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GERMINATION AND HORMONAL BALANCE *In vitro* OF *Astronium urundeuva* (M. Allemão) Engl.

GERMINAÇÃO E BALANÇO HORMONAL in vitro DE Astronium urundeuva (M. Allemão) Engl.

GERMINACIÓN Y EQUILIBRIO HORMONAL in vitro DE Astronium urundeuva (M. Allemão) Engl.

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ABSTRACT

Aroeira-do-sertão (Astronium urundeuva) has applicability for therapeutic purposes due to its pharmacological characteristics and the wood used due to its resistance and excellent durability. Due to the intense exploitation, it is included on the endangered species list. A micro propagation is a potential tool for in vitro propagation and species conservation. Therefore, this work aims to evaluate the germination and effect of different concentrations of ANA associated with TDZ and BAP on the in vitro multiplication of A. urundeuva. Explants were nodal segments obtained from seedlings germinated in vitro. They were inoculated in MS50% culture medium, supplemented with the concentrations of ANA (0.0; 0.025, and 0.050 mg. L-1), TDZ (0.0; 0.25; 0.50; 0.75, and 1.00 mg. L-1) and BAP (0.0; 0.25; 0.50; 0.75, and 1.00 mg. L-1). After 31 days, we evaluated the vigor of explants, calluses, and the number of sprouts. The in vitro germination of A. urundeuva seeds was 61%, demonstrating that micro propagation is a feasible technique to initiate. The explants supplemented only with BAP showed greater vigor. However, TDZ induced physiological disorders in the explants, such as intense callosities and subsequent tissue necrosis. The auxin/cytokine hormonal balance was not favorable for the in vitro multiplication of the species.

RESUMO

Aroeira-do-sertão (*Astronium urundeuva*) apresenta aplicabilidade para fins terapêuticos devido às suas características farmacológicas e para uso madeireiro em razão da sua resistência e com grande durabilidade. Isso acarretou a intensa exploração da espécie, levando-a a ser incluída na lista de espécies ameaçadas de extinção. Em decorrência desta situação, a micropropagação é tida como ferramenta em potencial para a propagação e conservação *in vitro* da espécie. Diante disso, o objetivo deste trabalho foi avaliar a germinação e o efeito de diferentes concentrações de ANA associada com TDZ e BAP na multiplicação *in vitro* de *A. urundeuva*. Segmentos nodais, obtidos a partir de plântulas germinadas *in vitro*, foram utilizados como explantes. Os mesmos foram inoculados no meio de cultura MS50%, suplementado com as seguintes concentrações de ANA (0,0; 0,025 e 0,050 mg.L-1) associadas com TDZ (0,0; 0,25; 0,50; 0,75 e 1,00 mg.L-1) e BAP (0,0; 0,25; 0,50; 0,75 e 1,00 mg.L-1). Transcorridos 31 dias, foi avaliado o vigor dos explantes, calejamento e número de brotações. O percentual de germinação *in vitro* das sementes de *A. urundeuva* foi de 61%, demostrando a viabilidade da técnica para iniciar a micropropagação. O maior vigor dos explantes foi obtido no meio de cultura suplementado somente com BAP, por sua vez, o TDZ induziu distúrbios fisiológicos nos explantes, como intenso calejamento e posterior necrose dos tecidos. Os resultados obtidos indicam a necessidade de maiores estudos em razão do balanço hormonal auxina/citocinas não foi favorável para a multiplicação *in vitro* da espécie.

RESUMEN

Aroeira-do-sertão (Astronium urundeuva) tiene aplicabilidad para fines terapéuticos debido a sus características farmacológicas y para uso de madera debido a su resistencia y con gran durabilidad. Esto llevó a la intensa explotación de la especie, lo que la llevó a ser incluida en la lista de especies en peligro de extinción. Como resultado de esta situación, la micropropagación se considera una herramienta potencial para la propagación in vitro y la conservación de la especie. Por lo tanto, el objetivo de este estudio fue evaluar la germinacion e el efecto de diferentes concentraciones de ANA asociadas con TDZ y BAP en la multiplicación in vitro de A. urundeuva. Los segmentos nodales, obtenidos de plántulas germinadas in vitro, se utilizaron como explantes. Se inocularon en el medio de cultivo MS50%, suplementado con las siguientes concentraciones de ANA (0,0, 0,025 y 0,050 mg. L-1) asociado con TDZ (0,0; 0,25; 0,50; 0,75 y 1,00 mg. L-1) y BAP (0,0; 0,25; 0,50; 0,75 y 1,00 mg. L-1). Después de 31 días, se evaluó el vigor de los explantes, el calloso y el número de brotes. El porcentaje de germinación in vitro de semillas de A. urundeuva fue del 61%, demostrando la viabilidad de la técnica para iniciar la micropropagación. El mayor vigor de los explantes se obtuvo en el medio de cultivo suplementado sólo con BAP, a su vez, el TDZ indujo trastornos fisiológicos en los explantes, tales como calloring intenso y posterior necrosis de los tejidos. Los resultados obtenidos indican la necesidad de estudios adicionales debido a que el equilibrio hormonal auxina/citoquina no es favorable para la multiplicación in vitro de la espécie.

