Research Article

In vitro shoot regeneration in *Myracrodruon urundeuva* Fr. All.[1]

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ABSTRACT

Myracrodruon urundeuva Fr. All. is a tree threatened with extinction, which has wood and medicinal potential. This study aimed to analyze the *in vitro* shoot regeneration in *M. urundeuva*, in order to increase the species multiplication. Two experiments were conducted: 1) concentrations of 6-benzylaminopurine (BAP) (0.0, 2.0, 4.0, 8.0 and 16.0 µM), in association with naphthaleneacetic acid (NAA) (0.0, 1.5 and 3.0 µM), in explants (cotyledon, hypocotyl and cotyledonary node); 2) concentrations of *meta*-topolin (*m*T) (0.0, 2.0, 4.0, 8.0, 16.0 and 32.0 µM) in explants (biaxillary, medial uniaxillary and apical basal nodal segment). The percentage of explants responsive to shoot regeneration, percentage of callus explants, number of shoots and shoot length were evaluated. In the first experiment, the shoot regeneration occurred only in explants of the cotyledonary node and hypocotyl type, with the highest responsiveness percentage (76.67 %) and number of shoots (1.97 and 1.63) obtained for the cotyledonary node in the presence of 3.0 µM of NAA in association with 2.0 (1.97 shoots/explant) and 4.0 µM (1.63 shoots/explant) of *m*T. In the second experiment, the resolution of the obtained quadratic equation indicates that the use of basal explant with 24.59 µM of *m*T added to the culture medium leads to the highest number of shoots (1.86). However, despite the *mT* having increased the mean number of shoots, all treatments containing this cytokinin showed callus formation. As a conclusion, it is possible to regenerate shoots in *M. urundeuva* from the cotyledonary node using BAP in association with NAA.

KEYWORDS: Aroeira-do-sertão, *in vitro* cultivation, medicinal plant.

RESUMO

Regeneração *in vitro* de brotações em *Myracrodruon urundeuva* Fr. All.

Myracrodruon urundeuva Fr. All. é uma árvore ameaçada de extinção, que possui potencial madeireiro e medicinal. Objetivou-se estudar a regeneração de brotos *in vitro* de *M. urundeuva*, visando aumentar a porcentagem de multiplicação da espécie. Foram realizados dois experimentos: 1) concentrações de 6-benzilaminopurina (BAP) (0,0; 2,0; 4,0; 8,0; e 16,0 µM), combinadas com ácido naftalenoacético (ANA) (0,0; 1,5; e 3,0 µM) em explantes (cotilédone, hipocótilo e nó cotiledonar); 2) concentrações de *meta*-topolina (*m*T) (0,0; 2,0; 4,0; 8,0; 16,0; e 32,0 µM) sob explantes (segmento nodal basal biaxilar, mediano uniaxilar e apical). Avaliaram-se a porcentagem de explantes responsivos para regeneração de brotações, porcentagem de explantes que formaram calos, número de brotos e comprimento da parte aérea. No primeiro experimento, observou-se regeneração de brotações apenas nos explantes do tipo nó cotiledonar e hipocótilo, sendo a maior porcentagem (76,67 %) de responsividade e número de brotos (1,97 e 1,63) obtidos com o nó cotiledonar na presença de 3,0 µM de ANA, combinado com 2,0 (1,97 brotos/explante) e 4,0 µM (1,63 brotos/explante) de *m*T. No segundo experimento, a resolução da equação quadrática obtida indica que o maior número de brotos (1,86) é alcançado utilizando-se o explante basal com 24,59 µM de *m*T adicionado ao meio de cultura. No entanto, apesar de a *m*T ter elevado o número médio de brotações, observou-se formação de calos em todos os tratamentos contendo essa citocinina. Conclui-se que é possível regenerar brotações em *M. urundeuva* a partir do nó cotiledonar com o uso de BAP combinado com ANA.

PALAVRAS-CHAVE: Aroeira-do-sertão, cultivo *in vitro*, planta medicinal.

