Impacts of different phytohormones on the vegetative propagation of seedlings of *Eucalyptus dunnii* Maiden and *Eucalyptus badjensis* Beuzev & Welch

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**ABSTRACT**

**Background:** The genus *Eucalyptus* has great potential in southern Brazil due to its diversity of uses and applications. The species can be easily propagated by seed, however, superior characteristics of selected individuals may only be maintained through vegetative propagation, which will enable increased productivity and generate quality wood to meet an increasingly demanding market. **Objective:** The objective of this study was to evaluate the effects of different phytohormones in the production of *Eucalyptus dunnii* and *Eucalyptus badjensis* by propagation through vegetative by mini-cuttings. In this experiment, we used AIB (indolebutyric acid) and AIA (indole acetic acid) as well as natural phytohormones obtained from aqueous extracts of purple nutsedge (*Cyperus rotundus*) and violet (Santpaulia ionantha). To evaluate the potential of these species as rooting enhancers, we used the following concentrations: 0; 2,000; 4000; 6,000 and 8,000 mg L⁻¹. The experiment consisted of a randomised block design in a factoral design, with two factors (phytohormone and different doses). The shoots were kept in the rooting substrates for 100 days; subsequently, we evaluated survival, height, diameter, dry shoot mass, dry root mass, total dry mass and height/diameter ratio. **Results:** There was no significant interaction between concentrations and phytohormones used for the evaluated parameters in the two species of *Eucalyptus*. Likewise, the concentration factor showed no significant difference in the assessments made in these species. For *E. dunnii*, there was no significant difference in mean height, diameter and dry mass of the aerial part and total dry mass as well as for the H/D ratio between the treatments. However, for the parameter root system dry mass, application of AIA promoted a greater volume of roots, although this differed statistically only from that in violet. It should be noted that root system mass is an important aspect in seedling production, since the roots are responsible for optimal seedling development and among the main parameters analysed to evaluate seedling quality. For *E. badjensis*, treatment with violet extract led to significantly different results compared to AIA and AIB treatments, but the results were similar to those obtained with purple nutsedge extract. In terms of diameter, treatment with purple nutsedge led to significantly different results compared to AIA and AIB treatment. In relation to dry mass of the aerial part, dry mass of the root system and total dry mass, treatment with violet extract obtained the highest values, significantly differing from those obtained by the other treatments. In contrast, overall H/D ratio did not differ between violet treatment and AIB/AIB treatment, with the exception of purple nutsedge. **Conclusion:** The two species responded differently to the used phytohormones. For *E. dunnii*, AIA promoted greater survival of cuttings, however, the...
INTRODUCTION

The production of forest seedlings represents an important segment of the Brazilian economy, due to the expansion of forestry in the country. It is estimated that currently, the commercial forest area is 6,000,000 ha, with a planting area of approximately 700,000 hectares per year (Abrasf, 2012).

It is noteworthy that the genus Eucalyptus is the main cultivated forest essence of the country, with approximately 4,500,000 ha planted (Abrasf, 2012). Therefore, Brazilian forestry research needs to focus on seedling production, improvements in planting and harvesting techniques and wood technological properties, among others.

Among the several species of Eucalyptus cultivated in Brazil, the species Eucalyptus dunnii Maiden deserves to be highlighted, as it presents rapid growth and resistance to mild frost, therefore being suitable for planting in the state of Santa Catarina at altitudes between 500 and 1,000m (Embrapa, 1988). Currently, studies on Eucalyptus badjensis Beuzev & Welch are also being developed, as this species presents significant resistance to frosts. However, there is little information on seedling production or even behaviour of this species in homogeneous plantations, and the seeds used are mainly obtained from areas of natural occurrence in Australia, presenting great heterogeneity. In this context, the production of seedlings via vegetative propagation can be seen as an alternative for multiplication of superior genotypes adapted to local conditions.

Currently, about 85% of eucalyptus seedlings produced in Brazil are obtained by vegetative propagation with the use of mini-cuttings from clonal mini-gardens (Silva et al., 2008). The intensification of the clonal production of seedlings, according to Alfenas et al. (2004), is mainly due to the multiplication of superior genotypes, which allows the maintenance of the characteristics of the selected matrix plant and therefore permits the implantation of uniform and highly productive stands.

