Plant Cell Culture & Micropropagation

http://pccm.ufla.br/index.php/plantcellculturemicropropagation/index

In vitro germination and micropropagation of *Myracrodruon urundeuva* Allemão (Anacardiaceae)

Augusto Vinicius de Souza Nascimento[1*], Ane Marcela das Chagas Mendonça[2], Carlos Dias da Silva Júnior[2], Marlucia Cruz de Santana[2], Paulo Augusto Almeida Santos[2]

ABSTRACT

Myracrodruon urundeuva is a native tree species that stands out for the use of its wood and for medicinal purposes. Due to the multiple uses and extractive exploitation, this species has been threatened with extinction. Thus, it is important to develop strategies in order to ensure conservation and long-term exploitation. This work aimed to establish a protocol of *in vitro* seed germination and micropropagation of *M. urundeuva*. Different support agents (vermiculite and agar) and presence of sucrose were tested for *in vitro* germination, while different concentrations of BAP (0.0, 0.5, 1.0, and 2.0 mg L^{-1}) and explant types (apical and cotyledonary nodes) were tested for multiplication. The use of vermiculite as a support agent favored *in vitro* germination of *M. urundeuva*, with germination percentage of 64.3% and 32% for vermiculite and agar, respectively. No significant differences were observed for the different BAP concentrations tested in multiplication and the treatment with 1.0 mg L^{-1} BAP presented higher values for most variables analyzed. Cotyledonary explants presented higher number of shoots (1.6 shoots) compared to the apical ones (1.1 shoots). Vermiculite may be used as a replacement for agar for *in vitro* seed germination and the combination of 1.0 mg L^{-1} BAP and cotyledonary explants may be used for *in vitro* multiplication of *M. urundeuva*. Furthermore, studies are needed to improve *in vitro* multiplication methods of this species.

Index terms: Aroeira-do-sertão; cytokinins; vermiculite; multiplication.

INTRODUCTION

Myracrodruon urundeuva Allemão is a native Brazilian tree species, popularly known as "aroeira-do-sertão", "aroeira-preta", and "urundeúva", that belongs to the Anacardiaceae family. This species is widely distributed in Brazil, co-occuring in different phytogeographic domains, such as Cerrado, Atlantic Forest, and mainly in Caatinga (Lorenzi, 2008; Lima, 2011; Maia, 2012). Its wood has a great economic importance, being used for resistant external constructions as sheds, bridges, fences, poles, and sleepers (Maia, 2012; Pareyn et al., 2018). In addition, the presence of flavonoids and chalcones in the bark and leaves provides anti-inflammatory, antimicrobial, antifungal, analgesic, and healing properties, enabling its pharmacological use (Matos et al., 2019; Galvão et al., 2018; Carvalho et al., 2017; Souza et al., 2007; Viana; Bandeira; Matos, 2003).

Considering the economic and pharmacological importance of *M. urundeuva*, an extractive exploitation has taken place and it represents a conservation problem. In this context, this practice has resulted in a severe reduction in natural populations of this species as well as its genetic variability (Pacheco et al., 2006; Monteiro et al., 2005). Due to its multiples uses and consequent risk of extinction, *M. urundeuva* species has been included on the Brazilian official list of endangered species (MMA, Normative Instruction n° 6, 2008) (BRASIL, 2008). Thus, studies about propagation are needed, in order to guarantee strategies for conservation and long-term exploitation of this species.

Although conventional propagation methods may be used for woody species, it is a difficult task developing uniform and numerous plants (Hung et al., 2016). In this context, *in vitro* methods are

https://doi.org/10.46526/pccm.2020.v16.156
Received in March 13, 2020 and approved in August 8, 2020
[1]Universidade Federal de Sergipe/UFS, São Cristóvão, SE, Brasil
[2]Universidade Federal de Sergipe/UFS, Departamento de Biologia, São Cristóvão, SE, Brasil
[*]Corresponding author: augustovinicius11@gmail.com

an alternative and a complement for traditional methods, so that micropropagation allows mass production of seedlings (Hung et al., 2016; Isah, 2016). *In vitro* seed germination is an important step widely used for micropropagation, and the development of reliable protocols enables the maintenance of genetic variability, besides providing contamination-free explants (Aragão et al., 2017; Nery et al., 2008; Nascimento; Franco; Frassetto, 2007).

