



## MINI-CUTTING SIZE, MINI-TUNNEL MANAGEMENT AND PHYTOHORMONES IN THE CLONAL PROPAGATION OF *Eucalyptus urophylla* AND HYBRIDS OF *Corymbia* spp.

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### ABSTRACT

The area planted with *Corymbia* spp. clones is expected to increase in the coming years, despite their propagation challenges. This study aimed to evaluate mini-cutting size, mini-tunnel opening time, and the use of growth regulators in the clonal propagation of *Corymbia* spp. Four experiments were conducted using three clones: *C. citriodora* × *C. torelliana*, *C. torelliana* × *C. citriodora*, and *E. urophylla*, where *E. urophylla* was used as a control due to its ease of propagation. The results from each experiment were used to optimize the methodology for the subsequent one. Initially, three mini-cutting sizes were tested: 5, 10, and 15 cm in length. The second experiment evaluated three collection times of mini-cuttings after mini-tunnel opening: 0, 24, and 48 hours. The third experiment tested five concentrations of indole-3-butyric acid (IBA): 0, 500, 1000, 1500, and 2000 mg kg<sup>-1</sup>. Finally, five concentrations of a formulated product containing different plant growth regulators (0, 1, 2, 3, and 4 mL L<sup>-1</sup>) were assessed. All experiments were arranged in a randomized block design in a factorial scheme according to genotype. The following variables were evaluated: final rooted mini-cutting yield, survival, plant height, root length, and root, shoot, and total dry mass. Mini-cuttings of 15 cm performed best in promoting the growth of both hybrids. Regarding mini-tunnel opening time, the 24-hour interval after opening was optimal for *C. citriodora* × *C. torelliana*. Applications of 2000 and 1000 mg kg<sup>-1</sup> IBA promoted rooting in *Corymbia* spp., and rooted mini-cutting quality was improved with the application of 4 mL L<sup>-1</sup> of the growth regulator formulation.

**Keywords:** Forest nursery; Plant growth regulators; Forestry

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# TAMANHO DE MINIESTACAS, MANEJO DO ESTUFIM E FITOHORMÔNIOS NA PROPAGAÇÃO CLONAL DE *Eucalyptus urophylla* E HÍBRIDOS DE *Corymbia* spp.

**RESUMO** A área plantada com clones do gênero *Corymbia* spp. aumentará nos próximos anos, apesar de sua difícil propagação. O objetivo então foi avaliar o tamanho de miniestacas, tempo de abertura do estufim, e uso de reguladores de crescimento na clonagem de *Corymbia* spp. Foram montados quatro experimentos com três clones: *C. citriodora* x *C. torelliana*, *C. torelliana* x *C. citriodora* e *E. urophylla*, que atuou como controle pela facilidade na propagação, o resultado do experimento anterior otimizou o próximo. Inicialmente foram testados três tamanhos de miniestacas: 5, 10 e 15 cm de comprimento. No segundo três tempos de coletas das miniestacas após abertura do estufim: 0, 24 e 48 horas. Para o terceiro experimento foram testadas cinco concentrações de ácido indolbutírico AIB (0, 500, 1000, 1500 e 2000 mg kg<sup>-1</sup>) e por fim cinco concentrações de um formulado com diferentes fitorreguladores (0, 1, 2, 3 e 4 mL L<sup>-1</sup>). Os experimentos foram conduzidos em blocos casualizados em esquema fatorial em função do genótipo. Em cada experimento avaliou-se: aproveitamento final de mudas, sobrevivência, altura de plantas, comprimento de raiz, e massa seca de raízes, parte aérea e total. Miniestacas de 15 cm foram melhores para o crescimento de ambos híbridos, em relação ao tempo de abertura do estufim, verificou-se que o intervalo de 24 horas após a abertura foi o melhor para as miniestacas de *C. citriodora* x *C. torelliana*. Aplicações de 2000 e 1000 mg kg<sup>-1</sup> de AIB favorecem o enraizamento de *Corymbia* spp., que pode ter a qualidade de mudas melhorada com aplicação de 4 mL L<sup>-1</sup> do formulado com diferentes hormônios.

**Palavras-Chave:** Viveiro florestal;  
Reguladores de Crescimento vegetal;  
Silvicultura

## 1. INTRODUCTION

By 2070, it is projected that approximately 66% of roundwood will be supplied by forest plantations (Nepal et al., 2019). The vegetative propagation of superior genotypes is the foundation of clonal forestry, enabling plantations with higher genetic gains (Ouyang et al., 2015). *Eucalyptus urophylla* and *E. grandis* are the most widely planted species for energy purposes (Massuque et al., 2023). In addition to *Eucalyptus* spp. wood, *Corymbia* spp. wood has also been extensively used for charcoal production.

Interspecific hybrids of *Corymbia* are considered potential substitutes for eucalyptus wood in the pulp and paper industry (Costa et al., 2022). The features of *Corymbia* that have attracted attention include higher drought resistance, greater wood density, faster growth, and increased tolerance to pests and diseases (Souza et al., 2020; Costa et al., 2022). Interspecific hybrids have been developed to combine desirable traits for example, *C. torelliana* is used for its good rooting capacity, while *C. citriodora* is selected for its higher wood volume increment and adaptability to diverse edaphoclimatic conditions (Reis et al., 2013).

The first step in the production of rooted mini-cuttings through mini-cutting is the collection of mini-cuttings, and their size can influence rooted mini-cutting development. The ideal size varies according to the plant species, which induces variations in the rhizogenic process and in the development of vegetatively propagated plants (Pimentel et al., 2021; Vigl et al., 2014).

Another important factor in rooted mini-cutting production is the condition of the mini-garden, such as the use of the mini-tunnel. This technology allows for increased productivity of mini-stumps and rooting of mini-cuttings (Rocha et al., 2023). The plastic prevents transpiration from the vegetative material from directly escaping into the external environment, creating a humid and warm microclimate (Rocha et al., 2022; Lima et al., 2022). However, there is limited literature on studies reporting the optimal timing for mini-tunnel opening before mini-cutting collection.

The use of hormones to enhance adventitious rooting is a common practice in



the forestry sector. This has enabled the commercial planting of species/clones with difficult rooting, such as *Corymbia* genotypes. However, their effectiveness can be highly variable, as adventitious root formation depends on the genotype, age, tissue lignification, and the concentration of endogenous hormones in the mother plant (Pant et al., 2022).

The response of woody species to hormone application and its impact on adventitious root formation is still not fully understood (Zhao et al., 2022). This highlights the need for continued research in this area. Alternatives to overcome rooting recalcitrance have been increasingly explored (Rocha et al., 2022), such as the application of indole-3-butyric acid (IBA) and other regulators that combine plant hormones.

IBA is a synthetic auxin widely used in forest nurseries to promote rooting of cuttings and adventitious root formation (Pant et al., 2022; Zhao et al., 2022). In the production of *Corymbia* and *Eucalyptus* rooted mini-cuttings, IBA is used to stimulate rooting, resulting in more vigorous plants (Abiri et al., 2020; Oliveira et al., 2024).

Combinations of growth regulators using different plant hormones, including auxin, gibberellin, and cytokinin, aimed at improving plant growth and development, may also be an alternative (Soares et al., 2023). These regulators have been used to enhance rooting and vegetative growth in various agricultural species. Despite their widespread application, they have not yet been used for treating *Eucalyptus* or *Corymbia* cuttings, representing a potential alternative for the forestry sector.

Thus, aiming to optimize the clonal rooted mini-cutting production process, the objective was to determine the mini-cutting size, mini-tunnel opening time, and growth regulator concentrations in the clonal propagation of *Corymbia* through Mini-cutting.

## 2. MATERIAL AND METHODS

### 2.1 Genotypes and clonal mini-garden formation

The experiments were conducted in a commercial nursery in the municipality of Itamarandiba, Minas Gerais, Brazil, with climatic conditions and mini-garden

establishment methodology by Rocha et al. (2023). Three genotypes were used: two *Corymbia* hybrids, *C. citriodora* × *C. torelliana* (C1) and *C. torelliana* × *C. citriodora* (C2), and a spontaneous hybrid of *E. urophylla* (C3) as a control.

