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Clonal propagation of *Eucalyptus urophylla* under effect of *Cyperus rotundus* extract and indole-3-acetic acid

Propagação clonal de *Eucalyptus urophylla* sob efeito do extrato de *Cyperus rotundus* e ácido indol-3-acético

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Cyperus rotundus tubers possess substances with allelopathic qualities. Some of these substances can act as synergists of plant hormones and be used for root induction in cuttings. This study aimed to shed light on the development and rooting of *Eucalyptus urophylla* cuttings treated with *C. rotundus* (nutgrass) tuber extract and IAA (indole-3-acetic acid). The experiments were conducted using a completely randomized design (0; 25; 50; 75 and 100% nutgrass extract and 0; 50; 250; 500; 750 and 1000 mg L^{-1} of IAA), with four repetitions per treatment and four cuttings per plot. Plantlet height, stem width and root length were measured. The greatest width and height values were recorded for the control (0% nutgrass extract and 0 mg L^{-1} of IAA), indicating that these substances did not contribute to the development of these variables. However, treatments of nutgrass extract up to 50% were associated with greater root length. *C. rotundus* extract concentrations of 53% can be used to improve root development in *E. urophylla* cuttings, and contribute to the production of higher quality plantlets.
Keywords: nutgrass, plant hormone, vegetative propagation.

Os tubérculos de *Cyperus rotundus* (tiririca) possuem substâncias com atividade alelopática, sendo algumas destas, capazes de atuar como hormônios vegetais e ser usados na indução de raízes em estacas. Este trabalho teve como objetivo elucidar o desenvolvimento e o enraizamento de estacas de *Eucalyptus urophylla* tratadas com extrato de tubérculo de *C. rotundus* e AIA (ácido indol-3-acético). Os experimentos foram conduzidos em delineamento inteiramente casualizado (0; 25; 50; 75 e 100% de extrato de tiririca e 0; 50; 250; 500; 750 e 1000 mg.L^{-1} de IAA), com quatro repetições por tratamento e quatro estacas por pote. As variáveis quantificadas foram altura das mudas, largura do caule e comprimento da raiz. Os maiores valores de largura e altura foram registrados na testemunha (0% extrato de tiririca e 0 mg L^{-1} de IAA), indicando que não contribuíram para o desenvolvimento dessas variáveis. No entanto, um aumento de até 50% no extrato de tiririca causou um aumento no comprimento da raiz, principalmente quando utilizado com 50 mg.L^{-1} de AIA. Concentrações de extrato de *C. rotundus* de 53% podem ser utilizadas para melhorar o desenvolvimento radicular em estacas de *E. urophylla*, contribuindo para a produção de mudas de melhor qualidade.
Palavras-chave: tiririca, hormônio vegetal, propagação vegetative.

1. INTRODUCTION

A significant percentage of lumber comes from planted forests (46%) with the highest production in tropical and subtropical areas [1]. The impact of planted forests on natural ecosystems is controversial. Nevertheless, preserving these ecosystems requires management practices that allow producers to meet rising market demand while taking into consideration the ecological, economic and social implications throughout the entire growth process [2].

The genus *Eucalyptus* is found in diverse ecological settings and is valued by the bioenergy industry for its short rotation, rapid growth, superior wood properties and high adaptability to different climatic conditions [3, 4]. In recent years, some *Eucalyptus* species have gained market attention as superior sources of lignocellulosic biomass and terpenes, and for their potential in the production of high-power and high-value biofuels [5].

Eucalyptus is the most planted forest species in Brazil, [6]. Within this genus, *Eucalyptus urophylla* and its hybrids are the most frequently used in commercial plantations and are obtained via vegetative propagation in clonal mini-gardens that produce commercially named clones [7, 8].

Eucalyptus clonal forestry programs provide genetic gains of approximately 25 to 50%, depending on how the clones are selected. In general, about 5 to 14 clones are typically used for new plantations, reaching annual average growth rates of 41 m^3 per hectare per year, according to optimized clonal propagation and forest management [9, 10].

