Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v41i1.46358

Embryogenic potential of the callus of gabirobeira, *Campomanesia adamantium* (Cambess) O. Berg

Marieli Rossato[1*], Pedro Vitor Schumacher[1], Antônio Paulino da Costa Netto[2], Vanessa Cristina Stein[3], Edésio Fialho dos Reis[2], Marlúcia Souza Pádua Vilela[4] and Luciano Vilela Paiva[4]

[1]Programa de Pós-Graduação em Fisiologia Vegetal, Universidade Federal de Lavras, Campus Universitário, Cx. Postal 3037, 37200-000, Lavras, Minas Gerais, Brazil. [2]Universidade Federal de Jataí, Jataí, Goiás, Brazil. [3]Universidade de São João Del-Rei, Divinópolis, Minas Gerais, Brazil. [4]Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. *Author for correspondence. E-mail: rossatomarieli@gmail.com

ABSTRACT. *Campomanesia adamantium* is a native plant species of Brazilian Cerrado with diverse economic potential and great medicinal importance. Its sexual propagation is impaired by the recalcitrance of its seeds, which prevents effective and profitable propagation. With the purpose of establishing commercial crops and minimizing the extractive use of vegetal resources, the aim of the present study was to induce embryogenic calli in nodal segments of gabirobeira, and to determine and characterize their embryogenic phase through the establishment of a growth curve based on cellular characteristics. Calli were induced using nodal segments inoculated in WPM culture medium without the addition of hormones (control) and with different concentrations of 2,4-D, IAA, IBA, NAA or picloram. Cytochemical and SEM analyses revealed cellular characteristics of the formation of meristematic centers that indicated 4.14 µM of picloram to be the best treatment for induction of embryogenic calli, and demonstrating their embryogenic potential. The treatment was used to establish a callus growth curve, from which it was inferred that calli should be transferred to new culture media on the 28th day to maintain cell viability.

Keywords: Somatic embryogenesis; Growth regulator; Callus.

Received on August 1, 2019.
Accepted on October 2, 2019.

Introduction

Campomanesia adamantium (Cambess) O. Berg, of the family *Myrtaceae*, is popularly known as gabirobeira or the gabiroba tree, and is of diverse economic importance. Its fruit can be consumed *in natura* or in the form of juice, ice cream, popsicles, candy, or liqueur (Bardiviesso, Maruyama, Reis, Modesto, & Rezende, 2011). In addition, studies have demonstrated the species to have medicinal properties including anti-inflammatory, antihyperalgesic and antidepressant actions (Souza et al., 2014), antimicrobial (Sá et al., 2018), as well as antidiarrheal (Lescano et al., 2016) and antileukemic activity, thus demonstrating therapeutic potential for diseases associated with the proliferation of tumor cells (Campos et al., 2017).

Gabirobeira is propagated sexually, however, the recalcitrance of its seeds hampers this process due to deterioration over time, resulting in a root protrusion rate of less than 50% (Dresch, Scalon, Masetto, & Mussury, 2014). Knowledge about the asexual propagation of this species is still incipient, but holds potential as a viable alternative for its entry into the consumer market and the preservation of the species it its natural state (Dresch, Masetto, Jeromini, & Scalon, 2017; Araújo & Souza, 2018; Oliveira, Silva, Costa Netto, Silva, & Silva, 2011).

Tissue culture techniques have been widely used for the propagation of recalcitrant and fruit-bearing species of the *Cerrado* (Brazilian tropical savanna) (Damiani, Silva, Goelzer, & Déo, 2016; Ribeiro, Pasqual, Villa, Pio, & Hilhorst, 2009; Sousa et al., 2017). Among the tissue culture techniques used for obtaining plants, organogenesis and somatic embryogenesis, both by the indirect process of callus formation, have proven particularly interesting for obtaining plants *in vitro* on a large scale (Smith, 2012).

During the indirect process of callus formation, cells undergo dedifferentiation, and later on, successive cell divisions, which form cell agglomerates (Ikeuchi, Sugimoto, & Iwase, 2013). Callus induction has been used in clonal propagation to study the culture conditions required by explants for growth (Lin & Zhang, 2005) and to understand cell development, using products that are generated by primary and secondary

metabolism (Shahzad et al., 2017). During this phase, cytological monitoring is necessary to identify cells that have embryogenic characteristics and to document abnormal formation of somatic embryos during plant regeneration (Gomes, Bartos, & Scherwinski-Pereira, 2017; Oliveira et al., 2017). Such cytological analyses have been used repeatedly for some species of the Myrtaceae family with the aim of describing the sequences of morphogenetic events involved in the development and formation of somatic embryos (Correia & Canhoto, 2010; Moura et al., 2017; Pescador, Kerbauy, Viviani, & Kraus, 2008; Pinto, Silva, Neves, Araújo, & Santos, 2010).

Information obtained from growth curves can also be valuable, particularly for determining the phase of greatest calli development, and thus differentiating its period of greatest embryogenic potential, and the phase during which it should be sub cultured so as to maintain embryogenic potential (Carvalho et al., 2013; Stein et al., 2010).

Campomanesia adamantium multiplication and rooting has been reported in the literature (Rossato et al., 2015). However, propagation by organogenesis or somatic embryogenesis in gabirobeira can facilitate asexual reproduction, multiplication of plants on a large-scale and maintenance of genetic material in germplasm banks. Therefore, the aim of the present study was to induce embryogenic calli in nodal segments of gabirobeira, and to determine and characterize the embryogenic phase using a callus growth curve based on cellular characteristics.