The troughs were covered by a mini-tunnel, which had a tubular structure with dimensions of 0.8 m × 16.3 m × 0.50 m, with a galvanized steel base covered by polyethylene plastic film, 150 µm thick. The first experiment was set up six months after the formation of the mini-garden.

### 2.2 Experimental Procedure

Four sequential experiments were conducted, with the results of each experiment used to optimize the methodology of the subsequent one. All were established under the same conditions. Mini-cuttings were planted 2 cm deep from the basal end in conical tubes with a capacity of 55 cm<sup>3</sup>. The substrate consisted of 70% coconut fiber and 30% rice husk, supplemented with: 1.0 kg m<sup>-3</sup> of single superphosphate, 1.0 kg m<sup>-3</sup> of MAP, and 2.0 kg m<sup>-3</sup> of Osmocote® (NPK 19-06-10). The leaves of the mini-cuttings were not trimmed.

After planting each experiment, the trays remained for 30 days in a greenhouse, covered with transparent polyethylene plastic film with a thickness of 150 µm, with an internal temperature of approximately 38 °C and relative humidity maintained between 80% and 90%. Irrigation was performed with a flow rate of 85 L h<sup>-1</sup> (1.4 L min<sup>-1</sup>), operating for 35 seconds every 40 minutes.

After the 30 days, the rooted mini-cuttings were transferred to the growth and hardening area. In this environment, weekly top dressing fertilizations were carried out. This fertilization was maintained until the rooted mini-cuttings reached a height of 20 cm, at which point the hardening process was initiated.

### Experiment 1 – Mini-cutting Size

The experimental design consisted of a randomized block design with nine treatments arranged in a 3 × 3 factorial scheme, comprising three clones (C1, C2, and C3) and three mini-cutting sizes (5, 10, and 15 cm in length). The mini-cuttings were collected from the clonal mini-garden (MG)

24 hours after the opening of the mini-tunnels, according to their respective sizes and clones. Four blocks were used, with 44 mini-cutting per plot, totaling 176 per treatment, the other experiments also followed this pattern of mini-cutting quantity.

### Experiment 2 – Mini-Tunnel Opening Time

The experimental design followed a randomized block design with nine treatments arranged in a  $3 \times 3$  factorial scheme, consisting of three clones (C1, C2, and C3) and three mini-cutting collection times (0 – immediately after the mini-tunnel opening, 24, and 48 hours after the mini-tunnel opening). Four blocks, each containing 176 mini-cuttings, were used per treatment. The mini-cutting size for the *Corymbia* hybrids was 15 cm, while for *Eucalyptus urophylla* it was 10 cm.

### Experiment 3 - Growth Regulator 1 (GR1)

The experiment followed a randomized block design (RBD), arranged in a  $3 \times 5$  factorial scheme corresponding to three genetic materials (C1, C2, and C3) and five concentrations of GR1 (0, 500, 1000, 1500, and 2000 mg kg<sup>-1</sup>).

The mini-cuttings from each genetic material were collected from the clonal mini-garden (MG) 24 hours after the opening of the mini-tunnels, with standardized sizes (15 cm for *Corymbia* genotypes and 10 cm for *E. urophylla*). They were then subjected to their respective treatments (0, 500, 1000, 1500, and 2000 mg kg<sup>-1</sup>). GR1 was applied in powder form to the basal portion of the mini-cuttings, which were planted immediately after the application.

### Experiment 4 - Growth Regulator 2 (GR2)

The experiment was conducted using a randomized complete block design (RCBD), with a  $3 \times 5$  factorial scheme, consisting of three genetic materials (C1, C2, and C3) and five concentrations of GR2 (0, 1, 2, 3, and 4 mL L<sup>-1</sup>), with four blocks and 176 mini-cuttings per treatment. The GR2 used was Stimulate® composed of 0.09 g L<sup>-1</sup> of kinetin, 0.05 g L<sup>-1</sup> of gibberellic acid, and 0.05 g L<sup>-1</sup> of 4-indole-3-butyric acid.

The mini-cuttings of each genetic material were collected from the clonal mini-

garden (MG) 24 hours after the opening of the mini-tunnel, with standardized sizes (15 cm for the *Corymbia* hybrids and 10 cm for *E. urophylla*). IBA was applied in powder form to the base of the mini-cuttings, at a concentration of 2000 mg kg<sup>-1</sup> for *C. citriodora* x *C. torelliana* and 1000 mg kg<sup>-1</sup> for *C. torelliana* x *C. citriodora* and *E. urophylla*. After the application of IBA, the mini-cuttings were planted.

The growth regulator was applied on the first day after the cuttings were planted, with a reapplication after 10 days. The applications were made using a sprayer, aiming to humidify the leaf surface of the mini-cuttings inside the greenhouse.

### Data Collection

After 45 days of rooting for the setup of each of the four experiments, survival percentage (SP) was counted, root length (RL) and plant height (PH) were measured using a millimeter ruler. The shoot and root parts were then separated, and the material was dried in a forced ventilation oven at 65 °C until reaching a constant weight, to determine the dry mass of the shoot (SDM), the roots (RDM) and total dry mass (TDM). For the variable survival and height, all plants within each replicate (block) were evaluated. For root length, root dry mass, shoot dry mass, and total dry mass, five rooted mini-cuttings per plot were assessed for each treatment and block.

At 90 days, the final rooted mini-cutting yield (FSY) was calculated, and suitable rooted mini-cuttings were those that: had a height equal to or greater than 20 cm; had good root development, considering the density of the root ball; collar diameter equal to or greater than 2 mm; had at least three pairs of leaves; and were free of diseases. The final rooted mini-cutting yield (FSY) was calculated considering the number of suitable rooted mini-cuttings (NSS) in relation to the total number of cuttings planted (TNMP) (Formula 1).

$$FSY(\%) = \frac{NSS}{TNMP \times 100} \quad (\text{Eq.1})$$

### Statistical Analysis

The data were subjected to verification of statistical assumptions, including error



independence (Durbin-Watson Test), normality (Shapiro-Wilk Test), homogeneity of variances (Bartlett's Test). Once these assumptions were met, the data were analyzed using analysis of variance (ANOVA), and when significant, the means of qualitative variables were grouped using the Scott-Knott test ( $p < 0.05$ ). For quantitative variables, regression analysis was performed ( $p < 0.05$ ). All analyses were conducted using R software, version 4.3.2 (R Core Team, 2023), using the ExpDes.pt package.

### 3. RESULTS

#### Experiment 1 - Mini-cutting Size

The clones and mini-cutting sizes significantly interacted in the survival percentage (SP), final rooted mini-cutting yield (FSY), root dry mass (RDM), shoot dry mass (SDM), and total dry mass (TDM) of *Corymbia* and *Eucalyptus* clones. Root length (RL) was the only variable that did not show a significant interaction between clones and mini-cutting sizes, but there was a difference between the clones.

In SP of the rooted mini-cuttings, there was a difference between the clones only at the 5 cm size, where the mini-cuttings of *E. urophylla* showed a reduction of approximately 3% in survival compared to the average of the *Corymbia* hybrids. The plants of *C. torelliana* x *C. citriodora* showed a reduction of approximately 1% in survival with the use of 15 cm mini-cuttings compared to the other sizes (Table 1).

Although not differing from the 10 cm length, all three clones had higher FSY with the use of 15 cm mini-cuttings, with the *C. citriodora* x *C. torelliana*, *C. torelliana* x *C. citriodora*, and *E. urophylla* clones showing FSY values of approximately 61%, 71%, and 77%, respectively, with the FSY of the *C. citriodora* x *C. torelliana* clone being lower than the others (Table 2).