INTRODUCTION

Myracrodruon urundeuva Fr. All. is a tree belonging to the Anacardiaceae family, popularly known in Brazil as 'aroeira-preta' or 'aroeira-do-sertão' (Lima 2011). The species is part of the *Caatinga* biome, which occupies about 11 % of the Brazilian territory and comprises herbaceous, shrub and arboreal plants (Drumond et al. 2016). It has a great potential for the recovery of degraded areas and for the composition of agroforestry systems (Maia 2012). Its wood is highly valued in external works and workpieces (Lima 2011). Moreover, the plant has medicinal use in the treatment of hemorrhages,

[1] Received: June 10, 2021. Accepted: Aug. 23, 2021. Published: Sep. 29, 2021. DOI: 10.1590/1983-40632021v5169269.
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respiratory and urinary infections, and digestive system disorders (Matos 1999).

The multiple uses of the species and the high indiscriminate exploitation of its resources threaten its genetic variability. Thus, methodologies and strategies for its conservation must be adopted (Cardoso et al. 2012).

Biotechnological processes such as plant tissue culture play an important role in the preservation and multiplication of species threatened with extinction (Suzuki et al. 2009, Oseni et al. 2019). One of the techniques for plant tissue culture is micropropagation, which allows obtaining seedlings from a large number of plants in a short space of time and at any time of the year, with adequate phytosanitary quality (Nagao et al. 1994).

Phytoregulators such as auxins and cytokinins are used to obtain *in vitro* morphogenic processes (Pasa et al. 2012, Soares et al. 2014). The main objective of these agents is to overcome possible deficiencies in the endogenous levels of hormones in the tissues of explants isolated from the production sites in the matrix plant (Grattapaglia & Machado 1998). Cytokinins are essential during the multiplication phase, as they control cell division and correlate with cell differentiation, especially during stem bud formation (Kerbauy 2008). In turn, auxins promote cell elongation and differentiation, in addition to being responsible for apical dominance and promoting *in vitro* rooting (Taiz & Zeiger 2017).

The cytokinin type and its concentration are the factors that most influence the success of *in vitro* multiplication (Grattapaglia & Machado 1998). Researchers have been using the cytokinin *meta*-topolin (*mT*) in *in vitro* propagation (Ahmad & Anis 2019, Nowakowska & Pacholczak 2020). Firstly isolated from poplar leaves (*Populus x canadensis* Moench, cv. Robusta), *m*T is an aromatic cytokinin that differs from isoprenoids in its biochemistry, receptors and biological activity (Strnad et al. 1997).

Except for 6-benzylaminopurine (BAP), purine cytokinins are chemically unstable (Vinayak et al. 2009). In turn, nonpurine types are generally stable and therefore advantageous in procedures that involve culture medium sterilization (Vinayak et al. 2009). Some authors have reported the use of *m*T as an alternative to reduce physiological disorders caused by other cytokinins (Bairu et al. 2008, Aremu et al. 2012, Souza et al. 2019). For example, Mirabbasi & Hosseinpour (2014), in a study with *Ulmus glabra* Huds, found that explants treated with *m*T produced more vigorous shoots than those treated with BAP.

In a study with *M. urundeuva*, Andrade et al. (2000) evaluated the effect of using concentrations of BAP in explants (nodal and apical segments) during *in vitro* multiplication via direct organogenesis. The authors reported explant regeneration; however, these explants developed in a single shoot. In this sense, the present study analyzes the *in vitro* shoot regeneration in *M. urundeuva*, aiming to increase the multiplication percentage of adventitious buds.

MATERIAL AND METHODS

Two experiments were conducted at the Universidade Estadual de Feira de Santana, Bahia state, Brazil, in 2015 and 2016. For *in vitro* culture establishment, *M. urundeuva* seeds from the Embrapa Semiárido (Petrolina, Pernambuco state, Brazil) were used.

The *in vitro* culture establishment relied on the methodology described by Andrade et al. (2000), with modifications. Fruit seeds of the species were superficially disinfested in running water with a few drops of neutral detergent, for 20 min. Then, the endocarp was removed by using a sieve. Finally, the fruit seeds remained for another 10 min in running water. The seeds were disinfested in a laminar flow chamber by immersion in 70 % ethanol for 30 sec, followed by immersion in sodium hypochlorite (1 % active chlorine) for 10 min, being then washed for three consecutive times with sterile distilled water. Subsequently, the seeds were inoculated in a culture medium to be used as an explant source at 30 and 60 days after sowing.

