SCIENTIA NATURALIS
Scientia Naturalis, v. 3, n. 5, p. 2174-2185, 2021
Home page: http://revistas.ufac.br/revista/index.php/SciNat
ISSN 2596-1640

Physiological responses of 6-benzylaminopurine on multiplication and indolebutyric acid on *in vitro* rooting of *Eucalyptus dunnii* Maiden

Karoline Gunther[1], Paulo Cesar Poeta Fermino Junior[2*]

[1]Graduação em Agronomia, Universidade Federal de Santa Catarina, Curitibanos, Santa Catarina, Brasil, [2]Professor da Universidade Federal de Santa Catarina, Departamento de Ciências Naturais e Sociais, Curitibanos, Santa Catarina, Brasil. *paulo.fermino@ufsc.br

Recebido em: 21/06/2021 **Aceito em: 23/11/2021** **Publicado em: 30/12/2021**

ABSTRACT

Eucalyptus dunnii Maiden is a wood species of wide interest and use in southern Brazil due to its adaptation to the cold climate. Low seed production favors use of plant tissue culture techniques for propagation. The objective of this work was to establish *in vitro* multiplication of *E. dunnii*, using micropropagation technique via direct organogenesis, evaluating different concentrations of 6-benzylaminopurine (BAP) in multiplication of the stem and cotyledon nodal segments, in addition to effect of different concentrations of indolebutyric acid (IBA) at rooting. After 30 days of culture, stem nodal segments had an increase in the percentage of shoots when subjected to low concentrations of BAP, while for the cotyledon nodal segments the absence of growth regulator and the use of a concentration of 2.0 mg L^{-1} of BAP showed the highest percentage of shoot formation. The increase in IBA concentration promoted increase in number of adventitious roots. *In vitro* propagation of *E. dunnii* using BAP and IBAare a viable protocol.

Keywords: Micropropagation. Growth regulators. Tree species.

Respostas fisiológicas do uso de 6-benzilaminopurina na multiplicação e do ácido indolbutírico no enraizamento *in vitro* de *Eucalyptus dunnii* Maiden

RESUMO

Eucalyptus dunnii Maiden é uma espécie madeireira de amplo interesse e uso no Sul do Brasil devido sua adaptação ao clima frio. A baixa produção de sementes favorece o uso de técnicas de cultura de tecidos vegetais para a propagação. O objetivo do trabalho foi estabelecer a multiplicação *in vitro* de *E. dunnii*, utilizando da técnica de micropropagação via organogênese direta, avaliando diferentes concentrações de 6-benzilaminopurina (BAP) na multiplicação dos segmentos nodais caulinares e cotiledonares, além do efeito das diferentes concentrações do ácido indolbutírico (AIB) no enraizamento. Após 30 dias de cultivo, segmentos nodais caulinares obtiveram aumento no percentual de brotos quando submetidos a baixas concentrações de BAP, enquanto que, para os segmentos nodais cotiledonares a ausência de regulador de crescimento e o uso da concentração de 2,0 mg L^{-1} de BAP apresentaram maior percentual de formação de brotos. O aumento da concentração de AIB promoveu o aumento do número de raízes adventícias. A propagação *in vitro* de *E. dunnii* com o uso de BAP e AIB é um protocolo viável.

Palavras-chave: Micropropagação. Reguladores de crescimento. Espécie arbórea.

INTRODUCTION

The *Eucalyptus* is considered one of the main wood species, a source of raw material for paper production, belonging to the Myrtaceae family, and originating in Australia and Indonesia with more than 600 species (IMA FLORESTAL, 2015). Among many, the species *Eucalyptus dunnii* Maiden has been standing out in the forest industry. One of the reasons is its adaptability to low temperatures, which is why it has been widely cultivated in the southern region of the country. In addition to being resistant to severe frosts, *E. dunnii* has another relevant characteristic, its low bioinvasion potential, which makes it difficult to randomly propagate, since it produces few seeds. For this reason, one of the alternatives for the production of seedlings is through micropropagation, as in addition to generating a clone of the mother plant, in vitro propagation guarantees homogeneous planting, on a large scale and in a short period of time. (ALMEIDA et al., 2008).

Many studies on plant tissue culture, especially by organogenesis, have been developed with Eucalyptus species in recent decades, requiring specific protocols (OLIVEIRA et al., 2017). Recently, investigations on *E. dunnii* have increased significantly with respect to *in vitro* propagation (OBERSCHELP et al., 2015; SOUZA et al., 2019), *in vitro* shoot elongation (NAVROSKI et al., 2015), and rooting adventitious (BRONDANI et al., 2018).