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1.INTRODUCTION

The plant flora of the Cerrado is one of the largest in the world, having more than 6,000 species (Brasil, 2009). In addition to having a high degree of endemism and occupying more than 23.3% of the Brazilian territory and 54% of the state of Minas Gerais (Biomas, 2019). The Cerrado is a domain that provides a variety of Ecosystem Services (SE), including cultural, provisional and regulatory services (Joly et al., 2019). Despite its importance, many of its ecosystems have given way to extensive agriculture and livestock, to the detriment of a biological diversity hitherto unknown and unaltered (Brasil, 2015; Martinelli et al., 2014; Strassburg et al., 2017). The pressure on the Cerrado flora caused several ecosystems to be altered by areas with degraded pastures, and many native species were extinguished due to disorderly exploitation.

In view of the current scenario, where concerns about climate change are growing, the Brazilian government made a new commitment at the United Nations Climate Change Conference 2021 (COP26), which concluded with the Glasgow Agreement, in which the country committed to restoring and reforesting 18 million hectares of forest by 2030 and restoring 30 million hectares of degraded pastures (Bichara et al., 2022). With this, study with native species has been done in order to assist and meet this proposed goal. Among the species in potential for recovery and restoration of the Cerrado is the Aroeira-do-sertão (*Astronium urundeuva*).

A. urundeuva is a species belonging to the family Anacardiaceae and endemic to Cerrado areas, occurring in the Midwest, North, Northeast, Southeast and South regions of Brazil (Silva-Luz & Pirani, 2016). Due to its characteristics as wood of great mechanical strength, it was intensely explored and, for this reason, it was included in the official list of Brazilian endangered species (Brasil, 2008). The species also has great medicinal potential, according to Nobre-Junior et al. (2009). Chalconas, a flavonoid found in *A. urundeuva* along with other therapies, can provide benefits to the treatment of patients with neurodegenerative lesions, such as Parkinson's disease. In addition, among the types of honeys sold in Brazil, aroeira honey, produced in the northern region of Minas Gerais, has been widely studied and valued for its remarkable characteristics, such as its dark color and accentuated flavor (Demier, 2018). Despite the multiple uses of the species and the economic interest in the species, exploration still occurs exclusively in an extractive way, compromising the conservation of genetic resources and the sustainability of production chains (Vieira et al., 2016).

In this context, the recovery and restoration of degraded areas, as well as the silvicultural exploitation of *A. urundeuva* depends on obtaining seeds and seedlings. Currently, the propagation of the species occurs exclusively seminally, and the market does not have available the amount of seedlings or the diversity required by environmental standards (Durigan et al., 2010). Therefore, the search for protocols of *in vitro* seed establishments can help in the difficulty of the natural propagation of the species.

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The advance in the improvement of vegetative propagation can facilitate the rapid multiplication of genotypes of interest, with the obtaining of quality seedlings and uniforms in sufficient quantity to meet the market demand (Xavier & Silva, 2010). Faced with the difficulties encountered in the sexual propagation of the Aroeira-do-sertão, an alternative to obtain seedlings of the species is micro propagation. The advantages of micro propagation include the ability to produce disease-free materials on a large scale and for shorter periods of time (Aitken-Christie et al., 1995; Arrigoni-Blank et al., 2011). In addition, *in vitro* cultivation techniques play a crucial role in the maintenance and sharing of germplasm with higher quality genes (Braun *et al*., 2010). *In vitro* cultivation often employs growth regulators in order to promote plant proliferation.

Among the various types of regulators, the class of cytokinins, which are used to promote plant growth, stimulating cell division, controlling the synthesis of proteins directly related to the formation of mytotic fibers and inhibiting the growth of the air and root systems, examples of regulators of this class are BAP (6-Benzylaminopurine) and TDZ [thiadizurom (N-phenyl-N-1, 2,3-tidiazol-5-tiurea)] (Castro et al., 2007). Another important class is that of auxins that support cell division, differentiation and stretching and are correlated with apical dominance, with the use of ANA (Acetic Naphthalene Acid), which has aided the *in vitro* production of native forest species.

The establishment of mature vegetative material collected from adult matrices trees presents high rates of fungal and bacterial contamination, in addition to phenolic oxidation. In this sense, a viable alternative has been the *in vitro* cultivation of seedlings obtained from seeds. The propagation material of juvenile origin presented higher *in vitro* response capacity due to the lower degree of cell differentiation and greater physiological vigor (Stuepp et al., 2018). In this sense, this study aimed to evaluate in (1) the *in vitro* germination of *Astronium urundeuva*, and (2) the multiplication of shoots of seminal origin under different concentrations of ANA in association with BAP and TDZ, in order to establish an efficient protocol of micro propagation to the species.

2. MATERIAL AND METHODS

2.1. Plant Material

The present study was conducted at the Forest Improvement Laboratory, located at the Center for Research in Agrarian Sciences (Centro de Pesquisa em Ciências Agrárias - CPCA) at the Institute of Agrarian Sciences of the Federal University of Minas Gerais (Instituto de Ciências Agrárias da Universidade Federal de Minas Gerais - ICA/UFMG), located in the city of Montes Claros, Minas Gerais. Diaspores of *A. urundeuva* were collected in September from 21 matrices located in a Cerrado fragment located in the county of Montes Claros - MG (latitude 16º40'59.7''S, longitude 43º50'21.9''W and altitude 680 m) (Figure 1). According to the Köppen climatic classification (Alvares et al., 2013) the region is characterized by having a tropical dry climate, with annual precipitation between 1,000 – 1,300 mm, dry winter and average temperature of 23.1ºC.

