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Scientific Article

Kinetin and 6-benzyladenine induce different morphogenetic responses in cotyledonary segments of royal poinciana

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Abstract

Understanding the *in vitro* performance of royal poinciana explants cultured in media supplemented with different types and concentrations of cytokinins may aid in the optimization of regeneration systems established for this woody species. In the present study we evaluate the *in vitro* performance of royal poinciana cotyledonary explants cultured in the presence of high concentrations of 6-benzyladenine (BA) and kinetin (KIN). Cotyledonary segments obtained from *in vitro* germinated seedlings were inoculated in Murashige and Skoog (MS) medium, supplemented with different concentrations (1, 2, 4, 8 mg L^{-1}) of BA or KIN. In the control treatment, no plant growth regulators (PGRs) were added. After 40 days of culture, regardless of the concentration used, the treatments supplemented with BA presented higher calli percentage and fresh mass compared to treatments supplemented with KIN. Adventitious shoots were mainly observed in BA-treatments. Histological analysis showed that adventitious shoots formed at the periphery of callus formed from mesophyll cells in the regions of the explant sectioning. The results obtained provide new information for the establishment of a micropropagation system for royal poinciana, an important ornamental tree species.
Keywords: *Delonix regia*, flamboyant, cytokinins, micropropagation, shoot regeneration

Resumo

Cinetina e 6-benziladenina induzem diferentes respostas morfogenéticas em segmentos cotiledonários de flamboyant

A compreensão do comportamento *in vitro* de explantes de flamboyant cultivados em meio de cultura suplementado com diferentes tipos e concentrações de citocininas pode auxiliar na otimização de sistemas de regeneração estabelecidos para essa espécie vegetal. Objetivou-se com o presente estudo avaliar o comportamento de explantes cotiledonares de flamboyant quando cultivados na presença de elevadas concentrações de 6-benziladenina (BA) ou cinetina (KIN). Segmentos cotiledonares obtidos de plântulas germinadas *in vitro* foram inoculados em meio de cultura Murashige e Skoog (MS), suplementado com diferentes concentrações (1, 2, 4, 8 mg L^{-1}) de 6-benziladenina (BA) ou cinetina (KIN). No tratamento controle não foi adicionado reguladores de crescimento. Após 40 dias de cultivo, independente da concentração utilizada, os tratamentos suplementados com BA apresentaram maiores porcentagens de formação de calos e massa fresca, em comparação aos tratamentos suplementados com KIN. A formação de gemas adventícias foi observada principalmente nos tratamentos suplementados com BA. Análises histológicas evidenciaram que a formação de gemas adventícias ocorreu na periferia de calos formados a partir de células do mesofilo, nas regiões de seccionamento do explante. Os resultados obtidos fornecem novas informações para o estabelecimento de um sistema de micropropagação de flamboyant, uma importante espécie arbórea ornamental.
Palavras-chave: *Delonix regia,* flamboyant, citocininas, micropropagação, organogênese *in vitro*

Introduction

Delonix regia (Bojer ex Hook) Raf., popularly known as royal poinciana or flamboyant, is a Fabaceae tree species widely distributed in tropical and subtropical regions. It is fast-growing and develops an umbrella-shaped cover with recognized agroforestry and ornamental relevance, being used mainly in urban landscapes (Neto and Souza, 2011). *D. regia* has also been used as a medicinal agent due its potential for production of antioxidant, antibacterial, anti-inflammatory, antidiarrheal, antidiabetic, antimicrobial and gastroprotective compounds (Shabir et al., 2011; Singh et al., 2002; Wang et al., 2016; Fatmawaty et al., 2017). In addition, the gum obtained from *D. regia* seeds is used as binder in the manufacture of tablets (Adetogun and Alebiowu, 2009; Rodriguez-Canto et al., 2019).

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Due to the ornamental and medicinal importance of this species, *in vitro* regeneration systems have been established to contribute to genotype multiplication and especially to assist bioprospection programs. Previous studies have reported the potential of embryonic *D. regia* explants to induce morphogenetic responses (Myers and Vendrame, 2004; Abdi and Hedayat, 2011).