Among the steps of micropropagation, the multiplication and elongation phase is one of the most important, and in these phases the objective is to obtain a high number of shoots or buds, with the least possible genetic variation, in the shortest time interval and free from contaminant (OBERSCHELP et al., 2015). The culture medium used at this stage will also vary according to the needs of the species, in addition, growth regulators called cytokinins are often used, so that there is an induction of budding, with 6-benzylaminopurine (BAP) being the most used in woody species (OLIVEIRA et al., 2013). *In vitro* rooting can be performed in different culture media with the addition of inducing auxins. Among the various existing auxins, indolebutyric acid (IBA) is the most used, as it has low phytotoxicity to explants, enabling in vitro rooting to show positive results (SOUZA et al., 2019). The objective of this work was to compare the effect of different concentrations of 6-benzylaminopurine (BAP) on the *in vitro* multiplication of cotyledonary and stem nodal segments and to evaluate the effect of

IBA concentrations on the in vitro rooting of regenerated *Eucalyptus dunnii* Maiden microshoots.

MATERIAL AND METHODS

The plant material used for this work came from *Eucalyptus dunnii* Maiden seeds donated by IPEF (Institute for Research and Forestry Studies). The seeds were stored in a refrigerator (5 °C) at the Laboratory of Biotechnology and Genetics of the Federal University of Santa Catarina, Campus de Curitibanos, where all steps of the present work were also carried out. The seeds were cleaned with detergent and running water to remove surface residues. Then, in a laminar flow chamber, they were disinfected in a commercial solution of sodium hypochlorite (2.5%) for 30 minutes, and then washed three times in distilled and sterilized water. Subsequently, ten seeds were inoculated in each glass flask containing 30 mL of medium MS (MURASHIGE; SKOOG, 1962), supplemented with 30 g L^{-1} sucrose and 6 g L^{-1} agar-agar (Vetec®), and sealed with PVC film for *in vitro* germination.

Seedlings germinated in vitro were excised in the portion of the cotyledonary nodal segments and young plants after 90 days, with approximately 7.0 cm in length, were excised in the portions of the stem nodal segments (1.0 cm) containing two buds. The nodal segments were transferred to glass flasks containing 30 mL of MS culture medium (MURASHIGE; SKOOG, 1962), supplemented with 30 g L-1 of sucrose, different concentrations of BAP (0.0; 0.5; 1, 0 and 2.0 mg L^{-1}), 6 g L^{-1} agar-agar. The pH of the culture medium was adjusted to 5.8 with NaOH and 0.5N HCl before sterilization for 15 min at 1.3 kgf cm^{-2}. Flasks were sealed with parafilm and cultures were maintained in a growth room at 25 ±2 ºC, luminous intensity of 60 µmol m^{-2} s^{-1} obtained with cold white Sylvania® fluorescent lamps, with 16 h photoperiod light, with a distance of 10-12 cm in height from the crops. After thirty days, the explants were evaluated in relation to the percentage of sprout formation, calluses, base oxidation, contamination and explant death.

Microshoots regenerated in the different multiplication treatments, with approximately 5.0 cm in height, were transferred to glass flasks containing rooting medium with 30 mL of WPM culture medium (LLOYD; MCCOWN, 1980), supplemented with 30 g L^{-1} of sucrose, different concentrations of IBA (0; 1.0; 2.0; 4.0

mg L^{-1}), 6 g L^{-1} agar-agar. After 30 days of cultivation, the microsprouts were evaluated for rooting percentage, number of regenerated roots and their average length.

The experimental design was completely randomized. The treatments for *in vitro* multiplication were organized in a 2x4 factorial scheme, using two types of explants (cotyledonary node and stem node) with four concentrations of BAP (0.0, 0.5, 1.0 and 2.0 mg L^{-1}), containing six repetitions, each repetition being composed of five nodal segments in two bottles referring to the stem node, and two nodal segments in each repetition of the cotyledonary node. In the rooting experiment, four treatments were organized, containing five repetitions each, with each repetition being composed of 10 microplants in two flasks. Data were subjected to analysis of variance (ANOVA), and linear regression analysis when significant differences were detected, followed by Tukey's mean separation test ($P < 0.05$), using the R computer program.

RESULTS AND DISCUSSION

The formation of adventitious shoots from different types of nodal segments of *Eucalyptus dunnii* occurred in all evaluated treatments (Figure 1), even in the absence of growth regulator (Table 1). Statistical comparison of the percentage of shoot formation between the different concentrations of BAP from cotyledonary nodal segments revealed the absence of significant differences ($p<0.05$). With the use of stem nodal segments as an explant source for multiplication, the highest sprouting percentages occurred in the absence of growth regulator and in the use of 0.5 mg L^{-1} of BAP.

