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Short communication

In vitro callogenesis of *Poincianella pyramidalis* (catingueira)

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ABSTRACT

This work describes the establishment of procedures to induce *in vitro* callogenesis from *Poincianella pyramidalis* (Tul.) L. P. Queiroz, Fabaceae, explants. Nodal, internodal and leaf segments were isolated from *in vitro* germinated seedlings and cultured in MS medium with 0, 2.5, 5 and 10 mg l^{-1} of 2,4-dichlorophenoxyacetic acid. After 30 days, the explants with induced callus showed a quadratic response for the segments nodal, internodal and leaf, with increasing the callus formation in 2,4-dichlorophenoxyacetic acid concentrations of 6.28, 6.49 and 4.91 mg l^{-1}, respectively. In 30 days there was a linear oxidation rise with the increase to the 2,4-dichlorophenoxyacetic acid. After 60 days, oxidation values were minimum, at 2,4-dichlorophenoxyacetic acid concentrations of 5.13 mg l^{-1} (internodal) and 3.98 mg l^{-1} (leaf). The highest callus production was observed after 30 days in the presence of 6.09 mg l^{-1}, 5.82 mg l^{-1} and 4.91 mg l^{-1} of 2,4-dichlorophenoxyacetic acid in nodal, internodal and leaf segments, respectively. After 60 days these segments showed peaks of production at 7.0 mg l^{-1} (nodal), 6.15 mg l^{-1} (internodal) and 5.08 mg l^{-1} (leaf) of 2,4-dichlorophenoxyacetic acid. For callus induction the intake of 2,4-dichlorophenoxyacetic acid was essential. The greater intensity in callus formation was observed in 4.91 mg l^{-1} in leaf segments after 30 days.

Introduction

Poincianella pyramidalis (Tul.) L. P. Queiroz, Fabaceae, botanical synonymous of *Caesalpinia pyramidalis* Tul., is an endemic tree occurring in Brazilian north and northeastern regions. It is an endemic plant of the "Caatinga" biome where it is known by several common names such as "pau-de-rato", "catinga-de-porco", "catingueira", among others, and it is widely used in folk medicine in the treatment of catarrhal infections, diarrhea, hepatitis and anemia (Silva et al., 2013). This species is a fast growing plant after the dry season and with good potential for regrowth, being attractive for the first and second phases of mixed reforestation of damaged area (Maia, 2012).

The leaves, roots and barks are rich in bioactive biflavonoids, flavonoids and gallic acid (Bahia et al., 2005; deOliveira et al., 2016a). Besides, previous studies on this species showed that the extract exhibited molluscicide, antimicrobial, anti-inflammatory and antinociceptive activities (deOliveira et al., 2016b).

This species propagates by seed and its reproduction has been hampered by inadequate practice of extractive use, since its leaves, flowers and stem bark are frequently used in folk medicine and also as wood for various purposes even as charcoal by locals (Maia, 2012). So, plant cell culture prospections provides alternative approaches which may be attractive when the plant is difficult to cultivate, produces significant bioactive compounds and, in some cases, mass cultivation of the source plant may not be possible due to environmental, ecological or climatic conditions may limit the cultivation processes. Depending on the nature of the plant, extraction of secondary metabolites directly from cultivated plant tissue may be an option. In this context, the callus cultures are the first stage in plant biomass cultivation processes (Yesil-Celiktas et al., 2010).

So, the development of techniques of micropropagation is important to species reproduction and also for production of bioactive compounds from callus. In this way, the use of auxins, such as 2,4-dichlorophenoxyacetic (2,4-D), are generally the most recommended compound for increasing of production of secondary metabolites. This is a synthetic auxin most commonly used for callus induction, the most active auxin and can replace the indolacetic acid natural auxin (IAA) in culture media because the latter is

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rapidly oxidized. The callus with different growth rates and differentiation levels (friable, compact) may differ in the ability to synthesize bioactive compounds (Navroski et al., 2012).

