BAP GROWTH REGULATOR EFFECT IN VITRO SHOOT MULTIPLICATION OF MELANOXYLON BRAUNA SCHOTT

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ABSTRACT

We report in this work an in vitro shoot multiplication method for Melanoxylon brauna Schott using nodal segments from germinated seeds in vitro. Plant material was cultivated on MS medium supplemented with 6-benzyladeninepurine (BAP: 1.11, 2.22 and 3.33 µM) and Naphthaleneacetic acid (NAA: 0.27 µM), combined with different amounts of subcultures for induction of shoot multiplication. Data were analyzed by non-parametric statistical Kruskal-Wallis test. Best results to shoot multiplication were obtained using 3.33 µM of BAP combined 0.27 µM of NAA in multiplication medium and performing one subculture, providing higher rates of shoots production per explant (average of 1.5) and high survival rate (94%). Although the impossibility to define a root induction medium, these results show a potential micropropagation to this Brazilian forest species.

KEYWORDS: Braúna; in vitro propagation; Organogenesis - Braúna; propagação in vitro; organogênese

INTRODUCTION

Know as Braúna, Melanoxylon brauna Schott, is originating in Brazilian Atlantic Forest biome, mainly in the south of Bahia, São Paulo and Minas Gerais, reaching 15 to 25m high. This species is known as one of the toughest and incorruptible, being used to several objectives, as building bridges, poles, fence posts, building constructions and manufacturing of musical instruments (SILVA et al., 2014). This biome has already undergone several phases of exploration, with logging was one of the main responsible for causing severe damage, destroying large areas of forest (FEARNSIDE, 2006; GONZAGA, 2006). Due to great difficulty of natural regeneration and exploitation, the species M. brauna is present in official documents of Brazilian government (IBAMA, 2016) classified as vulnerable. Brazilian government government have required of the logging companies more dedication to recover these degraded areas. Nevertheless, forest reminders still continue to suffer devastation.

In the last years, the interest in the propagation of native forest species has increased, aimed to restore the landscape and recover degraded areas, following the new environmental legislation requirements (ATAIDE et al., 2016). Some Brazilian native species have great potential in projects with environmental focus, as recovering degraded areas and to restore the landscape, due to better adaptation to the edaphoclimatic conditions and acting on the soil recovery (NIETSCHE et al., 2004). The species M. brauna presents great potential to be used in forest restoration in degraded areas. Seedlings of native woody species to be used in reforestation

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Therefore, the present work aimed to define a method for the in vitro multiplication of M. brauna for seedling production.

MATERIAL AND METHODS

The present research was conducted in the plant tissue culture laboratory at Instituto Federal do Sudeste de Minas Gerais, using M. brauna seeds from Forest Seeds Analysis Laboratory (LASF) at Universidade Federal de Viçosa – MG.

PLANT MATERIAL AND SURFACE STERILIZATION

The seeds were disinfected with 70% ethanol (1 min), sodium hypochlorite (NaOCl) 2% (15 min), washed 3 times with autoclaved distilled water in laminar air flow cabinet and then inoculated in test tube (with polypropylene cap) containing 10 mL of semi-solid MS half-strength medium (MURASHIGE & SKOOG, 1962), supplemented with 30 g L^{-1} of sucrose, 7 g L^{-1} of agar and 1.5 g L^{-1} of PVP (polyvinylpyrrolidone), with pH adjusted to 5.8 before autoclaving (120 °C for 15 minutes) and maintained at 25 ± 2 ºC, 16 hours of photoperiod and light intensity of 50 µ moles m^{-2} s^{-1} , for 30 days.

MULTIPLICATION OF SHOOTS

Nodal segments with one axillary bud (no leaves) from 30 days old plants (from in vitro germination) were inoculated into test tubes (20 x 150 mm) containing 10 mL of semi-solid MS medium supplemented with different concentrations of growth regulators (Table 1).

Table1. Concentration of growth regulators NAA and BAP to each treatment.

Treatment	NAA Concentration (µM)	BAP concentration (µM)
1	0	0
2	0.27	0
3	0.27	1.11
4	0.27	2.22
5	0.27	3.33

Due to limited plant material, was used just one concentration of NAA growth regulator. All experiment was maintained at 25 ± 2 ºC, 16 hours of photoperiod and light intensity of 50 µmol m^{-2} s^{-1}, for 90 days. Every 30 days, the explants were evaluated and subcultured (subculture 1, 2 and 3) in a fresh multiplication medium. Experimental design was completely randomized in a factorial scheme, with 5 replicates per treatment and 8 explants per replicate. The survival rate (shoots with healthy tissue), amount of buds formed and amount of leaves formed were evaluated at each subculture time.