Despite advances in the field, the rooting of clones can be challenging and demands attention for the induction and development of adventitious roots (AR) [11]. AR is a highly complex, multi-level regulated developmental process, that is affected by several endogenous and environmental factors [12].

Plant hormones participate in this process by increasing the percentage, speed, quality and uniformity of rooting. Auxins, such as indole-3-acetic acid (IAA), are related to the hormones that are most responsible for regulating AR formation [13]. Trueman (2018) [14] emphasized the necessity of finding optimal auxin doses for increasing adventitious root numbers, since higher doses can lead to defoliation and cutting death.

Cyperus rotundus L. (nutgrass or nutsedge) can cause 20–90% yield losses in various crops, and is considered the most significant weed worldwide [15]. Despite its negative effect on crops, nutgrass is an important medicinal plant, containing essential oils that have important compounds with antioxidant, anti-allergic, insecticidal, antimicrobial, hepatoprotective, anti-diabetic and anticonvulsant properties [16-18].

Nutgrass extract can influence some steps of plant metabolism, producing similar results to those of auxins. Allelochemicals are biochemicals produced plants that influence the development of surrounding organisms. These substances are useful in agriculture since they can improve plant growth in various ways, including the promotion of root development. These natural phytometabolites are free, environmentally friendly, biodegradable, and safe alternatives to synthetic plant growth regulators and phytohormones [19, 20].

IAA and Nutgrass extract have been used to stimulate rooting in various species [21-23]. Studies have shown that aqueous extracts of *Cyperus rotundus* tubers possess significant quantities of root promoting auxin, which is an effective root-promoting phytohormone for *Prunus persica* and *Handroanthus chrysotrichus* [24, 25]. Auxins are effective because they aid cell division and differentiation and regulate tropisms, apical dominance, root initiation and vascular tissue differentiation [26]. According to Delazeri et al. 2017 [27] despite the advantages of nutgrass extracts, related studies on eucalyptus are scarce.

Therefore, the present study aimed to assess shoot development and rooting in *Eucalyptus urophylla* cuttings after applications of *Cyperus rotundus* tuber extract and IAA.

2. MATERIAL AND METHODS

The experiment was carried out in a greenhouse, with lateral and overhead shading (50%), located at the Universidade Estadual de Goiás - University Unit of Ipameri (17º43’0” S, 48º8’38” W and 773 m altitude), Ipameri, Goiás, Brazil. The region has a tropical climate with a dry winter and wet summer (Aw) (Köppen).

Clonal *Eucalyptus urophylla* cuttings were obtained from parent plants at a commercial nursery. The eucalyptus mini-cuttings were produced in a mini-garden, harvested at 4 cm (height), refrigerated with water to maintain moisture, and transported to the laboratory for planting. *Cyperus rotundus* extracts were obtained from fresh tubers that had been washed, dried with paper towels and weighed, using 10 g of tubers per 200 ml of water. An exsicatti (dried specimen) of the plant sample was deposited in the Herbarium of Universidade Estadual de Goiás (HUEG) and registered as number 11770.

After processing, the samples were sieved and diluted in distilled water at the following concentrations: 0%; 25%; 50%; 75% and 100%. The extracts were prepared 24 hours before application to the cuttings and kept on ice until use. The commercial IAA concentrations used were 0 mg L^{-1}; 50 mg L^{-1}; 250 mg L^{-1}; 500 mg L^{-1}; 750 mg L^{-1} and 1000 mg L^{-1}.

The mini-cuttings were submitted to the different nutgrass and IAA treatments by immersing a stalk in the extract for 1 minute. The cuttings were then planted in polypropylene tubes (53 cm^3) containing a substrate of medium-grain vermiculite and then placed in a greenhouse with lateral and overhead shading. The temperature was monitored by thermometers (~ 25ºC) and the humidity was maintained by nebulization four times a day for 25 minutes. Nebulization was suspended overnight.