Test tubes (25 x 150 mm) containing 10 mL of woody plant medium (Lloyd & McCown 1980) supplemented with 87.64 mM of sucrose and solidified with 7 g L^{-1} of agar were used in the experiments. The pH was adjusted to 5.7 ± 0.1 (using 0.1N NaOH or HCl) before autoclaving at 121º C, for 15 min. After inoculation, the tubes were sealed with a polyvinylchloride (PVC) film.

In the first experiment, explants [cotyledon (whole), hypocotyl (± 10 mm) and cotyledonary node (± 10 mm)] from 30-day-old plants were inoculated in test tubes containing culture medium supplemented with concentrations of BAP (0.0, 2.0, 4.0, 8.0 and 16.0 µM), in association with

naphthaleneacetic acid (NAA) (0.0, 1.5 and 3.0 µM). The explants were inoculated vertically, except for the cotyledon (inoculated horizontally with the abaxial region in contact with the culture medium). The statistical design was completely randomized, in a 3 x 5 x 3 factorial arrangement (types of explants x BAP concentrations x NAA concentrations), totaling 45 treatments with 6 replications, each consisting of 5 plots (test tubes).

In the second experiment, explants of the basal nodal segment (biaxillary) (± 10 mm) (first pair of leaves), medial nodal segment (uniaxillary) (± 10 mm) and segment containing the apical bud (± 8 mm) from 60-day-old plants were excised in ascorbic acid solution (1.14 mM). The explants remained immersed in this solution for 10 min and were then inoculated vertically in culture medium containing concentrations of *m*T (0.0, 2.0, 4.0, 8.0, 16.0 and 32.0 µM). The statistical design was completely randomized, in a 3 x 6 factorial arrangement (explants x *m*T concentrations), totaling 18 treatments with 6 replications, each consisting of 5 plots (test tubes).

The cultures were kept in a growth room at a temperature of 25 ± 3 ºC, 16-h photoperiod and active photosynthetic radiation of 60 µmol m^{-2} s^{-1}, except for the material from the second experiment, which remained under dark conditions during the first seven days of culture. After 45 days of inoculation, the percentage of explants responsive to shoot regeneration, number of shoots, shoot length (mm) and percentage of callus explants were evaluated.

The data were statistically analyzed using the Sisvar software (Ferreira 2011). These were submitted to the Shapiro-Wilk normality test, followed by analysis of variance, being then transformed by the function $(x + 1)^{0.5}$, except for the percentage data, which were submitted to transformation into arcsine √%. When the "F" value was significant, qualitative data and adjustments were subjected to a comparison of means using the Tukey test, and quantitative data were submitted to polynomial regression analysis. The presented results are the original means obtained.

RESULTS AND DISCUSSION

The tests showed high oxidation percentages (data not shown). Tissue oxidation can cause direct death of explants or make the tissue unfeasible for morphogenesis. This problem is particularly serious in the isolation of explants of woody species. This is because their tissues are richer in secondary metabolism substances, more precisely phenolic compounds, which play an important role in defense against predators and microorganisms (Freitas et al. 2009). Other authors have reported the oxidation of explants of woody species such as blueberry (*Vaccinium* spp.) (Rosa et al. 2009), pomegranate (*Punica granatum* L.) (Dias et al. 2013), rosewood [*Dalbergia nigra* (Vell.) Allemão ex Benth.] (Sartor et al. 2013) and sabiá (*Mimosa caesalpiniifolia* Benth.) (Bezerra et al. 2014).

In the first experiment, the triggering of responses for shoot regeneration occurred only in the cotyledonary node and hypocotyl type explants. The lack of responsiveness of the cotyledon for shoot production may have occurred due to the lack of cellular competence to respond to the stimuli for dedifferentiation and redifferentiation with the aim to provide new abilities to explant cells. In this sense, the analyzed statistical data only regarded shoots originating from the hypocotyl and the cotyledonary node.

