

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

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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 4.44 μM of 6-benzylaminopurine (BAP) and supplemented with 0.0; 1.07; 2.14 and 3.21 μM 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 4.44 μM BAP supplemented with 3.21 μM NAA.*

Key words: *plant tissue culture, plant growth regulators, callus.*

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

RESUMO: *O objetivo do trabalho foi avaliar o potencial morfológico 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 4,44 μM de 6-benzilaminopurina (BAP) suplementado com 0,0; 1,07; 2,14 e 3,21 μM 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 4,44 μM de BAP, suplementado com 3,21 μM 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%

(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 Phytigel[®], 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±1°C 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±2°C, 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

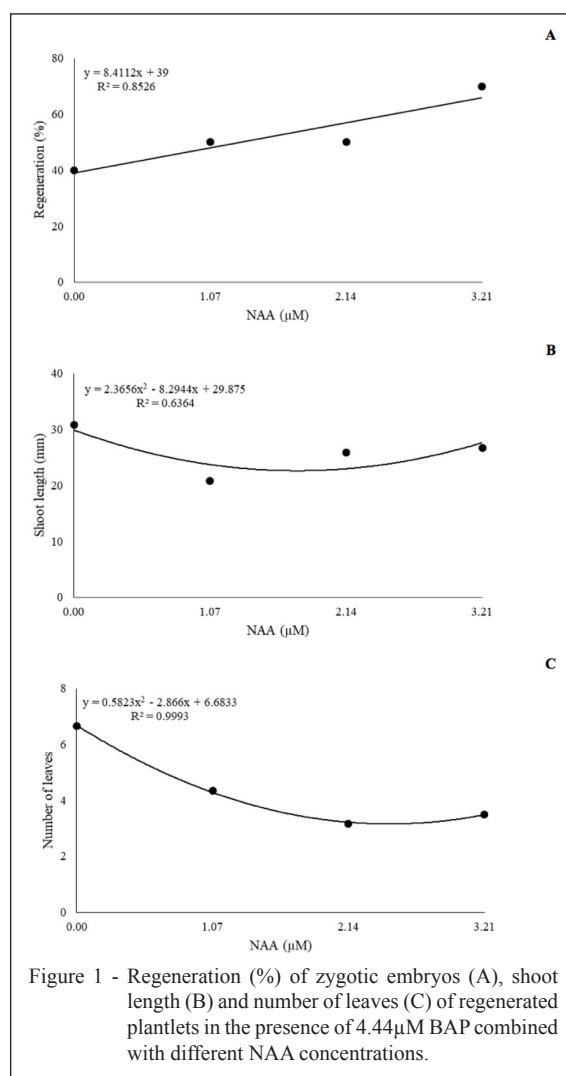


Figure 1 - Regeneration (%) of zygotic embryos (A), shoot length (B) and number of leaves (C) of regenerated plantlets in the presence of 4.44μM BAP combined with different NAA concentrations.

BAP and NAA. In addition, 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.

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