The aim of this study was to establish a protocol of *in vitro* seed germination and micropropagation for *M. urundeuva*. In order to fulfill this objective, we tested different sucrose concentration and different support agents for *in vitro* germination; and different BAP (6-benzylaminopurine) concentration and types of explants for *in vitro* multiplication.

MATERIAL AND METHODS

Plant material and seed disinfestation

Mature seeds of *M. urundeuva* were harvested from different trees in a Caatinga vegetation area in the municipality of Guanambi, Bahia state, Brazil (14° 12' 26'' S and 42° 46' 55'' W). The seeds were disinfested in 70% alcohol for 30 seconds in a laminar flow cabinet, immersed in a solution of sodium hypochlorite (1.25% active chlorine) and a drop of neutral detergent, for 15 minutes, and then washed three times in autoclaved distilled water. All the experiments were kept in a growth room (GR) at 25 ± 2 °C with a photoperiod of 12 hours, provided by an artificial light source (white fluorescent lamps) and a photosynthetically active radiation (PAR) of 40 µmol $m^{-2}s^{-1}$.

Experiment I: *In vitro* seed germination in different support agents and sucrose concentration

Seeds were inoculated in the following support agents: Vermiculite + water (V + W); Vermiculite + MS + sucrose (V + MS + S); Vermiculite + MS (V + MS); Agar + MS (A + MS); and Agar + MS + sucrose (A + MS + S) (Figure 1A and 1B). MS-based medium (pH 5.8) (Murashige and Skoog, 1962), sterilized by autoclaving for 20 minutes at 121 °C was supplemented with 100 mg L^{-1} myo-inositol, 30 g L^{-1} sucrose (for treatments that used sucrose), gelled with 7 g L^{-1} agar (for treatments that used agar) or added 30 mL vermiculite (for treatments that used vermiculite). During 30 days of experiment, percentage of germination (%), average germination time (days), germination speed index, and average germination speed were evaluated. These characteristics were calculated using the GerminaQuant version 1.0. The experiment was completely randomized, composed by five treatments and 12 replicates per treatment. Each replicate was composed by a 100 mL flask with six seeds, totalizing 72 seeds per treatment.

Experiment II: Effects of BAP concentrations on *in vitro* multiplication

M. urundeuva plants with 45 days of germination *in vitro* were used as explant source, from which were excised both apical and cotyledonary nodes (Figure 1C). For *in vitro* multiplication the following concentrations of 6-benzilaminopurine (BAP) were added to the medium: T1 (0.0), T2 (0.5), T3 (1.0) and T4 (2.0) mg L^{-1} BAP. MS medium supplemented with 30 g L^{-1} sucrose, 100 mg L^{-1} myo-inositol, 1 g L^{-1} activated charcoal, gelled with 7 g L^{-1} agar, and pH adjusted to 5.8 was used. After 30 days of experiment, number of shoots, number of leaves per shoot, shoot height (cm), shoot fresh weight (g), and survival (%) were quantified. The experiment was completely randomized and composed by four treatments. For number of shoots, number of leaves per shoot, shoot height, and shoot fresh weight 10 replicates were used. For survival, 13 replicates were used. Each replicate was composed by a test tube with one explant (Figure 1D).

Experiment III: Influence of different explants on *in vitro* multiplication

M. urundeuva plants 45 days after germination were used as a source of explants with two treatments: apical (T1) and cotyledonary (T2) nodes (Figure 1C). MS medium supplemented with 1 mg L^{-1} BAP, 30 g L^{-1} sucrose, 100 mg L^{-1} myo-inositol, 1 g L^{-1} activated charcoal, gelled with 7 g L^{-1} agar, and pH adjusted to 5.8 was used. After 30 days of experiment, number of shoots, number of leaves, shoot height (cm), and percentage of rooting (%) were evaluated. The experiment was completely randomized, composed by two treatments and 10 replicates, each being composed by a test tube with one explant (Figure 1D).

