In vitro germination of Abricó-de-Macaco (Couroupita guianensis Aubl.)
zygotic embryos in different culture media and light spectra

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ABSTRACT: Couroupita guianensis Aubl. is an Amazonian forest species with important medicinal and ornamental value. This study evaluated the effect of different culture media and light spectra on the in vitro germination and development of the zygotic embryos of C. guianensis. The culture media, MS and WPM, were evaluated without the addition of plant growth regulators and were associated with four LED light spectra: white (CW), 70% red + 30% blue (R2B), 100% red (R), and 100% blue (B). One hundred percent of the seeds successfully underwent in vitro germination, and the culture media did not interfere with embryo development. In addition to this, the different light spectra induced in vitro morphogenesis and R2B treatment significantly promoted the production of secondary roots. This effect may aid in the rooting and acclimatization of seedlings of this species.

Key words: morphogenesis, tissue culture, propagation, Couroupita guianensis Aubl.

INTRODUCTION

Couroupita guianensis Aubl., known in Brazil as the Monkey-Apricot, is a species of medicinal plant from the Amazon and is popularly used as a potent anti-inflammatory agent, administered through infusions made from its leaves, flowers, and bark (PINHEIRO et al., 2010). In some countries, such as India, it is widely used as an ornamental plant known as the Cannon Ball Tree. However, indiscriminate use in folk medicine by the Indians has drastically reduced its population (SHEKHAWAT & MANOKARI, 2016). In Brazil, deforestation is the persistent cause of the reduction in its native population year after year. In nature, the propagation of C. guianensis is exceptionally slow, being restricted to the germination of seeds that are recalcitrant and difficult to store (GOUSIA et al., 2013). In addition, low germination rate occurs frequently in seeds germinated directly in the soil (MUNISWAMY & SREENATH, 2000). Thus, tissue culture is a tool that could contribute to the multiplication of this species. The selection of the explant and the culture medium are basic conditions for establishing an in vitro propagation protocol or in vitro conservation method (JIANG et al., 2012). The cultivation medium, MS (MURASHIGE & SKOOG, 1962), has proven to be efficient and responsive in most herbaceous species such as banana, pineapple, and strawberry (GALAN et al., 2018; SOUZA et al., 2018; MOZAFARI et al., 2018). However, woody species have other needs, especially for basic salts. The Woody Plant Medium (WPM) used in culturing
(LLOYD & MCCOWN, 1980) has a more diluted composition, with 25% of the concentration of nitrate and ammonium, than the MS medium. For woody plants, the WPM medium has proven to be more responsive and resulted in better propagation rates (YEUNG et al., 2015; SHAHZAD et al., 2017). In tissue culture, different light spectra from light-emitting diodes (LEDs) have been widely studied and used in commercial and research laboratories. Light influences plant morphogenesis during in vitro development, which may cause a reduction in explant size, root emission and length, number of stomata in the leaves, and chlorophyll content (POUDEL et al., 2008; MACEDO et al., 2011). Therefore, this study investigated the effect of different culture media associated with different spectra of LED light on the in vitro germination and development of C. guianensis embryos.

Zygotic embryos from C. guianensis seeds, collected from ripe fruits, were used as the plant material. The collection took place 330 days after flowering. During collection, the fruits were opened, and the seeds were extracted, of which 48 were selected. The seeds were soaked in 50% (v/v) sodium hypochlorite solution (2.5% active chlorine) for 10 min to remove the pulp. The asepsis of the seeds was performed inside a laminar flow, using sodium hypochlorite solution (2.5% active chlorine) at 20% (v/v) for 15 min followed by three washes in sterile distilled water. Then, the embryos were excised and transferred to Pyrex® test tubes (15 cm long by 2.5 cm in diameter) with 25 mm diameter Kimble polypropylene caps, each containing 10 mL of two types of culture medium, MS and WPM. Both media were supplemented with 3.0% sucrose, gelled with 0.2% Phytagel®, and had their pH adjusted to 5.8. After inoculation, the embryos were kept in a growth room under four different spectra of LED light (GreenPower TLED-Philips™): white (CW), 70% red + 30% blue (R2B), 100% red 645-675 nm (R), and 100% blue 450–465 nm (B). The light intensity was adjusted to 50 µmol m⁻² s⁻¹ (LI-200 Liquor), at a temperature of 25 °C ± 2 °C, and photoperiod of 14/10 h (day/night). For each treatment, the test tubes were centralized and arranged in an equidistant manner to uniformly receive the evaluated spectrum. At the end of 30 days, the following variables were measured: seedling height (from the vegetative apex to the root neck in cm); root length (from the root neck to the tip of the root in cm); number of secondary roots (unit); chlorophyll contents a, b (ChlorofiLOG - Falker CFL-1030), and total; and total fresh weight (g) and total dry weight (g). The data were analyzed using a completely randomized design in a 4 × 2 factorial scheme (4 LED light spectra × 2 culture media). The trial comprised eight treatments with six replicates per treatment; each repetition was formed from four test tubes, totaling 192 tubes. Before applying analysis of variance, the data were submitted to exploratory analysis to verify whether the assumptions were being met. After analysis of variance, multiple comparisons were performed using Tukey’s HSD test. The analyses were performed using the R and SAS software, with an α significance level of 0.05. The level of significance adopted for the unfolding of interactions was equal to 0.25.