At concentrations of 0 and 2.0 mg L^{-1} of BAP, the percentage of shoot formation was greater in the cotyledonary nodal segments compared to the shoot nodal segments. In general, when working with the multiplication of *Eucalyptus*, cytokinin concentrations can vary from 0.1 to 5.0 mg L^{-1}, and these values vary according to the species and the cytokinin used. Furthermore, it is important to emphasize that the results of *in vitro* multiplication are directly influenced by the physiological state of the plant and the plant part used as an explant (GRATTAPAGLIA; MACHADO, 1998).

Table 1 - Percentage of sprouting, calluses, oxidation, death and contamination of cotyledonary nodal segments and shoot nodal segments of *Eucalyptus dunnii* Maiden in different concentrations of 6-benzylaminopurine (BAP) in mg L-1 in *in vitro* multiplication.

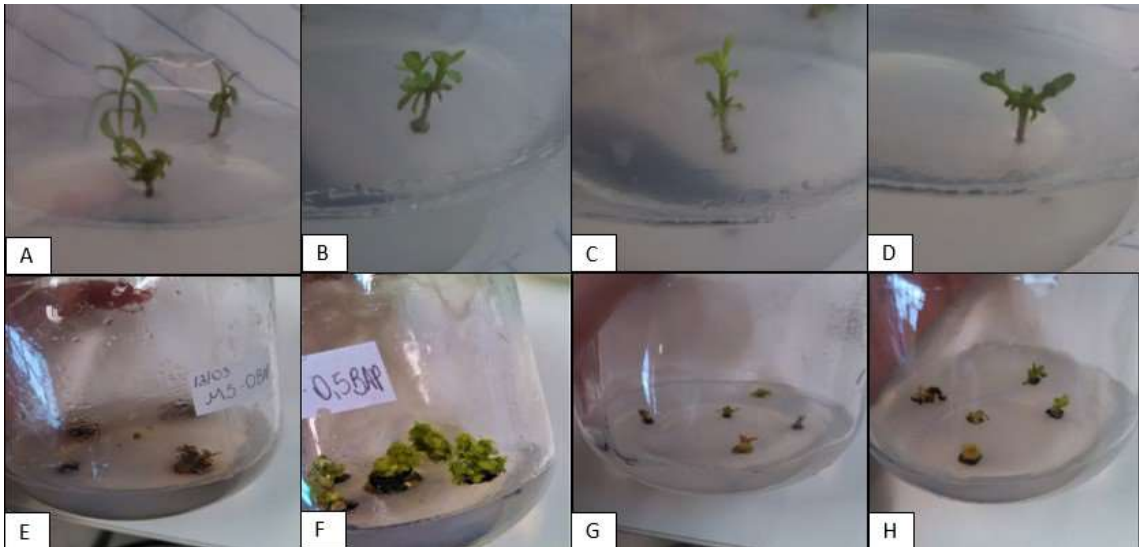
	Sprouting		**Calogenesis**		**Oxidation**		**Death**		**Contamination**	
	NCO	**NCA**	**NCO**	**NCA**	**NCO**	**NCA**	**NCO**	**NCA**	**NCO**	**NCA**
0	83.3Aa	60Ab	0Aa	90Ab	33.3Bb	100Aa	16.7Aa	23.3Aa	16.7Aa	16.7Aa
0.5	91.7Aa	83.8Aa	33.3Aa	83.3Ab	75Aa	100Aa	8.3Aa	16.7Aa	0Aa	0Aa
1.0	58.3Aa	43.3Aa	8.3Aa	33.3Cb	16.7Cb	100Aa	16.7Aa	16.7Aa	16.7Aa	16.7Aa
2.0	100Aa	53.3Ab	16.7Aa	46.7Bb	33.3Bb	100Aa	0Aa	33.3Aa	0Aa	33.3Aa
M	83.32	57.6	14.57	63.32	39.57	100	10.42	22.5	8.35	16.67
CV	35.5	57.25	17.8	49.43	87.06	0	29.94	17.35	34.41	23.38

Means followed by different uppercase letters vertically and lowercase letters horizontally differ by Tukey's test ($p<0.05$). NCO: Cotyledonary Node; NCA: Stem node; M: Average. CV: Coefficient of variation (%). M: mean.

The nodal segment to be used can influence organogenesis due to age or the type used, and it can be cotyledonary or stem. Fermino-Junior et al. (2009) in studies carried out with *Tectona grandis* L.f, showed that when younger explants are used, there is a more efficient induction of bud organogenesis, thus, the need for exogenous addition of BAP is smaller compared to adult explants. For the *Parapiptadenia* species, it was also observed that the best results for *in vitro* regeneration occurred in cotyledonary nodal segments (NASCIMENTO, 2008).