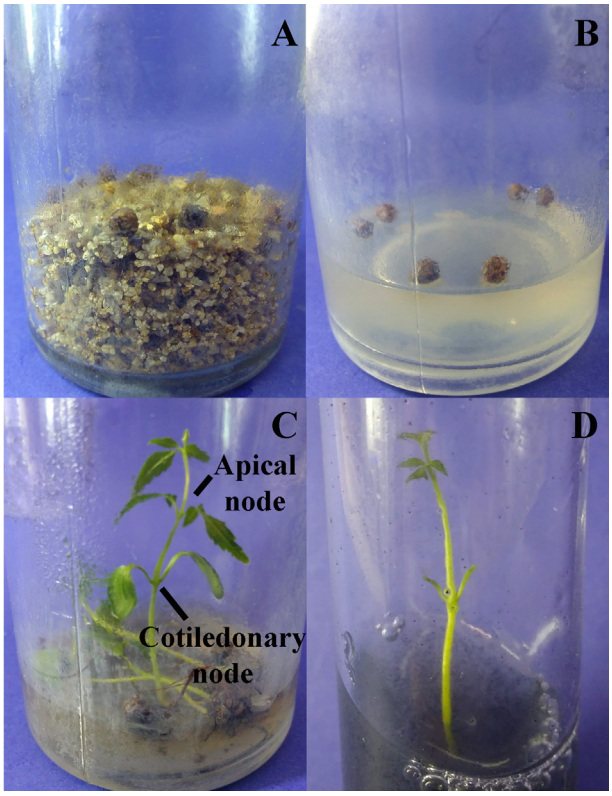

Figure 1: *M. urundeuva* seeds inoculated in different support agent: Vermiculite (A) and agar (B); 45-old days *in vitro* plants, sources of apical and cotyledonary nodes (C); explants inoculated in micropropagation medium (D).

Statistical analysis

The analyses were performed on the SISVAR software (Ferreira, 2011). These data were compared by Analysis of Variance (ANOVA) and pairwise differences were evaluated using Tukey's test ($p < 0.05$), with exception of Experiment III, which used the T test (LSD) ($p < 0.05$).

RESULTS AND DISCUSSION

Experiment I: *In vitro* seed germination in different support agents and sucrose concentration

Utilization of vermiculite in substitution of agar caused significant difference for germination of *M. urundeuva*. Higher percentage of germination was observed for the treatments that used vermiculite + water (V + W) and vermiculite + MS (V + MS), with results of 70.8 and 62.4%, respectively. For germination speed index, all treatments that used vermiculite in the composition had higher results, with averages of 0.69, 0.59, and 0.55 for vermiculite + water (V + W), vermiculite + MS (V + MS), and vermiculite + MS + sucrose (V + MS + S) (Figure 2 (a and b)). The treatments agar + MS + sucrose (A + MS + S) and agar + MS (A + MS) negatively affected the germination, with the lowest values of 27.7 and 37.5 for germination; 0.25 and 0.19 for germination speed index, respectively (Figure 2A and 2B).

The presence of sucrose negatively influenced the average germination time and the average germination speed only when agar was used as a support agent (A + MS + S), with results of 11.4 days and 0.078, respectively (Figure 2C and 2D).

The positive influence of vermiculite as a support agent for the *in vitro* germination of *M. urundeuva* is in accordance with resulted obtained by Oliveira et al. (2014) and Lameira et al. (2006) for *Hancornia speciosa* Gomes and *Swietenia macrophylla* King, respectively. For these woody species, higher percentage of germination and germination speed index were observed for the treatment that used vermiculite. Similar results were observed for *in vitro* germination of zygotic embryos and seeds of neem (*Azadirachta indica* A. Juss.), in which the use of vermiculite increased in more than 20% the rates of germination (Lédo et al., 2008).

In vitro germination may be negatively affected by toxicity and low water availability commonly related to the agar use (Golle et al., 2010). The presence of gelling agents together with salt concentration in culture medium alters the osmotic potential, and in consequence, affects soaking process of germination (Prudente et al., 2016; Nogueira et al., 2004). Thus, the increase of germination when vermiculite is used is possibly related to higher water availability to metabolism reactivation provided by this support agent (Golle et al., 2010). In addition, the low cost, uniform chemical composition, porosity, and capacity of water retention of vermiculite makes it a good option for this purpose (Ugarte et al., 2005; Martins et al., 2011).

Experiment II: Effects of BAP concentrations on *in vitro* multiplication

There was no difference for BAP concentrations used on *in vitro* multiplication of *M. urundeuva*. The treatments resulted in the following overall averages: number of shoots (1.2), higher shoot height (3.1

cm), shoot fresh weight (0.02), number of leaves per shoot (3.9), and survival (74.9%) (Table 1). Nevertheless, all treatments induced the formation of shoots, mainly in a single bud (Figure 3A and 3B). Possibly, the development of one shoot is responsible for inhibiting others (Andrade et al., 2000). This low rate of multiplication commonly observed in woody plants may have a relation with the plant morphogenetic characteristics (Costa; Nepomuceno; Santana, 2010; Cordeiro et al., 2004).

