present research was to develop a protocol for micropropagation of Anthurium bonplandii and Anthurium maricense by direct organogenesis. Nodal segments, with two or three nodes, were used as explants. The cultures were kept in a growth chamber at a temperature of 25±2ºC, under a photoperiod of 16 hours and a luminosity of 30*µmol m^{-2} s^{-1}*. At 60 days, the number of regenerated buds per explant (NBE) was counted. The experiment was carried out in an entirely randomised design consisting of six treatments for six different concentrations of 6-benzylaminopurine (6-BA) added to the P2 (Pierik) medium (0.0, 1.11, 2.22, 3.33, 4.44, and 5.55µM). All the treatments were performed in four repetitions with 10 culture tubes containing one explant each. The regression analyses were adjusted to a quadratic model, with R2 = 88.7% and 62.4% for A. maricense and A. bonplandii, respectively. The regressions indicate that the addition of 6-BA to the P2 medium resulted in larger values of NBE in both the species. The ideal 6-BA concentration for micropropagation varied depending on the species, with 2.5 and 1.7 NBE determined at 6-BA concentrations of 4.70 and 3.37µM for A. maricense and A. bonplandii, respectively.*
Key words: *Anthurium bonplandii*, *Anthurium maricense*, 6-BA, tissue culture.

Micropropagação por organogênese direta de duas espécies de antúrio para folhagem

RESUMO: *O objetivo do trabalho foi desenvolver um protocolo para micropropagação de Anthurium bonplandii e A. maricense por meio da organogênese direta. Foram utilizados como explantes, segmentos nodais contendo de dois a três nós. As culturas foram mantidas em sala de crescimento com temperatura de 25±2ºC, fotoperíodo de 16 horas e intensidade luminosa de 30µmol m^{-2} s^{-1}. Aos 60 dias, avaliou-se o número de brotações regeneradas por explante (NBE). O experimento foi em um delineamento inteiramente casualizado composto por seis tratamentos referentes ao meio P2 (Pierik) adicionado de seis concentrações de 6-benzilaminopurina (6-BA): 0,0; 1,11; 2,22; 3,33; 4,44 e 5,55µM, com quatro repetições de dez tubos de ensaio, contendo um explante cada. As análises de regressão se ajustaram ao modelo quadrático com R2=88,7% e 62,4% para A. maricense e A. bonplandii, respectivamente. As regressões indicaram que adições de 6-BA ao meio P2, promoveram maior número de NBE em ambas as espécies. A concentração de 6-BA estimada como ideal para a micropropagação varia de acordo com a espécie, sendo estimado 2,5 e 1,7 NBE, quando a concentração de 6-BA for igual a 4,70 e 3,37µM para as espécies de A. maricense e A. bonplandii, respectivamente.*
Palavras-chave: *Anthurium bonplandii, Anthurium maricense, 6-BA, cultura de tecido.*

Fresh plants of tropical foliage species, such as anthurium, have great potential to meet the demand of the floriculture segment (MORAIS et al., 2017). Anthurium plants are able to reproduce both sexually (via seeds) and asexually (DESAI et al., 2015). However, micropropagation is regarded as the best option for multiplication, allowing the production of anthurium plantlets on a commercial scale and for preserving the genetic identity of clones (ATAK & ÇELIK, 2009; FARSI et al., 2012; GU et al., 2012).

Commercially, micropropagation of anthurium occurs by indirect organogenesis; however, this approach is prone to somaclonal variation (PINHEIRO et al., 2009). Direct organogenesis using axillary buds from nodal segments can be used as an alternate approach to reduce callus formation and consequently the appearance of phenotypic variations (LOPES et al., 2012).

In micropropagation, the supplementation of culture medium with growth regulators plays an important role in the development and morphogenesis of plantlets (FLORES et al., 1998). In this context, the cytokine 6-benzylaminopurine (6-BA) has been widely used and is recommended in the micropropagation of

Received 11.11.17 Approved 05.04.18 Returned by the author 06.18.18
CR-2017-0817.R1

anthurium plants (MURILLO-GÓMEZ et al., 2014), for which the composition and concentration of these regulators in the medium are the key factors (BEZERRA et al., 2014).