The analysis of variance showed a highly significant effect ($p < 0.01$) of the triple interaction among the factors 'BAP x NAA x Explant' for percentage of explants responsive to shoot regeneration, number of shoots and shoot length (Table 1).

For the percentage of explants responsive to shoot regeneration, the use of the cotyledonary node in the presence of 3.0 µM of NAA, combined with 2.0 and 4.0 µM of BAP, led to the highest responsiveness percentage (76.67 %). These results corroborate Cordeiro et al. (2014), who studied *Eucalyptus globulus* Labill clones and found a positive effect of the auxin/cytokinin ratio for shoot regeneration. The hypocotyl explant showed a low percentage of regeneration in most of the treatments under study, reaching the highest percentage (36.67 %) with the use of 2.0 µM of BAP in the absence of NAA (Table 2).

Regarding the mean number of shoots, the use of the cotyledonary node in culture medium added with 3.0 µM of NAA, in association with 2.0 and 4.0 µM of BAP, led to the best results (1.97 and 1.63 shoots per explant, respectively) (Table 2). The values of the present study for number of shoots in *M. urundeuva* are higher than those observed by Andrade et al. (2000). These authors conducted a micropropagation study with the same species and observed a single shoot using nodal and apical segment explants in the presence of 4.5 µM of BAP.

Table 1. Summary of the analysis of variance for percentage of explants responsive to shoot regeneration (%RE), number of shoots (NS) and shoot length (SL), at 45 days after inoculation. Values obtained from the hypocotyl and cotyledonary node explants (EXP) of *Myracrodruon urundeuva* Fr. All. under concentrations of 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA).

Source of variation	DF	Mean square		
		%RE[x]	NS[z]	SL (mm)[z]
BAP	4	0.42**	0.08**	1.27**
NAA	2	0.91**	0.27**	2.05**
EXP	1	2.20**	0.89**	5.60**
BAP x NAA	8	0.49**	0.15**	1.09**
BAP x EXP	4	0.13[ns]	0.04[ns]	0.20[ns]
NAA x EXP	2	1.07**	0.26**	1.98**
BAP x NAA x EXP	8	0.69**	0.20**	1.61**
Residue	150	0.09	0.02	0.27
CV (%)		210.11	14.20	41.97

**, * and [ns]: significant at 1 % and 5 % of probability and not significant, respectively, by the F test. [z] Data transformed by the function $(x + 1)^{0.5}$. [x] Data transformed into arcsine √%.

Table 2. Percentage of explants responsive to shoot regeneration (%RE), number of shoots (NS) and shoot length (SL), at 45 days after inoculation. Values obtained from the hypocotyl (HYP) and cotyledonary node (CN) explants of *Myracrodruon urundeuva* Fr. All. under concentrations of 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA).

BAP (µM)	%RE		NS		SL (mm)	
	HYP	CN	HYP	CN	HYP	CN
			0.0 µM of NAA			
0.0	0.00 aA	13.33 aAB	0.00 aA	0.20 aAB	0.00 aA	1.50 aA
2.0	36.67 aA*	13.33 aAB	0.37 aA	0.13 aB	4.33 aA*	2.20 aA
4.0	0.00 aA	0.00 aB	0.00 aA	0.00 aB	0.00 aA	0.00 aA
8.0	0.00 aA	0.00 aB	0.00 aA	0.43 aAB	0.00 aA	1.17 aA
16.0	0.00 bA	46.67 aA*	0.00 bA	1.03 aA*	0.00 bA	3.37 aA
			1.5 µM of NAA			
0.0	0.00 aA	6.67 aA	0.00 aA	0.07 aA	0.00 aA	0.53 aA
2.0	0.00 aA	0.00 aA	0.00 aA	0.00 aA	0.00 aA	0.00 aA
4.0	0.00 aA	0.00 aA	0.00 aA	0.00 aA	0.00 aA	0.00 aA
8.0	0.00 aA	6.67 aA	0.00 aA	0.13 aA	0.00 aA	0.17 aA
16.0	0.00 aA	10.00 aA	0.00 aA	0.17 aA	0.00 aA	0.30 aA
			3.0 µM of NAA			
0.0	0.00 aA	0.00 aB	0.00 aA	0.00 aB	0.00 aA	0.00 aB
2.0	0.00 bA	76.67 aA*	0.00 bA	1.97 aA*	0.00 bA	8.67 aA*
4.0	0.00 bA	76.67 aA*	0.00 bA	1.63 aA*	0.00 bA	6.73 aA*
8.0	0.00 bA	26.67 aB	0.00 aA	0.43 aB	0.00 aA	0.97 aB
16.0	3.33 aA	0.00 aB	0.07 aA	0.00 aB	0.10 aA	0.00 aB