The highest RDM, SDM and TDM values for *Corymbia* clones were obtained using 15 cm minicuttings, without affecting *E. urophylla*. Although the RDM of the *E. urophylla* rooted mini-cuttings from 15 cm mini-cuttings did not differ from those from 5 cm and 10 cm mini-cuttings. For 10 cm and 15 cm lengths, the *C. torelliana* x *C. citriodora* rooted mini-cuttings had higher RDM, SDM, and TDM than the *C. citriodora* x *C. torelliana* and *E. urophylla* clones (Table 2).

The *C. torelliana* x *C. citriodora* rooted mini-cuttings, with an average RL of 27,5 cm, had the highest RL, followed by the *C. citriodora* x *C. torelliana* rooted mini-cuttings, and ultimately, *E. urophylla*, which reached an average RL of 20,3 and 16,8 cm, respectively (Figure 1).

#### Experiment 2 - Mini-Tunnel Opening Time

The eucalyptus clones and the mini-tunnel opening time (MTO) significantly interacted in the SP, FSY, RL, SDM and TDM. In RDM, the factors did not significantly interact, but there were effects from the individual factors, clones and MTO.

**Table 1.** Survival percentage (SP) and final rooted mini-cutting yield (FSY) in relation to *Corymbia* and *Eucalyptus* clones and mini-cutting sizes

**Tabela 1.** Porcentagem de sobrevivência (SP) e aproveitamento final de mudas (FSY) em relação aos clones de *Corymbia* e *Eucalyptus* e tamanhos de miniestacas

Clones	Mini-cutting size (cm)		
	5	10	15
	Survival (%)		
<i>C. citriodora</i> x <i>C. torelliana</i> (C1)	98,75 aA	98,13 aA	98,35 aA
<i>C. torelliana</i> x <i>C. citriodora</i> (C2)	98,98 aA	98,50 aA	97,35 aB
<i>E. urophylla</i> (C3)	95,40 bB	98,10 aA	97,93 aA
	Final rooted mini-cutting yield (%)		
<i>C. citriodora</i> x <i>C. torelliana</i>	47,81 bA	57,15 aA	60,72 bA
<i>C. torelliana</i> x <i>C. citriodora</i>	23,27 cB	61,24 aA	71,31 aA
<i>E. urophylla</i>	72,74 aA	71,08 aA	76,96 aA

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other according to the Scott-Knott test at 5% probability.

Médias seguidas pela mesma letra minúscula na coluna e pela mesma letra maiúscula na linha não diferem entre si, de acordo com o teste de Scott-Knott a 5% de probabilidade.

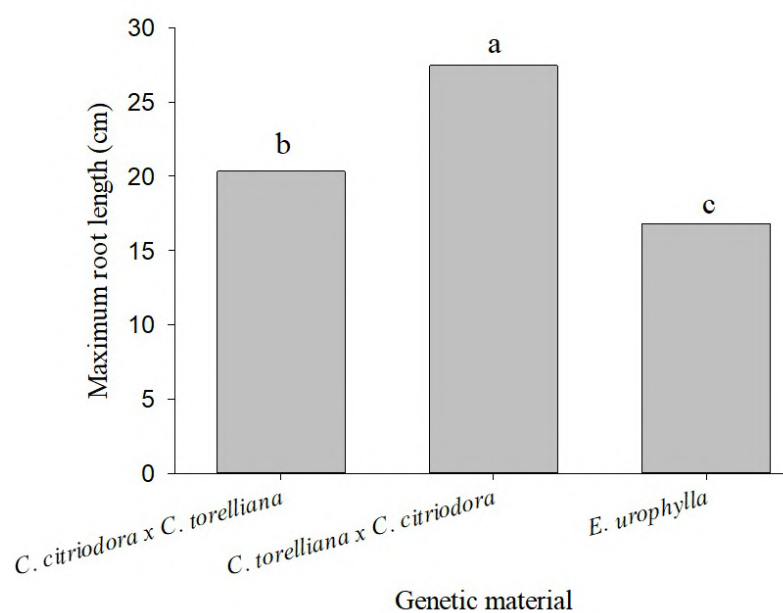
**Table 2.** Root (RDM), shoot (SDM), and total dry mass (TDM) in relation to *Corymbia* and *Eucalyptus* clones and mini-cutting sizes

**Tabela 2.** Massa seca de raízes (RDM), parte aérea (SDM) e total (TDM) de em relação aos clones de *Corymbia* e *Eucalyptus* e tamanho de miniestacas

Clones	Mini-cutting size (cm)		
	5	10	15
	Root dry mass (g)		
<i>C. citriodora</i> x <i>C. torelliana</i>	0,08 aC	0,10 bB	0,15 bA
<i>C. torelliana</i> x <i>C. citriodora</i>	0,09 aC	0,15 aB	0,20 aA
<i>E. urophylla</i>	0,07 aA	0,09 bA	0,10 cA
	Shoot dry mass (g)*		
<i>C. citriodora</i> x <i>C. torelliana</i>	0,23 bC	0,35 bB	0,54 bA
<i>C. torelliana</i> x <i>C. citriodora</i>	0,28 aC	0,48 aB	0,60 aA
<i>E. urophylla</i>	0,25 aC	0,32 bB	0,39 cA
	Total dry mass (g)		
<i>C. citriodora</i> x <i>C. torelliana</i>	0,30 aC	0,45 bB	0,69 bA
<i>C. torelliana</i> x <i>C. citriodora</i>	0,37 aC	0,63 aB	0,80 aA
<i>E. urophylla</i>	0,33 aC	0,41 bB	0,49 cA

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other according to the Scott-Knott test at 5% probability. Original means with statistics based on data transformed by  $\sqrt{x+1}$ .

Médias seguidas pela mesma letra minúscula na coluna e pela mesma letra maiúscula na linha não diferem entre si, de acordo com o teste de Scott-Knott a 5% de probabilidade. Médias originais com estatísticas baseadas em dados transformados por  $\sqrt{x+1}$ .



**Figure 1.** Length of the longest root of *Corymbia* and *Eucalyptus* plants. Means followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability

**Figura 1.** Comprimento máximo da raiz de plantas de *Corymbia* e *Eucalyptus*. Médias seguidas pela mesma letra não diferem entre si pelo teste de Scott-Knott a 5% de probabilidade

The SP of the clones did not differ among each other when the mini-cuttings were collected immediately after the mini-tunnel opening (0 hours), but when collected 24 and 48 hours after the mini-tunnel

opening (AAE), the mini-cuttings of *C. citriodora* x *C. torelliana* had lower survival. The MTO only influenced the survival of *C. citriodora* x *C. torelliana* plants, with the highest survival rate observed when mini-

cuttings were collected at the time of mini-tunnel opening (0 hours) and 48 hours AAE (Table 3).

Regarding the FSY, with a yield above 71%, the clones of *C. torelliana* x *C. citriodora* and *E. urophylla* at all mini-tunnel opening times had a higher FSY than the *C. citriodora* x *C. torelliana* rooted mini-cuttings, which remained below 57% (Table 3). The MTO again only influenced the FSY of *C. citriodora* x *C. torelliana*, with the highest yield (56.38%) observed when the mini-cuttings were collected 24 hours AAE.

The mini-cuttings of *C. torelliana* x *C. citriodora* at all mini-tunnel opening times had a higher RL than those of the other clones (Table 3). The mini-tunnel opening time influenced the RL of *C. torelliana* x *C. citriodora* and *E. urophylla*, with the *C. torelliana* x *C. citriodora* rooted mini-cuttings showing higher RL when the mini-cuttings were collected immediately after opening (AAE), and the *E. urophylla* rooted mini-cuttings showing higher RL when the mini-cuttings were collected 24 and 48 hours after opening (Table 3).