Studies on micropropagation have previously been conducted on several anthurium species (ATAK & ÇELIK, 2009; SILVA et al., 2015); however, the efficacy of this process in *Anthurium bonplandii* and *A. maricense* was evaluated for the first time in this study.

The *A. bonplandii and A. maricense* plantlets grown *in vitro* from seeds obtained from the Embrapa Agroindústria Tropical germplasm bank were used in the study. These plantlets were handled in a sterile environment of a laminar flow chamber; they were entirely defoliated and cut into nodal segments (microcuttings) of approximately 1.0cm length, with two or three nodes in each segment.

The microcuttings were inoculated in a horizontal position, in culture tubes containing 10 mL P2 (Pierik) culture medium (PIERIK, 1976), supplemented with 20.0gL^{-1} sucrose, and solidified with Gelrite® (1.8gL^{-1}); the pH of the medium was adjusted to 5.8 and it was autoclaved at 121ºC and 1 atm for 15 minutes. Culture tubes containing the explants were sealed and incubated in a growth chamber at 25±2ºC, under 16-h photoperiod, and 30µmol m^{-2} s^{-1} luminosity for 60 days to allow the regeneration of buds.

The experiment was performed based on an entirely randomized design, with four repetitions comprising 10 culture tubes containing one explant each, totalling 40 explants per treatment. The explants were subjected to six treatments, as follows: P2 without growth regulator (T1) or P2 supplemented with 6-benzylaminopurine (6-BA) at the concentrations of 1.11µM (T2), 2.22µM (T3), 3.33µM (T4), 4.44µM (T5), and 5.55µM (T6).

The evaluation was carried out 60 days after the inoculation of explants and consisted of counting the number of buds regenerated per explant (NBE). Results were subjected to regression analyses at a significance level of 5%. In both species, data fitted a quadratic model (Figure 1).

Based on the analyses, the addition of 6-BA to the culture medium at concentrations between 3.33 and 5.55µM resulted in the highest NBE in *A.*