Even today, studies dealing with *in vitro* morphogenesis of Brazilian native tree species are scarce (Oliveira et al., 2013) and for there are no previously reports with *P. pyramidalis*, only *Poincianella echinata* Lam and *P. bonduc* (L.) Roxb have been studied with this purpose (Werner et al., 2009, 2010; Cheruvathur et al., 2010; Silva et al., 2013). Thus, this study aimed to establish *in vitro* callus induction in *P. pyramidalis* explants targeting *in vitro* production protocol.

Materials and methods

Plant material

The seeds of *Poincianella pyramidalis* (Tul.) L. P. Queiroz, Fabaceae, were collected from native plants growing nearby the BR 110 highway at km 22, Jeremoabo-BA, Brazil (−10°04′33.80″ S, −38°20′28.00″ W) and they are kept at room temperature into brown paper.

In vitro *germination of seeds of* Poincianella pyramidalis

The seeds were cleaned with 70% alcohol for 1 min in laminar flow chamber, sequentially they were also treated with commercial 50% sodium hypochlorite solution for 8 min under stirring and then washed three times with sterile distilled water. Then, they are inoculated in test tubes containing 20 ml of MS medium (Murashige and Skoog, 1962) supplemented with 30 g l^{-1} sucrose and 7% agar. The pH of medium was adjusted to 5.8 before autoclaving at 120 °C for 20 min. After inoculation, the seeds were kept in a growth chamber at temperature of 25 ± 2 °C, in 16 h photoperiod, with light intensity of 50 μmol $^{-2}$ m^{-2} s^{-1} obtained by cool white fluorescent lamps.

Callus induction

After 45 days of *in vitro* germination the explants used for the study of 2,4-D effects on callus induction were nodal, internodal and leaf segments of seedlings. In laminar flow chamber, they were cut and inoculated in sterile and disposable Petri dishes (90 mm × 15 mm) containing 20 ml MS medium, supplemented with 30 g l^{-1} of sucrose and 5 g l^{-1} of agar. The 2,4-D (2,4-dichlorophenoxyacetic) was added to culture medium at concentrations of 0, 2.5, 5 and 10 mg l^{-1}. Then, the cultures were maintained in a growth room at temperature of 25 ± 2 °C in absence of light for 60 days. At 30 and 60 days after inoculation, the oxidation by means of a set rating scale based on percentage of explants with oxidation area was evaluated (grades 1: 0%; 2: 0–25%; 3: 25–50%; 4: 50–75%; 5: 75–100%) and the responsive explants percentage for callus formation by a rating scale based defined on the percentage of callus explant induction area (grades 1: 0; 2: 25–50%; 3: 50–75% and 4: 75–100%).

The experimental design was completely randomized in a 3 × 4 factorial scheme, three types of explants (nodal, internodal and leaf) and four 2,4-D concentrations, totaling 12 treatments with six replicates with four explants each.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and the means of the quantitative treatments evaluated by regression, with the Sisvar Statistical Analysis Program (Ferreira, 2011). Data expressed as percentages were previously processed according to arcsine $(\times/100)^{0.5}$.

Results and discussion

According to variance analysis was a significant interaction ($p < 0.05$) between the 2,4-D concentration and types of segments regardless of evaluation period, for all variables, except for the callus oxidation at 30 days culture where only 2,4-D showed a significant effect.

Regarding the callus oxidation percentage at 30 days of *in vitro* culture a positive linear correlation was observed with the increase of amounts of 2,4-D, with a significant increase in concentration of 10 mg l^{-1} (3.66%) as shown in Fig. 1A. There was a quadratic behavior callus oxidation percentage at 60 days depending on the 2,4-D concentrations (Fig. 1B) in which the results for the nodal segments were not significant. However, internodal and leaf segments had the minimum oxidation values with 5.13 and 3.98 mg l^{-1} of 2,4-D, respectively. The absence of antioxidants in culture medium and the presence of oxidation did not inhibit callus induction in all 2,4-D concentrations for callus of *P. pyramidalis*.