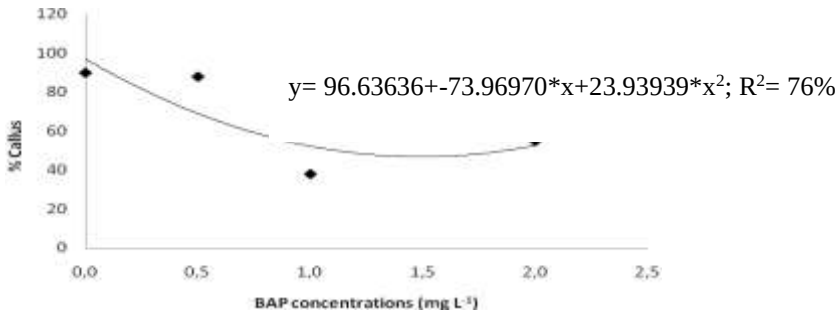
When it comes to the BAP phytoregulator, for the percentage of sprouts, studies carried out by Bennett et al. (1994) showed that when doses greater than 0.5 mg L^{-1} of BAP are used, the decrease in the percentage of yolks per multiplied explants is remarkable. For uvaieira (*Eugenia pyriformis* Cambess), the concentration of BAP that provides better development of buds and buds per explant was 1.0 mg L^{-1} (NASCIMENTO et al., 2008). Unlike these studies, for the tree species *Hancornia speciosa*, there is no need to use growth regulators for its *in vitro* multiplication, since, in the experiments performed, the absence of BAP showed the best results (OLIVEIRA et al., 2016).

Figure 1 – *In vitro* multiplication of *Eucalyptus dunnii* Maiden from cotyledonary nodal segments and non-cotyledonary nodal segments after 30 days of *in vitro* multiplication in MS culture medium, supplemented with different concentrations of BAP. A-D) Cotyledonary nodal segments. A) 0.0 mg L^{-1} BAP. B) 0.5 mg L^{-1} of BAP. C) 1.0 mg L^{-1} of BAP. D) 2.0 mg L^{-1} of BAP. E-H) Non-cotyledonary nodal segments. E) 0.0 mg L^{-1} of BAP. F) 0.5 mg L^{-1} of BAP. G) 1.0 mg L^{-1} of BAP. H) 2.0 mg L^{-1} of BAP.



The second parameter evaluated was the percentage of callus formation in the different types of nodal segments (Table 1). In cotyledonary nodal segments cultivated in a culture medium free of growth regulator, no callogenic mass was formed (Figure 1A). The addition of BAP at different concentrations in the culture medium promoted the formation of callus in cotyledonary nodal segments at all concentrations, showing no statistically significant differences ($p<0.05$) in the percentage of callogenesis. The percentage of callus formation in shoot nodal segments was higher in the absence of growth regulators (Figure 1E) and in the use of 0.5 mg L^{-1} of BAP (Table 1). The use of higher concentrations of BAP in the culture medium in stem nodal segments promoted a decrease in callogenesis until the concentration of 2.0 mg L^{-1} of BAP, expressed by quadratic equation in the regression analysis (Figure 2) with coefficient of determination (r^2) of 0.76.

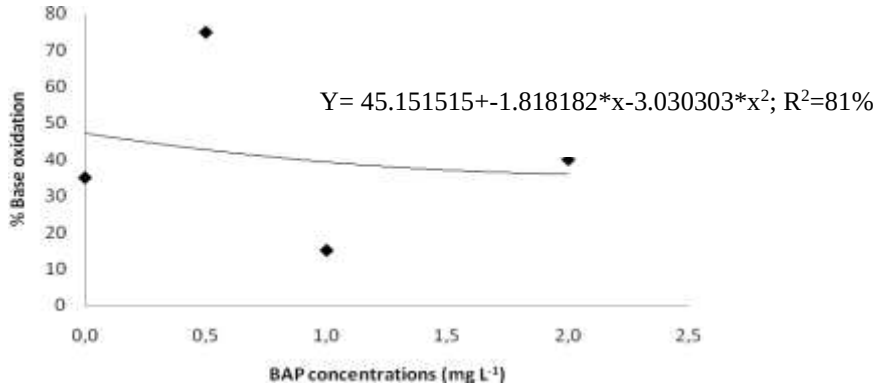
Figure 2 - Percentage of callus formation in shoot nodal segments of *Eucalyptus dunnii* Maiden subjected to different concentrations of BAP, after 30 days of *in vitro* multiplication.