Means followed by the same lowercase letter in the row and uppercase letter in the column (for each variable), regarding each NAA concentration, do not differ statistically by the Tukey test at 5 % of probability. Means followed by * differ from the others among NAA concentrations for the respective BAP and the same type of explant by the Tukey test at 5 % of probability.

The highest mean number of shoots occurring from the combination of BAP and NAA factors shows the importance of the synergistic effect of these regulators for regeneration from the cotyledonary node. Notwithstanding, the auxin-free medium also showed shoot formation, probably due to the way BAP works. This cytokinin can break the apical dominance and dormancy of lateral buds, thus forming new shoots (George 2008). This corroborates the results of other authors in micropropagation studies of woody species such as *Eremanthus erythropappus* (DC.) MacLeish (Prudente et al. 2016) and *Pterodon emarginatus* Vogel (Canatto et al. 2016).

For the variable shoot length, in the absence of NAA, the highest means occurred when using the hypocotyl explant in culture medium added with 2.0 µM of BAP (4.33 mm), and when using the cotyledonary node explant in culture medium added with 16.0 µM

of BAP (3.37 mm). The addition of 1.5 µM of NAA did not result in statistical differences between the resulting means. In turn, at the concentration of 3.0 µM of NAA, the highest mean values (8.67 and 6.73 mm) occurred with 2.0 and 4.0 µM of BAP, respectively, using the cotyledonary node (Table 2).

Using BAP in the culture medium in association with the highest NAA concentration under study significantly increased the shoot size, showing the importance of the hormonal interaction under this variable. This result differs from that of Silva et al. (2013), who verified that the addition of plant growth regulators [BAP, NAA, kinetin (KIN) and thidiazuron (TDZ)] to the culture medium reduces the shoot length in *Caesalpinia pyramidalis*. Ashraf et al. (2014) reported that high concentrations of cytokinins increase the shoot proliferation, although they can reduce the shoot size. This is because cytokinins stimulate sprouting by breaking apical dominance, thus reducing the plant size.

The analysis of variance for the second experiment showed a highly significant effect ($p < 0.01$) of the interaction '*m*T x explants' for the variables percentage of explants responsive to shoot regeneration, number of shoots and shoot length. Furthermore, the effect was significant ($p < 0.05$) for the variable percentage of callus explants (Table 3).

When evaluating the percentage of explants responsive to shoot regeneration in the absence of *m*T, the highest mean (92.5 %) occurred with the use of the apical segment explant. However, the mean did not differ statistically from the other means obtained with the use of the same explant as a function of *m*T concentrations (Table 4).

The shoot regeneration in *M. urundeuva* from the increase of *m*T in the culture medium may be due to cytokinins regulating cell division, i.e., acting on factors that govern the passage of the cell through the cell division cycle (Taiz & Zeiger 2017). Cytokinins are thus indispensable for breaking apical dominance and inducing axillary buds (Grattapaglia & Machado 1998), which possibly favored shoot proliferation.

The results of the present study for *M. urundeuva* corroborate those by Andrade et al. (2000), who also reported a high percentage of regeneration (90 %) for the same species, when using nodal and apical segment explants in the presence of 4.5 µM of BAP. Benmahioul et al. (2012) compared the cytokinins benzyladenine (BA), KIN and *m*T in cultures of *Pistacia vera* and observed the highest percentages of shoot regeneration in explants treated with BA and *m*T. For the latter, all concentrations under study led to 100 % of shoot regeneration.