Costa et al. (2019) observed adventitious shoot bud formation from the culture of cotyledonary segments in medium supplemented with cytokinin. According to the authors, the morphogenetic responses of the species increased linearly with the increase of the 6-benzyladenine (BA) concentrations (0.125 to 2.0 mg L^{-1}). The highest average number of adventitious shoots per explant was observed in the treatment supplemented with 2.0 mg L^{-1} BA although, a low rate of shoots has been reported. However, higher concentrations of this plant growth regulator (PGR), or even another type of cytokinin, were not tested.

Kinetin (KIN), for example, is capable of inducing cell proliferation and new adventitious shoot bud formation when added to the culture medium (Fráguas et al., 2004; Castilho et al., 2019), and is also recommended for micropropagation of some woody species, for example, *Salix humboldtiana* (Pereira et al., 2000), *Acacia auriculiformis* (Yadav et al., 2016) and *A. leucophloea* (Sharma et al., 2017).

Understanding of the *in vitro* performance of *D. regia* when cultured in medium supplemented with other types and concentrations of PGRs can help to optimize regeneration systems established for this plant species. The present study aims to evaluate the performance of *D. regia* cotyledonary explants when cultured in the presence of high BA or KIN concentrations to induce *in vitro* organogenesis to *D. regia*.

Materials and methods

D. regia seeds collected in Jataí (17°52'51"S, 51°42'0"W), in the state of Goiás, Brazil, were subjected to mechanical scarification, on the side opposite the hilum, using NORTON A257 saint-gobain® sandpaper. The scarified seeds were aseptically disinfected in ethanol 70% for 2 minutes, followed by immersion for 20 min in a non-diluted commercial sodium hypochlorite solution (2.5% active chlorine; Super Globo Química®, Contagem, Minas Gerais, Brazil). The disinfected seeds were then rinsed four times for 5 min in autoclaved deionized water and placed in 250 mL transparent glass flasks containing 20 mL culture medium consisting of half strength Murashige and Skoog (1962) basal salt solution (MS), sucrose (3% w/v), inositol (0.01% w/v) (Sigma Aldrich®), and agar (0.8% w/v) (Merck®, Darmstadt, Germany). The culture media pH was adjusted to 5.7 ± 0.1 and then autoclaved at 121 °C and 1.5 atm for 20 min. Two seeds were inoculated per flask. The flasks were conditioned in a growth room under 26 ± 2 °C, 16 h photoperiod and 36 µmol m^{-2} s^{-1} irradiance, supplied by two fluorescent lamps of 20 W (Osram® Daylight, Brazil).

Fifteen days after inoculation, cotyledon were removed from emerged seedlings and segmented into 4 fragments of approximately 2 cm² each. The cotyledonary fragments were inoculated in new 250 mL flasks containing 20 mL MS medium (total strength) as previously mentioned. However, at this stage the medium was also supplemented with different concentrations (1, 2, 4, 8 mg L^{-1}) of BA or KIN. PGRs were not added to the control treatment. The pH was adjusted to 5.7 ± 0.1 before autoclaving. Five flasks were prepared for each treatment. Four cotyledonary segments were placed in each flask with their abaxial surface in contact with the culture medium. After inoculation, the flasks were kept in the growth room under the same conditions mentioned above.

The experimental was conducted in a complete randomized design with nine treatments and five replicates. The experimental unit was a flask containing four cotyledonary segments. Cotyledonary responsiveness was evaluated by: (i) percentage of calli formation; (ii) fresh weight gain and (iii) number of shoots per explant. Fresh weight gain was obtained as the difference between explant fresh weight at inoculation (difference in flask weight before and after inoculation) and fresh weight after 40 days (4 explants from each replicate/flask were weighed directly). The percentage of calli formation and fresh weight gain data were submitted to analysis of variance and evaluated by regression using Sisvar 5.6 software. The number of shoots per explant was evaluated by the nonparametric Kruskal-Wallis tests with the Nemenyi-Damico-Wolfe-Dunn joint ranking test (Hollander and Wolfe, 1999) since this parameter did not meet normality and homogeneity.