According to studies, in woody species the formation of calluses is quite common, and these are formed when there is a balance between auxins and cytokinins (GRATTAPAGLIA; MACHADO, 1998). For *Schizolobium amazonicum*, studies showed that higher concentrations of cytokinin caused an increase in the percentage of calluses (BOTIN; CARVALHO, 2015). In cotyledonary nodal segments of *Parapiptadenia rigida*, when subjected to concentrations of BAP, callus formation was also observed, in this case, their occurrence can be explained by the fact that the species already has endogenous levels of auxins, which are sufficient for its multiplication, without the need for greater concentrations of auxins (NASCIMENTO, 2008). As common as callogenesis is, it is worth noting that in micropropagation it is undesirable, as it can affect the growth and development of explants.

Oxidation at the base of the cotyledonary and stem nodal segments occurred in all treatments used. The highest percentages of oxidation were observed in stem nodal segments (Table 1). In cotyledonary nodal segments, the highest percentage of oxidation occurred with the use of 0.5 mg L^{-1} of BAP. The increase in the concentration of BAP in the culture medium with cotyledonary nodal segments promoted a decrease in the percentage of oxidation, expressed by a quadratic equation of regression analysis (Figure 3) with a coefficient of determination (r^2) of 0.81. In stem nodal segments the percentage of oxidation was maximum (100%) in all treatments.

Figure 3 - Percentage of oxidation at the base of cotyledonary nodal segments of *Eucalyptus dunnii* Maiden at different concentrations of BAP, after 30 days of *in vitro* multiplication.



According to Silva et al., (2007) when working with the *in vitro* establishment of woody plants, one of the obstacles is the oxidation of explants. Several factors can influence oxidation, such as the plant's developmental stage, but what has the greatest

influence is the plant's genotype (WERNER et al., 2009). Furthermore, when excised angiosperms tend to secrete phenolic substances that can modify the culture medium, affect the absorption of metabolites and consequently cause oxidation (ANDRADE et al., 2000). The percentage of death (necrosis) and the percentage of contamination of the cotyledonary and stem nodal segments did not present statistically significant differences between treatments using BAP (Table 1).

The formation of adventitious roots in regenerated microsprouts occurred in all treatments, including in the absence of growth regulators (Table 2). The use of different concentrations of IBA did not promote statistically significant differences ($p<0.05$) in the percentage of rooting of microsprouts.

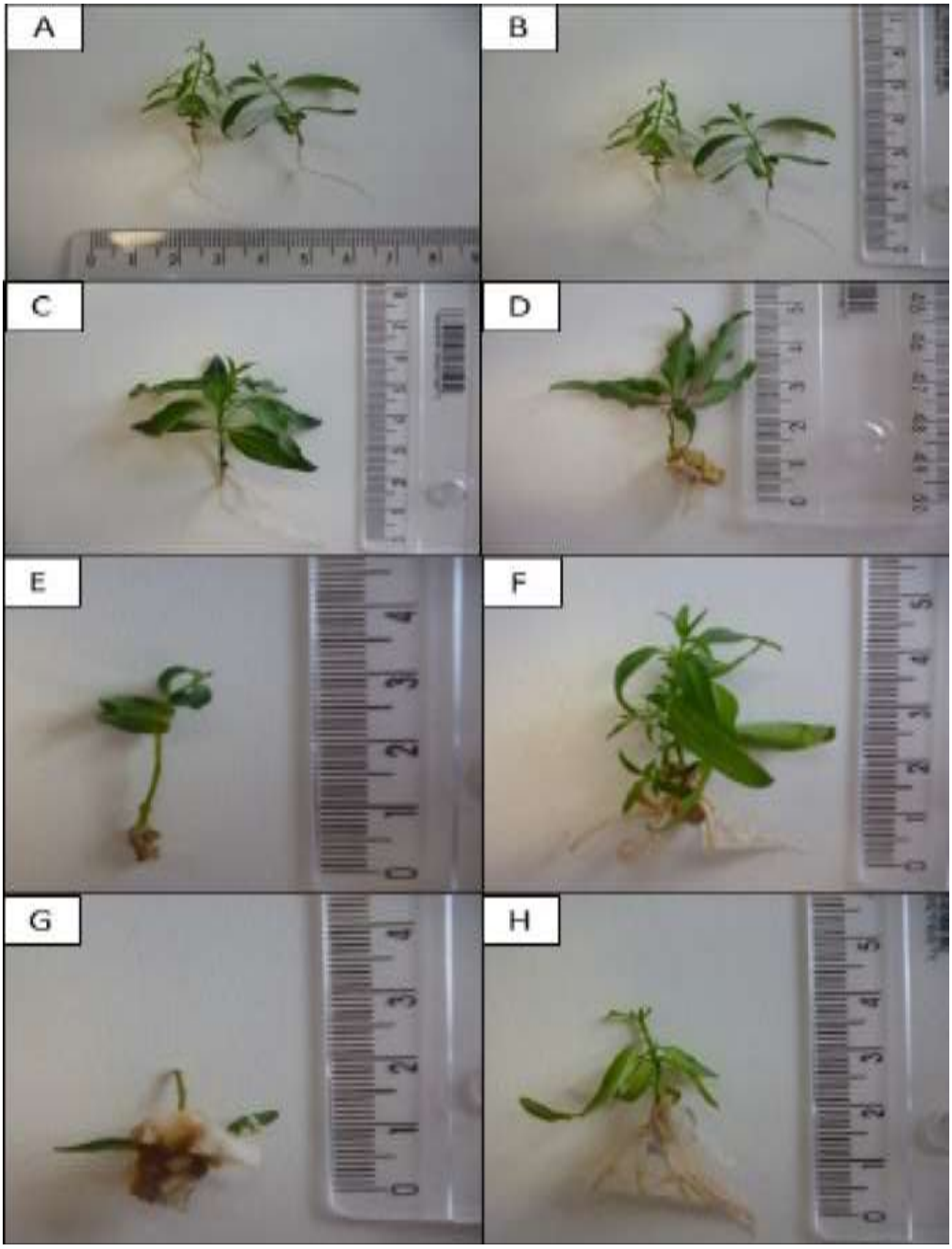
Table 2 – Percentage of roots, root number and length obtained through different concentrations of IBA in mg L^{-1} in the *in vitro* rooting of *Eucalyptus dunnii* Maiden.