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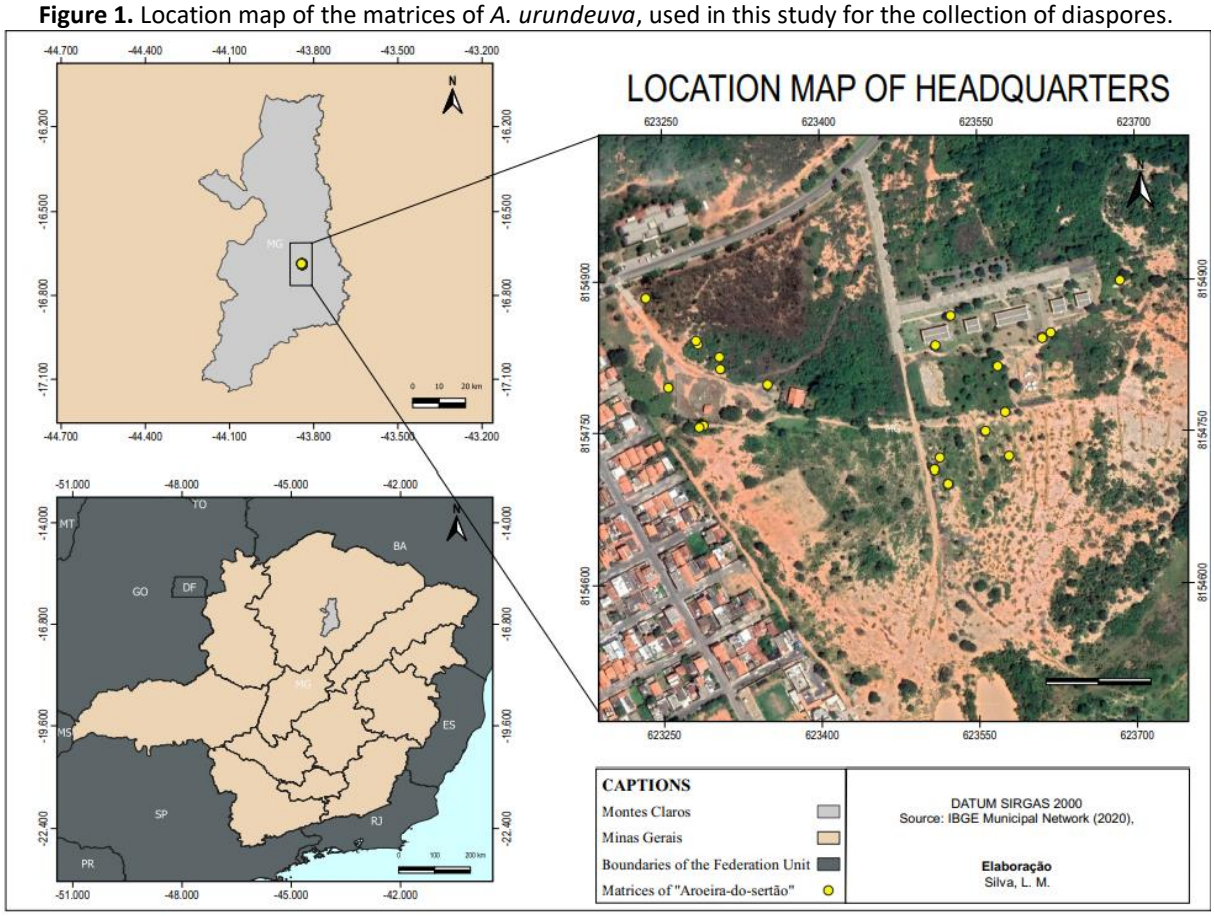
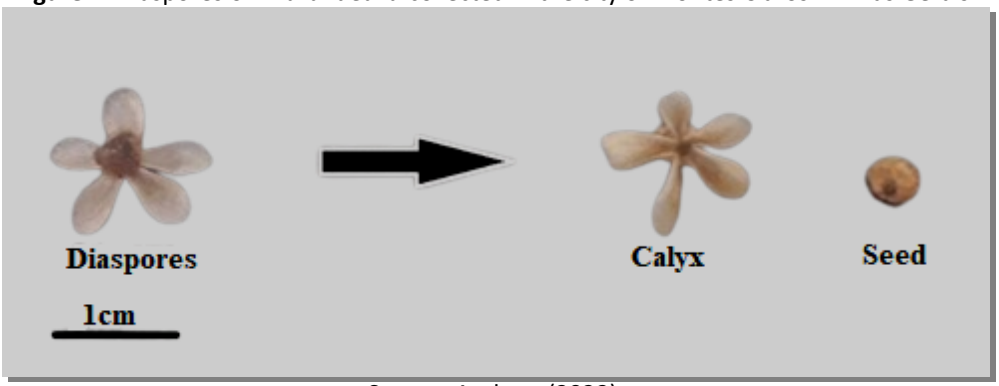
Figure 1. Location map of the matrices of *A. urundeuva*, used in this study for the collection of diaspores.



