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Callogenesis in ovaries of *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum

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ABSTRACT

Background: There are few studies concerning the action and concentration of growth regulators for callus induction in *Theobroma grandiflorum* explants. **Objective:** The objective of this work was to compare the effect of different concentrations of growth regulators for callus induction in ovaries of this species, aiming to contribute to the establishment of a micropropagation protocol. **Results:** The results showed that callogenesis occurs spontaneously in MS medium without growth regulators. 2,4-D intensifies callogenesis, while TDZ has a negative effect. The most efficient treatment was 4.0 mg.L^{-1} 2,4-D, which induced friable callus in 94.1% of the explants. **Conclusion:** The achievement of callogenesis in ovaries of *T. grandiflorum* indicates the potential of these explants for *in vitro* vegetative propagation of this species.

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INTRODUCTION

Theobroma grandiflorum is a native species to the Amazonian environment, traditionally known as Cupuassu. It belongs to the Malvaceae botanical family and is a fruit tree that is spread throughout the Brazilian Amazon and in parts of Colombia, Venezuela, Ecuador and Costa Rica (Esteves, 2013). Its fruit pulp is creamy white with an acidic flavor and a unique pleasant smell, being consumed *in natura* or as juice, liqueur, ice cream, jellies, yogurts, etc. Due to the interesting characteristics of the pulp and its easy industrialization, the fruit is one of the most attractive in the northern region of Brazil and draws national and international attention.

The main restraining factors of its expansion as a culture have been the absence of uniform and high quality genetic populations. The breeding programs have emphasized high production, pulp yield and resistance to *Crinipellis perniciosa* - its principal disease, that leads to hyperplasia and hypertrophy followed by a decrease in productivity (Souza, 2007).

The Codajas, Manacapuru, Belem and Coari clones of this species are the result of a selection work developed by Embrapa (Brazilian Agricultural Research Corporation) from a group of plants collected in northern Brazil in the decade of 1980, due to their high productivity and tolerance to *C. perniciosa* (Cruz and Alves, 2002). Alves and Ferreira (2012) have presented another variety, the BRS Carimbó, originated from those materials, presenting higher productivity of fruits and seeds and tolerance to *C. perniciosa*.

Micropropagation can make the breeding programs faster and therefore there is a great benefit in *in vitro* cloning of this material. However, efficient *T. grandiflorum* micropropagation has not been achieved yet.

Aiming to contribute to the settlement of a micropropagation protocol for *T. grandiflorum*, the objective of this work was to study different concentrations of growth regulators in the induction of callus in ovary explants.

MATERIAL AND METHODS

Codajas clonal plants cultivated in the Experimental Field of Embrapa Rondônia, in Porto Velho-RO, were used as the source of explants. Unopened 1.0 to 1.4 cm length flower buds were collected between 8 and 9 a.m. and washed with sponge, water and a detergent agent. In a laminar flow chamber the buds were disinfected by immersion in alcohol 70% (v/v) for 1 minute and in calcium hypochlorite 5% for 30 minutes (w/v) under shaking, followed by rinsing in sterile bidistilled water for three times. The ovaries were excised with scalpel and inoculated in test tubes containing 10 mL MS (Murashige and Skoog, 1962) medium with 16 g.L^{-1} agar, 60 g.L^{-1} sucrose and factorial combinations of 2,4-D (2,4-dichlorophenoxyacetic acid) (0, 2 and 4 mg.L^{-1}) and TDZ (thidiazuron) (0, 5

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and 10 mg.L^{-1}) and a further combination of kinetin (0.25 mg.L^{-1}) and 2,4-D (1 mg.L^{-1}). The pH medium was adjusted to 5.8±0.1 before autoclaving at 120°C and 1 atm for 20 minutes. After inoculation the cultures were kept in the dark, at 25±1ºC, for five weeks. The experimental design was randomized with five replications of four test tubes, each one containing one explant. The averages were compared by Tukey test at 5%.