IBA (mg L^{-1})	Rooting (%)	Root number	Lenght (cm)
0	43.20A	1.10D	2.24A
1.0	63.20A	3.70C	2.36A
2.0	100A	6.04A	0.94A
4.0	50A	4.94B	1.46A
Mean	64.10	3.94	1.75

Means followed by distinct uppercase letters on the vertical differ from each other by the Tukey test ($p<0.05$).

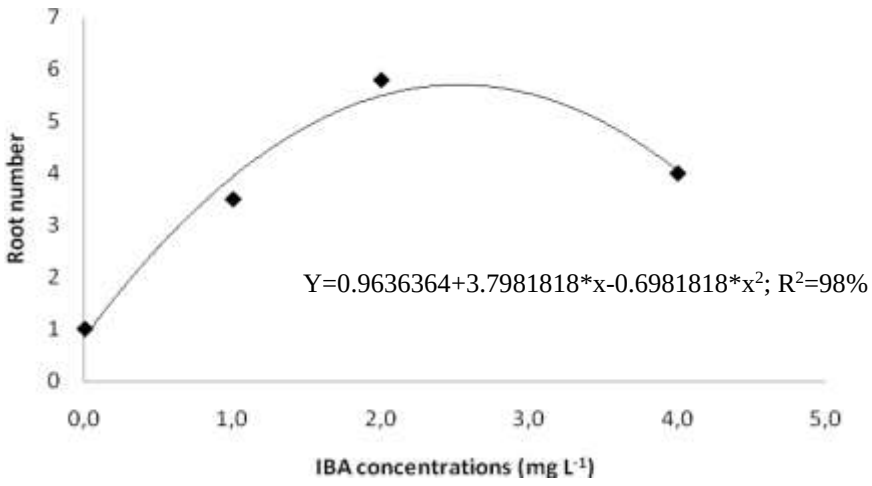
The number of regenerated adventitious roots (Figure 4A-H) in *E. dunnii* a presented statistically significant differences ($p<0.05$) between the different concentrations of IBA. The largest number of roots was observed with the use of 2.0 mg L^{-1} of IBA, the smallest number of roots in the absence of IBA.

Figure 4 – *In vitro* rooting of *Eucalyptus dunnii* Maiden microshoots at different concentrations of IBA in the rooting process, after 30 days of *in vitro* rooting. A-B) 0 mg L^{-1} of AIB. C-D) 1.0 mg L^{-1} of AIB. E-F) 2.0 mg L^{-1} of AIB. G-H) 4.0 mg L^{-1} of AIB.



The increase in the concentration of IBA promoted the increase in the number of adventitious roots, expressed by a quadratic equation of regression analysis (Figure 5), with a coefficient of determination (r2) of 0.98. The average length of adventitious roots did not show statistically significant differences ($p<0.05$) between treatments using IBA.