STATISTICAL ANALYSIS

The data were analyzed using R language (R Core Team, 2018) version 3.4.1 and using the package agricolae (DE MENDIBURU, 2014) to perform the statistical tests (ANOVA or Kruskal-Wallis).

RESULTS AND DISCUSSION

Normality and homogeneity of variances tests were performed using Shapiro-Wilk (SHAPIRO; WILK, 1965) and tests, respectively (data not shown).Since the data did not meet the statistical assumptions of an ANOVA, the non-parametric Kruskal-Wallis test was performed. Shoots multiplication data were analyzed in relation to treatments independent of the amount of subcultures and subcultures independent of the treatments (Figure 1A-F), treatments within each subculture (Figure 2A-I) and subcultures within each treatment (Figure 3A-O)..

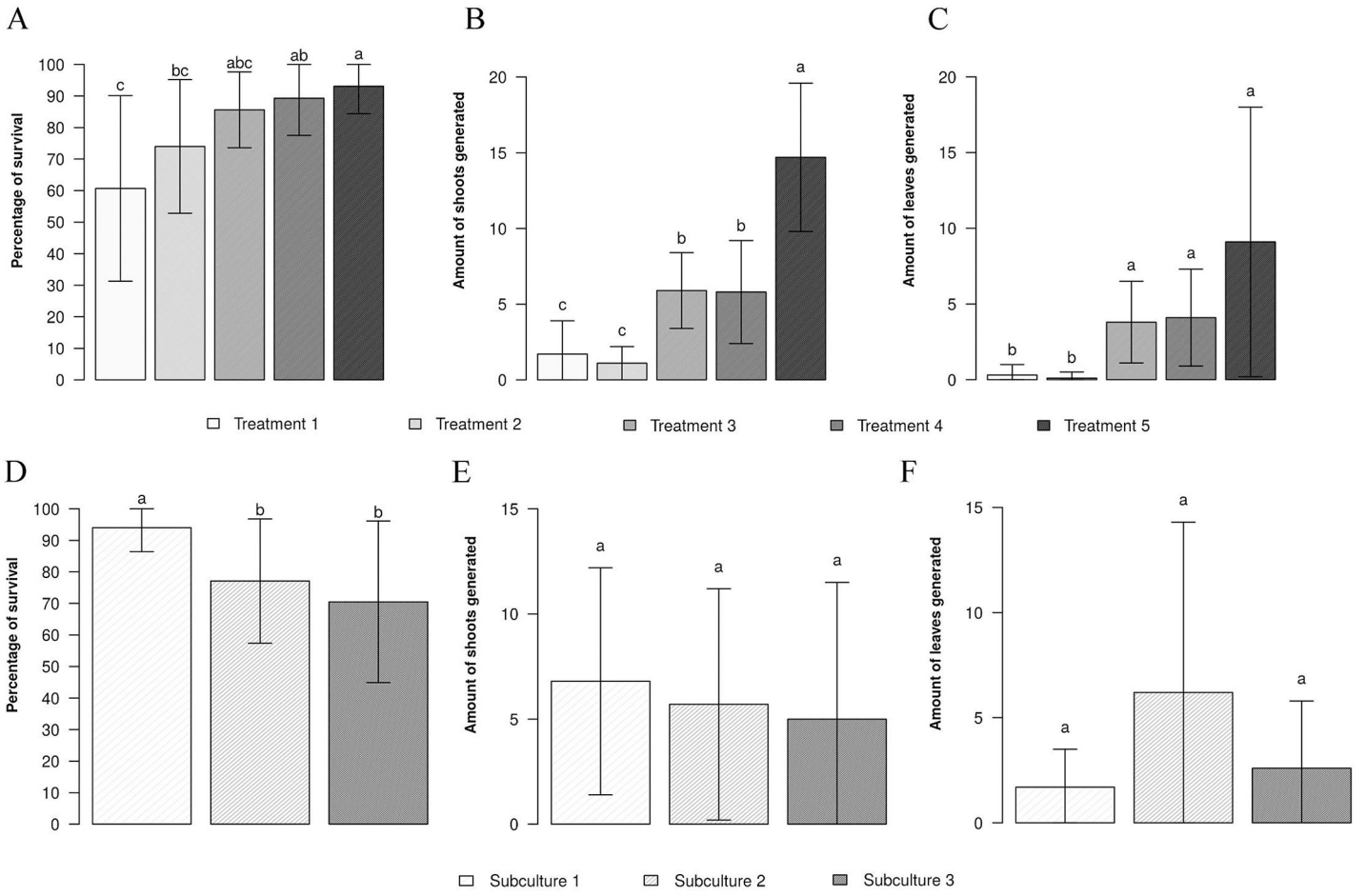

Figure 1. Evaluation of survival rate, shoots generated and leaves generated for explants of M. brauna under action of different treatments and amount of subculture to shoot multiplication. A-C, treatments comparison independent of subcultures; D-E, subcultures comparison independent of the treatments.