For structural characterization, cotyledonary explants cultured in medium without PGRs and in the presence of 2 mg L^{-1} BA and KIN were fixed in a solution of formaldehyde, acetic acid and 50% ethyl alcohol (FAA) for 72 h. Then, the samples were dehydrated with increasing serial ethanol concentrations and embedded in methacrylate resin (Historesin®, Leica Instruments, Heidelberg, Germany). Cross and longitudinal sections of 5 µm were produced using an automatic advance rotary microtome (RM2155, Leica Microsystems Inc., Buffalo Grove, IL) and stained with toluidine blue (pH 4.8) (O'Brien and McCully, 1981). Images were captured using a Zeiss Axioskope microscope equipped with a U-Photo Camera System (AxioCam HRc).

Results and discussion

The presence, concentration and type of cytokinin influenced the induction of morphogenetic responses in *D. regia* (Figure 1A). Organogenic calli were observed only in the presence of cytokinins. In the absence of PGRs, cotyledonary explants did not present any morphogenetic response (Figure 1B, C). Supplementation with cytokinin is essential for the induction of morphogenetic responses in *D. regia* cotyledonary explants (Costa et al., 2019). During *in vitro* organogenesis, molecular cytokinin signaling is necessary to induce cell proliferation and differentiate adventitious shoot buds promoting the differential gene expression essential for the formation of these organs (Müller and Leyser, 2011; Su et al., 2011).

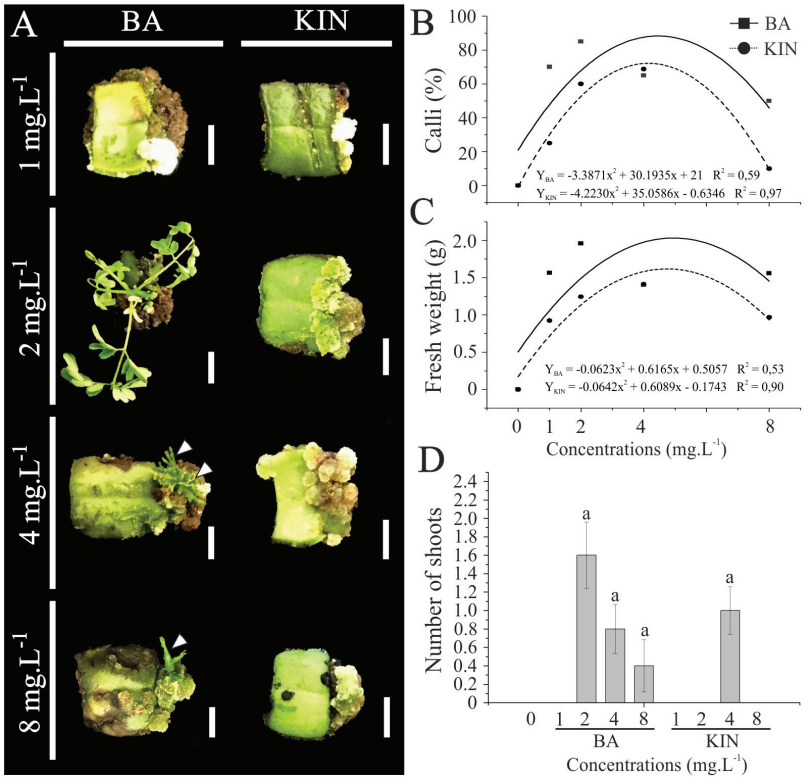

Figure 1. *In vitro* organogenesis induction from *Delonix regia* cotyledonary segments. (A) Cotyledonary explants cultured in medium supplemented with different concentrations of 6-benzyadenine (BA) or Kinetin (KIN). (B) Percentage of explants that produced calli; (C) Fresh weight gain per experimental unit; (D) Number of adventitious shoots per explant. Error bars denote the standard error of the mean. Equal letters refer that there is no statistical difference according to the Nemenyi-Damico-Wolfe-Dunn test ($p \leq 0.05$). *Abbreviations*: arrowhead – shoots buds. Bars = 0.5 cm.

In the present study, the percentage of callus fit a quadratic model (Figure 1B). Regardless of the concentration used, BA-treatments presented higher calli percentage compared to treatments supplemented with KIN (Figure 1B). The higher BA efficiency in inducing morphogenetic responses may be related to its lower susceptibility to enzymatic degradation (Magyar-Tabori et al., 2010). BA is a stable cytokinin that persists in the culture medium (Rahman, 2006). It is possible that the BA conjugated amount in the medium was lower than KIN, thus presenting a higher free form quantity readily available for the cotyledonary explants (Buah et al., 2010). This observation is in agreement with Klem et al. (2004) who reported that BA is chemically more stable than other purine-derived cytokinins.