Figure 5 – Root number of *Eucalyptus dunnii* Maiden formed at different concentrations of IBA in mg L^{-1}



The AIB concentrations have varied results according to the parameters evaluated, since each species needs a specific amount of auxin, as shown by studies carried out by Mercier (2004), that during the rooting process the need for auxin concentration it varies according to the organogenetic phase that it is in, as a higher concentration is more used in the induction phase, consequently there may be a reduction or even inhibition of the growth of these roots in high concentrations. According to Souza et al. (2011) these data may vary according to the internal concentration of auxin of the species under study and their genotype, in addition, the period in which it is exposed to these auxins can influence the result Final.

For root formation Radmann et al. (2014) evaluated that IBA concentrations were essential for root formation in trees, as there was no root formation, or there was a small number of roots without the addition of IBA. Another study by Soares and Miranda (2016) confirms that the best dose of IBA in citrus was 2.0 and 1.0 mg L^{-1} of IBA. It is also worth mentioning the studies by Silva et al. (2013) who carried out studies with *Psychotria ipecacuanha* and showed that the best IBA concentration for root formation was 1.5 mg L^{-1} IBA. Furthermore, according to studies by Pedrotti (2001), when using auxins in the culture medium, high doses can influence the formation of calluses. In this experiment, there was no callus formation only at the concentration of 0 mg L^{-1} of IBA, in the other concentrations (1.0; 2.0; 4.0 mg L^{-1} of IBA) there was the formation of calluses.

CONCLUSION

The addition of different concentrations of BAP in the culture medium does not increase the percentage of sprouting in cotyledonary and stem nodal segments. In stem nodal segments, low concentrations of BAP promote an increase in the percentage of sprouting of *Eucalyptus dunnii* Maiden. The use of different concentrations of IBA does not promote statistically significant differences in the percentage of rooting of microshoots. The increase in the concentration of AIB promotes the increase in the number of adventitious roots.

ACKNOWLEDGMENT

To IPEF (Institute for Forestry Research and Studies) for the donation of *Eucalyptus dunnii* seeds.

REFERENCES

ALMEIDA, J. R.; MARTINS, C. R.; DUTRA, L. F. Desinfestação de segmentos nodais de *Eucalyptusdunnii* visando estabelecimento *in vitro*. **Revista da Fzva,** v. 15, n. 1, p. 54-60, 2008.

ANDRADE, M. W.; LUZ, J. M. Q.; LACERDA, S. A.; MELO, P. R. A. Micropropagação da aroeira (*Myracrodruonurundeuva* Fr. All). **Revista Ciência e Agrotecnologia**, v. 24, n. 1, p. 174-180, 2000.

BENNETT, I. J.; MCCOMB, J. A.; TONKIN, C. M.; MCDAVID, M. A. I. Alternating cytokinins in multiplication media stimulates *in vitro* shoot growth and rooting of *Eucalyptus globulus*Labill. **Annals of Botany**, v. 74, p. 53-58, 1994.

BOTIN, A. A.; CARVALHO, A. Reguladores de crescimento na produção de mudas florestais. **Revista de Ciências Agroambientais,** v. 13, n. 1, p.1-14, 2015.

BRONDANI, G. E.; OLIVEIRA, L. S. de; KONZEN, E. R.; SILVA, A. L L. da; COSTA, J. L. Mini-incubators improve the adventitious rooting performance of *Corymbia*and *Eucalyptus* microcuttings according to the environment in which they are conditioned. **Anais da Academia Brasileira de Ciências**, v. 90, p. 2409-2423, 2018.

FERMINO JUNIOR, P. C. P.; NAGAO, E. O.; PEREIRA, J. E. S. Estabelecimento, germinação e multiplicação *in vitro* de teca (*Tectona grandis* L. f.) a partir de genótipos da Amazônia Sul-Ocidental. **Scientia Forestales**, v. 37, n. 84, p.427-435, 2009.

GRATTAPAGLIA D.; MACHADO, M. A. Micropropagação. In: TORRES, A. C.; CALDAS, L. S.; BUSO, J. A. (Ed.). **Cultura de tecidos e transformação genética de plantas**. Brasília, DF: EMBRAPA-SPI; EMBRAPA-CNPH. 1998. p. 183-260.

IMA FLORESTAL (Minas Gerais). **Eucalipto no Brasil.** 2015. Disponível em: http://www.imaflorestal.com/noticias/eucalipto-no-brasil-02-03-2015. Acesso em: 20 mar. 2017.

LLOYD, G.; MCCOWN, B. Commercially feasible micropropagation of mountain laurel, (*Kalmia latifolia*), by use of shoot tip culture. **Combined Proceedings**: **International Plant Propagator's Society**, n. 30, p. 421-427, 1980.