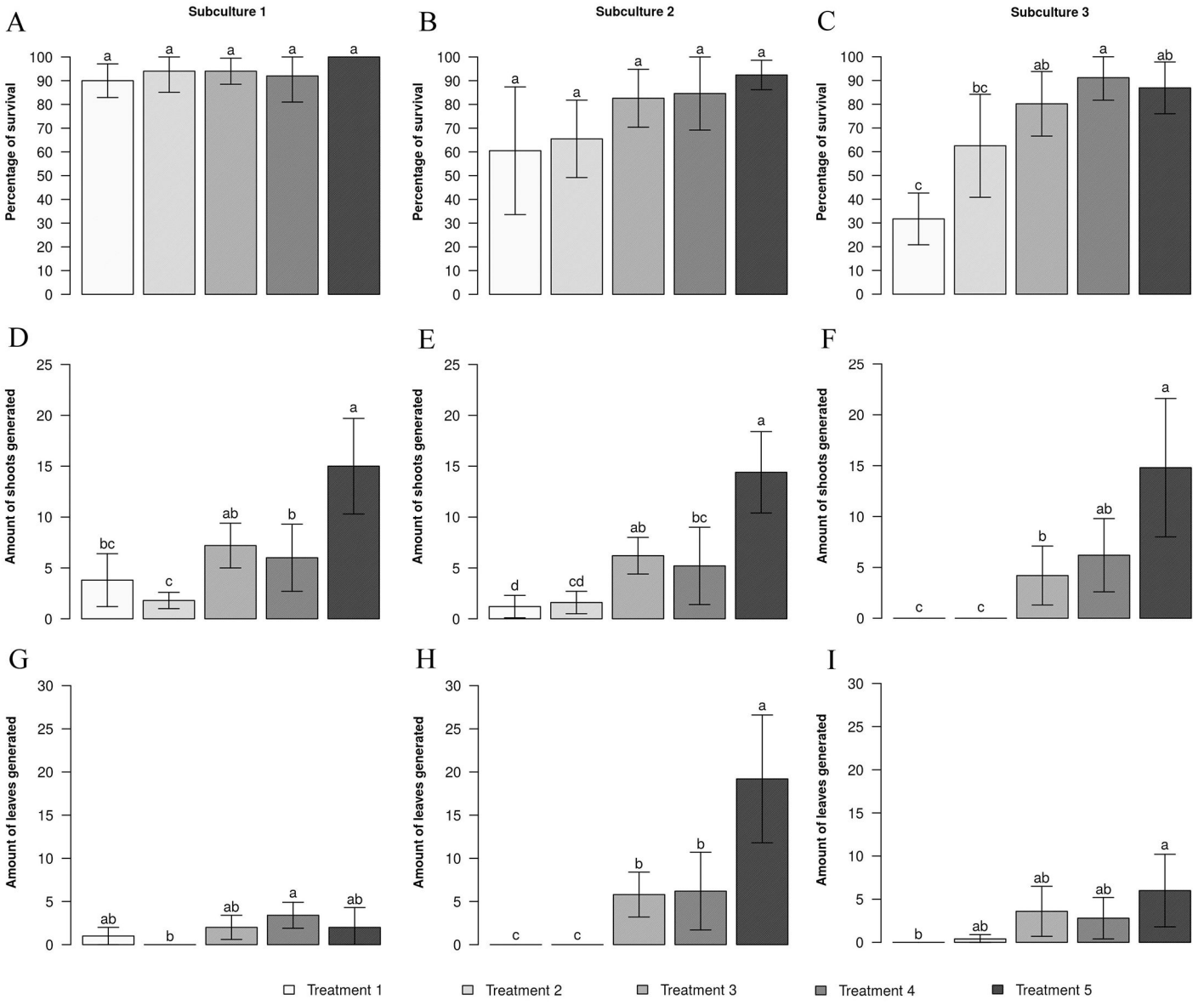

Figure 2. Evaluation of influence of the different treatments within each subculture on shoot multiplication of M. brauna; A-C, Survival rate comparison; D-F, Shoots generated comparison; G-I, Leaves generated comparison.