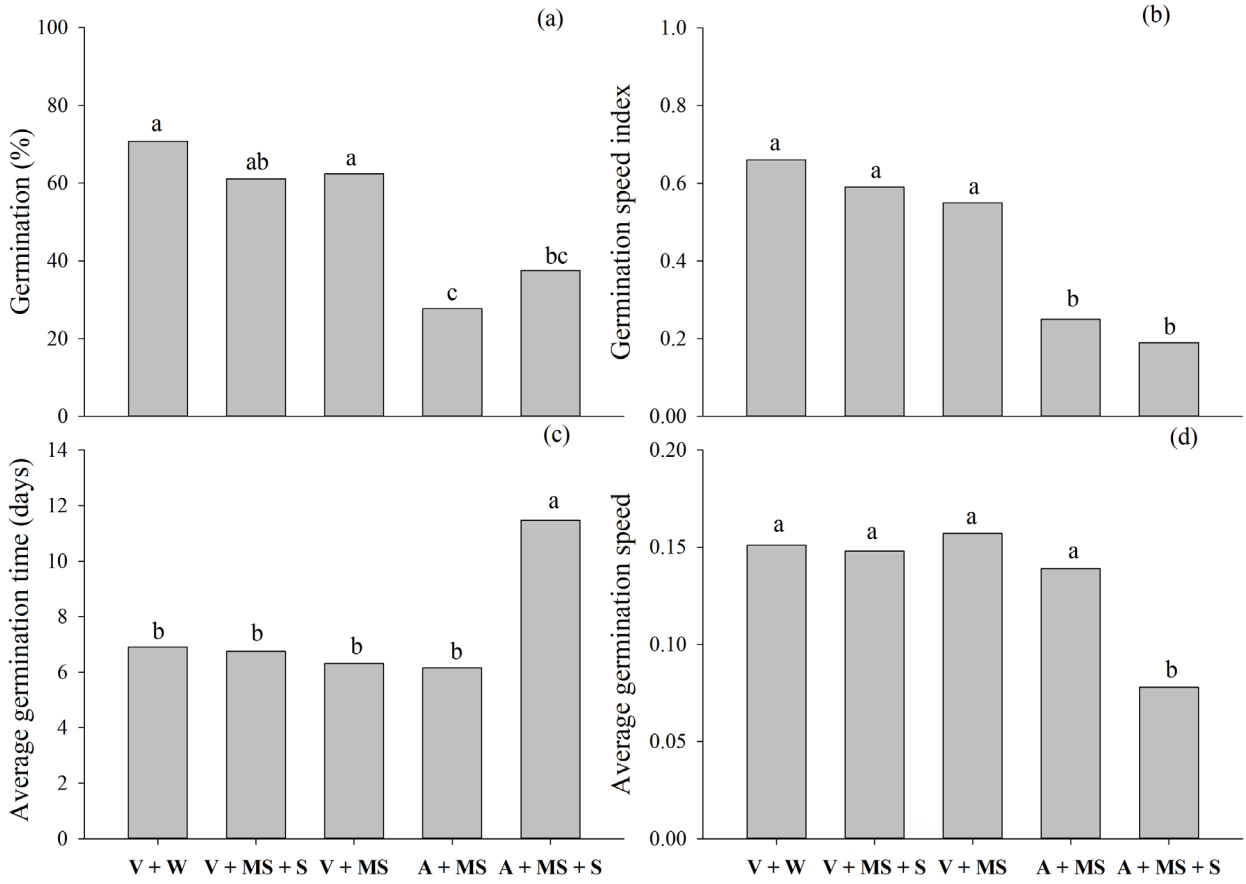

Figure 2: Percentage of germination (a), germination speed index (b), average germination time (c), and average germination speed (d) of *M. urundeuva* seeds inoculated in medium with different support agents and sucrose content. Values followed by the same letter do not show significant differences according to the Tukey's test ($p<0.05$).

Table 1: Number of shoots, shoot height, shoot fresh weight, number of leaves per shoot, and survival (%) of *M. urundeuva* explants micropropagated in different BAP concentrations.