A completely randomized design was used with a factorial arrangement of 5 nutgrass and 6 IAA concentrations, four repetitions per treatment in both experiments and four cuttings per plot. The cuttings were assessed 90 days after planting (DAP). The following variables were measured: plant height (cm) and root length (cm), measured with a ruler, and stem width (cm), determined using a caliper.

Data were submitted to analysis of variance (ANOVA) and a means test with splits. Afterwards, polynomial or linear regression analysis was used to assess the effects of the nutgrass and IAA doses. All statistical analyses were performed using Sisvar v.5.6. software [28].

3. RESULTS AND DISCUSSION

Clonal propagation of *Eucalyptus urophylla* can be optimized by applications of *Cyperus rotundus* tubercle extract and exogenous auxin (IAA), used either alone or in combination, to enhance the rooting of mini-cuttings. The propagules that did not receive applications of nutgrass extracts and/or IAA did not root within the time period of the experiment. The lowest concentrations of nutgrass extract and IAA produced the best rooting, while higher concentrations, greater than 70% for nutgrass extract and 500 mg L^{-1} for IAA, reduced rooting in correlation with rising doses.

The results indicated that nutgrass tuber extracts and IAA had a significant effect ($p < 0.05$) on stem height and diameter and root length. However, the interaction between the two did not significantly affect ($p>0.05$) seedling height (Table 1). The means test showed that rooting was optimized by combinations of low doses of IAA (between 50 and 250 mg L^{-1}) and nutgrass extract (from 25% to 50%). The combination of IAA 50 mg L^{-1} and nutgrass extract 50% produced roots that were 32% greater than those produced by IAA 50 mg L^{-1} in isolation. The effect was a 12% increase in stem height. Stem diameter was not significantly affected by the various applications, but in general, the smallest diameters were found in the treatments with the highest concentrations.

While some studies have shown that nutgrass extract increases rhizogenesis, and other studies have looked at combinations of the extract and various hormones, none have looked at interactions between nutgrass extract and IAI. The positive effect of nutgrass extract on plant growth has also never been explained. One study showed that the root length of *Duranta repens* cuttings was approximately 5% greater than it was with applications of 500 mg L^{-1} IBA; however, the number of roots was 15% greater than with IBA [29]. Thiesen et al. (2019) [26] studied grapevine cuttings and found that nutgrass extract promoted root elongation to the same degree as AIB and ANA. Another study examined the effect of nutgrass extract alone on *Handroanthus chrysotrichus* cuttings, and found that the cuttings that remained immersed in 50% extract had 31.7% and 40% higher rooting rate and root compliance, respectively, than did the control (without extract exposure) [25].

Enhanced root development at low doses of IAA and nutgrass extracts may be associated with the presence of phenolic acids that act as growth regulators and inhibit auxin oxidases and prevent the degradation of IAA in cuttings [30]. A phytochemical characterization of *Cyperus rotundus* showed the presence of two bioactive phenolic compounds, quercetin and chlorogenic acid, which can act as non-hormonal plant regulators [31].

Table 1: Analysis of variance and means test of the effects of Cyperus rotundus *extract concentrations and IAA doses on the cutting height and diameter and root length* Eucalyptus urophylla *cuttings.*

		Height (cm)		Diameter (cm)		Root (cm)	
FV	GL	QM	P	QM	P	QM	P
Extract	4	19.12	0.01	0.013	0.00	48.44	0.03
IAA	5	68.97	0.00	0.056	0.00	118.75	0.00
Extract x IAA	20	6.53	0.29	0.004	0.01	59.01	0.00
Residual	90	5.54		0.002		17.68	
CV%		26.23		3.8		47.7	

Height (cm)