Material and methods

Plant material

Explants were acquired from *in vitro* germination of gabiroba seeds inoculated in standard culture medium consisting of Woody Plant Medium salts (WPM) (Lloyd & McCown, 1980) with the addition of 30 g L^{-1} of sucrose and 7 g L^{-1} of agar, at pH 5.8. Seedlings were kept in a growth room at a temperature of 25 ± 2°C with a 16 hours photoperiod and photon irradiance of 43 µmol $m^{-2}s^{-1}$, and were sub cultured every 40 days.

Callogenesis induction

Seedlings at 40-days post germination were used for callus induction. The explants used were nodal segments of approximately 1 cm in length, which were inoculated in an induction medium consisting of WPM salts with the addition of 30 g L^{-1} of sucrose, 7 g L^{-1} of agar, and 0.9 mM of polyvinylpyrrolidone (PVP). The pH was adjusted to 5.8 prior to autoclaving at 120ºC for 20 minutes. Different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D: 4.52, 9.04 and 18.08 µM), indole-3-acetic acid (IAA: 5.7, 11.4 and 22.8 µM), indole-3-butyric acid (IBA: 4.92, 9.84 and 19.68 µM), naphthaleneacetic acid (NAA: 5.37, 10.74 and 21.48 µM), or 4-amino-3,5,6-trichloropicolinic acid (picloram: 4.14, 8.28 and 16.56 µM) were added to the culture medium, while a control was established without the addition of growth regulators. The treatments were maintained in the absence of light.

Evaluations were made every 30 days until reaching 90 days of inoculation (subcultures were not performed), at which time the cell mass percentage was evaluated according to the following scores: 1 = absence of callus, 2 = 25% of explant covered by callus, 3 = 50% of explant covered by callus, 4 = 75% of explant covered by callus, and 5 = 100% of explant covered by callus.

The results were submitted to analysis of variance by the Kruskal-Wallis non-parametric test, which was also used to compare the means at 5% probability (Kruskal & Wallis, 1952).

Growth curve

A growth curve was established based on the treatment with the highest average callus percentage in the shortest amount of time. Explants of plants sub cultured every 40 days under the same initial conditions were used by the fourth subculture for callus induction in the growth curve. Calli were obtained through treatment with induction medium supplemented with 4.14 µM of picloram. After inoculation, the explants were kept in a growth room at a temperature of 25 ± 2°C and without light.

Twenty-five replications were used to determine the fresh weight of calli, which were weighed every seven days until stabilization of the growth curve. Fresh matter gain as a function of each phase was defined by the ratio between the maximum weight reached by the callus and the weight of the callus in each of the phases.

The results were submitted to ANOVA, using the F test, and to regression analysis, also using the F test, both at 5% probability.

Cytochemical analysis and scanning electron microscopy (SEM)

Cytochemical analysis and SEM were performed with calli that were induced parallel to those used for determining the growth curve so as not to alter the number of explants used for determining weight. The calli were collected at 14-day intervals until stabilization of the curve.

The samples for cytochemical analysis were fixed according to Pádua, Paiva, Labory, Alves and Stein (2013), sectioned at a thickness of 5 µm using a rotary microtome, and stained with 0.05% toluidine blue and 2.5% Lugol's solution. Slides were viewed using a Zeiss photonic microscope connected to a digital camera. For SEM, samples were fixed according to Pádua et al. (2013), adapted the Bozzola and Russel (1998) protocol, and analyzed with a Zeiss EM 109 transmission electron microscope.

Results

Callogenesis induction

The presence of calli was observed in all treatments containing 2,4-D, IAA, IBA, NAA and picloram (Figure 1). The use of 4.14 µM picloran showed the best results for callus induction from nodal segments since it was possible to obtain calluses on 100% of the explant at 30 days. Doses of 8.28 and 16.56 µM picloram had similar results, but were only able to obtain 100% callus formation after 60 days, at the point oxidation began to be observed.

