Ciência Rural, Santa Maria, v.47: 10, e20161028, 2017 http://dx.doi.org/10.1590/0103-8478cr20161028
ISSNe 1678-4596
BIOLOGY

In vitro morphogenic response from zygotic embryos of *Genipa Americana*

Annie Carolina Araújo de Oliveira[1*] Caroline de Araújo Machado[1] Leila Albuquerque Resende de Oliveira[1] Ana Veruska Cruz da Silva[2] Ana da Silva Lédo[2]

[1]Programa de Pós-graduação em Agricultura e Biodiversidade (PPGAGRI), Universidade Federal de Sergipe (UFS), Av. Marechal Rondon s/n, Jardim Rosa Elze, 49100-000, São Cristóvão. SE, Brasil. E-mail: anniedeoliveira@hotmail.com. *Corresponding author.
[2]Empresa Brasileira de Pesquisa Agropecuária (Embrapa Tabuleiros Costeiros), Aracaju, SE, Brasil.

ABSTRACT: *The aim of this study was to evaluate the in vitro morphogenic potential of genipap (Genipa americana L.) zygotic embryos. Seeds obtained from ripe fruits had their zygotic embryos excised and inoculated in MS medium with 4.44µM of 6-benzylaminopurine (BAP) and supplemented with 0.0; 1.07; 2.14 and 3.21µM of naphthalene acetic acid (NAA). The potential of explants regeneration and the shoot length and number of leaves in plantlets were evaluated. The in vitro regeneration of genipap is possible from the conversion of zygotic embryos in a MS medium with 4.44µM BAP supplemented with 3.21µM NAA.*
Key words: *plant tissue culture, plant growth regulators, callus.*

Resposta morfogênica *in vitro* de embriões zigóticos de *Genipa americana*

RESUMO: *O objetivo do trabalho foi avaliar o potencial morfogênico in vitro de embriões zigóticos de jenipapeiro (Genipa americana L.). Sementes obtidas de frutos tiveram seus embriões zigóticos excisados e inoculados em meio MS com 4,44µM de 6-benzilaminopurina (BAP) suplementado com 0,0; 1,07; 2,14 e 3,21µM de ácido naftaleno acético (ANA). O potencial de regeneração dos explantes e o comprimento da parte aérea e o número de folhas nas plântulas formadas foi avaliado. Observou-se que é possível a regeneração in vitro de jenipapeiro a partir da conversão de embriões zigóticos em meio MS com 4,44µM de BAP, suplementado com 3,21µM de ANA.*
Palavras-chave: *cultura de tecidos vegetais, reguladores de crescimento vegetal, calos.*

Genipa americana L. (*Rubiaceae*), popularly known as genipap has an economic importance, both for its forestry and environmental potential (DURÃES et al., 2014). Predominantly exploited in an extractive way, this species has undergone accelerated genetic erosion. Conventional propagation occurs through seeds, but it is restricted due to non-uniformity of germination and rapidly loss of seed viability, since they are classified as intermediate (MAGISTRALI et al., 2013).

Application of tissue culture techniques works as a tool to overcome propagation problems of species such as genipap. These techniques use not only enables the maximization of production of seedlings with genetic fidelity, but also ensures the conservation of germplasm (REED et al., 2011). However, *in vitro* propagation is only possible when considering the regenerative potential that each genotype has, giving the morphogenic responses that different types of explants, medium composition and culture conditions may present (ELHITI & STASOLLA, 2011). As general rule, younger tissues have higher cell competence. Therefore, the cultivation of zygotic embryos has been used for the observation of processes which control *in vitro* morphogenesis (HU & FERREIRA, 1998). The aim of this study was to evaluate the effect of morphogenic potential of genipap zygotic embryos submitted to different concentrations of NAA in the presence of BAP.

Ripe fruits were collected from natural populations of Nucleo Bandeirante, Distrito Federal, Brazil. Extracted seeds were kept at room temperature for 24 hours, and disinfected under laminar flow chamber by immersion in 70% alcohol for 1min, followed by 2.5%

Received 11.17.16 Approved 06.25.17 Returned by the author 08.08.17
CR-2016-1028.R1

(v/v) solution of sodium hypochlorite plus Tween-20® for 20min and three times washed in distilled water. After that, the zygotic embryos were excised and inoculated in test tubes containing 20mL MS (MURASHIGE & SKOOG, 1962) medium with 30g L^{-1} sucrose, 4g L^{-1} de Phytagel®, 4.44µM of BAP and supplemented with 0.0; 1.07; 2.14 and 3.21µM of NAA. The pH of the culture medium was adjusted to 5.8, and autoclaved at 120±1ºC for 20min. Cultures were kept in darkness for 15 days, and then transferred to a growth room, where they remained for 45 days at 25±2ºC, under a photoperiod of 16/8 hours.