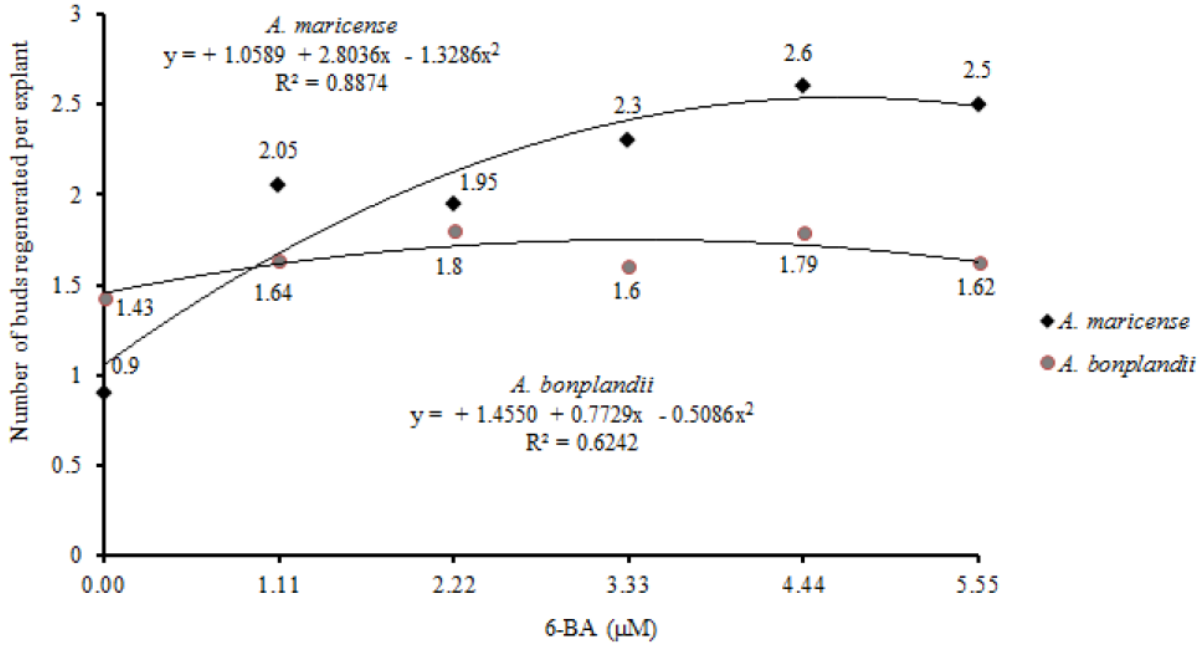

Figure 1 - Number of buds regenerated per explant after 60 days of *in vitro* cultivation, in the case of *Anthurium maricense* and *A. bonplandii*, as a function of 6-benzylaminopurine (6-BA) concentration added to the P2 culture medium. Source: Arlene Santisteban Campos.

maricense, with an NBE of 2.5 at 4.70µM 6-BA. In contrast, in *A. bonplandii*, the NBE was highest between T3 and T5, with an estimated NBE of 1.7 at 3.37µM 6-BA. The NBE values for the evaluated species in the treatments without cytokine (T1) were 1.06 for *A. maricense* and 1.46 for *A. bonplandii*, both of which were below the estimated maximum.

Such discrepancy in the NBE values between the evaluated species might be associated with the different concentrations of endogenous cytokines. According to PEREIRA et al. (2015), endogenous determinism is one of the factors that may affect *in vitro* multiplication. NOGUEIRA et al. (2007) stated that supplementation of the culture medium with cytokine may lead to an excess of this phytoregulator in the medium because of the cytokine already present in the explant (endogenous), eventually leading to reduced bud formation. Genotypic variation of the response to the distinct 6-BA concentrations between the two anthurium species evaluated in this study might be the result of different concentrations of the endogenous cytokines in the two species. This would explain both the higher value of NBE in *A. bonplandii* in T1 (1.46) and the lower concentration of cytokine (3.37µM) required to reach the maximum NBE value estimated for this species.

Therefore, it can be inferred that the ideal concentration of 6-BA for micropropagation varies with the species; an increase in the 6-BA concentration induces the regeneration of a larger number of buds per explants in *A. maricense* and *A. bonplandii*. The use of 6-BA at concentrations of 4.70 and 3.37µM is recommended for direct organogenesis in *A. maricense* and *A. bonplandii*, respectively.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

ACKNOWLEDGEMENTS

The author would like to thank Embrapa Agroindústria Tropical for the scholarships and the financial support.

REFERENCES

ATAK, Ç.; ÇELIK, Ö. Micropropagation of Anthurium andraeanum from leaf explants. **Pakistan Journal of Botany**, v. 41, p. 1155-1161, 2009. Available from: <http://www.pakbs.org/pjbot/PDFs/41(3)/PJB41(3)1155.pdf>. Acessed: Oct. 25, 2017.

BEZERRA, R.M.F. et al. Efeito de 6-benzilaminopurina sobre a propagação in vitro de Mimosa caesalpiniifolia Benth (Fabaceae). **Revista Árvore**, v.38, p.771-778, 2014. Available from: <http://www.redalyc.org/articulo.oa?id=48832704001>. Acessed: Oct. 25, 2017.

DESAI, C. et al. Micropropagation of Anthurium andraeanum -an important tool in floriculture. **Journal of Pharmacognosy and Phytochemistry**, v.4, p.112-117, 2015. Available from: <http://www.phytojournal.com/vol4Issue3/Issue_Sep_2015/4-3-5.1.pdf>. Acessed: Oct. 25, 2017.

FARSI, M. et al. Micropropagation of ***Anthurium andreanum*** cv. Terra. **African Journal of Biotechnology**, v.11, p.13162-13166, 2012. Available from: <http://www.academicjournals.org/AJB>. Acessed: Oct. 25, 2017. doi: 10.5897/AJB12.893.