Noteworthy, shoot regeneration occurred even in a cytokinin-free medium. This behavior indicates that there was no need for an exogenous source of cytokinin to stimulate the formation of shoots for the species. However, the regeneration of shoots from the basal and medial segments significantly increased with the addition of *m*T to the culture medium (Table 4). Dimitrova et al. (2016) did not obtain lateral shoots in cultures of *Pyrus communis* L. when using an *m*T-free medium, and the multiplication percentage increased as the concentrations of this cytokinin increased.

Other authors have also reported shoot formation in the absence of plant regulators in woody species such as *Hancornia speciosa* (Oliveira et al. 2016), *Caesalpinia pyramidalis* (Silva et al. 2013), *Tapirira guianensis* Aubl. (Gutiérrez et al. 2013) and *Luehea divaricata* (Flôres et al. 2011).

For number of shoots, the basal and medial nodal explants showed an increasing quadratic behavior ($p < 0.01$), as a function of *m*T concentrations.

Table 3. Summary of the analysis of variance for percentage of explants responsive to shoot regeneration (%RE), percentage of callus explants (%CE), number of shoots (NS) and shoot length (SL), at 45 days after inoculation. Values obtained from the basal nodal segment (first pair of leaves; biaxillary), medial nodal segment (uniaxillary) and apical segment explants (EXP) of *Myracrodruon urundeuva* Fr. All. under concentrations of *meta*-topolin (*m*T).

Source of variation	DF	Mean square			
		%RE[x]	NS[z]	SL(mm)	%CE[x]
*m*T	5	0.231[ns]	0.171**	48.373**	1.057**
EXP	2	2.984**	0.063[ns]	247.034**	0.993**
*m*T x EXP.	10	0.506**	0.095**	40.880**	0.281*
Residue	90	0.149	0.022	13.257	0.093
CV (%)		42.02	10.60	43.23	77.09

**, * and [ns]: significant at 1 % and 5 % of probability and not significant, respectively, by the F test. [z] Data transformed by the function $(x + 1)^{0.5}$. [x] Data transformed into arcsine √%.

Table 4. Means for percentage of explants responsive to shoot regeneration (%RE) and percentage of callus explants (%CE), at 45 days after inoculation. Values obtained from basal nodal, medial nodal and apical segment explants of *Myracrodruon urundeuva* Fr. All. under concentrations of *meta*-topolin (*m*T).

*m*T (µM)	Explant type		
	Basal segment	Medial segment	Apical segment
		%RE	
0.0	10.00 cB	55.83 bA	92.50 aA
2.0	76.39 abA	55.00 bA	87.50 aA
4.0	56.66 bA	70.00 abA	93.33 aA
8.0	47.22 bA	62.50 bA	94.44 aA
16.0	71.67 bA	83.33 aA	74.17 aA
32.0	73.33 aA	79.17 aA	82.50 aA
		%CE	
0.0	3.33 aC	10.83 aC	10.83 aA
2.0	55.56 aAB	65.83 aAB	20.83 bA
4.0	38.33 aBC	49.17 aABC	35.03 aA
8.0	13.89 aC	29.17 aBC	30.56 aA
16.0	63.33 aAB	46.67 abABC	19.17 bA
32.0	80.00 aA	75.00 aA	25.83 bA

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ statistically by the Tukey test at 5 % of probability.

However, the apical explant had no representative mathematical model. The quadratic equation obtained for the basal segment indicates that the use of 24.59 µM of *m*T reaches the highest estimate (1.86 shoots per explant) (Figure 1A). Considering the medial explant, the response curve suggests that the calculated value of 20.05 µM results in 1.30 shoots per explant. The mean values for this variable were 1.68 and 1.43 shoots/explant for the basal and medial explants, respectively (Figure 1A).

These results surpass those of a previous study with the same species by Andrade et al. (2000). The authors reported a single shoot from the nodal and apical segment explants with the use of the cytokinin BAP (4.5 µM).