The occurrence of oxidation in woody plant cultures is a common problem (Jaskani et al., 2008), which also affects the experiments with *P. pyramidalis* in both characteristics, the incidence and the intensity of explant type used. However, in this study, despite the oxidation explants variation, there is no limitation to callus development with 2.5 and 5.0 mg l^{-1} of 2,4-D. The callus induction in segments started among 7 and 21 days after inoculation and a formation of compact friable callus with a few areas was observed (Fig. 2). The scanning electron microscopy of the callus induction in nodal segments indicated small cells with a yellow color, characteristic of compact consistence callus. It also indicated rounded and nearby cells (Fig. 2B). The morphology of embryogenic and non-embryogenic callus were similar and they commonly are modified over time and change of growth regulators (Ribeiro et al., 2012).

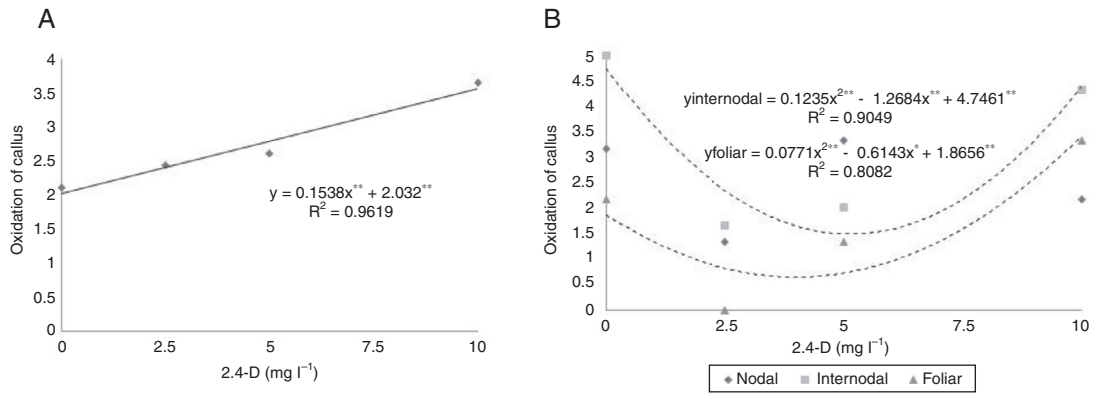

Fig. 1. Callus oxidation in *Poincianella pyramidalis* nodal, internodal and leaf explants at 30 (A) and 60 (B) days after inoculation.

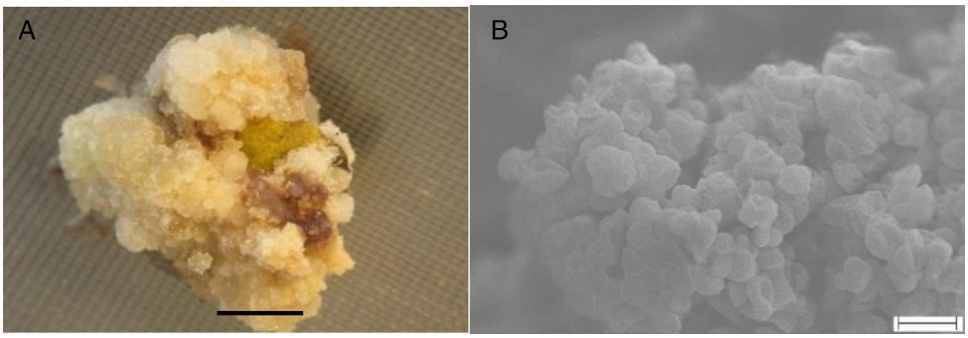

Fig. 2. (A) Appearance of compact callus formation with few friable areas of *Poincianella pyramidalis* at *in vitro* culture of 60 days; (B) Scanning electron micrograph showing *P. pyramidalis* calli. Bars (A) 0.5 cm, (B) 100 μm. Photos: Kicia Gomes-Copeland (A) Caroline Machado (B).