The excised embryos showed 100% in vitro germination in the two treatments of different culture media. Collecting the C. guianensis fruits at the ideal maturation stage was most likely the reason for the in vitro germination of 100% of the embryos. A similar result was obtained by MUNISWAMY and SREENATH (2000), who reported 95% germination of C. guianensis embryos while using MS culture medium with only half of the concentration of salts and was supplemented with 0.1 mg/L of the cytokinin, BA (benzyladenine). The use of exogenous cytokinin helps to facilitate successful in vitro germination; its promoting effects are mainly related to the relief of stress factors (NIKOLIĆ et al, 2006). The concentration of 0.1 mg/L BA probably contributed to the high germination percentage of the embryos, given that the fruits were harvested after only 240 days and not fully ripe as in the present study (330 days). Thirty days after inoculation, no significant differences were observed between the culture media for most of the characteristics evaluated in the C. guianensis embryos (Table 1). However, a different effect was observed in studies with native plants such as baru (Dipteryx alata Vog), cajú do cerrado (Anacardium othonianum Rizz), and caçari (Myrciaria dubia Kunth), which evaluated different concentrations of salts and culture media in the in vitro development of explants from these species. In baru, studies have indicated that the 100% MS medium showed a better performance than the WPM and lower concentrations of MS (25%, 50%, and 75% MS) (ARARUNA et al., 2017). In the trial with cajú do cerrado, the MS (50% and 25%) and WPM (100% and 50%) media were the most efficient in plant regeneration (ASSIS et al., 2012). Meanwhile, in caçari, 100% WPM was superior to the MS and JADS medium at concentrations of 25%, 50%, 75%, and 100% (ARAÚJO et al, 2016). Notably, for some woody plants, the higher concentration of nitrogen salts has a negative effect on in vitro germination.
development. Generally, NO$_3^-$ is the preferred form for N assimilation in most plants. In some woody plants, NH$_4^+$ is not necessary, and in large quantities, it can be toxic (POOTHONG & REED, 2016), as was observed in cajú do cerrado, where the complete MS medium was found to be harmful to the development of in vitro culture (ASSIS et al., 2012). In the present study, there was a significant difference in fresh weight under CW treatment using MS medium (Table 1). However, despite the greater accumulation of fresh weight in the MS medium, in the WPM, the explants showed good formation without the occurrence of oxidation or phytotoxicity.

Table 1 - Mean (standard deviation) of shoot length (cm), root length (cm), number of secondary roots, total fresh weight (g), total dry mass (g), Chlorophyll a, b and Total, of C. guianensis zygotic embryos as a function of the culture media, MS and WPM and different light spectra.