The highest percentage of callus formation for BA and KIN was 88.29% and 72.13% in the concentrations of 4.46 and 4.15 mg $L^{-1,}$ respectively (Figure 1B). On the other hand, the highest concentration of BA and KIN tested (8 mg L^{-1}) inhibited callus induction presenting the lowest calli percentages, 45.77% and 9.5%, respectively. The correct concentration of PGRs is essential for the specification and differentiation of morphogenetic responses and this is a species-dependent feature. For *Mimosa caesalpiniifolia*, another Fabaceae species, was also reported that the greatest number of morphogenetic responses was obtained in the presence of 4 mg L^{-1} BA and reduced responses in higher concentrations (> 6 mg L^{-1}) of this same PGR (Bezerra et al., 2014). Similarly, García-Angulo et al. (2018) showed that the increase over 5 mg L^{-1} BA significantly reduced the number of morphogenetic responses of *Populus* hybrids. For *D. regia*, previously studies recommended the use of low concentrations (0.125 - 2.0 mg L^{-1}) of PGRs (Myers and Vendrame, 2004; Abdi and Hedayat, 2011; Costa et al., 2019). However, the effect of higher concentrations (> 2 mg L^{-1}) of cytokinins to induce morphogenetic responses had not been tested until this present study.

The treatments supplemented with BA showed higher fresh weight values and fit a quadratic model (Figure 1C). According to the fited model, the highest value was observed in the treatment supplemented with 4.95 mg L^{-1} BA. For KIN-treatments the highest fresh weight value was observed at 4.74 mg L^{-1} although it has been lower than BA-treatments (Figure 1C). This variable seems to be related to the callus formation once the same overall pattern has been observed for both parameters (Figure 1B, C).

Adventitious shoot formation (Figure 1D) was observed in almost all BA-treatments (2, 4, and 8 mg L^{-1}) and only in the 4.0 mg L^{-1} KIN-treatment. The highest average of shoots was obtained in the treatment supplemented with

2.0 mg L^{-1} BA, although, no differences were observed among the treatments that induced shoots (Figure 1D). The efficiency of BA to induce shoots in comparison to other cytokinins was reported by Zarinjoei et al. (2014), using calli derived from cotyledon segments of *Gleditsia caspica*, another woody Fabaceae species. According to these authors, the highest bud formation was observed in MS medium supplemented with 1 mg L^{-1} BA. At this concentration, 94.3% of the cultivated calli produced shoot buds with an average number of 4.3 buds/explants. Similar results were also reported for *Citrus macrophylla* and *C. aurantium*, in which the number of shoots was superior in culture medium supplemented with 2 and 3 mg L^{-1} BA, respectively, compared to treatments supplemented in conjunction with KIN (Tallón et al., 2013). In *Punica granatum*, the greatest formation of adventitious shoots from cotyledonary explants was also obtained in a medium supplemented with 2 mg L^{-1} BA (Parmar, 2012). The number of adventitious shoots observed for *D. regia* in the present study was low. Previous studies with this species also reported the low morphogenetic potential of *D. regia* that presented few morphogenetic responses (Myers and Vendrame, 2004; Abdi and Hedayat, 2011). Further studies are still needed to optimize the induction and elongation stages of *D. regia* adventitious shoots.

The histological analysis corroborated the macroscopic observations showing that no morphogenetic response was observed in the explants cultured in the absence of cytokinin (Figure 2A). In treatments supplemented with KIN and BA, morphogenetic responses started from mesophyll cellular divisions, in the explant section region (Figure 2B, C). The cotyledonary explant mesophyll was dorsiventral consisting of 3-4 layers of palisade parenchyma and 10-15 layers of spongy parenchyma (Figure 2A).