Source: Authors (2022).

The diaspores collected were sent to the laboratory for manual processing, which consisted of the removal of the calyxes from the diaspores and the selection of those who did not visually present empty fruit-seed (absence of tissues), physical damage and/or caused by insects (Figure 2).

Figure 2. Diaspores of *A. urundeuva* collected in the city of Montes Claros - Minas Gerais.



Source: Authors (2022).

2.2. GERMINATION *IN VITRO*

The selected diaspores were placed in running water for 10 minutes and then immersed in a solution of sodium hypochlorite (NaClO) at 2.0 – 2.5% (v/v) of active chlorine for 15 minutes. At the end of the treatment, they were rinsed with deionized and autocled water three times and inoculated vertically in test tubes (2 x 10 cm), containing 5.0 mL of MS culture

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medium (Murashige & Skoog, 1962) with half the concentration of salts, supplemented with sucrose (30 g L^{-1}) and agar (6 g L^{-1}). The pH of the culture medium was adjusted to 5.8 and its sterilization was chemically performed with polybac 7DC biocide (2 ml L^{-1}). After inoculation, the explants were kept in a growth room under 16/8 hours photoperiod control (light and dark) and temperature of 27 ± 2ºC.

In the germination of *A. urundeuva*, 9 evaluations were performed in a period of 31 days after inoculation. During the evaluations, the number of root protrusion, number of seedlings and seeds not germinated were determined. Root protrusion was defined as radicle emission equal to or greater than 1 mm. Seedlings were classified as those that showed potential to continue their development and presented all their essential structures well developed, complete, proportional and healthy. On the other, the ungerminated seeds corresponded to those that presented absence of the embryo or some type of damage.

The experimental design adopted was completely randomized, containing 390 replicates, each experimental unit consisting of 1 seed. The data measured were analyzed for their normality and homogeneity ($P > 0.05$), respectively, by the Shapiro-Wilk and Bartlett tests. Then the data were submitted to variance analysis (ANOVA) and polynomial regression analysis ($P < 0.05$). Statistical analyses were performed using the package ExpDes.pt the statistical software RStudio®, version 3.5.3.

2.3. MULTIPLICATION *IN VITRO*

In multiplication of *A. urundeuva*, shoots were obtained from seeds germinated *in vitro*. For this, the seeds were placed in running water for 10 minutes and then immersed in an alcohol solution (70%) for 30 seconds and in sequence in sodium hypochlorite solution (NaClO) at 2.0 - 2.5% (v/v) of active chlorine for 15 minutes. At the end of the treatment, they were rinsed with deionized and autocled water three times and inoculated in test tubes (2 x 10 cm), containing 5.0 mL of MS culture medium (Murashige & Skoog, 1962) with half the concentration of salts, supplemented with sucrose (30 g L^{-1}), PVP (1.5 g L^{-1}) and agar (6 g L^{-1}). The pH of the culture medium was adjusted to 5.8 and its sterilization was chemically performed with polybac 7DC biocide® (2 ml L^{-1}). After inoculation, the explants were kept in a growth room under 16/8 hours photoperiod control (light and dark) and temperature of 27 ± 2 ºC.

After 25 days, the seedlings germinated *in vitro* were excised for root removal and the shoot without apical meristem was used as explant. The shoots were inoculated in culture medium in test tubes (2 x 10 cm), containing 5.0 mL of the MS culture medium with half the concentration of salts, supplemented with different concentrations of ANA associated with BAP and TDZ (Table 1), sucrose (30 g L^{-1}), PVP (1.5 g L^{-1}) and agar (6 g L^{-1}). The pH of the culture medium was adjusted to 5.8 and its sterilization was chemically performed with polybac 7DC biocide® (2 ml L^{-1}). After inoculation, the explants were kept in a growth room under 16/8 hours photoperiod control (light and dark) and temperature of 27 ± 2 ºC.

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Table 1. Description of the concentrations of growth regulators, acetic naphthalene acid (ANA), benzylaminopurine (BAP) and thidiazuron (TDZ) used in *in vitro* multiplication of shoots of *A.urundeuva*.

Treatments	ANA (mg L^{-1})	BAP (mg L^{-1})	TDZ (mg L^{-1})
T1	-	0.00	-
T2	-	0.25	-
T3	-	0.50	-
T4	-	0.75	-
T5	-	1.00	-
T6	0.025	0.00	-
T7	0.025	0.25	-
T8	0.025	0.50	-
T9	0.025	0.75	-
T10	0.025	1.00	-
T11	0.050	0.00	-
T12	0.050	0.25	-
T13	0.050	0.50	-
T14	0.050	0.75	-
T15	0.050	1.00	-
T16	-	-	0.00
T17	-	-	0.25
T18	-	-	0.50
T19	-	-	0.75
T20	-	-	1.00
T21	0.025	-	0.00
T22	0.025	-	0.25
T23	0.025	-	0.50
T24	0.025	-	0.75
T25	0.025	-	1.00
T26	0.050		0.00
T27	0.050	-	0.25
T28	0.050	-	0.50
T29	0.050	-	0.75
T30	0.050	-	1.00

Source: Authors (2022).