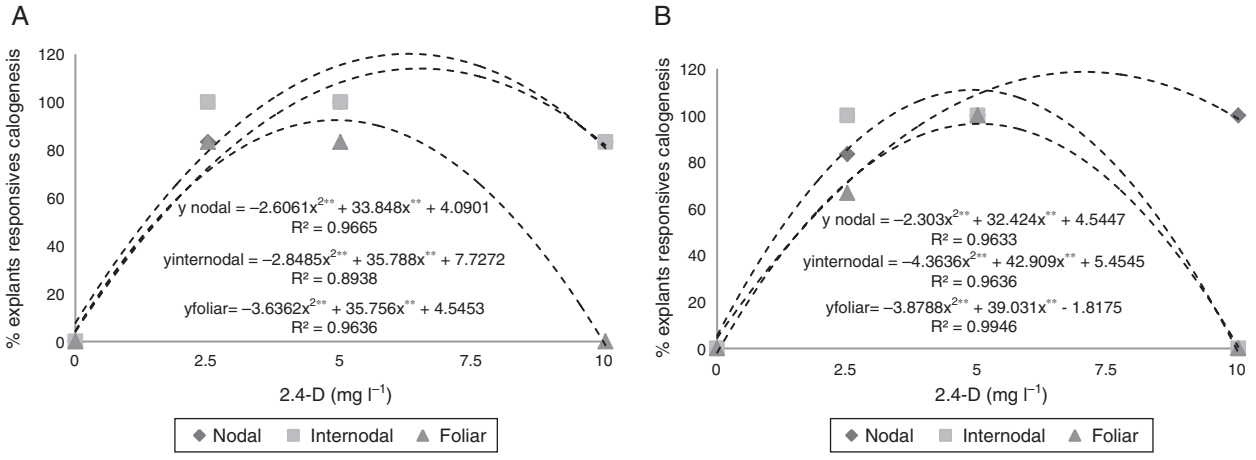

Fig. 3. Percentage of *Poincianella pyramidalis* nodal, internodal and leaf explants responsive to callus intensity in the presence of 2,4-D concentration of 30 (A) and 60 (B) days after inoculation.

There was no evidence of callus formation in the absence of 2,4-D (Fig. 3A and B). After 30 days of *in vitro* culture, the percentage of explants with callus induction showed a quadratic behavior for all segments (intermodal, nodal and leaf) with increase in callus formation in 2,4-D concentration of 6.28 mg l^{-1}; 6.49 mg l^{-1}, and 4.91 mg l^{-1}, respectively. Nevertheless, from these values, all samples showed a decrease for this variable (Fig. 3A). At day 60 (Fig. 3B) the behavior was similar, where the nodal, internodal and leaf segments kept continued on callus formation until 7.04 mg l^{-1}, 4.92 mg l^{-1} and 5.03 mg l^{-1} of 2,4-D, respectively, with a further decrease in callus formation.

However, from certain concentrations the auxins may have phytotoxic effects such as seen in Fig. 3A and B, inhibiting the callus formation, since the plant material used was originated from *in vitro* germinated seedlings provided the greater sensitivity to low concentrations 2,4-D due to the endogenous levels of auxin (Silva et al., 2013).