The vegetative propagation methodologies used today are mini-cutting and micro-cutting, which reduce the difficulties in the process of seedling production, which has previously been carried out via cuttings only (Xavier, 2002). Thus, micropropagation is a very useful tool in the forestry sector, because it allows the formation and maintenance of clonal clonal mini-gardens, from rejuvenated clones, allowing the propagation of genotypes of interest (Oliveira et al., 2016).

The advantages of mini-cutting are better rooting, higher root system quality, higher root emission velocity and reduction and rationalisation of operational activities; however, in some cases, it might be necessary to use growth regulators to increase rooting, such as AIB (indole butyric acid) and AIA (indole acetic acid) (Titon, 2001).

Among the growth-regulating substances, auxins have the greatest effects on the formation of adventitious roots (Hartmann et al., 2002). According to the same authors, the discovery of natural auxins, such as indole acetic acid (AIA), and synthetic auxins, such as indole butyric acid (AIB) and naphthaleneacetic acid (ANA), stimulated the highest production of adventitious rooting in stem and leaf cuttings and was a breakthrough in the history of vegetative plant propagation.

Auxin application results in a higher percentage, speed, quality and uniformity of rooting (Hartmann et al., 2002). Currently, research is being carried out to use natural phytohormones, such as extracts based on purple nutsedge (Cyperus rotundus) and violet (Saintpaulia ionantha), to increase rooting.

In this context, the objective of this study was to evaluate the effects of different phytohormones on the production of Eucalyptus dunnii and Eucalyptus badjensis seedlings via vegetative propagation by mini-cutting.

MATERIALS AND METHODS

For the establishment of the clonal mini-gardens of E. dunnii and E. badjensis, the sources of mini-cuttings used in this study were 120-day-old seedlings with a height of about 30 cm. The seedlings were transplanted to 2-L plastic vats containing commercial forest substratum based on pine bark and vermiculite, with four seedlings per vat.

After transplanting the seedlings to the pots, we pruned the aerial part to form the mini-stumps and, later, the clonal mini-gardens. Pruning was performed between 7 and 9 cm from the soil surface, leaving one pair of leaves to diminish stress and facilitate later budding.

In this experiment, indole butyric acid (AIB), indole acetic acid (AIA) and natural phytohormones, obtained by means of an aqueous extract of purple nutsedge (Cyperus rotundus) and violet (Saintpaulia ionantha), were applied to evaluate rooting potential. The following concentrations of AIB and AIA were evaluated: 0, 2,000, 4,000, 6,000 and 8,000 mg L⁻¹ (diluted to 50% in alcohol and 50% in v⁻¹ water). For the composition of extracts
with natural phytohormones, tubers of purple tea and violet leaves were used, crushed in a blender with 1,000 mL of distilled water. After processing, the suspension was sieved and diluted in distilled water in the following concentrations: 10, 25, 50 and 100%, these being the treatments used, to evaluate rooting potential. It should be noted that the aqueous extract was prepared on the same day as the treatment of the cuttings.

A randomised block design in a factorial scheme with two factors (one phytohormone and two different doses) was used in this experiment. The experiment presented eight mini-cuttings per experimental unit for each treatment, in four blocks. After 100 days of mini-cutting growth on the substrate, survival, height, diameter, shoot dry mass, dry mass of the root system, total dry mass and height/diameter ratio were evaluated. The results of this study were submitted to analysis of variance and Tukey's test (P≤0.05) in the statistical program Assistat 7.5.

Results:
There was no significant interaction (P <0.05) between the concentrations and phytohormones used for the parameters evaluated in the two *Eucalyptus* species; in addition, the concentration factor did not present significant differences in assessments. Analysis of the different phytohormones used in graphs 1 and 2 shows the survival percentages of *E. dunnii* and *E. badjensis* stem cuttings in the different phytohormones evaluated.

![Graph 1](image1.png)

*Means followed by the same letter do not differ statistically from each other by Tukey’s test (P < 0.05).*

**Fig. 1:** Survival means for *Eucalyptus dunnii* seedlings produced from stem cuttings treated with different phytohormones.

![Graph 2](image2.png)

*Means followed by the same letter do not differ statistically from each other by Tukey’s test (P < 0.05).*

**Fig. 2:** Survival means for *Eucalyptus badjensis* seedlings produced from stem cuttings treated with different phytohormones.
Table 1 shows the morphological parameters of *E. dunnii* seedlings in the evaluated treatments.