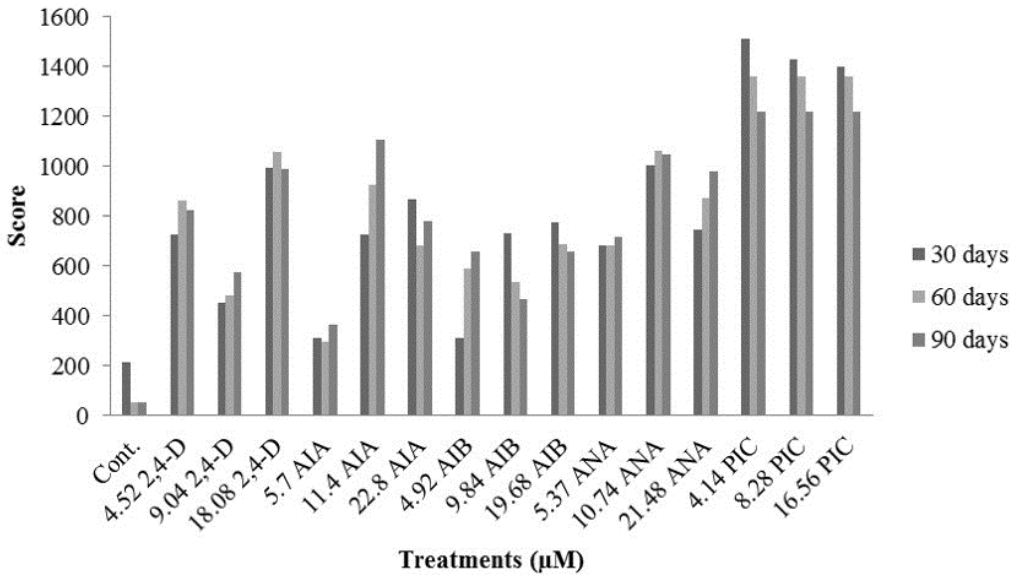

Figure 1. Evaluation of callus induction on nodal segments of gabiroba in WPM culture medium with different concentrations of 2,4-D, IAA, IBA, NAA, and Picloram at 30, 60, and 90 days. The percentage scores attributed to the calli were compared by the Kruskal-Wallis non-parametric test.

Based on these results, and according to the objective of the study, the dosage of 4.14 µM picloram was chosen for determining the growth curve and for cytochemical and SEM analyses.

Growth curve

The growth curve of calli on nodal segments of gabiroba exhibited a sigmoid pattern, with a gain of fresh matter during the period of cultivation. Five phases of growth were observed during the analyzed period (56 days from explant inoculation) due to the high callus growth rate (Figure 2).

The lag phase occurred up the 7th day of culture, resulting in a weight gain of 9% of the total. The exponential growth phase occurred between the 7th and 28th day of cultivation, and resulted in a weight gain of 66%. The linear growth phase, between the 28th and 35th day, resulted in a 17% gain of fresh mass. The deceleration period began on the 35th day and lasted until the 42nd day, resulting in 8% growth. Callus

growth reached the stationary phase on the 42nd day post-inoculation, after which a reduction of 5% of the fresh weight was observed, indicating the beginning of the decrease phase.

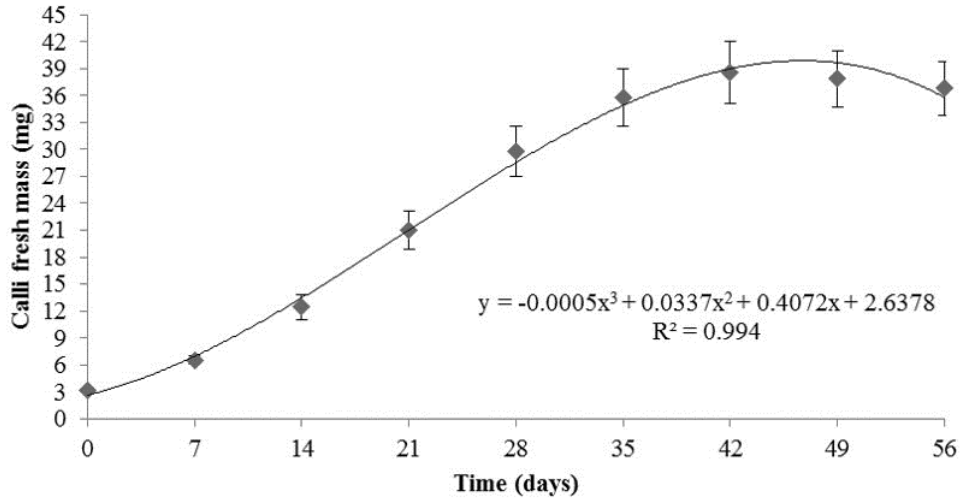

Figure 2. Growth curve of calli on nodal segments of gabiroba inoculated in WPM culture medium supplemented with 4.14 µM of picloram. I – lag phase (0-7 days); II – exponential phase (7-28 days); III – linear growth phase (28-35 days); IV – deceleration phase (35-42 days); V – stationary phase (after 42 days).

Cytochemical analysis and SEM

Cytochemical and SEM analyses revealed that calli induced with 4.14 µM picloram in nodal segments of gabirobeira possessed isodiametric cells at 14 and 28 days after inoculation (Figure 3A and B; Figure 4A and C), which corresponds to the exponential growth phase according to the growth curve.