30 days after the beginning of the experiment, the shoots were evaluated for their vegetative vigor, degree of heating and the number of shoots emitted. The vegetative vigor of the shoots and their degree of heating were evaluated based on a scale of notes (Figures 3 and 4).

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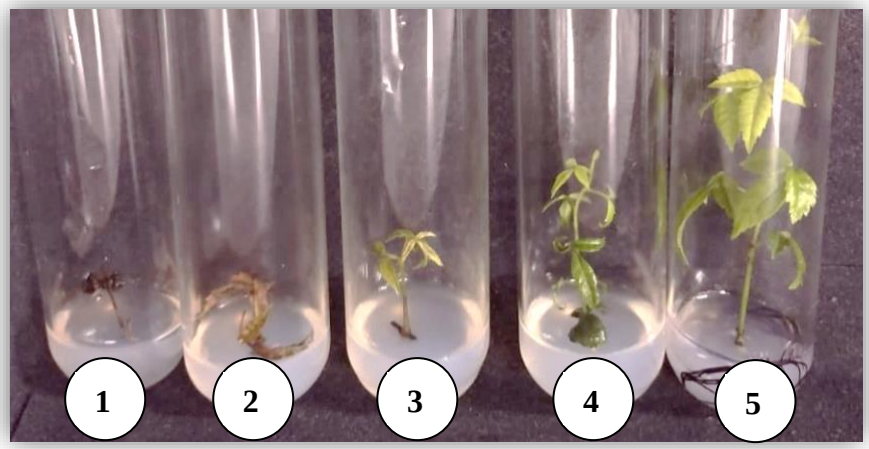
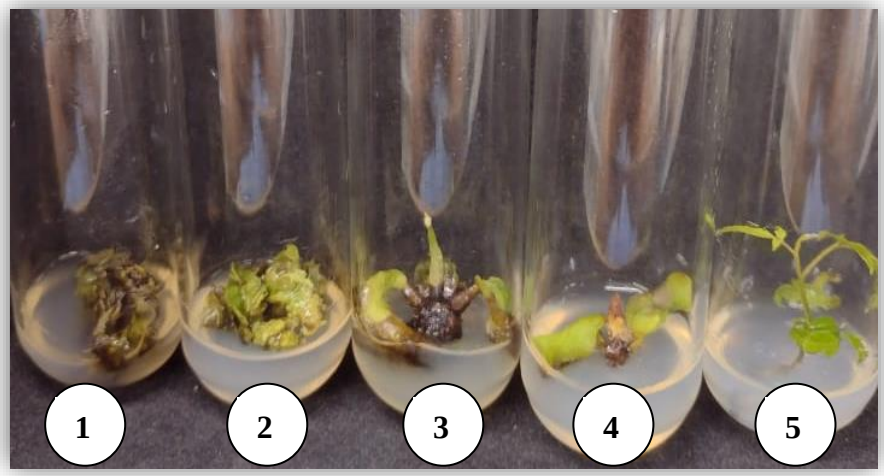
Figure 3. Scale of vegetative vigor classification scores of *A. urundeuva* shoots multiplied *in vitro* in MS 50% culture medium supplemented with different concentrations of ANA associated with BAP and TDZ concentrations. 1 - explant dead; 2 - explant in necrosis process; 3 - explant little vigorous; 4 - explants developed, but with physiological disorders; 5 - vigorous explants.



Source: Authors (2022).

Figure 4. Scale of classification notes of the degree of heating of *A. urundeuva* shoots multiplied *in vitro* in MS 50% culture medium supplemented with different concentrations of ANA associated with BAP and TDZ concentrations. 1 - Explant dead; 2 - Explant with large area of calluses; 3 - Explant with little presence of calluses; 4 - Explants developed, but with physiological disorders; 5 - Vigorous explants.



Source: Authors (2022).

The experiment was conducted in a completely randomized design, with 30 treatments, each composed of 10 replicates, with 1 sprouting per sampling unit. The data measured were analyzed for their normality and homogeneity ($P > 0.05$), respectively, by the Shapiro-Wilk and Bartlett test. Then the data were submitted to variance analysis (ANOVA) and polynomial regression analysis ($P < 0.05$). Statistical analyses were performed using the package ExpDes.pt the statistical software RStudio®, version 3.5.3.