	Extract (%)					
IAA (mg/L)	0	25	50	75	100	$\bar{x}$ IAA
0	10.91	10.7	11.1	10.0	5.87	9.71 B
50	11.66	12.3	12.39	12.61	10.67	11.9 A
250	11.64	11.01	10.7	8.1	7.38	9.76 B
500	7.83	8.0	6.31	6.81	5.4	6.87 C
750	7.71	6.79	6.15	8.39	9.29	7.67 C
100	9.29	7.53	8.1	8.18	6.25	7.87 C
$\bar{x}$ nutgrass	9.81 a	9.39 a	9.12 a	9.01 a	7.47 b	

Diameter (cm)

	Extract (%)				
IAA (mg/L)	0	25	50	75	100
0	1.4 aA	1.35 aA	1.38 aA	1.32 bB	1.26 aB
50	1.4 aA	1.36 bA	1.37 bA	1.46 aA	1.34 bA
250	1.38 aA	1.35 aA	1.38 aA	1.31 bB	1.3 bA
500	1.31 aB	1.27 aB	1.23 aB	1.29 aB	1.31 aB
750	1.33 aB	1.31 aA	1.24 bB	1.24 bB	1.29 aA
100	1.26 aB	1.24 aB	1.23 aB	1.25 aB	1.21 aB

Root (cm)

	Extract (%)				
IAA (mg/L)	0	25	50	75	100
0	0.0 bB	9.98 aB	12.58 aB	9.77 aA	3.2 bB
50	12.63 bA	13.32 bA	18.59 aA	12.72 bA	7.67 bB
250	11.59 aA	14.07 aB	13.61 aA	6.76 bB	4.98 bB
500	7.07 aA	8.52 aB	4.6 aB	8.21 aA	3.28 aA
750	6.93 bA	6.72 bB	2.04 bB	10.76 aA	12.1 aA
100	12.15 aA	6.59 aB	6.91 aB	9.26 aA	7.7 aA

Lower case letters within the same row and uppercase letters with the same column do not differ by the means test at 5% probability.

Regression analysis was used to assess the effect of the nutgrass concentrations and showed decreasing linear behavior for plantlet height ($R^2 = 0.61$; $p = 0.043$) and stem width ($R^2 = 0.84$; $p = 0.002$) and quadratic behavior for root length ($R^2 = 0.99$; $p = 0.000$) (Figure 1).

Plantlet height and stem width showed similar behavior in relation to the nutgrass concentrations used (Figures 1A and 1B). The highest values for these variables were observed at the lowest concentrations of the extract, indicating that the extract did not contribute to height or width. The 50% nutgrass dose increased root length, whereas higher doses did not (Figure 1C).

There is still a lack of consensus regarding the importance of nutgrass extract on promoting plantlet rooting. In this respect, Dias et al. (2012) [32] analyzed rooting in coffee plant cuttings immersed in aqueous nutgrass extract and suggested it was not a viable rooting alternative. Coltro et al. (2011) [33] studied the rooting of IAC 313 grapevine rootstock cuttings in nutgrass extract and concluded that the cuttings treated with 1% extract resulted in more roots per cutting and fewer cuttings without roots. The aqueous nutgrass extract was efficient at promoting rhizogenesis and produced a similar rooting percentage to that observed for AIB solution (1000 mg L^{-1}) in *Solanum lycopersicum* L. leaves [34].

The stimulatory effect of exogenous auxins on roots (synthetic or from plant extracts) depends on the concentration applied to the base of the cutting, where root stimulation increases up to a maximum dose, after which any additional increases are inhibitory [14, 35]. The effect also depends on the composition of the phenolic compounds as they are transferred to the plantlets, and how interactions of these metabolites with auxin and peroxidases affect the formation of adventitious roots. Phenolics are also important in modulating peroxidase activity and can also act as antioxidants, preventing auxin degradation at the base of the cuttings [36]. In the present study, nutgrass extract showed an inhibitory effect at the 53% concentration (Figure 1C).