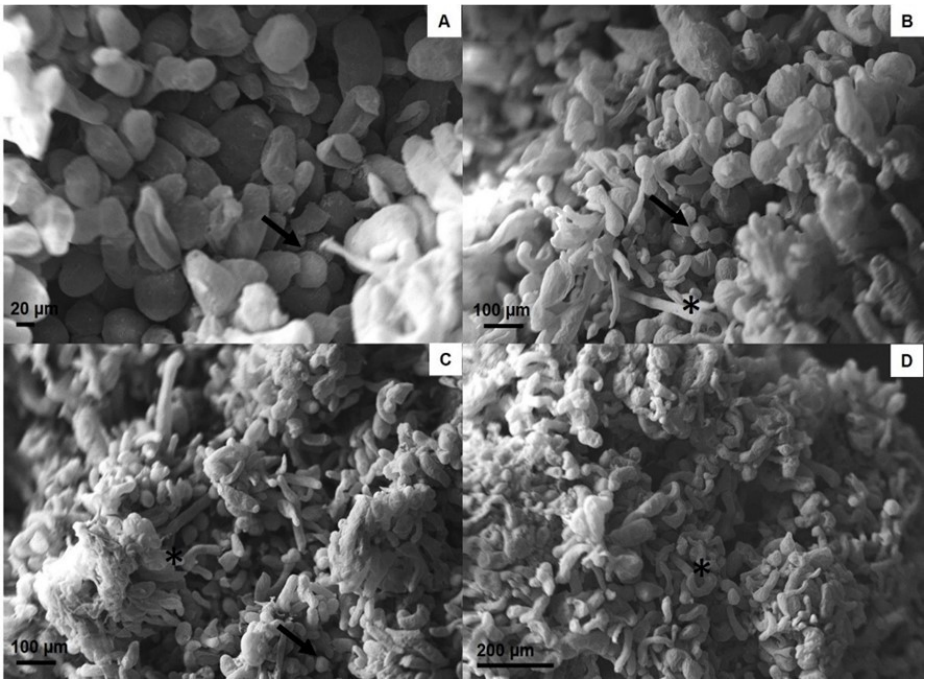

Figure 3. Scanning Electromicrograph of calli on nodal segments of gabiroba inoculated in WPM culture medium supplemented with 4.14 µM of picloram. Calli (A) at 14 days showing isodiametric cells (arrow); (B) at 28 days with isodiametric (arrow) and elongated (*) cells; (C) at 42 days with isodiametric (arrow) and elongated (*) cells; and (D) at 56 days with elongated cells (*).

At 14 days, during the exponential growth phase, the ratio between the number of elongated cells and the number of isodiametric cells was low. However, at 28 days, still in the exponential stage, and at 42 and 56 days, in the deceleration and decrease phases, respectively, the ratio between the number of elongated and isodiametric cells increased, thus characterizing a negative relationship between increasing numbers of elongated cells and decreasing numbers of isodiametric cells over time (Figure 3B, C and D).

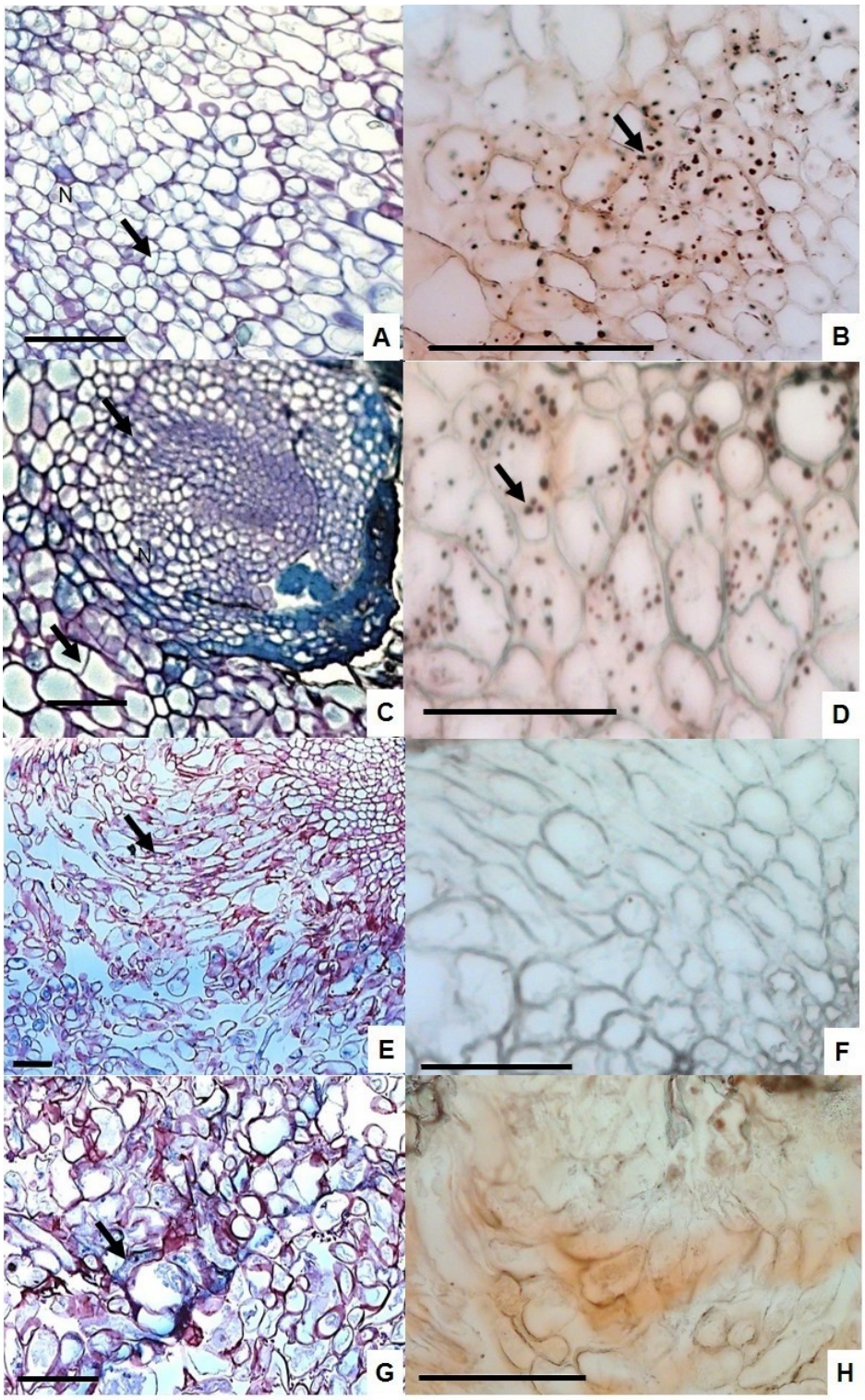

Figure 4. Cytochemical analysis of calli in WPM culture medium supplemented with 4.14 µM of picloram on nodal segments of gabiroba – A, C, E, G; calli stained with toluidine blue - B, D, F, H; calli stained with Lugol's solution – A and B. Calli at 14 days showing: A) cells with prominent nuclei (N) and intense cell division, and B) presence of starch grains (arrow). C and D) Calli at 28 days showing: C) cells in division and the formation of pro-embryogenic mass with cells with prominent nuclei (N), and D) presence of starch grains (arrow). E and F) Calli at 42 days showing: E) elongated cells and F) cells without the presence of starch grains (arrow). G and H) Calli at 56 days showing: G) elongated and dispersed cells and H) cells without the presence of starch grains. Bar: 100 µm.