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3. RESULTS AND DISCUSSION

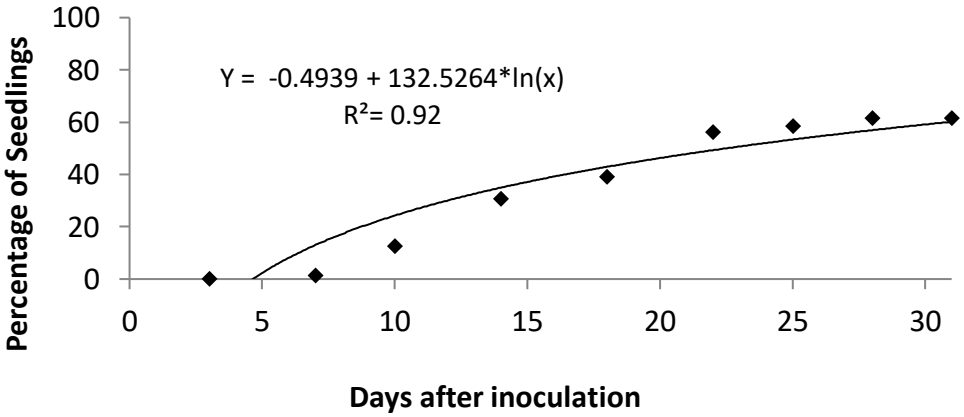
3.1. GERMINATION *IN VITRO*

Native forest species, such as *A. urundeuva*, which are still in a pre-breeding stage, present difficulties in their propagation whether seminal or clonal. In most native stalones, the low germination rate is a factor that impairs its perpetuation. Therefore, the results obtained from *in vitro* germination in the present study were satisfactory when compared to the seed propagation method (Nascimento *et al*., 2022). At the end of the 31 days of *in vitro* cultivation, a percentage of 61.53% of seedlings was observed; 34.10% of ungerminated seeds and 4.37% of seeds with root protrusion.

The beginning of the germination process was observed from the third day of *in vitro* cultivation, where there was a total of 14.35% of seeds with the presence of radicle. The rapid *in vitro* germination of *A. urundeuva* seeds is due to its adaptability to the environmental conditions of the natural occurrence sites of the species. For this, there is rapid water absorption and, physiologically, a sharp increase in respiratory intensity, which is responsible for the production of energy that will be used in biochemical reactions. Among the biochemical reactions, we have the degradation of reserve substances, which will nourish the embryonic axis until the development of the root system (Alonso, 2018).

In the germination process of *A. urundeuva* seeds, for so-called normal seedlings, there was a peak after 7 days of *in vitro* cultivation that extended to the 23rd, in which it observed a germination stabilization, totaling 240 seedlings (Figure 5). This behavior can be explained because the Aroeira diaspores have an average seedling emergence rate after 5 days of sowing (Paula *et al*., 2022). Over time, diaspores that are not viable, even if provided all the ideal conditions, there is no seedling formation.

Figure 5. Percentage of seedlings (%) of *A. urundeuva* germinated *in vitro* in MS 50% culture medium, after 31 days of inoculation.



Source: Authors (2022).

The *in vitro* germination of *A. urundeuva* is related to some factors, including the genetic variability of seeds. The literature reports that the germinated diaspores of *A. urundeuva* range from 20% to 80% (Dorneles et al., 2005). In the present study, 34.10% of diaspores were classified as unfeasible and this genetic factor makes it difficult to determine the

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possible causes of this unviability, because each seed will respond independently. In addition, another decisive factor that can directly affect diaspore conditions is the health of the mother plant at fruition time. The edaphoclimatic conditions of the site of the matrices plants can alter the transport of nutrients for the entire plant and consequently impact on the formation of quality seeds.

Table 2. Percentages of root protrusions and ungerminated diaspores of *A. urundeuva* germinated *in vitro* in MS 50% culture medium after 31 days of inoculation.

Evaluation days	Root protrusion (%)	Non-germinated diaspores (%)
3	14.35	85.64
7	41.28	57.43
10	34.35	53.07
14	31.,28	38.20
18	23.07	37.94
22	8.71	35.12
25	6.41	35.12
28	4.35	34.10
31	4.35	34.10

Source: Authors (2022).

Root protrusion is a variable of paramount importance since it is indicative that seeds when emitting roots have a high probability of developing complete seedlings. Generally, low percentages for this index are tied to numbness or incapacitation of the roots to break the integuments, which routinely occurs in native species. However, Dorneles et al., (2005) testing different methods of dormancy overcoming in *A. urundeuva*, observed that the main methods that help in this process reduced the viability of the seed and consequently promotes the reduction of its germination rate, and it is not recommended for this species the use of any dormancy breakage methods, as verified in the present study. In this context, even those diaspores (4.35%) that emitted radicle after 28 days of *in vitro* cultivation developed the aerial part in sequence (Table 2).

The results obtained for *in vitro* germination of *A. urundeuva* showed that its *in vitro* propagation is feasible, with a satisfactory percentage of normal seedlings (61%). This demonstrates that the micro propagation of the species from juvenile material is a potential tool for further studies, whether for the multiplication of genotypes or for germplasm conservation.

3.2. MULTIPLICATION *IN VITRO*

In vitro multiplication aims at the proliferation of plant material at a marked growth rate, with the emission of the largest number of shoots in each subculture. However, several woody species, especially native forests, have presented problems at this stage (Choudhary *et al*., 2020). Several factors may be linked to the reduced multiplicative rate of shoots, such as genotype, culture medium, *in vitro* cultivation conditions (Ferrari et al., 2004). In the present study, the follow-up of the experiment through periodic observations opened an indication that *A. urundeuva* presents a prominent apical dominance. Therefore, the