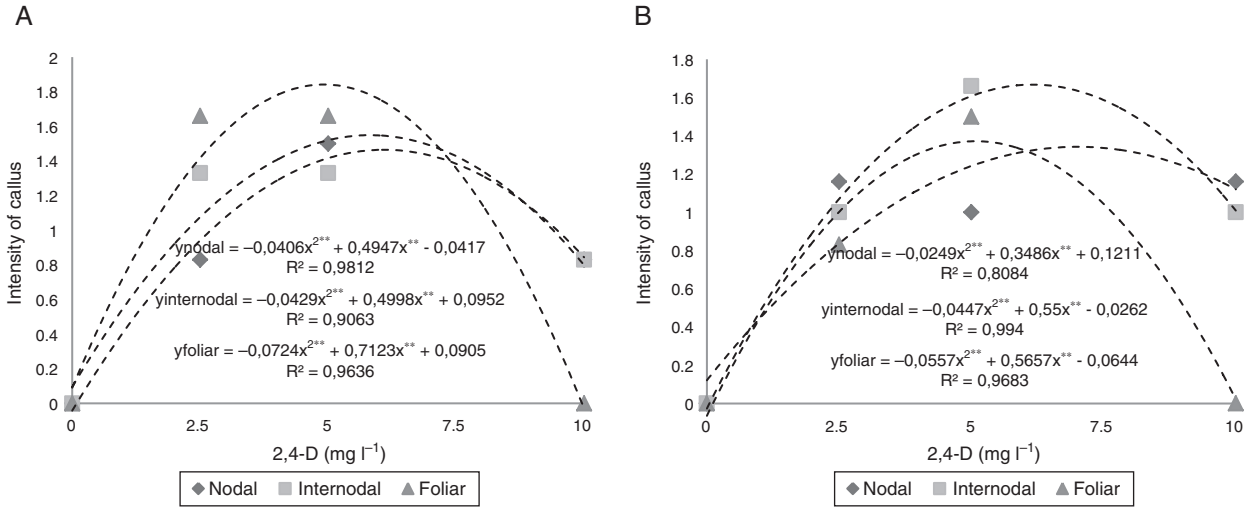

Fig. 4. Callus formation intensity notes in *Poincianella pyramidalis* nodal, internodal and leaf explants at 30 (A) and 60 (B) days in presence of 2,4-D.

The callus formation intensity shown a significant interaction between explants and 2,4-D concentrations at 30 and 60 days after inoculation after the quadratic curve adjustments. Fig. 4A shows that the maximum callus induction was with 2,4-D concentrations of 6.09, 5.82 and 4.91 mg l^{-1} in the nodal, internodal and leaf segments, respectively. From these values was noticed a callous mass decrease, especially in leaf explants with 10 mg l^{-1} of 2,4-D. Probably the high auxin concentration in the culture medium promoted a greater oxidation and tissue death due to the presence of high phenolic compounds concentrations that oxidize and deteriorate the plant material (Payghamzadeh and Kazemitabar, 2011).

After 60 days, the nodal, internodal and leaf segments had producing callus peaks in 2,4-D concentrations of 7.0, 6.15 and 5.08 mg l^{-1}, respectively. A decrease in the production of callus on foliar compared to 30 days after inoculation was observed increased 2,4-D concentrations for callus induction at 60 days after inoculation (Fig. 4B). The foliar segment had higher intensity of callus formation at 30 days and the internodal at 60 days.

These findings are understandable since there are differences in segments development regarding *in vitro* morphogenetic process. Previous works reported that the choice of medium, explant type, and subsequent callus type strongly influenced plant regeneration rates (Monfort et al., 2012; Perera et al., 2015).

It is noteworthy that there is no work involving *in vitro* callus formation for the *P. pyramidalis*, just for the same genus and, still have different physiological behaviors, but corroborating each other. Our results contribute for further studies since it confirms the 2,4-D effects on "catingueira" callus production.

Conclusions

The 2,4-dichlorophenoxyacetic induce callus on nodal, leaf and internodal explants of *P. pyramidalis*. The 6.28, 6.49 and 4.91 mg l^{-1} concentrations of 2,4-D in nodal, internodal and leaf segments, respectively, induced at 30 days the highest callus formation. The presence of 10 mg l^{-1} 2,4-D increased the callus oxidation after 30 days of *in vitro* culture. However the higher callus formation in leaf explant occurring in the presence of 4.91 mg l^{-1} 2,4-D at 30 days of *in vitro* culture. These findings are indicative the method employed is adequate for micropropagation of this Brazilian medicinal plant.