BAP (mg L^{-1})	Number of shoots	Shoot height (cm)	Shoot fresh weight (g)	Number of leaves	Survival (%)
0.0	1.2a	2.2a	0.017a	3.4a	66.6a
0.5	1.0a	2.7a	0.016a	2.8a	55.5a
1.0	1.5a	4.1a	0.026a	5.2a	88.8a
2.0	1.2a	3.4a	0.021a	4.4a	88.8a
Overall average	1.2	3.1	0.02	3.9	74.9
CV (%)	57	59	62	55	57.7

* Values followed by the same letter do not show significant differences according to the Tukey's test ($p<0.05$).

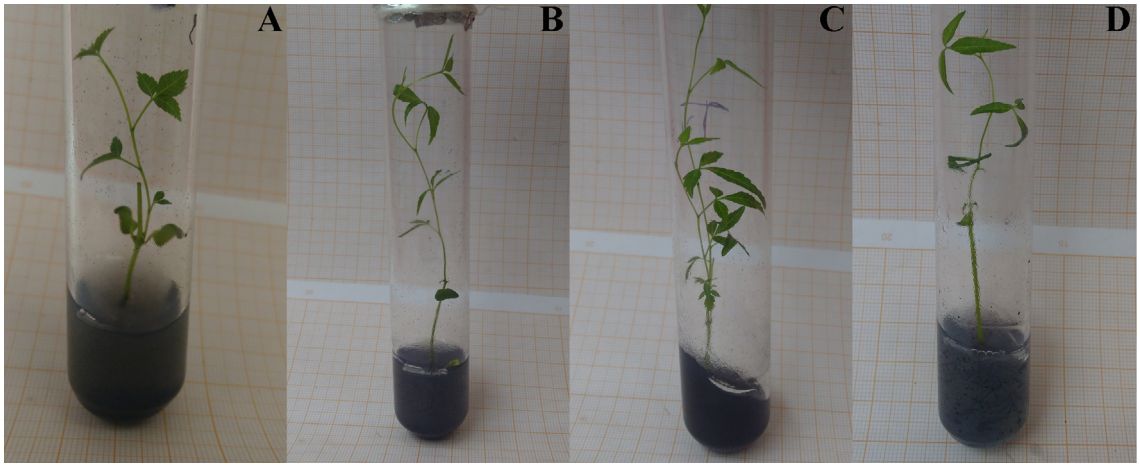

Figure 3: Single shoot (A and B), shoots from cotyledonary explants (C), and shoots from apical explants (D) of *M. urundeuva* micropropagated in different BAP concentrations and from different type of explants.

Andrade et al. (2000) evaluated the *in vitro* multiplication of *M. urundeuva* and observed a highest number of shoots for 1.0 mg L^{-1} BAP, so that most of the shoots developed in a single bud, which confirm our results. Similar results were observed by Paiva and Aloufa (2009) with *Schinus terebinthifolius*; however, there was two shoots in each nodal segment. The organogenesis of shoots in all concentrations of BAP, including in the absence of this growth regulator, may indicate that endogenous cytokinins induce apical and cotyledonary shoots, considering this hormone are synthetized in the roots and transported in the xylem vessels (Ishibashi et al., 2017; Bezerra et al., 2014).

Experiment III: Influence of different explants on *in vitro* multiplication

Explants from cotyledonary nodes presented higher number of shoots (1.6 shoots), showing they are more responsive when compared to the ones from apical nodes (1.1 shoots) (Figure 3 (C and D)). Although cotyledonary showed higher means for the other traits, there was no significant difference for the shoot height, number of leaves per shoot, and percentage of rooting (Table 2).

In fact, Aragão et al. (2017) and Costa, Nepomuceno and Santana (2010) observed that cotyledonary segments have a higher number of shoots for *Cariniana legalis* and *Erythrina velutina*, respectively, showing a high responsiveness when compared to apical nodes. Kielse et al. (2009) also found a positive interaction for shoot development in *Parapiptadenia rigida* using cotyledonary and nodal explants. The same study pointed that cotyledonary explants had a higher percentage of rooting, with value of 71%.

Table 2: Number of shoots, shoot height, number of leaves per shoot, and rooting (%) of apical and cotyledonary explants of *M. urundeuva*.