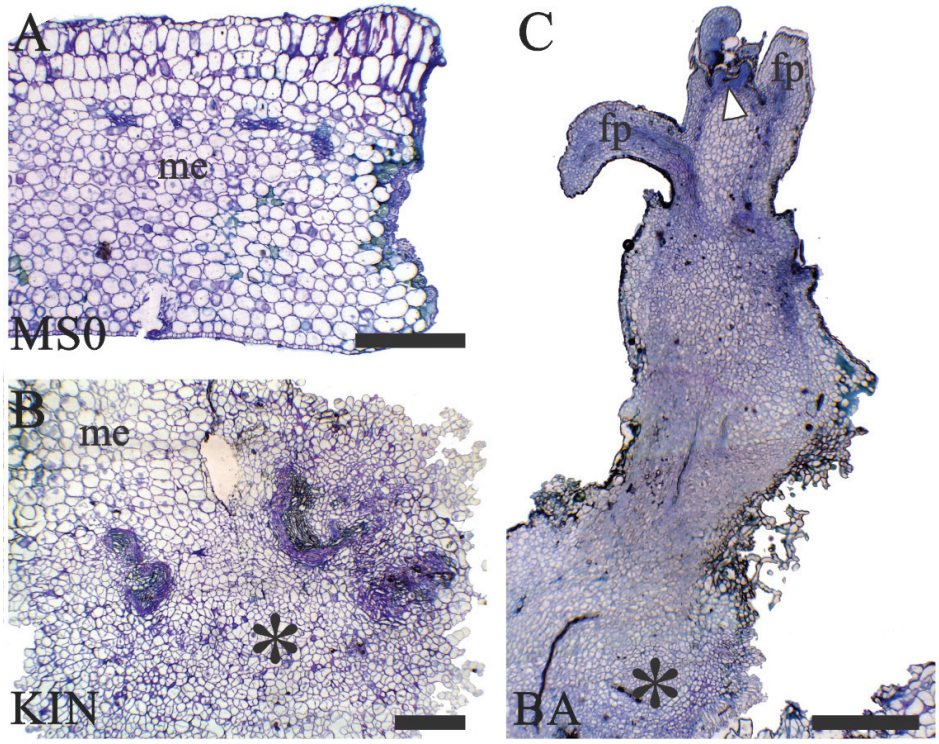

Figure 2. Histological characterization of *D. regia* cotyledonary explants after 40 days of culture. (A) Cotyledonary explant cultured in MS medium without plant growth regulator. (B, C) Cotyledonary explants cultured in media supplemented with 2.0 mg L^{-1} of KIN (B) and BA (C). Abbreviations: arrowhead – promeristem; asterisk – callus; fp foliar primordium; me mesophyll. Bars = 500 µm.

The mesophyll cells of cotyledonary explants cultured in media supplemented with cytokinins presented an intense process of cell division (Figure 2B, C). However, in the treatment supplemented with KIN, a progressive effect of cell proliferation was observed (Figure 2B). Most peripheral cells divided and hypertrophied, appearing voluminous and vacuolized, the typical appearance of callus cells (Figure 2B). No other morphogenetic response, except for the callus, was observed. Calli were also observed in the treatment supplemented with BA. However, in the callus periphery, adventitious shoots were histologically structured consisting of leaf beginnings and a promeristem, consistent with the organogenic regeneration pathway (Figure 2C). The results obtained suggest there are differences in the recognition or action mechanism of BA and KIN, since adventitious shoot differentiation was observed mainly in the presence of BA, even if both constitute purine-derived cytokinins.

Conclusions

Cytokinin supplementation was essential to induce *in vitro* morphogenetic responses from *D. regia* cotyledonary explants. All treatments supplemented with BA showed higher percentage of calli and fresh weight gain in comparison to KIN. Adventitious shoots were observed mainly in BA-treatments too. Although the conversion of shoots into plants was not observed, we believed that the results obtained may contribute to the establishment and optimization of *in vitro* regeneration systems of this important ornamental woody species.

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Author Contribution

A.O.C. 0000-0002-4481-129X and **D.I.R.** 0000-0001-6683-0961 designed the research project; **A.O.C., L.A.S.S.** 0000-0003-0450-1809, **V.F.S.** 0000-0001-8043-7584 and **M.M.** 0000-0002-8491-5691 established the in vitro cultures; **A.O.C., L.A.S.S.** and **I.M.D.** 0000-0003-2012-0838 carried out the microscopy analyses; **A.O.C., M.M.** and **G.Z.S.** 0000-0002-6380-1599 carried out the statistical analyses; **A.O.C.** and **D.I.R.** wrote the paper; **M.M.R., G.Z.S.** and **M.L.S.** 0000-0001-6928-285X revised the paper.