The SDM and TDM of *C. torelliana* x *C. citriodora* and *E. urophylla* rooted mini-cuttings at all MTO were higher than those of *C. citriodora* x *C. torelliana* (Table 4). At 48 hours after mini-tunnel opening (AAE), the

*C. torelliana* x *C. citriodora* rooted mini-cuttings had the highest SDM. The MTO influenced the SDM and TDM of *C. torelliana* x *C. citriodora* and *E. urophylla*, with these clones showing higher TDM when the mini-cuttings were collected 24 hours AAE (Table 4). The *C. torelliana* x *C. citriodora* rooted mini-cuttings had their TDM influenced only by the MTO, with the rooted mini-cuttings collected 24 hours AAE having the highest TDM.

The RDM of *E. urophylla* rooted mini-cuttings was the highest (0.17 g), followed by *C. torelliana* x *C. citriodora* (0.15 g), and then *C. citriodora* x *C. torelliana*, which had an average RDM of 0.13 g (Figure 2A). Rooted mini-cuttings from mini-cuttings collected 24 hours after mini-tunnel opening (AAE) had 0.17 g of RDM, which was higher than those collected at 0 and 48 hours AAE, which had RDM values of 0.14 g (Figure 2B).

### Experiment 3 - Growth Regulator 1 (GR1)

The genetic materials differed significantly in survival, FSY, PH, RL, RDM, SDM and TDM. The concentrations of GR1 also influenced the FSY, RDM, SDM, and total dry mass TDM (Table 5). A significant interaction was observed between the genetic materials and GR1 concentrations in RDM, SDM, and TDM.

**Table 3.** Survival percentage (SP), final rooted mini-cutting yield (FSY), and Length of the longest root (RL) in relation to *Corymbia* and *Eucalyptus* clones and mini-tunnel opening times

**Tabela 3.** Porcentagem de sobrevivência (SP), aproveitamento final (FSY), e comprimento da maior raiz (RL) em relação aos clones de *Corymbia* e *Eucalyptus* e tempos de abertura do estufim

Clones	Mini-Tunnel Opening Time (Hours)		
	0	24	48
	Survival (%)		
<i>C. citriodora</i> x <i>C. torelliana</i>	99,11 aA	97,15 bB	98,16 bA
<i>C. torelliana</i> x <i>C. citriodora</i>	99,04 aA	100 aA	100 aA
<i>Eucalyptus urophylla</i>	98,60 aA	98,73 aA	99,47 aA
	Final rooted mini-cutting yield (%)		
<i>C. citriodora</i> x <i>C. torelliana</i>	46,54 bB	56,38 bA	45,00 bB
<i>C. torelliana</i> x <i>C. citriodora</i>	80,15 aA	71,33 aA	79,92 aA
<i>Eucalyptus urophylla</i>	77,21 aA	75,17 aA	82,84 aA
	Length of the longest root (cm)		
<i>C. citriodora</i> x <i>C. torelliana</i>	13,88 bA	14,98 cA	13,50 cA
<i>C. torelliana</i> x <i>C. citriodora</i>	37,93 aA	31,93 aB	33,55 aB
<i>Eucalyptus urophylla</i>	15,63 bB	18,08 bA	19,85 bA

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other according to the Scott-Knott test at 5% probability.

Médias seguidas pela mesma letra minúscula na coluna e pela mesma letra maiúscula na linha não diferem entre si, de acordo com o teste de Scott-Knott a 5% de probabilidade.

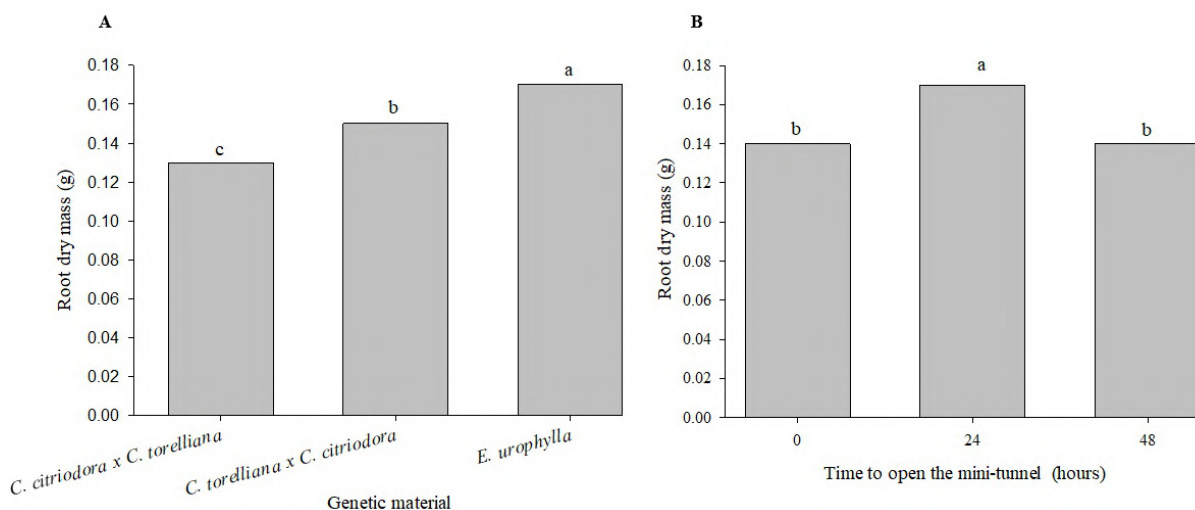
**Table 4.** Shoot (SDM) and total dry mass (TDM) in relation to *Corymbia* and *Eucalyptus* clones and mini-tunnel opening times prior to mini-cutting collection

**Tabela 4.** Massa seca da parte aérea (SDM) e total (TDM) em relação aos clones de *Corymbia* e *Eucalyptus* e tempos de abertura do estufim antes da coleta das miniestacas

Clones	Mini-Tunnel Opening Time (Hours)		
	0	24	48
Shoot dry mass (g)			
<i>C. citriodora</i> x <i>C. torelliana</i>	0,48 bB	0,54 bA	0,46 cB
<i>C. torelliana</i> x <i>C. citriodora</i>	0,58 aA	0,59 aA	0,58 aA
<i>E. urophylla</i>	0,57 aA	0,60 aA	0,52 bB
Total dry mass (g)			
<i>C. citriodora</i> x <i>C. torelliana</i>	0,59 bB	0,68 bA	0,58 cB
<i>C. torelliana</i> x <i>C. citriodora</i>	0,72 aB	0,77 aA	0,72 aB
<i>E. urophylla</i>	0,74 aB	0,78 aA	0,67 bC

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other according to the Scott-Knott test at 5% probability.

Médias seguidas pela mesma letra minúscula na coluna e pela mesma letra maiúscula na linha não diferem entre si, de acordo com o teste de Scott-Knott a 5% de probabilidade.



**Figure 2.** Root dry mass of *Corymbia* and *Eucalyptus* as a function of clones (A) and mini-tunnel opening times before mini-cutting collection (B). Means followed by the same letter do not differ significantly from each other according to the Scott-Knott test at a 5% probability level

**Figura 2.** Massa seca de raízes de *Corymbia* e *Eucalyptus* em função dos clones (A) e dos tempos de abertura dos minitúneis antes da coleta das miniestacas (B). Médias seguidas pela mesma letra não diferem significativamente entre si, de acordo com o teste de Scott-Knott a um nível de probabilidade de 5%

The cuttings of *C. torelliana* x *C. citriodora* and *E. urophylla* had survival rates of 95.16% and 96.8%, respectively (Table 5), with no significant difference between them but higher than the survival rate of *C. citriodora* x *C. torelliana* (90.40%). Despite the lower survival of the *C. citriodora* x *C. torelliana* cuttings, the FSY of rooted mini-cuttings (50.66%) was higher than that of *C. torelliana* x *C. citriodora* (34.44%). The *E. urophylla* clone, with 69.23%, had the highest FSY of rooted mini-cuttings (Table 5).