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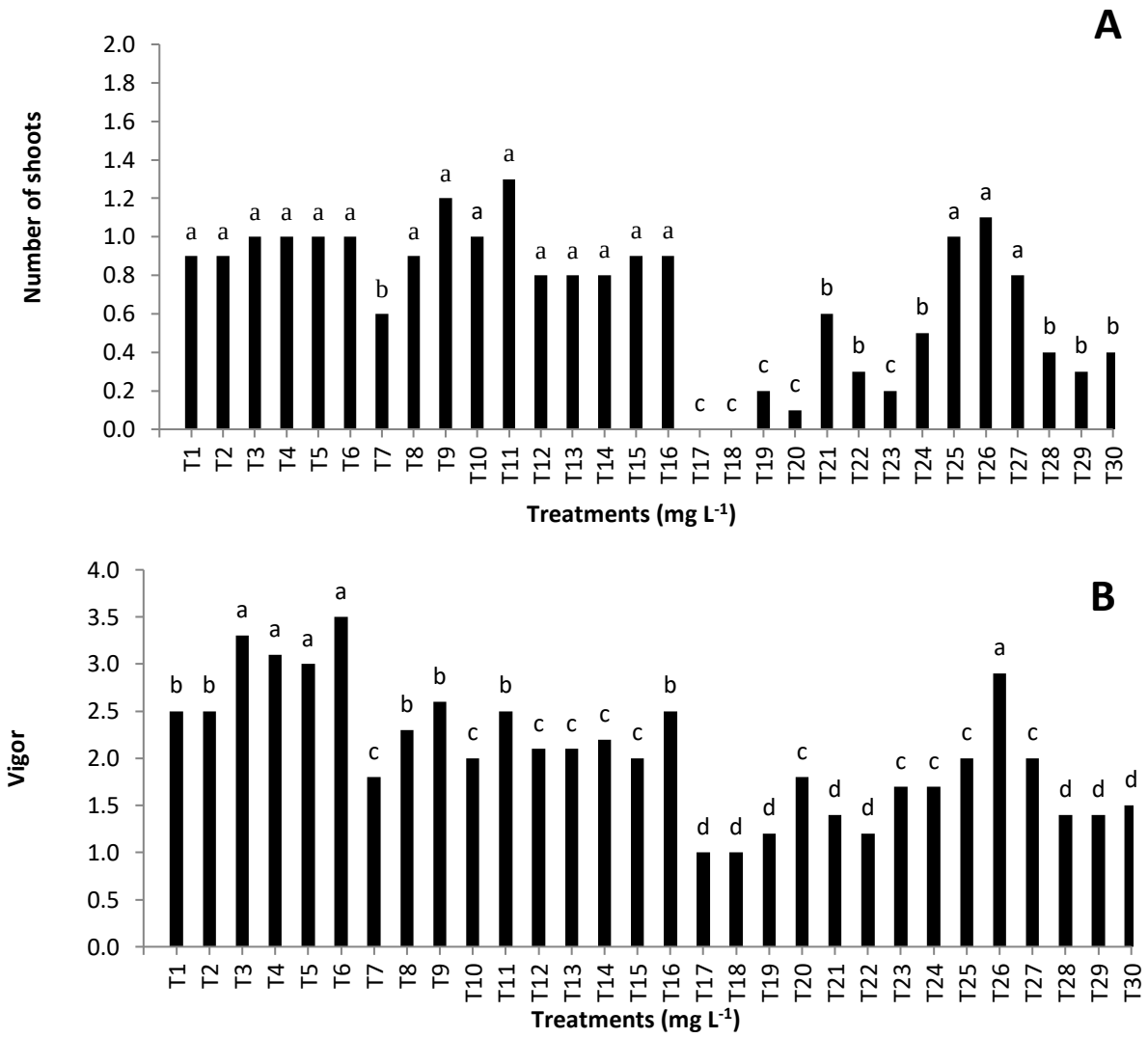
alteration of the auxin/cytokinin hormonal balance of explants by supplementation of the culture medium with growth regulators may be decisive for the breakdown of this dominance and induce the multiplication of new shoots.

Regarding the number of shoots, the best results were obtained in those treatments with the presence of ANA (Figure 6A). The positive effect of ANA for the multiplication of the shoots of *A. urundeuva* is probably linked to its contribution to the maintenance of an endogenous balance in explants that favored the breakdown of apical dominance and the induction of the multiplication of axillary shoots (Nascimento et al., 2022). The association of ANA and BAP promoted the multiplication of shoots only in treatments T11 and T9. In general, the association of BAP and ANA was not effective in the proliferation of *A. urundeuva* shoots.

Figure 6. Average values of the number of shoots (A), vigor (B) and callot (C) of *A. urundeuva* in the *in vitro* multiplication phase. Means followed by distinct letters differ from each other by the Scott-Knott test at 0.05 significance.