Characteristics	Apical	Cotyledonary	CV (%)
Number of shoots	1.1b	1.6a	31.7
Shoot height (cm)	2.2a	3.1a	42.7
Number of leaves	3.2a	3.8a	36.4
Rooting (%)	60a	90a	57

* Values followed by the same letter do not show significant differences according to the T test ($p<0.05$).

The higher values observed for cotyledonary explants may be related to their better performance during the phase of induction of shoots, considering that auxins and cytokinins act synergistically during the induction of roots, mainly in young leaves and buds (Kielse et al., 2009). Indeed, it is described that cotyledonary explants could have endogenous auxin, in an enough quantity to induce the development of shoots and adventitious roots (Pacurar; Perrone; Bellini, 2014).

CONCLUSIONS

Vermiculite can be used as a support agent for *in vitro* seed germination of *M. urundeuva*, once it was observed higher percentage of germination and

germination speed index. The presence of sucrose only influenced the germination in combination with agar, presenting a longer germination time and a minor average germination speed.

The addition of different concentration of BAP did not increase the number of shoots, so that most of them developed in a single bud; however, the use of cotyledonary explants was more efficient in the formation of shoots when compared to apical explants.

REFERENCES

ANDRADE, M. W. et al. Micropropagação da aroeira (*Myracrodruon urundeuva* Fr. All). *Ciência e Agrotecnologia*, 24(1):174-180, 2000.

ARAGÃO, V. P. M. et al. Micropropagation of *Cariniana legalis* (Martius) O. Kuntze, an endangered hardwood tree from the Brazilian Atlantic Forest. *Plant Cell Culture & Micropropagation*, 13(2):41-50, 2017.

BEZERRA, R. M. F. et al. Efeito de 6-benzilaminopurina sobre a propagação *in vitro* de *Mimosa caesalpiniifolia* Benth. (Fabaceae). *Revista Árvore*, 38(5):771-778, 2014.

BRASIL. Ministério do Meio Ambiente. *Instrução Normativa n°. 006 de 23 de setembro de 2008*. Disponível em: http://www.mma.gov.br/ Acesso em 03 set. 2018.

CARVALHO, C. E. S. et al. Anti-Leishmania activity of essential oil of *Myracrodruon urundeuva* (Engl.) Fr. All.: Composition, cytotoxity and possible mechanisms of action. *Experimental Parasitology*, 175:59-67, 2017.

CORDEIRO, I. M. C. C. et al. Efeito do BAP sobre a proliferação de brotos *in vitro* de *Schizolobium amazonicum* Huber ex Ducke (paricá). *Cerne*, 10(1):118-124, 2004.

COSTA, G. M.; NEPOMUCEMO, C. F.; SANTANA, J. R. F. Propagação *in vitro* de *Erythrina velutina. Ciência Rural*, 40(5):1090-1096, 2010.

FERREIRA, D. F; Sisvar: A computer statistical analysis system. *Ciência e Agrotecnologia*, 35(6):1039-1042, 2011.

GALVÃO W. R. A. et al. Gastroprotective and anti-inflammatory activities integrated to chemical composition of *Myracrodruon urundeuva* Allemão - A conservationist proposal for the species. *Journal of Ethnopharmacology*, 222:177-189, 2018.

GOLLE, D. P. et al. Substratos alternativos e tratamentos pré-germinativos na germinação *in vitro* de *Pinus taeda* L. *Revista Árvore*, 34(1):39-48, 2010.

HUNG, C. D. et al. LED light for *in vitro* and *ex vitro* efficient growth of economically important highbush blueberry (*Vaccinium corymbosum* L.). *Acta Physiologiae Plantarum*, 8:152, 2016.

ISAH, T. Induction of somatic embryogenesis in woody plants. *Acta Physiologiae Plantarum*, 38:118, 2016.

ISHIBASHI, V. et al. Estabelecimento *in vitro* de *Acacia mearnsii* De Wild. (Fabaceae). *Plant Cell Culture & Micropropagation*, 13(1)15-20, 2017.

LAMEIRA, O. A. et al. Efeito de substratos na germinação *in vitro* de mogno (*Swietenia macrophylla* King). *Plant Cell Culture & Micropropagation*, 2(1):15-19, 2006.

LÉDO, A. S. et al. Germinação *in vitro* de embriões zigóticos e sementes de nim indiano (*Azadirachta indica* A. Juss.). *Revista Brasileira de Plantas Medicinais*, 10(3):1-5, 2008.