The rooted mini-cuttings of *Corymbia* clones, with an average height of approximately 38 cm, had a higher average height than *E. urophylla*, whose average height was 35.87 cm (Table 5). The rooted mini-cuttings of *C. torelliana* x *C. citriodora*, with 21.89 cm, had a greater root length (RL) than the other genetic materials used, whose heights were approximately 17 cm (Table 5).

The FSY of rooted mini-cuttings in relation to the concentrations of GR1 followed a linear increasing model, with the



maximum dose used (2000 mg kg<sup>-1</sup>) resulting in an FSY of 60.81% (Figure 3A). On the other hand, the RL followed a quadratic polynomial model, with the maximum response point at a dose of 1220 mg kg<sup>-1</sup>, which promoted an average root length of approximately 20 cm (Figure 3B).

The RDM differed among the genotypes

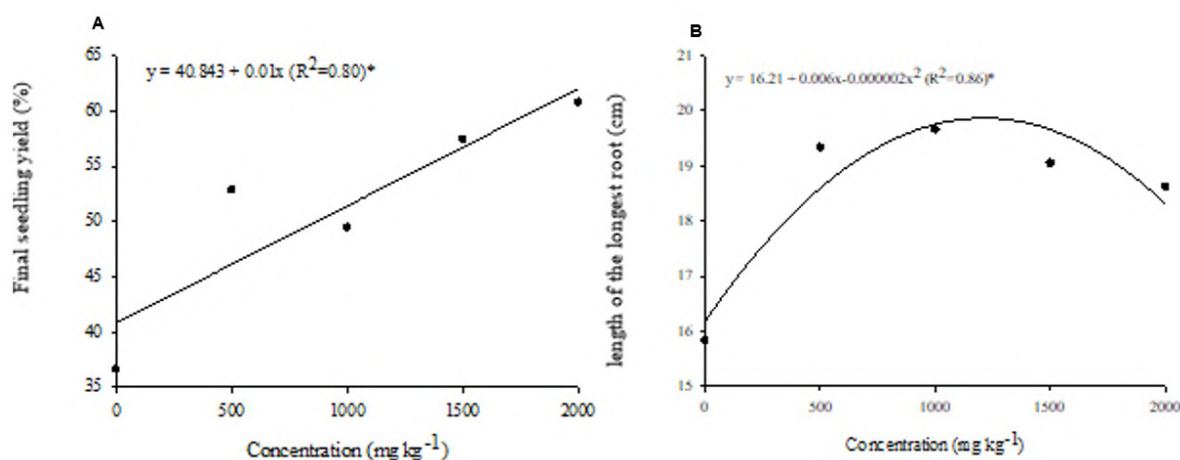
only at the doses of 500 and 1000 mg kg<sup>-1</sup> of GR1. At the dose of 500 mg kg<sup>-1</sup>, the rooted mini-cuttings of *C. torelliana* x *C. citriodora* (0.069 g) and *E. urophylla* (0.067 g) had higher RDM than *C. citriodora* x *C. torelliana* (0.056 g), while at the dose of 1000 mg kg<sup>-1</sup>, the *C. torelliana* x *C. citriodora* clone, with 0.073 g, had the

**Table 5.** Percentage of survival (SP), final seedling utilization (FSY), plant height (PH) and root length (RL) of *Corymbia* and *Eucalyptus* clones as a function of the general average concentrations of growth regulator 1 (GR1)

**Tabela 5.** Percentual de sobrevivência (SP), aproveitamento final de mudas (FSY), altura de plantas (PH) e comprimento de raiz (RL) de clones de *Corymbia* e *Eucalyptus* em função da média geral das concentrações do regulador de crescimento 1 (GR1)

Genetic materials	SP (%)	FSY (%)	PH (cm)	RL (cm)
<i>C. citriodora</i> x <i>C. torelliana</i>	90,40b	50,66 b	37,82 a	16,71 b
<i>C. torelliana</i> x <i>C. citriodora</i>	95,16 a	34,44 c	37,48 a	21,89 a
<i>E. urophylla</i>	96,98 a	69,23 a	35,87 b	17,01 b

Means followed by the same letter in the column do not differ from each other according to the Scott-Knott test ( $p < 0.05$ ). Médias seguidas pela mesma letra na coluna não diferem entre si de acordo com o teste de Scott-Knott ( $p < 0,05$ ).



**Figure 3.** Final rooted mini-cutting yield (A) and length of the longest root (B) of *Eucalyptus* plants as a function of growth regulator 1 concentration

**Figura 3.** Aproveitamento final de mudas (A) e comprimento da maior raiz (B) de plantas de eucalipto em função da concentração do regulador de crescimento 1

highest RDM compared to the others.

The responses of the genetic materials for SDM and TDM were variable across the concentrations of GR1. Without the growth regulator (0 mg kg<sup>-1</sup>), the rooted mini-cuttings of *E. urophylla* had higher SDM and TDM than the *Corymbia* clones. At the concentration of 500 mg kg<sup>-1</sup>, the rooted mini-cuttings of *C. torelliana* x *C. citriodora* had SDM and TDM similar to those of *E.*

*urophylla*. At the concentration of 1000 mg kg<sup>-1</sup>, the rooted mini-cuttings of *C. torelliana* x *C. citriodora* had higher SDM and TDM than the other genotypes. At the concentration of 1500 mg kg<sup>-1</sup>, the cuttings of *E. urophylla* had the highest average SDM and TDM, at 0.35 and 0.42 g, respectively. At the concentration of 2000 mg kg<sup>-1</sup>, both *Corymbia* clones had higher SDM and TDM than the rooted mini-cuttings of *E. urophylla*.

The concentrations of GR1 significantly differed in the RDM of *C. citriodora* x *C. torelliana*, following a quadratic polynomial model, with the best responses at the concentration of 2000 mg kg<sup>-1</sup> (Figure 4A). For the plants of *C. torelliana* x *C. citriodora*, there was no significant difference between the GR1 concentrations in RDM, and for *E. urophylla*, no model adjustment nor significant difference was observed between the GR1 concentrations (Figure 4A).

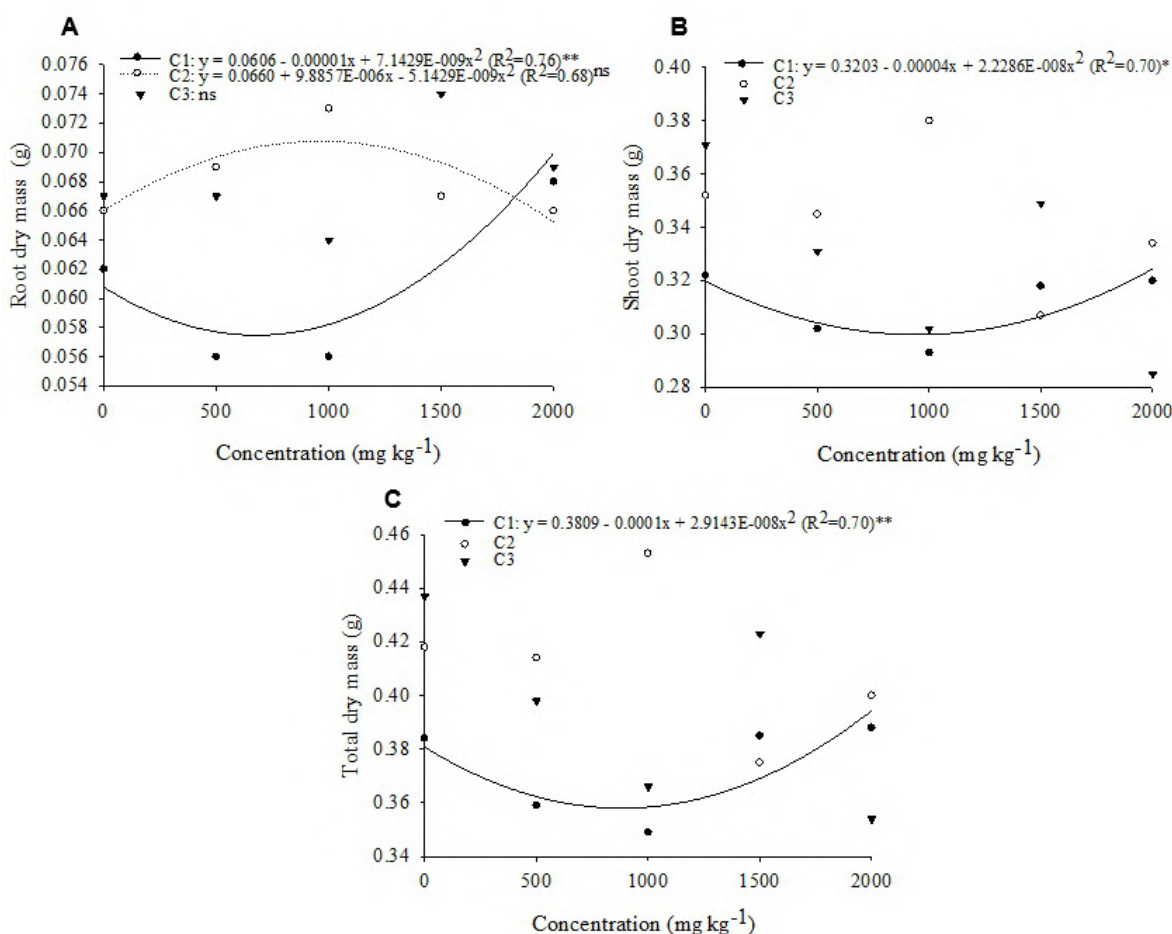
For the rooted mini-cuttings of *C. torelliana* x *C. citriodora* and *E. urophylla*, no model adjustment was observed for the effect of GR1 concentrations on SDM (Figure 4B) and TDM (Figure 4C). However, for the *C. citriodora* x *C. torelliana* clone, the concentrations followed a quadratic