Author contributions'

KKPGC, AGA and FTCA designed the experiments and carried them out. KKPGC and JPC wrote the manuscript drafts. JPC proposed the work and is the KKPCC's advisor. ASL helped in the project development and did the statistical analyses.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Bahia, M.V., Santos, J.P.D., David, J.P., David, J.M., 2005. Biflavonoids and other phenolics from *Caesalpinia pyramidalis* (Fabaceae). J. Braz. Chem. Soc. 16, 1402–1405.
Cheruvathur, M.K., Britto, J., Thomas, T.D., 2010. Callus induction and shoot regeneration from epicotyl explants of ethnomedicinally important *Caesalpinia bonduc* (L.) Roxb. Iran. J. Biotechnol. 8, 263–269.
deOliveira, J.C., David, J.M., David, J.P., 2016a. Chemical composition of root barks and flowers of *Poincianella pyramidalis* (Fabaceae). Quim. Nova 39, 189–193.
deOliveira, J.C., David, J.M., David, J.P., 2016b. Biflavonoids from the bark roots of *Poincianella pyramidalis* (Fabaceae). Phytochem. Lett. 16, 18–22.
Ferreira, D.F., 2011. Sisvar: a computer statistical analysis system. Cienc. Agrotec. 35, 1039–1042.
Jaskani, M.J., Abbas, H., Sultana, R., Khan, M.M., Qasim, M., Khan, I.A., 2008. Effect of growth hormones on micropropagation of *Vitis vinifera* L. cv. Perlette. Pak. J. Bot. 40, 105–109.
Maia, G.N., 2012. Caatinga: árvores e arbustos e suas utilidades. Ed. Leitura & Arte, São Paulo.
Monfort, L.E.F., Pinto, J.E.B.P., Bertolucci, S.K.V., Rossi, Z.T.T., Santos, F.M., 2012. Efeito do BAP no cultivo *in vitro* de *Ocimum selloi* Benth. Rev. Bras. Pl. Med. 14, 458–463.
Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473–497.
Navroski, M.C., Waldow, D.A.G., Pereira, M.O., Pereira, A.O., 2012. Calogênese *in vitro* de segmentos aplicais caulinares e intermodais em segurelha (*Satureja hortensis* L.). Rev. Agro@mbiente On-line 6, 228–234.
Oliveira, L.S., Dias, P.C., Brondani, G.E., 2013. Micropropagação de espécies florestais brasileiras. Pesq. Flor. Bras. 33, 439–453.
Payghamzadeh, K., Kazemitabar, S.K., 2011. *In vitro* Propagation of Walnut. Afr. J. Biotechnol. 10, 290–311.
Perera, D., Barnes, D.J., Baldwin, B.S., Reichert, N.A., 2015. Direct and indirect *in vitro* regeneration of *Miscanthus* × *giganteus* cultivar Freedom: effects of explant type and medium on regeneration efficiency. In vitro Cell. Dev. Biol. Plant 51, 294–302.
Ribeiro, L.O., Paiva, V.P., Padua, M.S., Santos, B.R., Alves, E., Stein, V.C., 2012. Morphological and ultrastructural analysis of various types of banana callus, cv. Prata anã. Acta Sci. 34, 423–429.
Silva, T.S., Nepomuceno, C.F., Borges, B.P.S., Alvim, B.F.M., Santana, J.R.F., 2013. *In vitro* multiplication of *Caesalpinia pyramidalis* (Leguminosae). Sitientibus 13, 2–6.
Werner, E.T., Cuzzuol, G.R.F., Pessotti, K.V., Lopes, F.P., Roger, J.A., 2009. Controle da calogênese do pau-brasil *in vitro*. Rev. Arvore 33, 987–996.
Werner, E.T., Pessotti, K.V., Mengarda, L.H.G., Vendrame, W.P., Cuzzuol, G.R.F., 2010. Meios de cultura, reguladores de crescimento e fontes de nitrogênio na regulação da calogênese do Pau-brasil (*Caesalpinia echinata* Lam.). Acta Bot. Bras. 24, 1046–1051.
Yesil-Celiktas, O., Gurel, A., Vardar-Sukan, F., 2010. Large Scale Cultivation of Plant Cell and Tissue Culture in Bioreactors. Transworld Research Network, Kerala, India.