**Table 1**: Averages of height, diameter, dry mass of the aerial part, dry mass of the root system, total dry mass and height/diameter ratio for *Eucalyptus dunnii* seedlings produced from stem cuttings treated with different phytohormones.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Height (cm)</th>
<th>Diameter (Mm)</th>
<th>MAS (g)</th>
<th>MSR (g)</th>
<th>MST (g)</th>
<th>H/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIB</td>
<td>25.53 a</td>
<td>2.68 a</td>
<td>1.56 a</td>
<td>0.65ab</td>
<td>2.21 a</td>
<td>9.55 a</td>
</tr>
<tr>
<td>AIA</td>
<td>26.79 a</td>
<td>2.84 a</td>
<td>1.79 a</td>
<td>0.79a</td>
<td>2.59 a</td>
<td>9.46 a</td>
</tr>
<tr>
<td><em>Saintpaulia ionantha</em></td>
<td>23.83 a</td>
<td>2.46 a</td>
<td>1.69a</td>
<td>0.54 b</td>
<td>2.23 a</td>
<td>9.31 a</td>
</tr>
<tr>
<td><em>Cyperus rotundus</em></td>
<td>25.14 a</td>
<td>2.47 a</td>
<td>2.05 a</td>
<td>0.63 ab</td>
<td>2.69 a</td>
<td>9.79 a</td>
</tr>
<tr>
<td>CV%</td>
<td>23.77</td>
<td>23.80</td>
<td>38.97</td>
<td>42.72</td>
<td>37.85</td>
<td>21.80</td>
</tr>
</tbody>
</table>

MAS = dry mass of the aerial part, MSR = dry mass of the root system, MST = total dry mass, H/D = height/diameter ratio. * Means followed by the same letter in the column do not differ statistically from each other by Tukey's test (P <0.05).

Table 2 shows the average values of the morphological parameters evaluated for *E. badjensis*.

**Table 2**: Averages of height, diameter, dry mass of the aerial part, dry mass of the root system, total dry mass and height/diameter ratio for *Eucalyptus badjensis* seedlings produced from stem cuttings treated with different phytohormones.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Height (cm)</th>
<th>Diameter (mm)</th>
<th>MAS (g)</th>
<th>MSR (g)</th>
<th>MST (g)</th>
<th>H/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIB</td>
<td>13.10c</td>
<td>1.50 b</td>
<td>0.29 c</td>
<td>0.09 c</td>
<td>0.38 c</td>
<td>8.54 ab</td>
</tr>
<tr>
<td>AIA</td>
<td>14.13 c</td>
<td>1.65 b</td>
<td>0.36 c</td>
<td>0.13 c</td>
<td>0.47 c</td>
<td>8.17 ab</td>
</tr>
<tr>
<td><em>Saintpaulia ionantha</em></td>
<td>19.90 a</td>
<td>2.09 a</td>
<td>1.04 a</td>
<td>0.63 a</td>
<td>1.67 a</td>
<td>9.10 a</td>
</tr>
<tr>
<td><em>Cyperus rotundus</em></td>
<td>16.67ab</td>
<td>2.25 a</td>
<td>0.74 b</td>
<td>0.40 b</td>
<td>1.14 b</td>
<td>7.36 b</td>
</tr>
<tr>
<td>CV%</td>
<td>24.29 %</td>
<td>20.94 %</td>
<td>43.59 %</td>
<td>25.85 %</td>
<td>25.13 %</td>
<td>19.61 %</td>
</tr>
</tbody>
</table>

MAS = dry mass of the aerial part, MSR = dry mass of the root system, MST = total dry mass, H/D = height/diameter ratio. * Means followed by the same letter in the column do not differ statistically from each other by Tukey's test (P < 0.05).