polynomial model, with the best responses at concentrations of 0 and 2000 mg kg<sup>-1</sup> for both variables (SDM and TDM).

#### Experiment 4 - Growth Regulator 2 (GR2)

The genetic materials used differed in SP, FSY, PH, RL, RDM, SDM and TDM. There was an effect of GR2 concentrations and a significant interaction between the genetic materials and GR2 concentrations for RL, RDM, SDM, and TDM.

The mini-cuttings of *C. torelliana* x *C. citriodora* and *E. urophylla* showed higher SP (86.99% and 79.69%, respectively), with no significant difference between them, but both were superior to the SP of *C. citriodora* x *C. torelliana*, which averaged 58.41%. The highest FSY was obtained in *E. urophylla*, with a mean value of 66.68% (Table 6). The



**Figure 4.** Root (A), shoot (B), and total (C) dry mass of *Corymbia citriodora* x *Corymbia torelliana* (C1), *C. torelliana* x *C. citriodora* (C2), and *Eucalyptus urophylla* (C3) plants as a function of growth regulator 1 concentrations. \* Significant at 5%, \*\* Significant at 1%, ns not significant

**Figura 4.** Massa seca de raízes (A), parte aérea (B) e total (C) de plantas de *Corymbia citriodora* x *Corymbia torelliana* (C1), *C. torelliana* x *C. citriodora* (C2), e *Eucalyptus urophylla* (C3) em função das concentrações do regulador de crescimento 1. \* Significativo a 5%, \*\* Significativo a 1%, ns não significativo

mini-cuttings of *C. citriodora* × *C. torelliana* had the lowest FSY (31.22%).

The rooted mini-cuttings of *C. torelliana* × *C. citriodora* showed the greatest mean PH (42.53 cm), followed by *C. citriodora* × *C. torelliana* (40.31 cm) and *E. urophylla*, which had a mean PH of 37.12 cm (Table 6).

Without the application of the growth regulator (0 mL L<sup>-1</sup>), rooted mini-cuttings of *C. torelliana* × *C. citriodora* and *E. urophylla* exhibited greater root length (RL), with mean values of 23.20 cm and 21.42 cm, respectively. At the concentration of 1 mL L<sup>-1</sup> of GR2, the RL of the genetic materials did not differ significantly. However, at concentrations of 2, 3, and 4 mL L<sup>-1</sup>, the rooted mini-cuttings of *C. torelliana* × *C. citriodora* had the highest mean RL, ranging from 26 to 28 cm.

Rooted mini-cuttings of *E. urophylla* showed higher RDM than the *Corymbia* clones at GR2 concentrations of 0, 1, 2, and 3 mL L<sup>-1</sup>. At the concentration of 4 mL L<sup>-1</sup>, the highest mean RDM was observed in rooted mini-cuttings of *C. torelliana* × *C. citriodora*. The SDM and TDM were higher in *C. torelliana* × *C. citriodora* rooted mini-cuttings at all tested concentrations.

For RL, it was not possible to fit a model that adequately explained the biological behavior of any of the genetic materials used (Figure 5).

The RDM of *E. urophylla* followed a decreasing linear model, declining from 0.13 g in untreated rooted mini-cuttings (0 mL L<sup>-1</sup> of GR2) to 0.11 g at 4 mL L<sup>-1</sup>, representing a reduction of approximately 20%. For the *Corymbia* clones, RDM fitted a quadratic polynomial model: in *C. citriodora* × *C. torelliana*, the best response was observed without the use of the regulator, with an

RDM of approximately 0.10 g; in contrast, *C. torelliana* × *C. citriodora* showed a more pronounced response at 4 mL L<sup>-1</sup> of GR2, reaching a mean RDM of 0.11 g (Figure 6A).

GR2 concentrations did not significantly affect the SDM of *C. citriodora* × *C. torelliana*. In *C. torelliana* × *C. citriodora*, no model could be fitted, but the best response was observed at 4 mL L<sup>-1</sup> of GR2, with an SDM of 0.59 g (Figure 6B). In *E. urophylla* rooted mini-cuttings, SDM decreased with increasing GR2 concentrations, fitting a decreasing linear model. There was a reduction of approximately 7% in SDM when comparing rooted mini-cuttings treated with 4 mL L<sup>-1</sup> to those not treated with the growth regulator (Figure 6B).

For TDM, there was no significant difference among GR2 concentrations and no model fit for *C. citriodora* × *C. torelliana*. In *C. torelliana* × *C. citriodora*, no model could be fitted either, but the best response was obtained with the application of 4 mL L<sup>-1</sup>, resulting in a mean TDM of 0.70 g. In *E. urophylla*, GR2 concentrations followed a decreasing linear model, leading to a reduction of approximately 10% at the highest concentration compared to the treatment without the growth regulator (Figure 6C).

#### 4. DISCUSSION

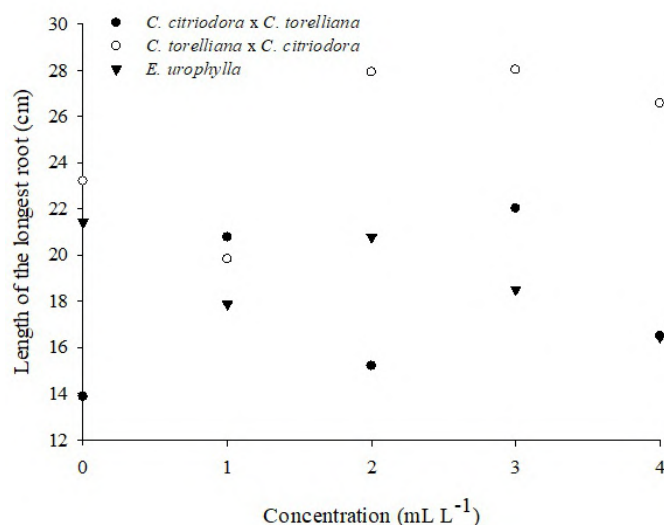
The size of the mini-cutting influences the development of the plants, and the ideal size varies according to the species, which leads to variations in the rhizogenic process and the development of vegetatively propagated (Pimentel et al., 2021; Vigl et al., 2014). The superiority of medium and large mini-cuttings compared to smaller ones may