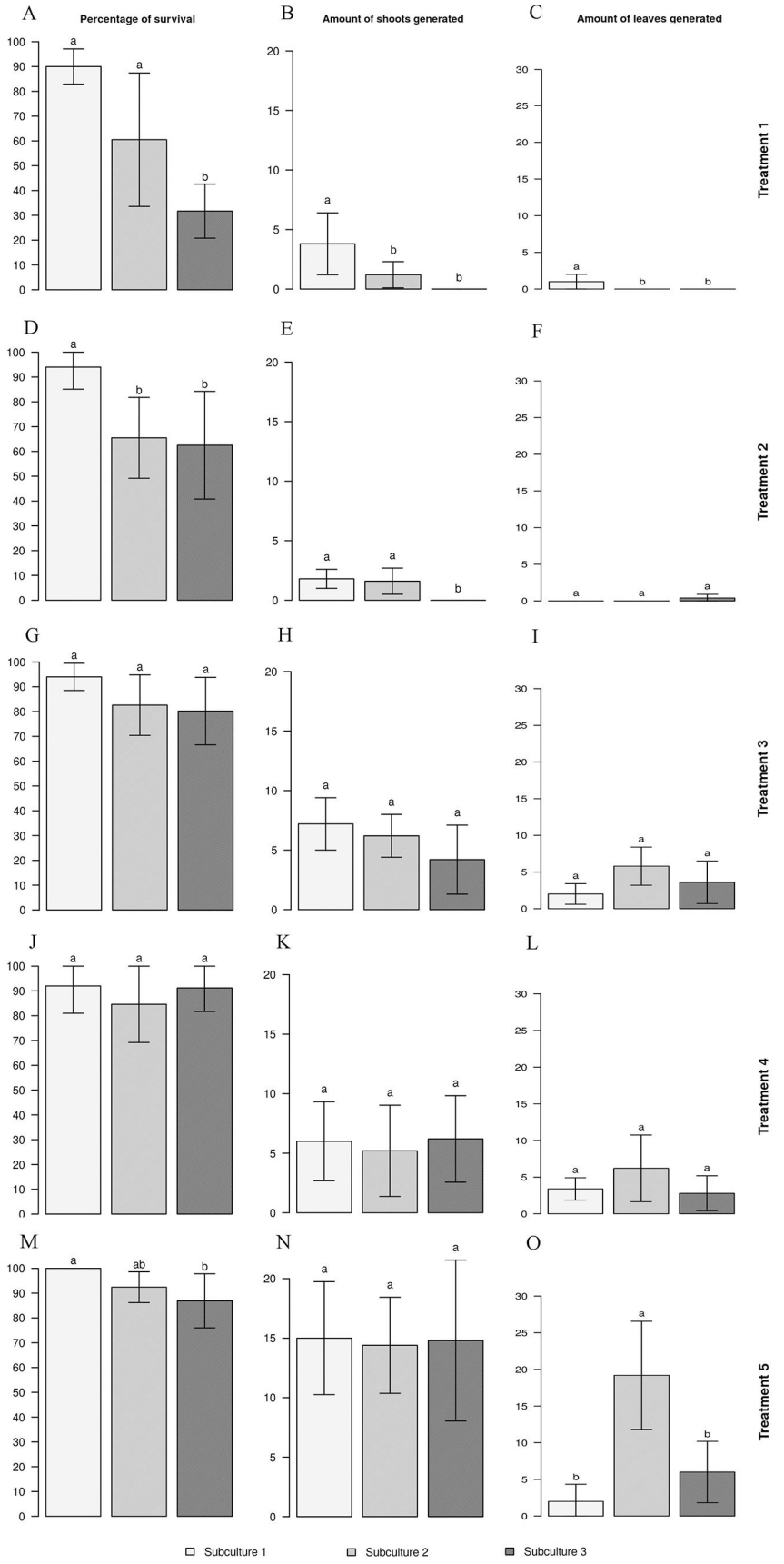

Figure 3. Evaluation of influence of the amount of subculture performed within each different treatments on shoot multiplication of M. brauna; A, D, G, J and M, Survival rate comparison; B, E, H, K and N, Shoots generated comparison; C, F, I, L and O, Leaves generated comparison.