Results of the cytochemical analysis of calli were consistent with those of the SEM analysis. At 14th and 28th days, isodiametric cells could be seen undergoing division and positioned juxtaposed, while on the 28th day cellular agglomerates were observed forming pro-embryogenic masses (PEM) that were intensely stained with toluidine blue. However, at 42th and 56th days after inoculation, elongated and dispersed cells were observed (Figure 4E and G).

Staining with Lugol's solution revealed the presence of starch grains in the callus cells at 14th and 28th days (Figure 4B and D), but none at 42th and 56th days (Figure 4F and H), indicating greater embryogenic potential at beginning of cultivation (at 14th and 28th days).

Discussion

Tissue culture techniques, including callus induction, are important viable alternatives for the vegetative propagation of native Cerrado species (Pinhal et al., 2011). In the present study, nodal segments of gabiroba were found to be efficient sources of explants for the induction of calli with the presence of pro-embryogenic masses.

According to Titon, Xavier, Otoni, and Motoike (2007), picloram is used for the induction of calli or the maintenance of cultures in suspensions, as well as for inducing the formation of embryogenic calli. Most studies have indicated that calli are achieved primarily through the use of 2,4-D (Bajpai, Kalim, Chandra, & Kamle, 2016; Fehér, 2015; Rocha et al., 2016; Winkelmann et al., 2015), including in the induction of calogenesis in *Campomanesia rufa* leaf explants (Sant'Ana, Paiva, Reis, Silva, & Silva, 2018), which was not found in the present study. Satisfactory results have been achieved using picloram for embryogenic callus induction in *Eucalyptus grandis* x *E. urophylla* (Moura et al., 2017) and *Acca sellowiana* (Cangahuala-Inocente, Caprestano, & Pierre, 2007), both from the same family, Myrtaceae, as gabiroba.

It is essential to know the behavior and development of the cells that are in intense multiplication after the induction of callus formation in order to maintain embryogenic characteristics. The callus growth curve of the present study presented five developmental stages of cell growth: lag, exponential growth, linear, deceleration and stationary (Santos, Ferreira, & Sarubo, 2010). The analysis of the growth curve of the present study revealed rapid growth and development of calli in comparison with that of other species (Nogueira et al., 2008; Santos, Paiva, Paiva, & Paiva, 2008; Santos et al., 2010; Santos & Souza, 2016; Stein et al., 2010; Vasconcelos et al., 2012), demonstrating that growth pattern varies among explants of different species. In the present case, the rapid growth and development of calli exhibiting embryogenic characteristics is extremely advantageous for producing plants on a large scale in a short period of time.

The exponential growth phase, characterized by maximum cell division (Stein et al., 2010), and the linear growth phase, with a decrease in cell division and an increase in cell size (Santos et al., 2008), exhibit varying temporal patterns among species. In leaf explants of *Coffea canephora* var. Conilon, growth occurred between the 16th and the 34th day after inoculation (Santos et al., 2010). For calli obtained from leaf explant of *inga* (*Inga vera* Willd. Subsp. Affinis (DC.) T.D. Penn), this phase started on the 40th day and continued until the 50th day (Stein et al., 2010). The linear growth phase of calli of leaf explants of murici-pequeno (*Byrsonima intermedia* A. Juss.) occurred between the 40th and 60th days of culture (Nogueira et al., 2008). In calli obtained from both leaf and nodal segments of *Coffea canephora* L. cv. Apoatã, linear growth was obtained from the 63rd to 70th day (Santos et al., 2008).

In the present study, the deceleration period was observed at 35 days, and was characterized mainly by a reduction in nutrient content in the culture medium, drying of the agar, and accumulation of toxic substances in the growth medium (Smith, 2012). Thus, it is appropriate for calli to be transferred from the culture medium prior to the 28th day in order to maintain exponential growth.

According to the results presented in the growth curve (Figure 2), the cytochemical (Figure 4) and SEM (Figure 3) analyses identified isodiametric cells at 14 and 28 days after inoculation, which corresponded to the exponential growth phase, as determined by the growth curve. Isodiametric cell shape is characteristic of meristematic cells, calli composed of cells of its size, with small dimensions and a dense cytoplasm with high embryogenic potential (Peña-Ramírez et al., 2011; Vejsadová, Matiska, Obert, Ürgeová, & PreŤová, 2016). The presence of elongated cells, seen on the 28th day, indicates vacuolization, which, along with the rupture of cell membranes, is one of the first signs of cell death (Hatsugai, Yamada, Goto-Yamada, Hara-Nishimura, & Beers, 2015).