**Table 6.** Survival percentage (SP), final rooted mini-cutting yield (FSY) and plant height (PH) of *Corymbia* and *Eucalyptus* clones as a function of growth regulator 2 (GR2) concentrations

**Tabela 6.** Percentual de sobrevivência (SP), aproveitamento final de mudas (FSY) e altura de plantas (PH) de clones de *Corymbia* e *Eucalyptus* em função das concentrações do regulador de crescimento 2 (GR2)

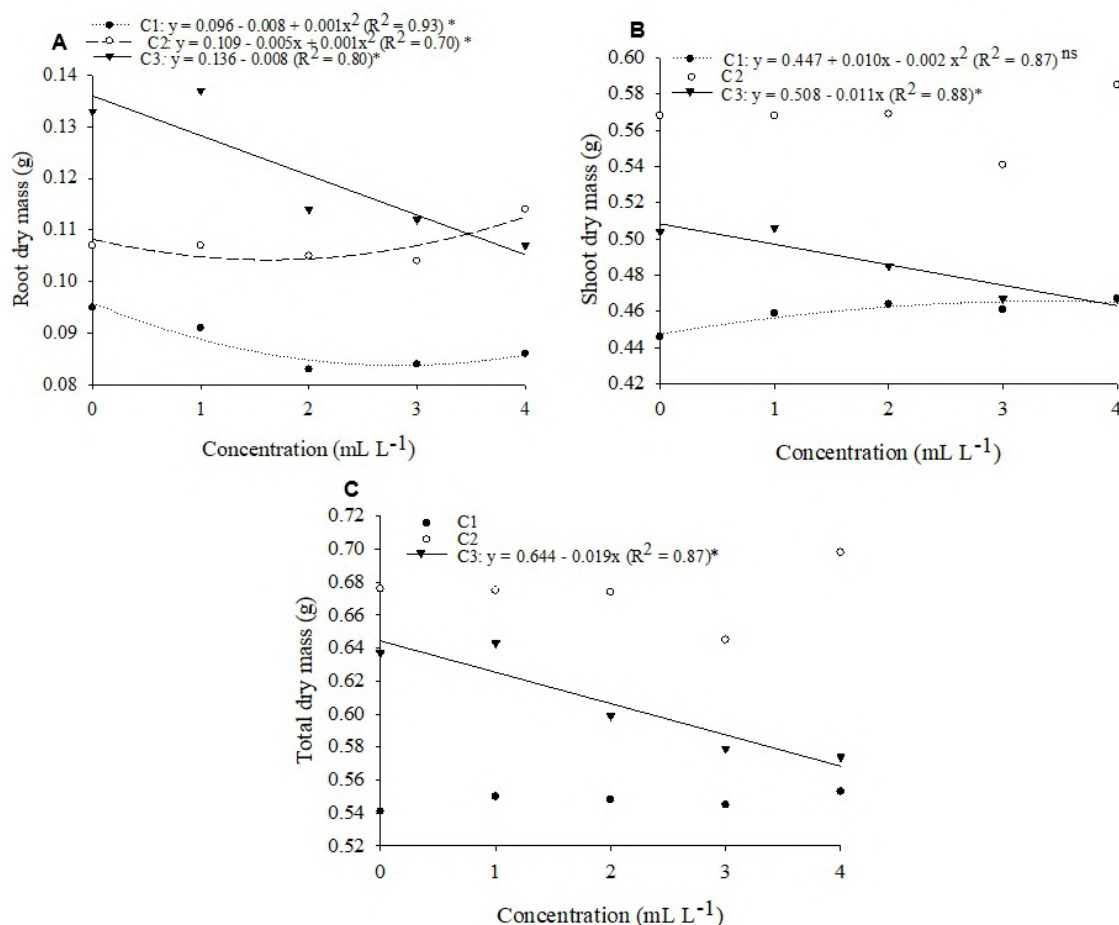
Genetic materials	SP (%)	FSY (%)	PH (cm)
<i>C. citriodora</i> x <i>C. torelliana</i>	58,41 b	31,22 c	40,31 b
<i>C. torelliana</i> x <i>C. citriodora</i>	86,99 a	44,60 b	42,53 a
<i>E. urophylla</i>	79,69 a	66,68 a	37,12 c

Means followed by the same letter in the column do not differ from each other according to the Scott-Knott test (p<0.05). Médias seguidas pela mesma letra na coluna não diferem entre si de acordo com o teste de Scott-Knott (p < 0,05).



**Figure 5.** Length of the longest root of *Corymbia* and *Eucalyptus* plants as a function of growth regulator 2 concentrations

**Figura 5.** Comprimento da maior raiz de plantas de *Corymbia* e *Eucalyptus* em função das concentrações do regulador de crescimento 2



**Figure 6.** Root (A), shoot (B), and total (C) dry mass of *Corymbia citriodora* × *Corymbia torelliana* (C1), *C. torelliana* × *C. citriodora* (C2), and *Eucalyptus urophylla* (C3) plants as a function of growth regulator 2 concentrations. \* Significant at 5%, ns not significant

**Figura 6.** Massa seca de raízes (A), parte aérea (B) e total (C) de plantas de *Corymbia citriodora* × *Corymbia torelliana* (C1), *C. torelliana* × *C. citriodora* (C2), e *Eucalyptus urophylla* (C3) em função das concentrações do regulador de crescimento 2. \* Significativo a 5 %, ns não significativo



be due to their greater number of buds and leaves on the vegetative propagules, which enhances the synthesis of soluble sugars responsible for supplying energy (Pimentel et al., 2021; Shao et al., 2018).

Another factor is that the *Corymbia* hybrid with *C. torelliana* as the female parent (*C. torelliana* × *C. citriodora*) had a higher FSU than the hybrid with *C. citriodora* as the female parent (*C. citriodora* × *C. torelliana*) (Table 2). This higher FSU is partly attributed to the superior rooting capacity of *C. torelliana* × *C. citriodora*, since FSU is determined based on plant height ( $\geq 20$  cm), stem diameter ( $\geq 2$  mm), rooting, plant health, and the exclusion of rooted mini-cuttings that do not meet the minimum requirements. Given that *C. torelliana* exhibits greater rooting ability compared to other species of the *Corymbia* genus, this species has become an important component in hybrid composition for clonal selection purposes, which is currently its main role in forest breeding programs (Miranda et al., 2023).

Overall, the effects of mini-cutting sizes on FSU (Table 2), RDM, SDM, and TDM (Table 3) in *E. urophylla* rooted mini-cuttings were less pronounced than in *Corymbia* hybrids. The *E. urophylla* clone used is already well-established in the market and was used as a control to compare the development of the *Corymbia* hybrids. Because there was no difference in the final yield of rooted mini-cuttings between 10 and 15 cm, 10 cm mini-cuttings were used for *E. urophylla* due to their greater abundance in the mini-garden.

The benefits of using a mini-tunnel are attributed to the changes promoted in the production microenvironment, such as carbon dioxide concentration, solar irradiance, temperature, and relative humidity, which increase shoot production (Lima et al., 2022).

The *C. citriodora* × *C. torelliana* hybrid was the only one for which FSU was influenced by the opening time, showing the highest FSU when the mini-cuttings were collected 24 hours after opening (AAE) (Table 5). These rooted mini-cuttings also exhibited the highest TDM at the same collection time. Although the clones of *C. torelliana* × *C. citriodora* and *E. urophylla*

were not significantly influenced by the mini-tunnel opening time, they showed higher FSU percentages at 0 and 48 hours AAE, respectively (Table 5). During the experiment, it was observed that at 48 hours AAE, the mini-cuttings of the *Corymbia* clones were more hardened and had shed their leaves, which is one of the symptoms of powdery mildew caused by *Podosphaera pannosa*, a cosmopolitan species that affects plants from different families (Fonseca et al., 2017). This attack was more pronounced in the *Corymbia* clones than in *E. urophylla*.