The experimental design was completely randomized with four treatments, and which treatment composed by twenty replications with one explant per tube. After 30 days, regeneration (%) was evaluated. Shoot length and number of leaves were scored in plantlets regenerated at 60 days. For the effect of NAA concentration, regression equations were estimated, using the SISVAR statistical software (FERREIRA, 2011).

Regeneration percentage showed a positive linear behavior (Figure 1A). In the absence of NAA, regeneration percentage was 40% with increase up to 70% in response of the presence of this regulator. The combination between 4.44µM BAP and NAA may promote an initial stimulus for the development of embryos. Similar results were reported by LÉDO et al. (2002) and LEITE et al. (2014) in cultivations with *Euterpe oleracea* Mart. and *Orbignya oleifera* Burret, respectively. Also, cytokinins are known for regulate several cellular processes, including the expansion of cotyledons into dicotyledons (TAIZ & ZEIGER, 2013). Cotyledons act as nutritional reserves which will maintain the embryo until its development into plantlet, which becomes autotrophic (LÉDO et al., 2008).

Shoot length presented positive quadratic behavior in the presence of NAA (Figure 1B). Reduction in shoot length was observed with the increase of NAA concentration, resulting in higher growth (30.83mm) in the absence of this phytohormone. Auxins are important for maintenance the apical dominance and higher endogenous concentrations may result in inhibition of shoot emission (TAIZ & ZEIGER, 2013). This result agrees with REZENDE et al. (2011), who reported higher shoot growth (24.40mm) in *Coffea arabica* L. somatic embryos in the absence of plant growth regulators. The same positive quadratic behavior was observed for number of leaves with minimum point of 3.16µM NAA (Figure 1C). Increasing NAA concentration promoted a reduction in the number of leaves. Otherwise, rooting was observed in 10% of plantlets in MS medium with all NAA concentrations.

Direct organogenesis was not observed but the progressive increase in concentrations of NAA with 4.44µM BAP resulted in a considerable number of plantlets with compact callus formation at the explants base. Callus formation is associated with the balance between auxin and cytokinin (TAIZ & ZEIGER, 2013). In recent studies on genipap, ALMEIDA et al. (2015) have reported callus formation in nodal and leaf segments inoculated in MS medium with 18.10 and 36.20µM 2,4-D (dichlorophenoxyacetic acid) and in the presence of 7.86µM BAP for leaf explants. Although, it is not the preferred route to plant regeneration, callus formation has been the basis for studies related to somatic embryogenesis and production of *in vitro* secondary metabolites (NOGUEIRA et al., 2007).

It is possible to obtain *in vitro* genipap regeneration from the conversion of zygotic embryos on MS medium supplemented with 4.44µM

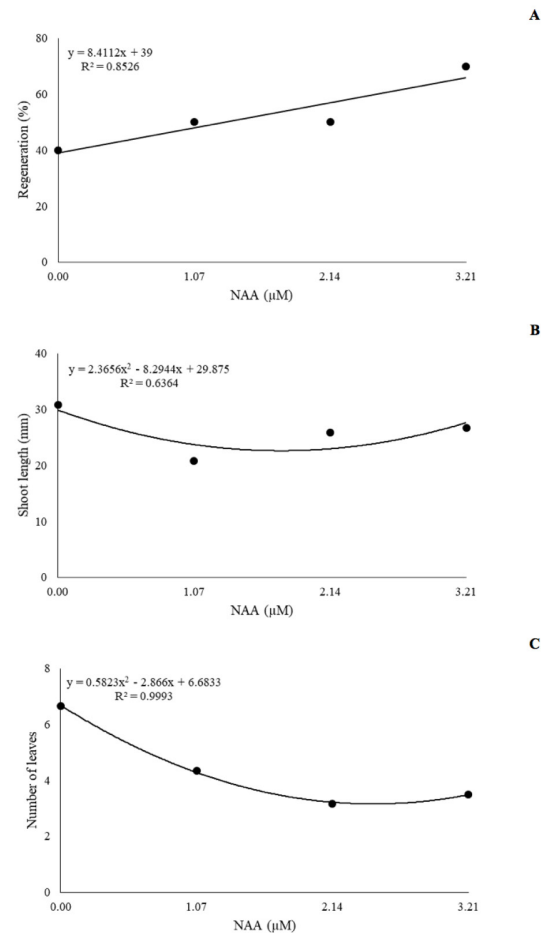

Figure 1 - Regeneration (%) of zygotic embryos (A), shoot length (B) and number of leaves (C) of regenerated plantlets in the presence of 4.44µM BAP combined with different NAA concentrations.