Another indication of embryogenic competence is the presence of starch grains produced prior to embryo formation, which serves to initiate and sustain embryo development (Hazubska-Przybył, Kalemba, Ratajczak, & Bojarczuk, 2016). Analysis of starch content during development of somatic embryos of goiabeira-serrana (*Acca sellowiana* (O. Berg.) Burret) revealed an initial content six times greater than that at the end of embryogenic development (Cangahuala-Inocente, Steiner, Maldonado, & Guerra, 2009). In the present study, starch grains could be observed until the 28th day of culture, after which cell masses lose their embryogenic characteristics (Figure 4F and H).

Embryogenic structures do not continue their development during the stationary phase because cells go into decline and lose their intracellular organization due to the process of senescence (Santos et al., 2013). Therefore, transfer to a new culture medium can maintain the continuity of development, resulting in a greater proliferation of cells with embryogenic potential.

Conclusion

The use of 4.14 µM picloram was shown to be the most efficient treatment tested at inducing cell masses with MPE formation. Calli must be transferred to a new culture medium at 28 days for the maintenance of their growth, thus preventing oxidation and lack of nutrients.

Reference

Araújo, E. F. L., & Souza, E. R. B. (2018). Fenologia e reprodução de *Campomanesia adamantium* (Cambess.) O. Berg (Myrtaceae). *Scientific Eletronic Archives, 11*(2), 166–175. doi: 10.36560/1122018414

Bajpai, A., Kalim, S., Chandra, R., & Kamle, M. (2016). Recurrent somatic embryogenesis and plantlet regeneration in *Psidium guajava* L. *Brazilian Archives of Biology and Technology*, *59*, 1–11. doi: 10.1590/1678-4324-2016150170

Bardiviesso, D. M., Maruyama, W. I., Reis, L. L., Modesto, J. H., & Rezende, W. E. (2011). Diferentes substratos e recipientes na produção de mudas de guabiroba (*Campomanesia pubescens* O.Berg). *Revista Científica Eletrônica de Agronomia*, *18*(1), 52–59.

Bozzola, J. J., & Russel, L. D. (1998). *Electron microscopy* (2nd ed.). Boston, MA: Jones & Bartlett.

Campos, J. F., Espindola, P. P. T., Torquato, H. F. V., Vital, W. D., Justo, G. Z., Silva, D. B., … Santos, E. L. (2017). Leaf and root extracts from *Campomanesia adamantium* (Myrtaceae) promote apoptotic death of leukemic cells via activation of intracellular calcium and caspase-3. *Frontiers in Pharmacology*, *8*, 1–16. doi: 10.3389/fphar.2017.00466

Cangahuala-Inocente, G. C., Caprestano, C. A., & Pierre, J. (2007). Competência embriogenética em tecidos florais de *Acca sellowiana* (Myrtaceae). *Revista Brasileira de Biociências*, *5*(S2), 87–89.

Cangahuala-Inocente, G. C., Steiner, N., Maldonado, S. B., & Guerra, M. P. (2009). Patterns of protein and carbohydrate accumulation during somatic embryogenesis of *Acca sellowiana*. *Pesquisa Agropecuária Brasileira*, *44*(3), 217–224. doi: 10.1590/S0100-204X2009000300001

Carvalho, M. A. F., Paiva, R., Alves, E., Nogueira, R. C., Stein, V., Castro, E. M. d., … Vargas, D. P. (2013). Morphogenetic potential of native passion fruit (*Passiflora gibertii* NE Brown.) calli. *Brazilian Journal of Botany*, *36*(2), 141–151. doi: 10.1007/s40415-013-0015-4

Correia, S. M., & Canhoto, J. M. (2010). Characterization of somatic embryo attached structures in *Feijoa sellowiana* Berg . (Myrtaceae). *Protoplasma*, *242*, 95–107. doi: 10.1007/s00709-010-0130-z

Damiani, C. R., Silva, L. D., Goelzer, A., & Deo, T. G. (2016). Seed origin, storage conditions, and gibberellic acid on in vitro germination of *Campomanesia adamantium* (Cambess.) O. Berg. *African Journal of Biotechnology*, *15*(32), 1731–1737. doi: 10.5897/AJB2016.15277

Dresch, D. M., Masetto, T. E., Jeromini, T. S., & Scalon, S. P. Q.(2017). Reduced sensitivity of *Campomanesia adamantium* (Cambess.) O.Berg seeds to desiccation : effects of polyethylene glycol and abscisic acid. *American Journal of Plant Sciences*, *8(10)*, 2501–2515. doi: 10.4236/ajps.2017.810170

Dresch, D. M., Scalon, S. P. Q., Masetto, T. E., & Mussury, R. M. (2014). Storge of *Campomanesia adamantium* (Cambess.) O. Berg seeds : influence of water content and environmental temperature. *American Journal of Plant Science*, *5*(17), 2555–2565. doi: 10.4236/ajps.2014.517269

Fehér, A. (2015). Somatic embryogenesis—stress-induced remodeling of plant cell fate. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, *1849*(4), 385–402. doi: 10.1016/j.bbagrm.2014.07.005

Gomes, H. T., Bartos, P. M. C., & Scherwinski-Pereira, J. E. (2017). Dynamics of morphological and anatomical changes in leaf tissues of an interspecific hybrid of oil palm during acquisition and development of somatic embryogenesis. *Plant Cell, Tissue and Organ Culture*, *131*(2), 269–282. doi: 10.1007/s11240-017-1282-8

