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Effect of growth regulators BAP and NAA for in vitro multiplication and elongation of *Corymbia citriodora* juvenile explants

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ABSTRACT: *Corymbia citriodora is a species of great importance to Brazilian forestry. Vegetative propagation of selected Corymbia genotypes has been a challenge due to low rooting percentages. Micropropagation represents an alternative to advance the cloning techniques. This study aimed to evaluate the use of growth regulators BAP and NAA in vitro elongation and multiplication of Corymbia explants. A completely randomized experimental design was adopted, with six treatments defined by two culture medium compositions and three types of explants (MS I + base; MS I + intermediate; MS I + apical and MS II + base; MS II + intermediate; MS II + apical), with eight replications (tubes) with one explant per replication. The statistical analysis comprises of a residual normality test (Shapiro-Wilk), followed by an analysis of variance (ANOVA) using the F-test. We did not find a significant difference between treatments for the multiplication and elongation of Corymbia citriodora.*

Keywords: Vegetative propagation, cloning, micropropagation

Introduction

Vegetative propagation is widely employed in the forestry to increase production in planted forests (Mazette et al., 2020). High-quality seedlings, particularly of eucalyptus, favor final products with greater economic value (García-Quincoces et al., 2023). However, some species, such as *Corymbia citriodora*, exhibit recalcitrance to rooting (Lima et al., 2022), which can hinder or even make it unfeasible to propagate high-value genotypes for the industry through cuttings (Vilasboa et al., 2022).

Among the factors that influence the success of *in vitro* multiplication, the appropriate balance of growth regulators is crucial, with cytokinins and auxins being commonly employed (Asmar et al., 2011). Each micropropagation stage, species, and type of explant require different concentrations of these growth regulators (Miranda et al., 2016; Porfírio et al., 2019).

The use of the cytokinin 6-benzylaminopurine (BAP) and the auxin α-naphthaleneacetic acid

(NAA) in culture medium formulations for multiplication and elongation is common in *in vitro* cultivation (Esyanti et al., 2019). Our study aimed to evaluate the effect of those two growth regulators, as well as types of explants, on the *in vitro* elongation and multiplication of juvenile explants of *Corymbia citriodora.*

Material and Methods

The explants came from basal, intermediate and apical segments containing axillary buds. They were obtained from shoot buds of *Corymbia citriodora* seedlings produced via seminal and maintained *in vitro* on MS (Murashige and Skoog, 1962) culture medium with 50% salts and vitamins. Seeds were obtained from a forestry company located in Minas Gerais. Segments of approximately 2 cm were excised and established *in vitro* on MS medium supplemented with 30 g L-1 sucrose, 800 mg L-1 polyvinylpyrrolidone (PVP), and 6.5 g L-1 agar. In the first culture (MS I), concentrations of 0.5 mg L-1 BAP and 0.01 mg L-1 NAA were used, while in the second medium (MS II), concentrations of 0.1 mg L-1 BAP and 0.05 mg L-1 NAA were used. The pH was adjusted to 5.7±0.1 before adding the agar. The cultures grew in test tubes (150 mm long and 25 mm in diameter) containing 10 mL of culture medium and sealed with polypropylene caps. Sterilization was carried out in an autoclave at 121ºC and 1 atm pressure for 15 minutes. After inoculation, the explants were maintained under a photoperiod of 16 hours with a light intensity of 40 µmol m-2 s-1 and a temperature of 25±2ºC.

The experimental design was completely randomized with six treatments (MS I + basal, MS I + intermediate, MS I + apical, MS II + basal, MS II + intermediate, MS II + apical) and eight replicates (tubes) with one explant per replicate. After 26 days, the multiplication was evaluated by counting the number of lateral shoots emitted by each explant in each treatment. For elongation, the height of the shoots was measured using a graduated ruler. A residual normality test (Shapiro-Wilk) and analysis of variance (ANOVA) using the F-test were performed with the Exp.Des.pt package (Ferreira et al., 2021) in R software (R Development Core Team, 2014), and descriptive statistics using Excel software.

Results and discussion

According to the ANOVA, there was no significant difference between the treatments ($p > 0.05$) for the height of the shoot buds. The seedlings grown in the MS II culture medium had the greatest height growth (Table 1). These results may be explained by the fact that the MS II culture

medium has a lower cytokinin/auxin ratio compared to the MS I medium (Xavier et al., 2013). The lower ratio favors the direction of explant metabolism towards elongation rather than multiplication (Oliveira et al., 2011, Falcón-Bautista et al., 2018).

Table 1. Descriptive statistics for shoot height of *Corymbia citriodora*.

Treatment	Min	Max	Mean	Standard deviation
MS I + base	0.7	2.7	2.037	0.6760
MS I + intermediate	0.9	2.7	1.825	0.4950
MS I + apical	1	2.6	1.800	0.4899
MS II + base	1.7	3.9	2.475	0.8031
MS II + intermediate	1.1	2.6	1.962	0.5317
MS II + apical	1.5	2.2	1.875	0.2435

For the trait of number of shoots emitted per explant, the treatments did not differ statistically, as shown by ANOVA ($p > 0.05$). It was observed that the treatment with MS I medium and intermediate explant showed the highest mean value of multiplication, with 3.625 shoots per explant (Table 2). The higher ratio of cytokinin/auxin in the MS I medium, together with the use of the intermediate explant in which apical dominance is interrupted, are some of the factors that explain this superior response compared to the other treatments (Kaviani, 2015; Falcón-Bautista et al., 2018).

Table 2. Descriptive statistics for the number of shoots per explant of *Corymbia citriodora.*

Treatment	Min	Max	Mean	Standard Deviation
MS I + base	1	6	3.000	1.6036
MS I + intermediate	2	5	3.625	1.3025
MS I + apical	1	4	2.500	1.0690
MS II + base	2	4	3.250	1.3887
MS II + intermediate	2	5	3.125	1.3562
MS II + apical	2	5	2.875	1.3562

The results of this study refer to the first subculture of the multiplication phase. However, it was possible to verify that the growth regulators BAP and NAA, as well as the type of explant, can interfere in the *in vitro* multiplication and elongation process of *C. citriodora* (Nyunt and Aye, 2019).

Conclusion

The different treatments did not show statistically significant differences for the multiplication and elongation of *Corymbia citriodora* explants. Evaluation in successive multiplication subcultures is recommended.

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