M. urundeuva probably has a strong apical dominance, and the use of *m*T increased its mean number of shoots. This represents a positive result for the micropropagation protocol of the species, given the importance of this variable for this purpose

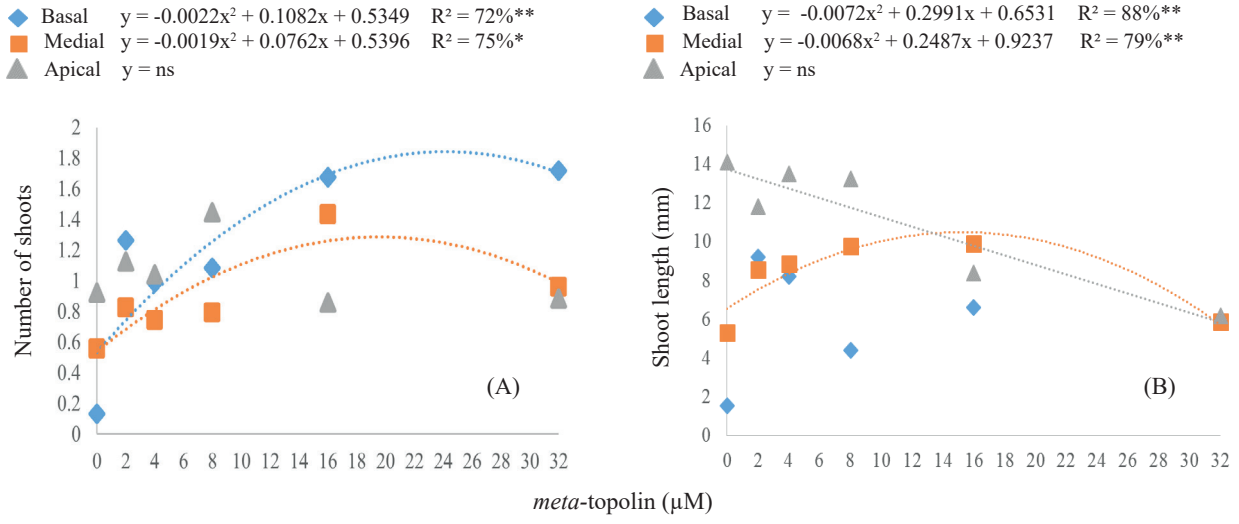

Figure 1. Number of shoots (A) and shoot length (B), at 45 days after inoculation. Values obtained from the basal nodal segment, medial nodal segment and apical segment of *Myracrodruon urundeuva* Fr. All. under concentrations of *meta*-topolin (*m*T). ^{ns}, * and **: not significant and significant at 5 % and 1 % of probability, respectively, by the F test.

(Figure 2). This result corroborates Gentile et al. (2017), who reported an increase in the multiplication percentage of *Corylus column* with the use of *m*T.

In turn, when studying *Ulmus glabra*, Mirabbasi & Hosseinpour (2014) found that explants treated with *m*T produced more vigorous shoots than those treated with BAP. Notwithstanding, unlike what occurred for *M. urundeuva*, *m*T did not increase the multiplication percentage, with the highest values occurring in the absence of this cytokinin in medium added with 1.78 µM of BAP (5.05 and 4.39 shoots per explant, respectively).

Gentile et al. (2014) compared concentrations of *m*T with 2.1 µM of benzyladenine in rootstocks of *Prunus domestica* L. and *Prunus insititia* x *domestica* and did not record an increase in the number of shoots with the addition of *m*T. However, when evaluating *P. insititia x domestica* alone and using BA and *m*T at the same concentration (2.1 µM), these authors realized that, despite not having improved shoot proliferation, *m*T positively influenced the shoot growth and quality.

In the assessment of shoot length, the regression model showed an increasing quadratic behavior ($p < 0.01$) for the medial nodal explant. This explant is estimated to reach the maximum length value (10.50 mm) with 15.25 µM of *m*T. Cytokinin increments greater than the estimated maximum value tend to disfavor shoot length (Figure 1B). In turn, the apical explant showed a decreasing linear behavior ($p < 0.01$). For the basal explant, the authors could not obtain a mathematical model with biological significance.