**Discussion:**

As seen in Figures 1 and 2, the two species responded differently to the treatments. For *E. dunnii*, the application of AIA promoted the highest percentage of rooting at the end of the study. However, this did not differ significantly from AIB. Both treatments showed higher results than natural rooting. For *E. badjensis*, the natural phytohormones were superior to AIA and AIB, and rooting in *S. ionantha* differed statistically from that in the other species. Languinottiet al. (2011), evaluating the rooting of *Eucalyptus benthamii* cuttings with the use of AIA, AIB and purple nutsedge extract, observed that there was no significant difference in treatments in relation to the percentage of rooted cuttings for the different phytohormones used. In addition, the different concentrations did not result in different outcomes. However, in our study, the pattern was different, and AIA and AIB resulted in higher rooting rates when compared to *C. rotundus*.

Almeida et al. (2007), studying the effects of AIB and ANA on the rooting of *Eucalyptus cloeziana* cuttings, found significantly higher rates for AIB compared to ANA. Analysing the doses of indolebutyric acid used in rooting and growth experiments on *Eucalyptus urophylla* cuttings, Lana et al. (2008) observed that indolebutyric acid resulted in higher rooting and seedling growth rates, irrespective of the application form (paste or powder).

In a study by Titon et al. (2003), regarding the effect of AIB on the rooting of mini-cuttings and micro-cuttings of *Eucalyptus grandis* clones, the use of AIB did not influence the survival of the micro-shoots at the exit of the greenhouse. Pires, Wendling and Brondani (2013), evaluating indolebutyric acid and orthotropism in mini-cuttings of *Araucaria angustifolia*, have shown that auxin can limit rooting. Survival occurred in approximately 50% of the mini-cuttings, but rooting was low, only reaching up to 26%.

Thus, the influence of the different phytohormones used in the rooting of *Eucalyptus* cuttings varies according to the evaluated species. In addition, the application form (paste or liquid) can be another factor that directly influences the survival of the cuttings. Wilson (1994) points out that in vegetative propagation of *Eucalyptus* by cutting, indolebutyric acid (AIB) is the most commonly used auxin, mainly with dosages of 6,000 to 8,000 mg L⁻¹. However, Titon et al. (2003), evaluating the effect of AIB on the rooting of mini-cuttings of *Eucalyptus grandis* clones, observed that with the application of 1,000 to 2,000 mg L⁻¹ of AIB, optimum survival and rooting rates were obtained for most clones in the study.

In addition to the application of phytohormones, the applied doses also led to varying results in different studies. In this work, we observed no differences between doses, similar to the results found by Languinottiet al. (2011), evidencing the need for further studies evaluating the doses, as the application form for the species *E. badjensis*, which does not present research in this line of study. Based on the results of our study, using natural phytohormones to induce rooting leads to the highest percentage survival of *Eucalyptus* cuttings.

However, studies on the use of natural phytohormones as rooting enhancers are still scarce. Analysing the effect of *C. rotundus* on the rooting of coffee cuttings, Santos et al. (2011) verified that treatments based on watery extracts of purple nutsedge had a significant influence on the number of rooted cuttings. In contrast,
Languinottiet al. (2011) did not observe any effects of *C. rotundus* extract on the rooting of *E. benthamii* cuttings. This is another indicator that species respond differently to the application of phytohormones.

To date, violet extract is not commonly used as a rooting enhancer. Although its application has resulted in high rooting rates, its behaviour as a phytohormone is still unknown. In this study, violet extract had a beneficial effect on the rooting of *E. badjensis* seedlings.

For *E. dunnii*, there was no significant difference in mean height, diameter and dry mass of the aerial part and total dry mass as well as for the H/D ratio between the treatments (Table 1). However, for the parameter root system dry mass, application of AIA promoted a greater volume of roots, although this differed statistically only from that in *S. ionantha*. It should be noted that root system mass is an important aspect in seedling production, since the roots are responsible for optimal seedling development and among the main parameters analysed to evaluate seedling quality, Languinottiet al. (2011), analysing the influences of different phytohormones on morphological parameters in *E. benthamii* stakes, did not find a significant difference between the analysed variables. This is in agreement with the results found by Borges et al. (2005), who evaluated the rooting of mini-clones of *Eucalyptus globulus* and did not find significant effects of AIB concentrations on the morphological characteristics of the evaluated plants. Similarly, Lana et al. (2008) state that the use of phytoregulators in any concentration and application form does have no impact on the dry shoot and shoot biomass of *Eucalyptus urophylla*. Silva et al. (2011), evaluating the growth of coffee seedlings immersed in purple nutsedge extract, showed that the aqueous extract of purple nutsedge had no influence on the growth of the root system and on other morphological variables.