RESULTS AND DISCUSSION

The explants swelling began seven days after inoculation indicating the callus initiation. Similar results were obtained by Ferreira *et al.* (2005), employing young leaves of cupuassu completely expanded, aiming to standardize an adequate culture medium to rapid vegetative propagation by somatic embryogenesis.

As shown in Fig. 1 callogenesis occurred spontaneously in MS medium without growth regulators in 66.7% of the explants. Callogenesis does not necessarily always depend on the exogen application of cytokinins, therefore when a tissue cultivated *in vitro* has suffered physical or chemical injuries may give origin to a callus (Rosal, 2004). Some authors mention that certain tissues have the ability to synthesize hormones in *in vitro* conditions, even in primary cultures. Lima et al. (2002) also observed callus initiation in cassava explants cultivated in medium without regulators in only 14 days. During the callogenesis process, the growth regulators act, synergistically or not, upon organized and differentiated tissue cells, causing the expression of a cell mass with meristematic characteristics that very often grows fast and irregularly (Santos 1998). In this context, the balance of cytokinins and auxins promotes the tissue growth, especially 2,4-D and TDZ, 2,4-D being the most widely used (Palu et al, 2004).

TDZ had a negative influence on callus induction, with necrosis of virtually all the explants submitted to this regulator. From the six treatments containing this regulator, only one resulted in callus formation, and in only 26.7% of the explants. Nogueira et al. (2007) also observed necrosis caused by addition of TDZ in stem fragments of *Byrsonima intermedia* A. Juss. causing the inhibition of bud formation. These results contradict those found by Silva et al. (2006), that obtained positive results working with staminodes and ligules of *T. grandiflorum*, which produced greater amounts of callus using TDZ (10 µg1^{-1}), indicating that improved results can be obtained by increasing the concentration of TDZ.

The results found indicated no synergistic interaction between 2,4-D and TDZ on morphogenetic expression. Even at lower concentrations, TDZ did not provide induction or growth of undifferentiated cells, causing necrosis. However, treatments employing 2,4-D without TDZ produced differentiated significant responses, and growing in proportion to the increase of its concentration. Cavalcante (2001) observed a reverse situation in leaf explants of cupuassu: the combination of TDZ and 2,4-D did not result in callus induction, but TDZ alone, at 2 and 3 µM, resulted in high callus formation.

2,4-D intensified callogenesis. The most efficient treatment was 4 mg.L^{-1} 2,4-D, which induced friable callus in 94.1% of the explants and differed significantly from the other treatments. Although not measured, the calluses obtained in this treatment were more developed and friable. Treatments containing 1 mg.L^{-1} 2,4-D + 0,25 mg.L^{-1} kinetin had been tested by Bragado (2009), who achieved 82% of callus induction in ovaries of a seedless variety of cupuassu. Ledo et al. (2002) evaluated the morphogenetic responses from different cupuassu tree explants under various conditions of *in vitro* culture. The authors affirm that the absence of embryogenic callus induction observed in cultures may be related to several factors such as type and development stage of explants, medium culture and type and growth regulators concentration.

The results show that callogenesis occurs spontaneously in ovaries of *T. grandiflorum* in MS medium without growth regulators; 2, 4-D intensifies the process, while TDZ has a negative influence; the most efficient treatment for friable callus induction being 4 mg.L^{-1} 2,4-D.