References

ABDI, G.; HEDAYAT, M. Induction of somatic embryogenesis from immature zygotic embryo and immature seed of royal poinciana (*Delonix regia*). **World Applied Sciences Journal**, v.13, n.3, p.391-395, 2011.

ADETOGUN, G.E.; ALEBIOWU, G. Properties of *Delonix regia* seed gum as a novel tablet binder. **Acta Polica Pharmacology**, v.66, p.433-438, 2009.

BEZERRA, R.M.F.; ALOUFA, M.A.I.; FREIRE, F.A.M.; SANTOS, D.D. Efeito de 6-benzilaminopurina sobre a propagação *in vitro* de *Mimosa caesalpiniifolia* Benth. (Fabaceae) **Revista Árvore**, v.38, n.5, p.771-778, 2014

BUAH, J.N.; DANSO, E.; TAAH, K.J.; ABOLE, E.A.; BEDIAKO, E.A.; ASIEDU, J.; BAIDOO, R. The effects of different concentrations cytokinins on the *in vitro* multiplication of plantain (*Musa* sp.). **Biotechnology**, v.9, n.3, p.343-347, 2010.

CASTILHO, C.V.V.; LEITÃO, S.G.; SILVA, V.D.; MIRANDA, C.O.; SANTOS, M.C.S.; BIZZO, H.R.; SILVA, N.C.B. *In vitro* propagation of a carvacrol-producing type of *Lippia origanoides* Kunth: A promising oregano-like herb. **Industrial Crops and Products**, v.130, n.1, p.491-498, 2019.

COSTA, A.O.; SILVA, L.A.S.; DUARTE, I.M.; ROCHA, M.M.; SILVA, G.Z.; SILVA, D.F. P.; NETTO, A.P.C.; ROCHA, D.I. Shoot proliferation and *in vitro* organogenesis from shoot apex and cotyledonary explants of royal poinciana (*Delonix regia*), an ornamental leguminous tree. **Trees**, 2019. (Submited)

FATMAWATY, R.; AMANDA, A.; INNAYAH, S.; VIVITRI, D.P. Antimalarial Effect of Flamboyant (*Delonix regia*) Bark and Papaya (*Carica papaya* L.) Leaf Ethanolic Extracts against *Plasmodium berghei* in Mice. **Biomedical & Pharmacology Journal**, v.10, n.3, p.1081-1089, 2017.

FRÁGUAS, C.B.; PASQUAL, M.; PEREIRA, A.R. Multiplicação *in vitro* de *Ficus carica* L.: efeito da cinetina e do ácido giberélico. **Ciência e Agrotecnologia**, v.28, n.1, p. 49-55, 2004.

GARCÍA-ANGULO, P.; VILLAR, I.; GINER-ROBLES, L.; CENTENO, M.L. *In vitro* regeneration of two *Populus* hybrid clones. The role of pectin domains in cell processes underlying shoot organogenesis induction. **Biologia Plantarum**, v.62, n.4, p.763-774, 2018.

HOLLANDER, M.; WOLFE, D.A. **Nonparametric Statistical Methods**, 2nd edition. John Wiley and Sons, New York. 1999.

KLEM, M.; BALLA, J.; MACHACKOVA, B.I.; EDER, J.; PROCHAZKA, S. The uptake and Metabolism of Benzylaminopurine in tobacco (*Nicotiana tabacum* L.) and Cucumber (*Cucumis sativus* L.) explants. **Plant Growth Regulator**, v.31, n.1 p.135-142, 2004.

MAGYAR-TÁBORI, K.; DOBRÁNSZKI, J.; SILVA, J.A.T.; BULLEY, S. M.; HUDÁK, I. The role of cytokinins in shoot organogenesis in apple. **Plant Cell Tissue Organ Culture**, v.101, p.251-267, 2010.