KIELSE, P. et al. Regeneração *in vitro* de *Parapiptadenia rigida. Ciência Rural*, 39(4):1098-1104, 2009.

LIMA, B. G. *Caatinga:* Espécies lenhosas e herbáceas. Mossoró-RN, Editora Universitária da UFERSA, 2011. 316p.

LORENZI, H. *Árvores Brasileiras:* Manual de Identificação e cultivo de plantas arbóreas nativas do Brasil. Vol.01. 5ª edição. Nova Odessa: Instituto Plantarum de Estudos da Flora Ltda, 2008. 381p.

MAIA, G. N. *Caatinga:* Árvores e arbustos e suas utilidades. 2 ed. Fortaleza-CE. Printcolor Gráfica e Editora, 2012. 413p.

MARTINS, C. C. et al. Vermiculita como substrato para o teste de germinação de sementes de barbatimão. *Ciência Florestal*, 21(3):421-427, 2011.

MATOS, A. A. et al. An extract from *Myracrodruon urundeuva* inhibits matrix mineralization in human osteoblasts. *Journal of Ethnopharmacology*, 237(192-201), 2019.

MONTEIRO, J. M. et al. Teor de taninos em três espécies simpátricas da caatinga. *Revista Árvore*, 29(6):999-1005, 2005.

NASCIMENTO, P. K. V.; FRANCO, E. T. H.; FRASSETTO, E. G. Desinfestação e germinação *in vitro* de sementes de *Parapiptadenia rigida* Bentham (Brenam). *Revista Brasileira de Biociências*, 5(2):141-143, 2007.

NERY, M. C. et al. Germinação *in vitro* e *ex vitro* de embriões/sementes de *Tabebuia serratifolia* (VAHL) NICH. *Cerne*, 14(1):1-8, 2008.

NOGUEIRA, C. N. et al. Germinação *in vitro* de murici-pequeno (*Byrsonima intermedia* A. Juss.). *Ciência e Agrotecnologia*, 28(5):1053-1059, 2004.

OLIVEIRA, K. S. et al. Efeito de diferentes meios de cultura na germinação *in vitro* de sementes de mangabeira (*Hancornia speciosa* Gomes). *Revista Árvore*, 38(4):601-607, 2014.

PACHECO, M. V. et al. Efeito de temperaturas e substratos na germinação de sementes de *Myracrodruon urundeuva* Fr. All. (Aanacardiaceae). *Revista Árvore*, 30(3):359-367, 2006.

PACURAR, D. I.; PERRONE, I.; BELLINI, C. Auxin is a central player in the hormone cross-talks that control adventitious rooting. *Physiologia Plantarum*, 151(1):83-96, 2014.

PAIVA, A. M. S.; ALOUFA, M. A. I. Estabelecimento *in vitro* de aroeira da praia (*Schinus terebinthifolius* Raddi) em diferentes concentrações de 6-benzilaminopurina (BAP). *Revista Brasileira de Plantas Medicinais*, 11(3):300-304, 2009.

PAREYN, F. G. C. et al. *Myracrodruon urundeuva* - Aroeira. In: CORADIN, L.; CAMILLO, J.; PAREYN, F. G. C. (Ed.). *Espécies Nativas da Flora Brasileira de Valor Econômico Atual ou Potencial* - Plantas para o Futuro: Região Nordeste. Brasília - DF. MMA, 2018. p.766-772.

PRUDENTE, D. O. et al. Germinação *in vitro* e criopreservação de paineira-rosa. *Scientia Agraria Paranaenses*, 15(3):272-276, 2016.

SOUZA, S. M. C. et al. Antiinflammatory and antiulcer properties of tannins from *Myracrodruon urundeuva* Allemão (Anacardiaceae) in rodents. *Phytotherapy Research*, 21(3):220-225, 2007.

UGARTE, J. F. O.; SAMPAIO, J. A.; FRANÇA, S. C. A. Vermiculita. In: LUZ, A. B.; LINS, F. A. F. *Rochas & Minerais Industriais*. 2.Ed. - Rio de Janeiro: CETEM - Centro de Tecnologia Mineral /MCT, 2005. p.677-698.

VIANA, G. S. B.; BANDEIRA, M. A. M.; MATOS, F. J. A. Analgesic and antiinflammatory effects of chalcones isolated from *Myracrodruon urundeuva* Allemão. *Phytomedicine*, 10(2-3):189-195, 2003.