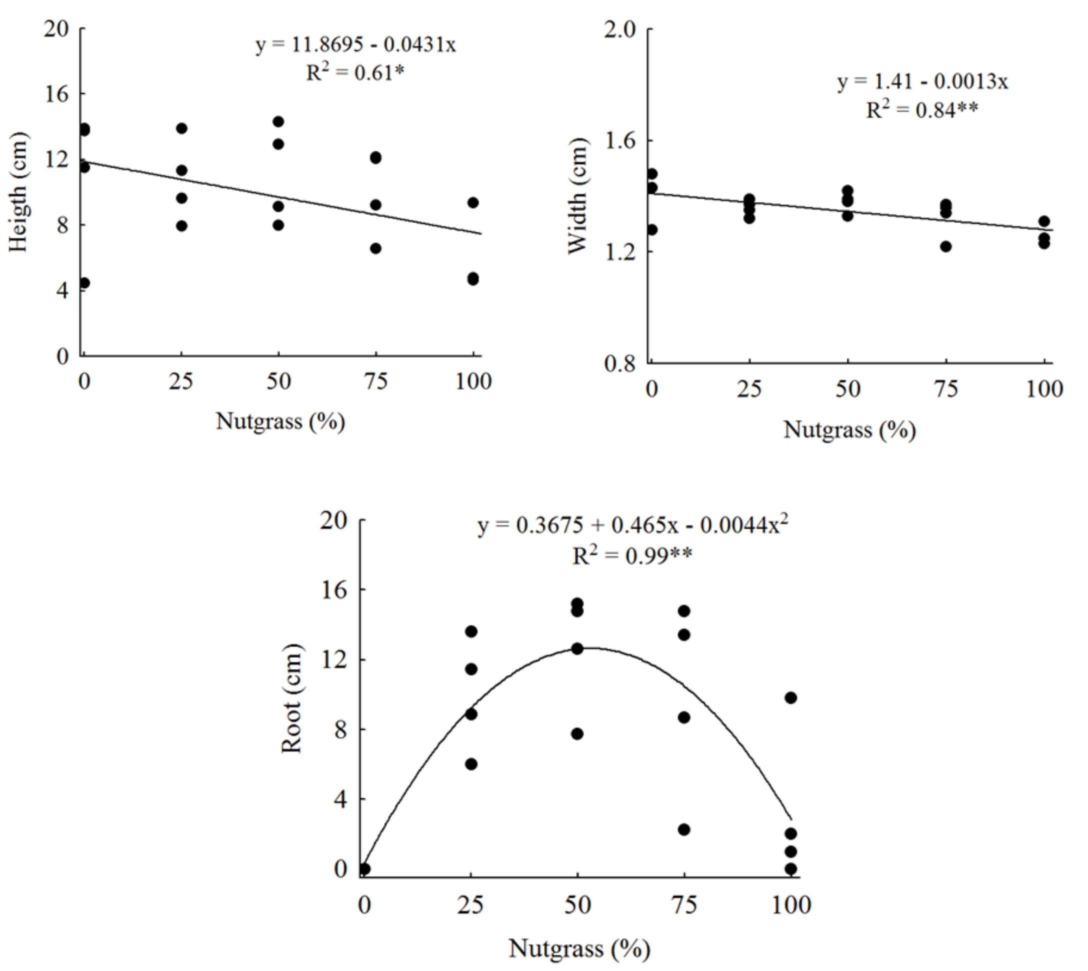

Figure 1: Regression analysis of the effect of Cyperus rotundus *extract on the variables studied. Effect of* C. rotundus *extract on (A) plantlet height, (B) stem width and (C) root length. Legend: * = significant regression (0.01 < p ≤ 0.05); ** = significant regression (p ≤ 0.01).*

Regression analysis of the effect of IAA demonstrated a linear decline for plantlet height ($R^2 = 0.52$; $p = 0.026$) and stem width ($R^2 = 0.90$; $p = 0.001$) and nonsignificant regression ($p > 0.05$) for root length. The highest values for plantlet height and stem width were obtained in the absence of IAA (Figure 2). An increase in IAA concentration had no effect on these variables (Figure 2A and 2B).