| Culture media | CW (g) | R2B (g) | R (g) | B (g) |
|---------------|--------|---------|-------|-------|
| MS            | 9,07 (0,90) Aa | 7,52 (3,06) Aa | 7,72 (1,27) Aa | 7,98 (2,89) Aa |
| WPM           | 8,27 (1,17) Aa | 8,13 (1,02) Aa | 7,65 (1,28) Aa | 7,20 (0,75) Aa |
| VC (%)        | 22,14 |
| MS            | 4,03 (3,32) Aa | 6,37 (2,49) Aa | 5,07 (2,92) Aa | 7,38 (5,50) Aa |
| WPM           | 4,60 (2,05) Aa | 7,70 (3,28) Aa | 4,42 (2,46) Aa | 5,53 (2,62) Aa |
| VC (%)        | 29,92 |
| MS            | 6,50 (4,51) ABa | 12,00 (6,36) Aa | 5,00 (5,62) Ba | 6,33 (3,50) ABa |
| WPM           | 9,83 (6,65) ABA | 18,33 (7,53) Aa | 7,67 (5,89) Ba | 9,17 (8,82) ABA |
| VC (%)        | 40,91 |
| MS            | 2,02 (0,26) Aa | 1,80 (0,57) Aab | 1,52 (0,46) Ab | 1,80 (0,72) Aab |
| WPM           | 1,47 (0,31) Bb | 1,94 (0,24) Aa | 1,46 (0,44) Ab | 1,69 (0,42) Aab |
| VC (%)        | 26,56 |
| MS            | 0,32 (0,07) Aa | 0,30 (0,09) Aa | 0,24 (0,03) Aa | 0,29 (0,05) Aa |
| WPM           | 0,27 (0,02) Aa | 0,28 (0,05) Aa | 0,28 (0,02) Aa | 0,24 (0,02) Aa |
| VC (%)        | 18,14 |
| MS            | 24,28 (9,53) Aa | 26,93 (8,66) Aa | 16,58 (3,38) Aa | 19,52 (8,90) Aa |
| WPM           | 21,78 (7,51) Aa | 26,95 (7,77) Aa | 19,50 (5,52) Aa | 28,73 (8,32) Aa |
| VC (%)        | 33,37 |
| MS            | 5,70 (3,02) Aa | 6,55 (3,53) Aa | 3,47 (1,11) Aa | 4,70 (2,90) Aa |
| WPM           | 5,33 (2,48) Aa | 6,47 (2,40) Aa | 4,13 (1,73) Aa | 7,83 (4,60) Aa |
| VC (%)        | 31,15 |

Means followed by different letters differ (P ≤ 0.05), uppercase letters horizontally and lowercase letters vertical, within each culture media.
vitro germination of C. guianensis, regardless of the culture medium used. Using Heliconia cultivar in an in vitro conservation study using slow-grown storage with different wavelengths of the LED light spectra, the largest number of roots was observed in the mixed light treatment (70R30B) followed by the white light treatment (RODRIGUES et al., 2018). Nevertheless, proportions of 1:1 and 3:1 of the red and blue spectra did not show satisfactory results in the rooting of Protea cynaroides and Brassica napus (WU & LIN, 2012; LI et al., 2013). Therefore, it is evident that the effects of the available light spectra on the explant are probably specific to each species or cultivar. The higher occurrence of secondary roots in R2B may be a promising characteristic for the in vitro germination of mature embryos, thereby reducing losses in the acclimatization phase and optimizing this technique of producing C. guianensis seedlings. As reported by GOUSIA et al. (2013), the slow germination speed of C. guianensis and the recalcitrance of its seeds justify the application of this technique.

In conclusion, the culture media MS and WPM can be applied for the in vitro germination of zygotic embryos of C. guianensis. The R2B spectrum favors the formation of secondary roots in germinated embryos.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS’ CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

REFERENCES

ARARUNA, E. C.; RIBEIRO, O. J. P, et al. Salt concentrations in culture media for the development of Diplopterys alata in vitro. Pesquisa Agropecuária Brasileira, 2017; 52(12), 1295-1300. Available from: <https://www.scielo.br/pab/a/GWit/aK6h0bCGF-FKwwv8NhF/?lang=en>. Accessed: May, 25, 2021. doi: 10.1590/s1000-204x2017001200020.

ARAÚJO, M. C. R.; CHAGAS, E. A, et al. Micropropagation of caçari under different nutritive culture media, antioxidants, and levels of agar and pH. African Journal of Biotechnology, 2016; 15(33): 1771-1780. Available from: <https://academicjournals.org/journal/AJB/article-abstract/55E05EC01118>. Accessed: May, 25, 2021. doi: 10.5897/AJB2016.15417.

ASSIS, K. C.; PEREIRA, F. D, et al. In vitro cultivation of Anacardium othonianum Rizz.: effects of salt concentration and culture medium volume. Acta Scientiarum Agronomy, 2012; 34(1): 77-83. Available from: <https://periodicos.uerj.br/index.php/ActaSciAgrom/article/view/10968>. Accessed: May, 25, 2021. doi: 10.4025/actasciagron.v34i1.10968.