The highest FSU was observed in the *E. urophylla* clone at 48 hours AAE (Table 5). Notably, the clones of *C. torelliana* × *C. citriodora* and *E. urophylla* did not significantly differ in FSU at any of the collection times, despite that hybrids of *Corymbia* are known for their difficulty in rooting. The *Eucalyptus* clones have been more extensively studied and utilized (Massuque et al., 2023).

In the same site, Rocha et al. (2023) observed that exposing stock plants to the mini-tunnel for 45 days resulted in higher mini-cutting productivity, increased height and dry biomass, which was advantageous for the management operation of the mini-garden. Canguçu et al. (2022) found that the use of the mini-tunnel in the clonal mini-garden had different effects on the productivity of the mother plant mini-cuttings, depending on the season of propagule collection (cold or warm). In beds covered with mini-tunnels, the highest mini-cutting productivity observed mainly in the cold season may be related to the temperature changes that occur near the canopy of the mother plants (Canguçu et al., 2022).

The *C. torelliana* × *C. citriodora* hybrid exhibited the greatest root length (Figure 1 and Table 5). Larger roots can assist plants in water and nutrient absorption, improving rooted mini-cutting development and resulting in better shoot development and TDM. This may make the clone more resistant to water stress and more tolerant to pest and disease attacks, as previously reported (Souza et al., 2020; Costa et al., 2022).

Lima et al. (2022) reported that the benefits of using the mini-tunnel for rooting *C. torelliana* × *C. citriodora* are greater

during the winter, attributing this to more favorable temperatures. Future studies may explore the relationship between mini-tunnel opening time and seasons of the year, as climatic conditions vary throughout the year. Wetter periods may reduce powdery mildew infestation, allowing the mini-tunnel to remain open for a longer period to favor the hardening of mini-cuttings.

The *Corymbia* clones showed the highest FSY at 2000 mg kg<sup>-1</sup>. IBA can be rapidly metabolized in eucalyptus plant tissues (Sharma et al., 2023), where it stimulates root formation by modulating the expression of specific genes related to root development (Chen et al., 2024). The use of IBA promotes the formation of a robust root system that is efficient in nutrient absorption, which is essential for the establishment of young plants in the field (Yao et al., 2021).

In RL, the concentration that resulted in the best response was 1500 mg kg<sup>-1</sup> (Figure 3), with a reduction beyond this dose. Plants also possess endogenous hormones, so the application of higher doses can lead to hormonal imbalance, reducing root development. However, this reduction in RL beyond the 1500 mg kg<sup>-1</sup> dose was not sufficient to affect plant development, as the FSY was higher at the 2000 mg kg<sup>-1</sup> dose.

The SDM and TDM of the clones were influenced by IBA concentrations, with *E. urophylla* exhibiting higher SDM and TDM at lower concentrations. However, at the 2000 mg kg<sup>-1</sup> concentration, both *Corymbia* clones had higher SDM and TDM. This demonstrates that the mini-cuttings of *Corymbia* clones are more dependent on auxins for their development than those of *E. urophylla*, and furthermore, higher concentrations inhibited the development of *E. urophylla*. It is worth noting that *Eucalyptus* clones have been exploited for decades, undergoing various breeding programs (Maciel et al., 2022). It has been shown that the efficacy of IBA in promoting adventitious rooting can be strongly influenced by the genotype. A study with *Prunus* rootstocks, Garnem and GF 677, demonstrated that root induction by IBA was genotype-dependent (Justamante et al., 2022).

In addition to responses genetic material, they can also be influenced by

factors such as the propagation environment and the leaf area of the cuttings (Vallejos-torres et al., 2021). Efficacy may also vary depending on the plant's ability to metabolize these auxin compounds and on species-specific factors, such as the expression of genes related to auxin transport and biosynthesis (Ludwig-müller, 2000; Sun et al., 2023).

It was observed that the performance of clonal rooted mini-cuttings of *E. urophylla* and *Corymbia* hybrids varied significantly due to their genetic and GR1 concentration. While *E. urophylla* stood out for its higher FSY, due to its superiority for adventitious rooting, *Corymbia* clones, particularly *C. torelliana* × *C. citriodora*, showed higher average height and root length, desirable characteristics. The concentration of 2000 mg kg<sup>-1</sup> of GR1 was the most efficient for FSY, although higher concentrations inhibited the growth of *E. urophylla*, indicating a lower dependence of this species on exogenous auxins.

In the experiment with GR2, there was a strong influence of the genetic material (Table 6). GR2 did not influence rooted mini-cutting survival and FSY (Table 6). The formation of adventitious roots from stems, as used in cutting propagation, is crucial in clonal propagation and is primarily controlled by the balance of endogenous and exogenous hormones (Lakehal & Bellini, 2019). However, the mechanism by which endogenous and environmental factors interact to control adventitious root formation is still poorly understood (Geiss et al., 2018).

The reduction in RL (Figure 5), RDM (Figure 6A), SDM (Figure 6B), and TDM (Figure 6C) of *E. urophylla* plants with increasing concentrations of GR2 may have occurred due to a hormonal imbalance in the plants. *E. urophylla* is a species that develops well without the application of hormones. This can be observed by analyzing rooted mini-cutting survival and final rooted mini-cutting yield, which were only affected by the clone factor, indicating that regardless of the GR2 concentration used, the best responses for these characteristics come from *E. urophylla*. At the concentration of 1 mL L<sup>-1</sup>, *E. urophylla* plants show a response similar to or even better than when no growth

regulator was applied (0 mL L<sup>-1</sup>) for RDM, SDM, and TDM.

Negishi et al. (2014), studying the hormonal levels of *E. globulus*, reported that auxins and cytokinins play an important role in the formation of adventitious roots, and that the interaction between auxin and cytokinin levels and their metabolism for root formation is complex. It can be assumed that the optimal concentrations of these hormones for *E. urophylla* may be different from those in the product. Auxins generally have a beneficial effect on root formation, but cytokinins can inhibit rooting if applied in excess (Kurepa & Smalle, 2022).

For the *Corymbia* hybrids used, in general, GR2 tended to benefit RL, SDM and TDM, reinforcing the idea that these clones are more responsive to plant growth regulators. This may be due to rooting recalcitrance, as observed, with low final rooted mini-cutting productivity, below 45% (Table 6). Thus, it is observed that GR2 proved to be more beneficial for the *Corymbia* hybrids, with optimal concentrations ranging between 3 and 4 mL L<sup>-1</sup>. The GR2 can be a valuable tool to enhance the robustness and quality of *Corymbia* rooted mini-cuttings, while its use in *E. urophylla* should be more carefully adjusted.

## 5. CONCLUSION

Mini-cuttings of 15 cm were better for the growth of *Corymbia* hybrids. Regarding the time of opening of the mini-tunnel, it was found that the interval of 24 hours after opening was the most suitable for *C. citriodora* x *C. torelliana* mini-cuttings, while the other clones did not show significant differences. The use of growth regulators had variable positive effects, with the *Corymbia* hybrids benefiting more from higher concentrations (2000 and 1000 mg kg<sup>-1</sup> of IBA). For the compound growth regulator, the use of 4 mL L<sup>-1</sup> increased rooting and rooted mini-cutting quality for *C. torelliana* x *C. citriodora*. This work describes an effective protocol for the clonal propagation of *Eucalyptus urophylla* and *Corymbia* hybrids.

## 6. ACKNOWLEDGEMENTS

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## AUTHOR CONTRIBUTIONS

Trindade, R.N.R.: Conceptualization, Data curation, Investigation, Project administration, Resources, Writing – original draft; Andrade, G.F.P. de: Visualization, Writing – review & editing; Oliveira, A.M. de: Formal analysis; Titon, M.: Methodology, Supervision; Costa, M.R. da: Methodology, Supervision.

## DATA AVAILABILITY

The entire dataset supporting the findings of this study has been published within the article.

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