Best results are observed using 2.22 and 3.33 µM of BAP (treatment 4 and 5, respectively) and performing one subculture, obtaining an average of 6 shoots and 3.4 leaves (using 2.22 µM of BAP) and an average of 15 shoots and 2 leaves (using 3.33 µM of BAP). Similar results were reported by Silva (2019), using different explants of M. brauna under influence of growth regulator BAP, obtaining high bud production and shoots using high BAP concentrations (4.44 and 8.88 µM). In fact, BAP is known as to induce the development of the superior part of the plant, showing high efficiency for production of shoots to Ceropegia noorjahaniae (CHAVAN et al., 2014, ADSUL et al., 2019, GREENWELL & RUTER, 2018) and development of shoots, leaves and buds (TRIPATHI & KUMARI, 2010, SANT'ANA et al., 2018).

Survival rate of the explants (absence of oxidation) shows a significant difference, reach best values (94%) using one subculture (30 days on multiplication medium). High survival rates of nodal segments in the M. brauna multiplication phase show high vigor of the selected tissues. PVP is used as antioxidant agent in plant tissue culture, and in micropropagation of woody species have a significant role in the low oxidation rate, as reported by Nikam et al. (2013), Pequeño-Granado et al. (2015) and Sant'Ana et al. (2018), working with Boswellia serrata Roxb, Jatropha curcas L. and Campomanesia rufa, respectively. Without use PVP in the medium, high auxin concentration can increase the tissue oxidation (Gomes-Copeland et al., 2017). The manipulation of explants in this work, each cultivation influenced the survival rate, increasing the tissue oxidation and consequently decreasing the number of surviving explants. Since the excision of new shoots to posterior subculture leaves explants subject to stress, phenolic compounds are generated, increasing the explant oxidation (PHULWARIA et al., 2011).

Shoots were produced in treatment 1, even without having ANA or BAP growth regulator in the culture medium, probably due to the endogenous concentrations of growth regulators in the explants. The same was reported by Ribas et al. (2005) in Aspidosperma polyneuron and Fermino Júnior and Scherwinski-Pereira (2012) in Amburana acreana explants. In our present study, multiplication rate of the plant material reached a maximum value of 1.5 shoots (average of treatment) per explant (Supplemented material T1). Working with different BAP concentrations to in vitro multiplication of Tectona grandis, Fermino Júnior et al. (2009) were able to obtain 1.75 shoots per explant, while Hubner et al. (2007) reached from 1.8 to 3.0 shoots per explant, working with different concentrations of BAP and NAA to in vitro multiplication of Aspidosperma ramiflorum.

Rooting process was not observed in all treatments, show that NAA concentration was not effective. Similar results were reported by Thuzar et al. (2011), obtaining just shoots formation when used a combination of NAA and BAP to regenerate zygotic embryos of oil palm. Nevertheless, Pádua et al. (2017) obtained success to rooting zygotic embryos of oil palm, had greater rooting rates (87%) using the auxin IBA (Indole Butyric Acid).

CONCLUSION

Although was not possible to define a complete micropropagation protocol due to absence success of root treatment, this work shows a possibility to multiplicate Melanoxylon brauna Schott from in vitro seeds germinated. The explants subculture decreases the micropropagation of this species. Using 3.33 µM of BAP provide best results to shoot multiplication, while 0.27 µM of NAA was not effective to induct rhizogenesis. More study is necessary to define an efficient NAA concentration capable to induct root process or use

others auxins, like IBA (Indole Butyric Acid) or IAA (Indole Acetic Acid).

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Resumo

Neste trabalho, relatamos um método de multiplicação in vitro para Melanoxylon brauna Schott, utilizando segmentos nodais oriundos de sementes germinadas in vitro. O material vegetal foi cultivado em meio MS suplementado com 6-benziladeninapurina (BAP: 0; 1,11, 2,22 e 3,33 µM) e ácido naftalenoacético (ANA: 0,27 µM), combinado com diferentes números de subcultivos para indução e multiplicação de brotos. Os dados coletados foram analisados pelo teste de Kruskal-Wallis (análise estatística não paramétrica). Os melhores resultados para indução e multiplicação de brotos foram obtidos utilizando meio MS suplementado com 3,33 µM de BAP combinado com 0,27 µM de ANA e realizando um subcultivo, fornecendo altas taxas de produção de brotos por explante (média de 1,5) e alta taxa de sobrevivência (94%). Apesar da impossibilidade de definir um meio de enraizamento, os resultados apresentados mostram o potencial de realização da multiplicação in vitro desta espécie florestal nativa.

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