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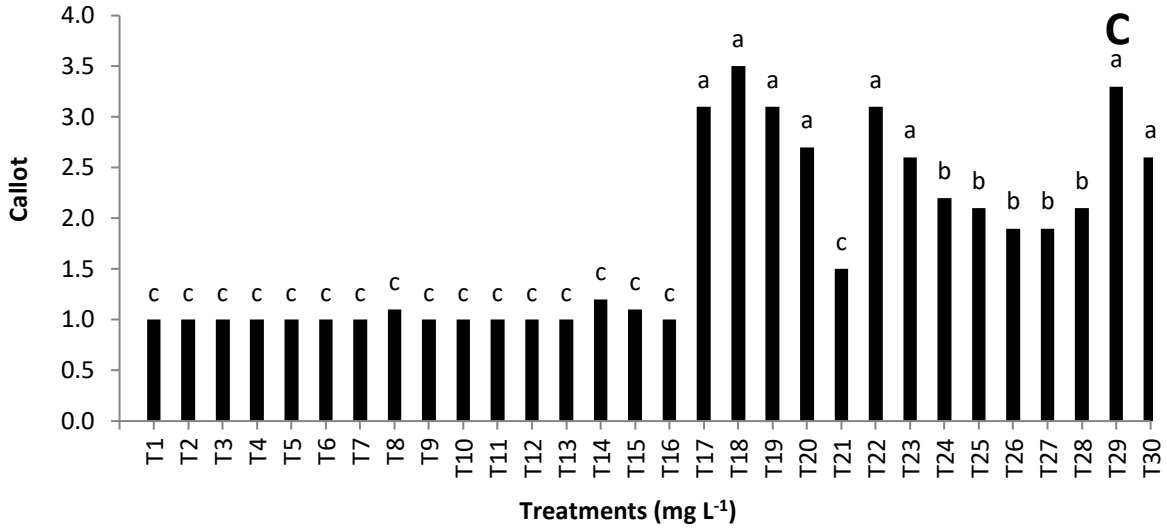
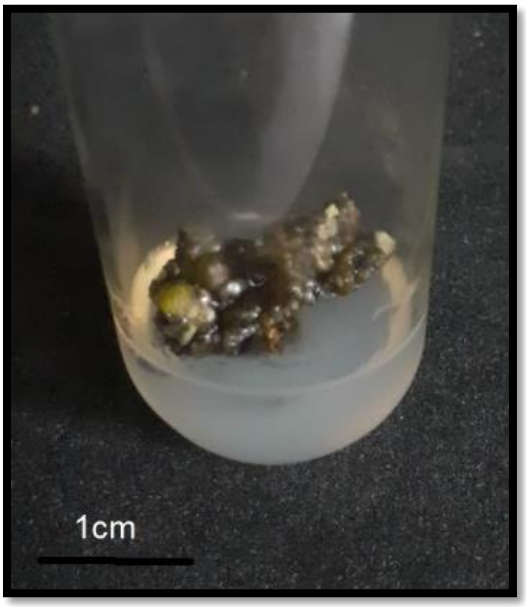


The results related to the multiplication of shoots in the culture medium supplemented with ANA associated with TDZ were unsatisfactory, since in most treatments there was intense heat of the explants (Figure 7). Most explants showed death or inhibition of the development of apical meristem. This response is probably due to the hormonal effect of TDZ that induces an intense cellular dedifferentiation, which contributed to the inhibition of the development of the apex of explants, promoting the breakdown of apical dominance (Máximo et al., 2020). On the other hand, the intense heating promoted by the action of TDZ in the culture medium provided the formation of well-characteristic structures of meristhematic tissues, with the formation of a sprouting, most likely adventitious. Thus, this result opens perspectives for the use of TDZ as an inducer of organogenesis and/or somatic embryogenesis in *A. urundeuva*. The morphogenic potential of TDZ has already been proven for different forest species (Dhiman et al., 2018).

Figure 7. Visual aspect of nodal segment-induced heating in treatment with ANA associated with TDZ in MS 50% culture medium, after 31 days of inoculation of *A. urundeuva*.



Source: Authors (2022).

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It is emphasized that, in the treatments in which ANA was associated with BAP, there was no heating of the shoots (Figure 6C). Therefore, in the *in vitro* multiplication of *A. urundeuva*, the type of cytokinin used as a growth regulator has a differentiated effect on the morphogenesis of explants. BAP is the main cytokinin used in *in vitro* culture media of forest species (Sant'ana et al., 2018). The vegetative vigor of the shoots was obtained for those treatments in which only BAP was supplemented to the culture medium (Figure 6B). In turn, the treatments with TDZ all presented mostly the death of the shoots, due to the intense heat observed.

In general, the shoots that developed presented mild symptoms of nutritional deficiency, with the yellowing of the leaf limbus of some shoots. These results reveal the need for further studies related to *in vitro* nutrition, such as evaluation of culture media and concentrations of their components. As an alternative to the MS medium, the use of the WPM culture medium can show good results in Brazilian native species such as: *Hancornia speciosa* and *Dalbergia nigra* (Pires et al., 2019; Pessanha et al., 2022)

In general, based on the results obtained in the present study, further investigations in the micropropagation of *A. urundeuva* are recommended, especially with modifications of the culture medium, as found for Rubus sp. and Eucalyptus Globulus Labill. (Villa et al., 2010; Cordeiro et al., 2014). Further studies in order to determine the ideal concentration of growth regulators for *in vitro* multiplication of *A.urundeuva* shoots with the association of ANA x BAP correspond to the best alternative to optimize the production of propagules *in vitro*. Moreover, due to the results obtained with intense callot explants in treatments with TDZ, it can be used in new research with organogenesis and/or embryogenesis of the species in order to determine possible changes in its germination rate and multiplication *in vitro*.

4. CONCLUSIONS

In vitro germination of *A.urundeuva* is an alternative to conventional propagation methods, obtaining 61% of viable seedlings for subsequent stages of micro propagation.

The hormonal balance established between ANA x BAP and ANA x TDZ in none of the treatments tested was effective to induce *in vitro* multiplication of *A. urundeuva*.

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