Hatsugai, N., Yamada, K., Goto-Yamada, S., & Hara-Nishimura, I. (2015). Vacuolar processing enzyme in plant programmed cell death. *Frontiers in Plant Science*, *6*, 1–11. doi: 10.3389/fpls.2015.00234

Hazubska-Przybył, T., Kalemba, E. M., Ratajczak, E., & Bojarczuk, K. (2016). Effects of abscisic acid and an osmoticum on the maturation, starch accumulation and germination of *Picea* spp. somatic embryos. *Acta Physiologiae Plantarum*, *38*(2), 59. doi: 10.1007/s11738-016-2078-x

Ikeuchi, M., Sugimoto, K., & Iwase, A. (2013). Plant Callus : Mechanisms of induction and repression. *The Plant Cell*, *25(9)*, 3159–3173. doi: 10.1105/tpc.113.116053

Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, *47*(260), 583–621. doi: 10.2307/2280779

Lescano, C. H., Oliveira, I. P., Zaminelli, T., Baldivia, D. S., Silva, L. R., Napolitano, M., … Sanjinez-Argandoña, E. J. (2016). *Campomanesia adamantium* peel extract in antidiarrheal activity : the ability of inhibition of heat-stable enterotoxin by polyphenols. *PLoS ONE*, *11*(10). doi: 10.1371/journal.pone.0165208

Lin, Y. J., & Zhang, Q. (2005). Optimising the tissue culture conditions for high efficiency transformation of indica rice. *Plant Cell Reports*, *23*(8), 540–547. doi: 10.1007/s00299-004-0843-6

Lloyd, G., & McCown, B. (1980). Commercially feasible micropropagation of mountain laurel *Kalmia latifolia* by use of shoot-tip culture. *International Plant Propagation Society Proceedings*, *30*, 421–427.

Moura, L. C., Xavier, A., Cruz, A. C. F., Gallo, R., Gatti, K. C., Miranda, N. A., & Otoni, W. C. (2017). Effects of explant type, culture media and picloram and dicamba growth regulators on induction and proliferation of somatic embryos in *Eucalyptus grandis* x *E. urophylla*. *Revista Árvore*, *41*(5), 1–10. doi: 10.1590/1806-90882017000500002

Nogueira, R. C., Paiva, R., Lima, E. C., Soares, G. A., Oliveira, L. M., Santos, B. R., … Castro, A. H. F. (2008). Curva de crescimento e análises bioquímicas de calos de murici-pequeno (*Byrsonima intermedia* A. Juss.). *Revista Brasileira de Plantas Medicinais*, *10*(1), 44–48.

Oliveira, L. J. d., Silva, S.M.d., Costa Netto, A. P., Silva, S. M. d., & Silva, F. M. d. S. M. d. (2011). Características agronômicas e atividade da redutase do nitrato em plantas de *Campomanesia* sp. sob estresse hídrico. *Revista Agrarian*, *4*(11), 43–53.

Oliveira, E. J., Koehler, A. D., Rocha, D. I., Vieira, L. M., Pinheiro, M. V. M., Matos, E. M., … Otoni, W. C. (2017). Morpho-histological, histochemical, and molecular evidences related to cellular reprogramming during somatic embryogenesis of the model grass *Brachypodium distachyon*. *Protoplasma*, *254*(5), 2017–2034. doi: 10.1007/s00709-017-1089-9

Pádua, M. S., Paiva, L. V, Labory, C. R. G., Alves, E., & Stein, V. C. (2013). Induction and characterization of oil palm (*Elaeis guineensis* Jacq.) pro-embryogenic masses. *Anais da Academia Brasileira de Ciências*, *85*(4), 1545–1556. doi: 10.1590/0001-37652013107912

Peña-Ramírez, Y. J., García-Sheseña, I., Hernández-Espinoza, A., Domínguez-Hernández, A., Barredo-Pool, F. A., González-Rodríguez, J. A., & Robert, M. L. (2011). Induction of somatic embryogenesis and plant regeneration in the tropical timber tree Spanish red cedar [*Cedrela odorata* L.(Meliaceae)]. *Plant Cell, Tissue and Organ Culture*, *105*(2), 203–209. doi: 10.1007/s11240-010-9853-y

Pescador, R., Kerbauy, G. B., Viviani, D., & Kraus, J. E. (2008). Anomalous somatic embryos in *Acca sellowiana* (O . Berg) Burret (Myrtaceae). *Brazilian Journal of Botany*, *31*(1), 155–164. doi: 10.1590/S0100-84042008000100014

Pinhal, H. F., Anastácio, M. R., Carneiro, P. A. P., Silva, V. J., Morais, T. P., & Luz, J. M. Q. (2011). Aplicações da cultura de tecidos vegetais em fruteiras do Cerrado. *Ciência Rural*, *41*(7). doi: 10.1590/S0103-84782011005000089

Pinto, G., Silva, S., Neves, L. O. d., Araújo, C., & Santos, C. (2010). Histocytological changes and reserve accumulation during somatic embryogenesis in *Eucalyptus globulus*. *Trees*, *24*(4), 763–769. doi: 10.1007/s00468-010-0446-5

Ribeiro, M. N. O., Pasqual, M., Villa, F., Pio, L. A. S., & Hilhorst, H. W. M. (2009). *In vitro* seed germination and seedling development of *Annona crassiflora* Mart. *Scientia Agricola*, *66*(3), 410–413. doi: 10.1590/S0103-90162009000300017

Rocha, D. I., Pinto, D. L. P., Vieira, L. M., Tanaka, F. A. O., Dornelas, M. C., & Otoni, W. C. (2016). Cellular and molecular changes associated with competence acquisition during passion fruit somatic embryogenesis: ultrastructural characterization and analysis of SERK gene expression. *Protoplasma*, *253*(2), 595–609. doi: 10.1007/s00709-015-0837-y

Rossato, M., Schumacher, P. V., Costa Netto, A. P., Souza, G. C., Reis, E. F., & Stein, V. C. (2015). Multiplicação e enraizamento *in vitro* de Gabirobeira. *Plant Cell Culture & Micropropagation*, *11*(2), 70–77.