MÜLLER, D.; LEYSER, O. Auxin, cytokinin and the control of shoot branching. **Annals of Botany**, v.107, n.7, p.1203-1212, 2011.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia plantarum**, v.15, n.3, p.473-497, 1962.

MYERS, A.R.; VENDRAME, W.A. Propagation of yellow kampong royal poinciana via somatic embryogenesis. **Proceedings of the Florida State Horticultural Society**, v.117, n.1, p.338-341, 2004.

NETO, E.M.L.; SOUZA, R.M. Comportamento e características das espécies arbóreas nas áreas verdes públicas de Aracaju, Sergipe. **Scientia Plena**, v.7, n.1. p.1-10, 2011.

O'BRIEN, T.P.; MCCULLY, M.E. **The study of plant structure principles and selected methods**. 1981.

PARMAR, N.; KANWAR, K.; THAKUR, A.K. *In Vitro* Organogenesis from Cotyledon Derived Callus Cultures of *Punica granatum* L. cv. Kandhari Kabuli. **National Academy Science Letters**, v.35, n.3, p.215-220, 2012.

PEREIRA, A.M.S.; BERTONI, B.W.; MORAES, R.M.; FRANCA, S.C. Micropropagation of *Salix humboldtiana* Hild. **Revista Brasileira de Plantas Medicinais**, v.2, n.2, p.17-21, 2000.

RAHMAN, A. Allelopathic potential of *Parthenium hysterophorus* L. on *Cassia* sp. **Allelopathy Journal**, v.18, n.2, p.345-354, 2006.

RODRIGUEZ-CANTO, W.; CHEL-GUERRERO, L.; FERNANDEZ, V.V.A.; AGUILAR-VEJA, M. *Delonix regia* galactomannan hydrolysates: Rheological behavior and physicochemical characterization. **Carbohydrate polymers**, v.206, p.573-582, 2019.

SHABIR, G.; ANWAR, F.; SULTANA, B.; KHALID, Z.M.; AFZAL, M.; KHAN, Q.M. Antioxidant and antimicrobial atributes and phenolics of different solvent extracts from leaves, flowers and bark of Gold Mohar [*Delonix regia* (Bojer ex Hook.) Raf]. **Molecules**, v.16, n.9, p.7302-7319, 2011.

SHARMA, P.; BABEL, P.; GOSWAMI, N.; PUROHIT, S.D. Micropropagation of *Acacia leucophloea* (Roxb.) Willd. A multi-purpose legume tree. **Proceedings of the National Academy of Sciences, India Section B: Biological Sciences**, v.88, n.4, p.1329-1335, 2017.

SINGH, A.K.; CHAND, S.; PATTNAIK, S.; CHAND, P.K. Adventitious shoot organogenesis and plant regeneration from cotyledons of *Dalbergia sissoo* Roxb.; a timber yielding tree legume. **Plant Cell Tissue Organ Culture**, v.68, n.2, p.203-209, 2002.

SU, Y.H.; LIU, Y.B.; ZHANG, X.S. Auxin-cytokinin interaction regulates meristem development. **Molecular Plant**, v.4, n.4, p.616-625, 2011.

TALLÓN, C.I.; PORRAS, I.; TORNERO-P, O. High efficiency *in vitro* organogenesis from mature tissue explants of *Citrus macrophylla* and *C. aurantium*. ***In Vitro* Cellular & Developmental Biology-Plant**, v.49, n.2, p.145-155, 2013.

WANG, L-S.; LEE, C-T.; SU, W-L.; HUANG, S-C.; WANG, S-C. *Delonix regia* leaf extract (DRLE): a potential therapeutic agent for cardioprotection. **PloS One**, v.11, n.12, p.1-17, 2016.

YADAV, R.; YADAV, N.; KUMAR, S. An improved micropropagation and assessment of genetic fidelity in multipurpose medicinal tree, *Acacia auriculiformis*. **Proceedings of the National Academy of Sciences, India Section B: Biological Sciences**, v.86, n.4, p.921-929, 2016.

ZARINJOEI, F.; RAHMANI, M. S.; SHABANIAN, N. *In vitro* plant regeneration from cotyledon-derived callus cultures of leguminous tree *Gleditsia caspica* Desf. **New Forest**, v.45, n.6, p.829-841, 2014.