These results are inferior to those by Andrade et al. (2000), in micropropagation studies with the same species, who reported shoots with a mean height of 13 mm from nodal and apical segments inoculated in culture medium supplemented with 4.5 µM of BAP.

Gentile et al. (2017) also recorded mean values higher than those of the present study in cultures of *Corylus colurna*. For this species, the authors observed shoots 30 mm long in the presence of the highest concentration of *m*T under study (8.2 µM). Moreover, Gentile et al. (2014), using the cytokinins *m*T and benzyladenine at a concentration of 2.1 µM in *Prunus insititia x domestica*, found an increase in shoot length, which reached 31.9 mm with the use of *m*T.

All treatments under study showed callus formation. When the culture medium was added with 32 µM of *m*T, 80 % of the explants formed calluses with the use of the basal nodal segment; however, without statistical difference from the medial explant (75 %) in the same *m*T concentration. The value for the first explant did not differ from the percentages (55.56 and 66.33 %) for the same explant at 2.0 and 16 µM of *m*T, respectively (Table 4).

The increase in the percentage of explants that formed calluses with the addition of the cytokinin to the culture medium, especially at the highest concentrations, is possibly due to hormonal imbalances. Noteworthy, the explants used for shoot regeneration came from plant material germinated *in vitro*, possibly containing high concentrations of endogenous auxins.

Callus formation in *M. urundeuva* explants negatively influenced the shoot quality, especially for callus formed in explant regions very close to the buds (data not shown). Callus formation at the base of explants during the multiplication phase may compromise axillary bud proliferation and shoot elongation, affecting the *in vitro* development (Pereira et al. 2015).

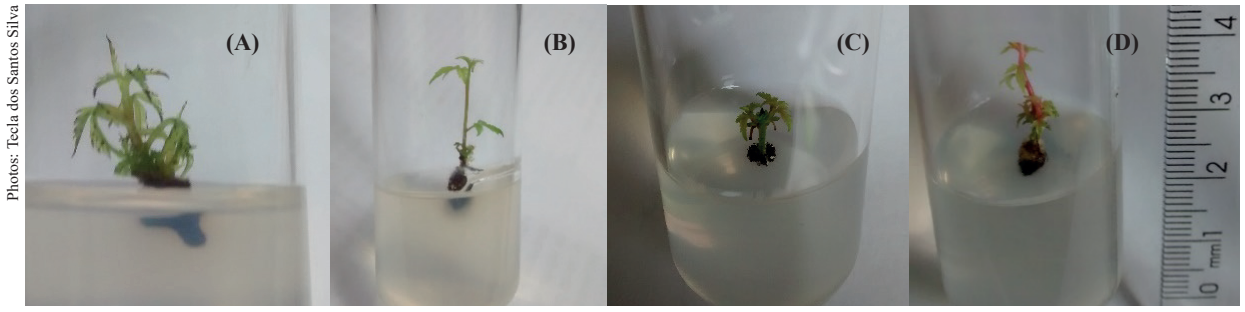

Figure 2. *Myracrodruon urundeuva* Fr. All. shoots at 45 days after inoculation. Values obtained from explants under concentrations of *meta*-topolin (*m*T). A) 16.0 µM of *m*T (medial segment); B) 2.0 µM of *m*T (basal segment); C) 16.0 µM of *m*T (basal segment); D) 32.0 µM of *m*T (basal segment).

Some authors reported callus formation in explants during *in vitro* propagation in cultures of other species, including *Mimosa caesalpiniifolia* Benth. (Bezerra et al. 2014) and *Handroanthus chrysotrichus* (Pereira et al. 2015).

The results of this research can be applied to the *in vitro* propagation of the species under study, also serving as a basis for further research seeking to optimize micropropagation protocols for *M. urundeuva* and other woody plants.

CONCLUSIONS

1. It is possible to regenerate shoots in *Myracrodruon urundeuva* from cotyledonary node explants using BAP in association with NAA;
2. The use of *m*T favors the shoot proliferation in basal and medial nodal segments; however, the large formation of calluses influences the shoot quality.

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