CHUNG, J. P.; HUANG, C. Y.; DAI, T. E. Spectral Effects on embryogenesis and plantlet growth of Oncidium ‘Gower Ramsey’. Scientia Horticulturae, 2010; 124: 511–516. Available from: <https://www.sciencedirect.com/science/article/pii/S0304423810004407?via%3Dihub>. Accessed: May, 25, 2021. doi: 10.1016/j.scienta.2010.01.028.

GALAN, V. et al. Propagación del banano: técnicas tradicionales, nuevas tecnologías e innovaciones. Revista Brasileira de Fruticultura, 2018; 40 (4): e-574. Available from: <https://www.scielo.br/j/rbf/a/2a10j9h4y53v5v57v999pGmGz/?lang=en>. Accessed: May, 25, 2021. doi: 10.1590/0103-900420180574.

GOUSIA, S. K.; ASHOK, K. K, et al. Biological activities and medicinal properties of Couroupita guianensis. International Journal of Pharmacy Pharmaceut Sci Res, 2013; 3(4):140–143.

JIANG, Q.; ZHANG, Y. et al. Establishment of an in vitro plant regeneration protocol for Casuarina cunninghamiana Miq. Via indirect organogenesis. New For, 2012; 43:143–154.

LI, H.; TANG, C.; XU Z. The effects of different light qualities on raapsedes (Brassica napa L.) plantlet growth and morphogenesis in vitro. Scientia Horticulturae, 2013; 150: 117-124. Available from: <https://www.sciencedirect.com/science/article/pii/S0304423812004955?via%3Dihub>. Accessed: May, 25, 2021. doi: 10.1016/j.scienta.2012.10.009.

MACEDO, A. F.; LEAL-COSTA, M. Y, et al. The effect of light quality on leaf production and development of in vitro-cultured plants of Alternanthera brasiliense Kunze. Environmental and Experimental Botany, 2011; 70: 43-50. Available from: <https://www.sciencedirect.com/science/article/pii/S0098847210001231?via%3Dihub>. Accessed: May, 25, 2021. doi: 10.1016/j.envexpbot.2010.05.012.

MULEO, R.; MORINI, S. Light Quality regulates shoot cluster growth and development of MM106 Apple genotype in in vitro culture. Scientia Horticulturae, 2006; 108: 364–370. Available from: <https://www.sciencedirect.com/science/article/pii/S0304423806000999?via%3Dihub>. Accessed: May, 25, 2021. doi: 10.1016/j.scienta.2006.02.014.

MUNISWAMY, B.; SREENATH, H. L. In-vitro development of plants from cultured embryos of cannon ball tree (Couroupita
In vitro germination of Abricó-de-Macaco (Couroupita guianensis Aubl.) zygotic embryos in different culture media and light spectra.

Ciência Rural, v.52, n.1, 2022.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 1962; 15: 473-497.

NIKOLIĆ, R., MITIĆ, N., MILETIĆ, R. et al. Effects of Cytokinins on *In Vitro* Seed Germination and Early Seedling Morphogenesis in *Lotus corniculatus L.*. *J Plant Growth Regul*, 2006; 25:187-194. Available from: <https://link.springer.com/article/10.1007%2Fs00344-005-0129-4>. Accessed: May, 25, 2021. doi: 10.1007/s00344-005-0129-4.

POUDEL, P. R.; KATAOKA, I.; MOCHIOKA, R. Effect of red- and blue-light-emitting diodes on growth and morphogenesis of grapes. *Plant cell, Tissue and Organ Culture*, 2008; 92: 147-153. Available from: <https://link.springer.com/article/10.1007%2Fs11240-007-9317-1>. Accessed: May, 25, 2021. doi: 10.1007/s11240-007-9317-1.

RODRIGUES, P. H. V.; ARRUDA, F.; FORTI, V. A. Slow-grown in vitro conservation of *Heliconia champneiana* cv. Splash under different light spectra. *Scientia Agricola*, 2018; 75(2), 163-166. Available from: <https://www.scielo.br/j/sa/a/pb7mszYd4rdsb4THSdgMHNP?lang=en>. Accessed: May, 25, 2021. doi: 10.1590/1678-992x-2016-0394.

Yeung, E. C. T.; Stasolla, C.; Sumner, M. J.; Huang, B. Q. (Ed.). *Plant microtechniques and protocols*. Nova York: Springer, 2015. 576p. Available from: <https://link.springer.com/book/10.1007%2F978-3-319-19944-3>. Accessed: May, 25, 2021. doi: 10.1007/978-3-319-19944-3.