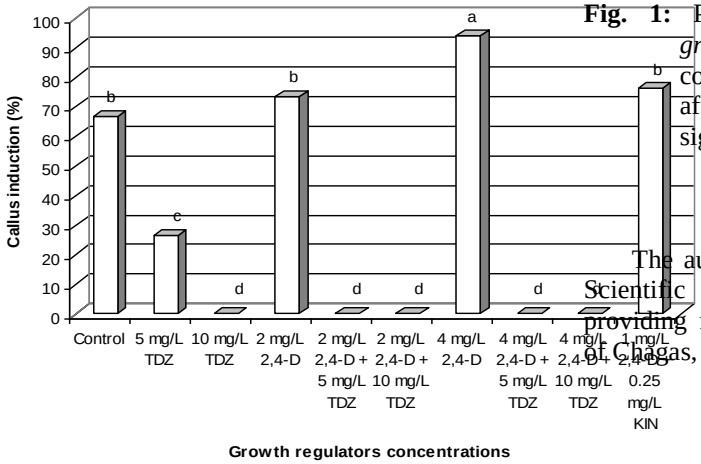

Fig. 1: Percentages of callus induction in *T. grandiflorum* ovaries in different concentrations of growth regulators, 35 days after inoculation. *Letters indicate significance by Tukey test, at 5%.

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REFERENCES

Alves, R.M. and F.N. Ferreira, 2012. BRS Carimbó – A nova cultivar de cupuaçuzeiro da Embrapa Amazônia Oriental. Embrapa.

Bragado, A.C., 2009. Avaliação do potencial de explantes florais de *Theobroma grandiflorum* Schum para propagação *in vitro.* FARO - Faculdade de Ciências Humanas, Exatas e Letras de Rondonia.

Cavalcante, A.S.L., 2001. Respostas morfogenéticas *in vitro* de acaizeiro (*Euterpe oleracea* Mart.) e de cupuaçuzeiro (*Theobroma grandiflorum* (Wild. ex Spreng.) Schum.). Universidade Federal do Ceará.

Cruz, E.D. and R.M. Alves, 2002. Clones de cupuaçuzeiro tolerantes à vassoura-de-bruxa. Embrapa Amazônia Oriental.

Esteves, G., 2013. Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro.

Ferreira, M.G.R., C.H.S. Carvalho, A.A. Carneiro and C.F. Damião Filho, 2005. Indução de embriogênese somática em cupuaçu (*Theobroma grandiflorum* Schum**.**). Revista Brasileira de Fruticultura, 27(3): 500-503.

Ledo, A.S., O.A. Lameira and A.K. Benbadis, 2002. Explantes de cupuaçuzeiro submetidos a diferentes condições de cultura *in vitro*. Revista Brasileira de Fruticultura, 24(3): 604-607.

Lima, G.P.P., C. Barsalobres, I.M.T. Piza and M.P. Cereda, 2002. Efeito do BAP e ANA e atividade da peroxidase em mandioca (*Manihot esculenta* crantz cv mcol 22) cultivada *in vitro*. Revista Brasileira Agrociência, 8(2): 107-110.

Murashige, T. and F.A. Skoog, 1962. Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15(3): 473-497.

Nogueira, R.C., R. Paiva, L.M. Oliveira, G.A. Soares, F.P. Soares, A.H.F. Castro and P.D.O. Paiva, 2007. Indução de calos em explantes foliares de murici-pequeno (*Byrsonima intermedia* A. Juss.). Ciencia e Agrotecnologia, 31(2): 366-370.

Palu, E.G., A.B. Silva and M. Pasqual, 2004. Calogênese *in vitro* em anteras de *Coffea arabica* L. Ciencia e Agrotecnologia, 28(4): 736-742.

Rosal, L.F., 2004. Germinação, indução de calos, micropropagação e anatomia foliar da candeia *(Eremanthus erythropappus* (DC.) Mac Leish). Universidade Federal de Lavras.

Santos, M.R.A., 1998. Germinação, calogênese e caracterização de saponinas em *Smilax japecanga* Grisebach. Universidade Federal de Lavras.

Silva, M.B., A.R. Ramos and G.A. Venturieri, 2006. Indução de calos em espécies Amazônicas do gênero *Theobroma*. Ciencia e Agrotecnologia, 30(2): 257-265.

Souza, A.G.C., 2007. Boas práticas agrícolas da cultura do cupuaçuzeiro. Embrapa Amazônia Oriental.