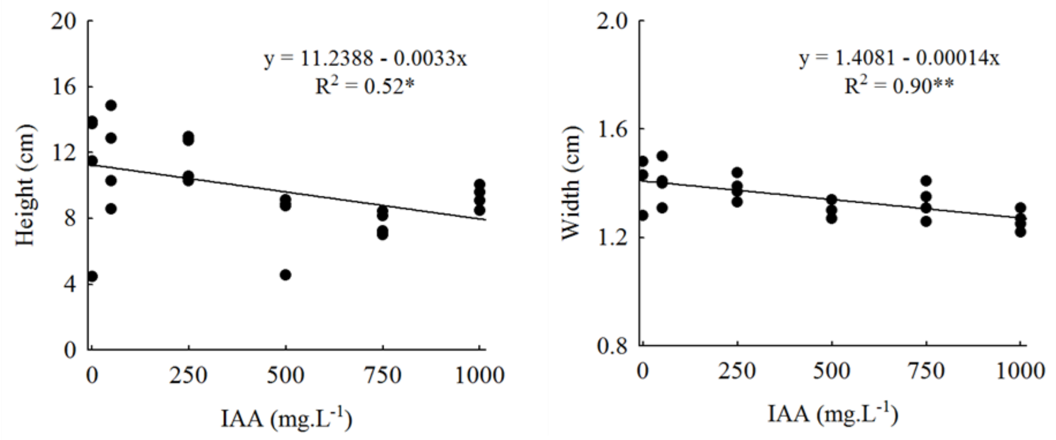

*Figure 2: Regression analysis of the effect of IAA on the variables studied. Effect of IAA on (A) plantlet height, and (B) stem width. Legend: * = significant regression (0.01 < p = 0.05); ** = significant regression (p = 0.01).*

Use of an exogenous auxin can promote root formation provided sufficient quantities are supplied [37]. This generally does not occur since, according to Negishi et al. (2014) [21], IAA is relatively unstable, despite being the primary endogenous auxin.

In a study on the effects of the plant growth regulators IBA and NAA on adventitious rooting in mini-cuttings of four *Eucalyptus grandis* x *E. urophylla* clones, Goulart et al. (2008) [38] found that the cuttings responded more efficiently to IBA than to NAA. IBA doses greater than 500 mg L^{-1} were more efficient in all four clones, whereas a degree of toxicity was observed in some clones at concentrations at or above 2000 mg L^{-1}. Scariot et al. (2017) [24] evaluated the effect of an aqueous extract of *C. rotundus* and IAA on hardwood peach cuttings and found greater rooting at 500 mg L^{-1} of IAA, but no improvement in rooting from nutgrass.

Câmara et al. (2016) [39] reported positive influences on the survival and sprouting rates of mini-cuttings of acerola with *C. rotundus* applications. Silva et al. (2011) [40] also detected beneficial results using *C. haspan* for rooting sugarcane. Azevedo et al. (2020) [41] evaluated *C. haspan* extract on coffee plants and found improvements when the extract was associated with humic acid, which may have resulted from the combined effect of the auxins present in both the humic acid and extract.

Delazeri et al. 2017 [27] evaluated applications of violet (*S. ionantha*) and nutgrass (*C. rotundus*) on *E. dunnii* and *E. badjensis* and reported that the species respond differently to the application of phytohormones and that more study was needed to find optimal species and doses. The authors also emphasized that natural phytohormones induce rooting, which leads to better survival rates in *Eucalyptus* cuttings.

4. CONCLUSION

Cyperus rotundus extract concentrations of 53% can be used to improve root development in *E. urophylla* cuttings, which contributes to higher quality plantlets. An IAA concentration of 50 mg L^{-1} was the most beneficial for rooting. This benefit was even greater when combined with optimal doses of nutgrass extract.

Plant extracts show promising results and may be more effective than commercially available synthetic options. The findings of the current study underscore the impact and relevance of studies on plant extracts that shed light on the behavior of species and potential extracts, the use of naturally occurring resources and leveraging synergies within these systems.

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