BAP and NAA. In additional, more regenerative response is possible in the presence of 3.21µM NAA. Considering the different responses obtained in this study, further researches should be carried out aiming to improve the morphogenetic expression of genipap zygotic embryos.

ACKNOWLEDGMENTS

Universidade Federal de Sergipe (PPGAGRI/UFS), Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and fellowship.

REFERENCES

ALMEIDA, C.S. et al. Morphogenetic responses of jenipapo in different *in vitro* culture conditions. **Revista Caatinga**, v.28, n.1, p.58-64, 2015. Available from: <http://periodicos.ufersa.edu.br/revistas/index.php/sistema/article/view/3069>. Accessed: Sept. 14, 2016.

DURÃES, M.C.O. et al. Floristic survey of arboreal stratum of three fragments of riparian forest as a subsidy to 'Rio Cedro' vegetation recomposition in Montes Claros – MG. **Ciência Florestal**, v.24, n.1, p.47-58, 2014. Available from: <http://cascavel.ufsm.br/revistas/ojs-2.2.2/index.php/cienciaflorestal/article/view/13322>. Accessed: July 14, 2016. doi: 10.5902/1980509813322.

ELHITI, M.; STASOLLA, C. The use of zygotic embryos as explants for *in vitro* propagation: an overview. In: THORPE, T.A.; YEUNG, E.C. **Plant embryo culture**: methods and protocols. New York: Humana, 2011. Cap.17, p.229-255.

FERREIRA, D.F. SISVAR: a computer statistical analysis system. **Ciência e Agrotecnologia**, v.35, n.6, p.1039-1042, 2011. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413-70542011000600001. Accessed: Oct. 04, 2016. doi: 10.1590/S1413-70542011000600001.

HU, C.Y.; FERREIRA, A.G. Cultura de embriões. In: TORRES, A.C. et al. **Cultura de tecidos e transformação genética de plantas**. Brasília: Embrapa – SPI/Embrapa – CNPH, 1998. p.371-393.

LÉDO, A.S. et al. Germinação *in vitro* de embriões zigóticos e sementes de nim indiano (*Azadirachta indica* A. Juss.). **Revista Brasileira de Plantas Medicinais**, v.10, n.3, p.1-5, 2008. Available from: <http://www.sbpmed.org.br/download/issn_08_3/artigo1_v10n3.pdf>. Accessed: July 22, 2015.

LÉDO, A.S. et al. Somatic embryogenesis from zygotic embryos of *Euterpe oleracea* Mart. **Revista Brasileira de Fruticultura**, v.24, n.3, p.601-603, 2002. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-29452002000300004>. Accessed: Sept. 08, 2016. doi: 10.1590/S0100-29452002000300004.

LEITE, M.S. et al. In vitro germination of babassu: influence of growth regulators in zygotic embryos. **Acta Scientiarum**, v.36, n.4, p.449-456, 2014. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1807-86212014000400009>. Accessed: Sept. 10, 2016. doi: 10.4025/actasciagron.v36i4.15484.

MAGISTRALI, P.R. et al. Physiological behavior of *Genipa americana* L. seeds regarding the capacity for desiccation and storage tolerance. **Journal of Seed Science**, v.35, n.4, p.495-500, 2013. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S2317-15372013000400011>. Accessed: Aug. 12, 2016. doi: 10.1590/S2317-15372013000400011.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum**, v.15, n.3, p.473-479, 1962. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1399-3054.1962.tb08052.x/abstract>. Accessed: Oct. 24, 2016. doi: 10.1111/j.1399-3054.1962.tb08052.x.

NOGUEIRA, R.C. et al. Calli induction from leaf explants of murici-pequeno (*Byrsonima intermedia* A. Juss.). **Ciência e Agrotecnologia**, v.31, n.2, p.366-370, 2007. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413-70542007000200015>. Accessed: Sept. 21, 2016. doi: 10.1590/S1413-70542007000200015.

REED, B.M. et al. Biodiversity conservation and conservation biotechnology tools. ***In vitro* Cellular & Developmental Biology – Plant**, v.47, n.1, p.1-4, 2011. Available from: <http://link.springer.com/article/10.1007%2Fs11627-010-9337-0>. Accessed: Aug. 20, 2016. doi: 10.1007/s11627-010-9337-0.

REZENDE, J.C. et al. Effects of auxins and cytokinins on the development of Coffea arabica L. somatic embryos. **Plant Cell Culture & Micropropagation**, v.7, n.1, p.1-8, 2011. Available from: <http://www.abctp.org.br/revista/v7n1.pdf>. Accessed: Aug. 01, 2016.

TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. Porto Alegre: Artmed, 2013. 5v. 918p.