FLORES, R. et al. Regeneração *in vitro* de espinheira-santa (***Maytenus ilicifolia*** Mart.). **Revista Brasileira de Agrociência**, v.4, p.201-205, 1998. Available from: <https://periodicos.ufpel.edu.br/ojs2/index.php/CAST/article/view/224>. Acessed: Mai. 08, 2017. doi: 10.18539/cast.v4i3.224.

GU, A. et al. Regeneration of ***Anthurium andraeanum*** from leaf explants and evaluation of microcutting rooting and growth under different light qualities. **Hortscience**, v.47, p.88-92, 2012. Available from: <https://www.researchgate.net/publication/279654032>. Acessed: Mai. 08, 2017.

LOPES, L.C. et al. Cultura de embrião e indução de brotos in vitro para micropropagação do pinhão-manso. **Pesquisa Agropecuária Brasileira**, v.47, p.900-905, 2012. Available from: <http://www.scielo.br/scielo.php?pid=S0100-204X2012000700004&script=sci_abstract&tlng=pt>. Acessed: Mai. 08, 2017. doi: 10.1590/S0100-204X2012000700004.

MORAIS, E. B. et al. Evaluation of potential use of native *anthurium* foliage. **Ornamental Horticulture**, v. 23, n. 1, p. 07-14, 2017. Available from: <https://ornamentalhorticulture.emnuvens.com.br/rbho/article/viewFile/949/691>. Acessed: Apr. 03, 2018. doi: 10.14295/oh.v23i1.949.

MURILLO-GÓMEZ, P.A. et al. Micropropagation of the native species *Anthurium antioquiense* Engl. for conservation purposes. **Agronomía Colombiana**, v.32, p.334-340, 2014. Available from: <https://revistas.unal.edu.co/index.php/agrocol/article/view/46809/52445>. Acessed: Apr. 03, 2018. doi: 10.15446/agron.colomb.v32n3.46809.

NOGUEIRA, R. C. et al. Indução de calos em explantes foliares de murici-pequeno (*Byrsonima intermedia* A. Juss.). **Ciência e Agrotecnologia**, v.31, n.2, p.366- 370, mar./abr.2007. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413-70542007000200015>. Acessed: Apr. 03, 2018. doi.org/10.1590/S1413-70542007000200015.

PEREIRA, M.O. et al. Multiplicação in vitro de ipê-amarelo (***Handroanthus chrysotrichus***). **Nativa**, v.3, p.59-63, 2015. Available from: <http:// periodicoscientificos.ufmt.br/ojs/index.

php/nativa/article/download/1879/pdf>. Acessed: Mai. 08, 2017. doi: 10.14583/2318-7670.v03n01a10.

PIERIK, R.L.M. ***Anthurium andraeanum*** plantlets produced from callus tissues cultivated *in vitro*. **Physiologia Plantarum**, v.37, p.80-82, 1976. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1111/j.1399-3054.1976.tb01876.x>. Acessed: Mai. 08, 2017. doi.org/10.1111/j.1399-3054.1976.tb01876.x.

PINHEIRO, M.V.M. et al. Micropropagação de antúrio 'IAC Eidibel' por meio da indução ao estiolamento e regeneração de plantas**. Revista Brasileira de Horticultura Ornamental**, v.15, p.133-142, 2009. Available from: <https://ornamentalhorticulture. emnuvens.com.br/rbho/article/viewFile/494/362>. Acessed: Mai. 08, 2017. doi: 10.14295/rbho.v15i2.494.

SILVA, J.A.T. et al. Genetic transformation and molecular research in ***Anthurium***: progress and prospects. **Plant Cell Tissue and Organ Culture**, v.123, p.205- 219, 2015. Available from: <https://link.springer.com/content/pdf/10.1007%2Fs11240-015-0832-1.pdf> Acessed: Mai. 08, 2017. doi: 10.1007/s11240-015-0832-1.