For *E. badjensis*, treatment with violet (*S. ionantha*) extract led to significantly different results compared to AIA and AIB treatments, but the results were similar to those obtained with purple nutsedge (*C. rotundus*) extract. In terms of diameter, treatment with purple nutsedge led to significantly different results compared to AIA and AIB treatment. In relation to dry mass of the aerial part, dry mass of the root system and total dry mass, treatment with violet extract obtained the highest values, significantly differing from those obtained by the other treatments. In contrast, overall H/D ratio did not differ between violet treatment and AIB/AIB treatment, with the exception of *C. rotundus*.

Rossarolla et al. (2013), evaluating the use of purple nutsedge extract in the induction of sprouting in mini-cuts of acerola (*Malpighia emarginata* DC), observed that its use induced greater sprouting in cuttings of this species. Silva et al. (2011), evaluating the growth of coffee seedlings immersed in purple nutsedge extract, observed that, in relation to shoot growth, aqueous extracts of 800 and 400 g L$^{-1}$ resulted in higher amounts of dry matter and greater seedling height.

In rooting tests using IAC 313 on vine cuttings with extracts of nutsedge, Coltro et al. (2011) concluded that the cuttings treated with 1% *C. rotundus* extract had a higher number of roots per stem and a lower number of stems without roots, indicating the efficiency of this treatment. Once again, evaluating the morphological parameters, we notice that the two *Eucalyptus* species responded differently to the application of phytohormones. Specifically, *E. badjensis* responded positively to natural phytohormones, mainly to *S. ionantha* extract. However, further studies are recommended to prove the efficiency of these substances.

**Conclusion:**

The two *Eucalyptus* species evaluated in this study responded differently to the used phytohormones. For *E. dunnii*, the application of AIA promoted the highest percentage of rooting at the end of the study. However, this did not differ significantly from AIB. Both treatments showed higher results than natural rooting. For *E. badjensis*, the natural phytohormones were superior to AIA and AIB, and rooting in violet (*S. ionantha*) differed statistically from that in the other species.

To date, violet extract is not commonly used as a rooting enhancer. Although its application has resulted in high rooting rates, its behaviour as a phytohormone is still unknown. In this study, violet extract had a beneficial effect on the rooting of *E. badjensis* seedlings.

For *E. dunnii*, there was no significant difference in mean height, diameter and dry mass of the aerial part and total dry mass as well as for the H/D ratio between the treatments. However, for the parameter root system dry mass, application of AIA promoted a greater volume of roots, although this differed statistically only from that in violet (*S. ionantha*). It should be noted that root system mass is an important aspect in seedling production, since the roots are responsible for optimal seedling development and among the main parameters analysed to evaluate seedling quality.

For *E. badjensis*, treatment with violet (*S. ionantha*) extract led to significantly different results compared to AIA and AIB treatments, but the results were similar to those obtained with purple nutsedge (*C. rotundus*) extract. In terms of diameter, treatment with purple nutsedge (*C. rotundus*) led to significantly different results compared to AIA and AIB treatment. In relation to dry mass of the aerial part, dry mass of the root system and total dry mass, treatment with violet extract obtained the highest values, significantly differing from those obtained by the other treatments. In contrast, overall H/D ratio did not differ between violet treatment and AIB/AIB treatment, with the exception of purple nutsedge (*C. rotundus*).
Thus, the influence of the different phytohormones used in the rooting of *Eucalyptus* cuttings varies according to the evaluated species. In addition, the application form (paste or liquid) can be another factor that, nutrition, and the intrinsic characteristics of each species, present directly influences in the survival of the cuttings.

The importance of this work is emphasized, since the *Eucalyptus badjensis* species, resistant to intense cold is promising for the southern region of Brazil, however, it presents few studies, including basic studies on seedling production, and adaptation to different types of soil by example. In addition, this study proposes the use of an alternative form of rooting that deals with natural phytohormones such as violet (*S. ionantha*) and purple nutsedge (*C. rotundus*), which are very common in Brazil, and are efficient but do not yet have many studies with eucalyptus species. The use of natural phytohormones may be an alternative to the use of synthetic rooting, thus constituting an alternative of low cost, contributing to the reduction of production costs and consequently the final value of the seedlings produced.

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