MERCIER, H. Auxinas. In: KERBAUY, G. B. (ed.). **Fisiologia vegetal**. Guanabara Koogan, Rio de Janeiro, p. 217-249, 2004.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. **Physiologia Plantarum**, v. 15, p. 473-497, 1962.

NASCIMENTO, A. C.; PAIVA, R.; NOGUEIRA, R. C.; PORTO, J. M. P.; NOGUEIRA, G. F.; SOARES, F. P.; BAP e AIB no cultivo *in vitro* de *Eugenia pyriformis* Cambess. **Revista Acadêmica**: **Ciências Agrárias e Ambientais**, v. 6, n. 2, p. 223-228. 2008.

NAVROSKI, M. C.; REINIGER, L. R. S.; PEREIRA, M. O. Alongamento *in vitro* de rebentos de *Eucalyptusdunnii* em função de diferentes genótipos e concentrações de ácido 1-naftil-acético (ANA). **Revista de Ciências Agrárias**, v. 38, n. 1, p. 79-86, 2015.

OBERSCHELP, G. P. J.; GONÇALVES, A. N.; MENEGHETTI, E. C.; GRANER, E.; ALMEIDA, M. de. *Eucalyptus dunnii* Maiden plant regeneration via shoot organogenesis on a new basal medium based on the mineral composition of young stump shoots. **In Vitro Cellular and Developmental Biology - Plant,** v. 51, p. 626-636, 2015.

OLIVEIRA, C.; DEGENHARDT-GOLDBACH, J.; BETTENCOURT, G.M.F.; AMANO, E.; FRANCISCON, L.; QUOIRIN, M. Micropropagation of *Eucalyptus grandis* x *E. urophylla* AEC 224 clone. **Journal of Forestry Research**, v. 28, n. 1, p. 29-29, 2017.

OLIVEIRA, L. S.; DIAS, P. C.; BRONDANI, G. E. Micropropagação de espécies florestais brasileiras. **Pesquisa Florestal Brasileira**, v. 33, n. 76, p. 439-453, 2013.

PEDROTTI, E. L.; VOLTOLINI, J. A**.** Enraizamento *ex vitro* e aclimatação do porta-enxerto de macieira M.9. **Revista Brasileira Fruticultura**, v. 23, n. 2, p. 234-239, 2001.

RADMANN, E. B.; GALLO, C. M.; RITTERBUSCH, C. W.; BIANCHI, V. J.; FERNANDO, J. A.; PETERS, J. A. Enraizamento *in vitro* e aclimatização do porta-enxerto de ameixeira 'MR. S. 2/5'. **Plant Cell Culture and Micropropagation,** v. 10, n. 2, p. 1-11, 2014.

SILVA, L. C.; SCHUCH, M. W.; SOUZA, J. A.; ERIG, A. C.; ANTUNES, L. E. C. Efeito da iluminação e pré-lavagem das brotações de mirtilo cv. Florida no estabelecimento *in vitro*. **Revista Brasileira de Agrociência**, v. 13, n. 1, p. 127-129, 2007.

SILVA, M. L.; AZEVEDO, A. A.; SILVA, G. M.; C, I. F.; ROSSI, A. A. B.; OTONI, W. C. Germinação *in vitro* e organogênese direta em explanteshipocotiledonares com polaridade invertida de *Psychotria ipecacuanha* (Brot.) Stokes**. Revista Fitos**, v. 8, n.1, p. 5-11, 2013.

SOARES, B. O. MIRANDA, V. S**.** Enraizamento *in vitro* e aclimatização *ex vitro* de cultivares de citros. **Revista de Ciências Agrarias – Amazon Journal of Agricultural And Environmental Sciences,** v. 59, n. 2, p. 144-151, 2016.

SOUZA, A. V.; BERTONI, B. W.; FRANCA, S. C., PEREIRA, A. M. S. Conservação e enraizamento *in vitro* de infalível (*Mandevilla velutina* K. Schum.), uma planta medicinal do Cerrado. **Revista Brasileira de Plantas Medicinais**, v. 13, n. 1, p. 319-327, 2011.

SOUZA, D. M. S. C.; FERNANDES, S. B; AVELAR, M. L. M.; FRADE, S. R; P.; MOLINAR, L. V; GONÇALVES, D. S.; BRONDANI, G. E. Mixotrophism effect on *in vitro* elongation and adventitious rooting of *Eucalyptus dunnii*. **CERNE**, v. 25, n. 4, p.394-401, 2019.

WERNER, E. T.; CUZZUOL, G. R. F.; PESSOTTI, KAMILA, V.; LOPES, F. P.; ROGER, J. A. Controle da calogênese do pau-brasil *in vitro*. **Revista Árvore**, v. 33, n. 6, p. 987-996, 2009.