Sá, S., Chaul, L. T., Alves, V. F., Fiuza, T. S., Tresvenzol, L. M. F., Vaz, B. G., … Paula, J. R. (2018). Phytochemistry and antimicrobial activity of *Campomanesia adamantium*. *Revista Brasileira de Farmacognosia*, *28*(3), 303–311. doi: 10.1016/j.bjp.2018.02.008

Sant'Ana, C. R. O., Paiva, R., Reis, M. V., Silva, D. P. C., & Silva, L. C. (2018). In vitro propagation of *Campomanesia rufa*: an endangered fruit species. *Ciência e Agrotecnologia*, *42*(4), 372–380. doi: 10.1590/1413-70542018424011018

Santos, C. G., Paiva, R., Paiva, P. D. O., & Paiva, E. (2008). Indução e análise bioquímica de calos em segmentos foliarese nodais de *Coffea canephora* L. cv. Apoatã. *Magistra*, *20*(1), 22–29.

Santos, D. N., Nunes, C. F., Soares, J. D. R., Valente, T. C. T., Alves, E., Labory, C. R. G., & Pasqual, M. (2013). Cytological characterization of *Jatropha curcas* callus in different periods of cultivation. *Crop Breeding and Applied Biotechnology*, *13*(4), 228–233. doi: 10.1590/S1984-70332013000400002

Santos, M. R. A., Ferreira, M. G. R., & Sarubo, V. (2010). Determination of callus growth curve in conilon coffee. *Revista Caatinga*, *23*(1), 133–136.

Santos, M. R. A., & Souza, C. A. (2016). Dedifferentiation of leaf cells and growth pattern of calluses of *Capsicum annuum* cv . Etna. *Australian Journal of Basic and Applied Sciences*, *10*(12), 362–368.

Shahzad, A., Sharma, S., Parveen, S., Saeed, T., Shaheen, A., Akhtar, R.,… Ahmad, Z. (2017). *Historical perspective and basic principles of plant tissue culture*. Singapore: Springer. doi: 10.1007/978-981-10-2961-5

Smith, R. (2012). *Plant tissue culture: techniques and experiments* (3rd ed.). Cambridge, MA: Academic Press.

Sousa, Í. D., Sousa, J. B., Pereira, F. D., Santana, J. G., Neto, A. R., & Assis, E. S. (2017). Composição do meio de cultivo para produção de microplantas de caju-de-árvore-do-Cerrado (*Anacardium othonianum* RIZZ.). *Revista Científic@ - Multidisciplinary Journal*, *4*(1), 1–11. doi: 10.29247/2358-260X.2017v4i1.p01-11

Souza, J. C., Piccinelli, A. C., Aquino, D. F. S., Souza, V. V., Schmitz, W. O., Traesel, G. K., … Arena, A. C. (2014). Toxicological analysis and antihyperalgesic, antidepressant, and anti-inflammatory effects of *Campomanesia adamantium* fruit barks. *Nutritional Neuroscience*, *20*(1), 1–9. doi: 10.1179/1476830514Y.0000000145

Stein, V. C., Paiva, R., Vargas, D. P., Soares, F. P., Alves, E., & Nogueira, G. F. (2010). Ultrastructural calli analysis of *Inga vera* Willd. subsp. *Affinis* (DC.) TD Penn. *Revista Árvore*, *34*(5), 789–796. doi: 10.1590/S0100-67622010000500004

Titon, M., Xavier, A., Otoni, W. C., & Motoike, S. Y. (2007). Efeito dos reguladores de crescimento dicamba e picloram na embriogênese somática em *Eucalyptus grandis*. *Revista Árvore*, *31*(3), 417–426. doi: 10.1590/S0100-67622007000300007

Vasconcelos, J. N. C., Cardoso, N. S. N., Oliveira, L. M., Santana, J. R. F., Fernandez, L. G., Bello Koblitz, M. G., & Silva, M. L. C. (2012). Indução, caracterização bioquímica e ultra-estrutural de calos de aroeira-do-sertão (*Myracrodruon urundeuva* Fr. All.). *Revista Brasileira de Plantas Medicinais*, *14*(4), 592–597. doi: 10.1590/S1516-05722012000400004

Vejsadová, H., Matiska, P., Obert, B., Ürgeová, E., & PreŤová, A. (2016). Somatic embryogenesis in *Phlox paniculata*–histological analysis. *Biologia*, *71*(7), 763–768. doi: 10.1515/biolog-2016-0100

Winkelmann, T., Ratjens, S., Bartsch, M., Rode, C., Niehaus, K., & Bednarz, H. (2015). Metabolite profiling of somatic embryos of *Cyclamen persicum* in comparison to zygotic embryos, endosperm, and testa. *Frontiers in Plant Science*, *6*, 597. doi: 10.3389/fpls.2015.00597