Nativa, Sinop, v. 10, n. 1, p. 117-124, 2022.
Pesquisas Agrárias e Ambientais
DOI: https://doi.org/10.31413/nativa.v10i1.13351 ISSN: 2318-7670

Seedling production of *Mimosa calodendron* Mart. ex Benth. in a temporary immersion bioreactor

Denys Matheus Santana Costa SOUZA[1], Andressa Rosa MARTINS[1], Sérgio Bruno FERNANDES[1], Juscelina Arcanjo dos SANTOS[1], Gilvano Ebling BRONDANI[1]*

[1] Laboratory of *In Vitro* Culture of Forest Species, Department of Forestry Sciences, Federal University of Lavras, Lavras, MG, Brazil.
*E-mail: gilvano.brondani@ufla.br
(ORCID: 0000-0003-4356-7163; 0000-0002-7578-0916; 0000-0001-8685-1268; 0000-0003-4731-2610; 0000-0001-8640-5719)

Received in 24/01/2022; Accepted in 08/03/2022; Published in 26/03/2022.

ABSTRACT: Micropropagation is one technology to propagate endemic species of the Ferruginous Rupestrian Grasslands when *in vitro* genetic conservation is sought. The present study aimed to assess the breaking of dormancy, *in vitro* establishment, multiplication, elongation, rooting, and acclimatization of *Mimosa calodendron* from culture in a temporary immersion bioreactor system. The seeds used for the experiments were from plants originating from the Ferruginous Rupestrian Grasslands. The percentage of contamination, oxidation, unresponsive seeds, germination, number of buds per explant, shoot length, senescence, percentage of adventitious rooting, and acclimatization were assessed. The breaking of dormancy was most successful by mechanical scarification (80% germination). Immersion in sodium hypochlorite for 5 minutes was the most efficient treatment for *in vitro* establishment (90%). For the in *vitro* multiplication and elongation phase, the use of liquid culture medium from cultivation in a temporary immersion bioreactor was the most suitable for the characteristics number of buds per explant (2.55 buds), vigor (1.1), oxidation (1.3) and senescence (1.3) according to the score's scale. Regardless of the *in vitro* cultivation method, the percentages of rooting and acclimatization were satisfactory, and it was possible to obtain complete plants in 190 days.
Palavras-chave: *in vitro* propagation; Ferruginous Rupestrian Grasslands; endemic species.

Produção de mudas de *Mimosa calodendron* Mart. ex Benth. em biorreator de imersão temporária

RESUMO: A micropropagação é uma alternativa para a propagação de espécies endêmicas do Campo Rupestre Ferruginoso, quando se busca a conservação genética *in vitro*. O trabalho teve como objetivo avaliar a superação de dormência, estabelecimento *in vitro*, multiplicação, alongamento, enraizamento e aclimatização de *Mimosa calodendron* a partir do cultivo em sistema de biorreator de imersão temporária (BIT). As sementes utilizadas para os experimentos foram provenientes de plantas oriundas do Campo Rupestre Ferruginoso. A porcentagem de contaminação, oxidação, sementes não responsivas, germinação, número de gemas por explante, comprimento de brotos, senescência, porcentagem de enraizamento e aclimatização foram avaliados. A superação de dormência por escarificação mecânica (80% de germinação) foi a mais adequada para a superação de dormência. A imersão em hipoclorito de sódio por 5 minutos foi o tratamento mais eficiente para o estabelecimento *in vitro* (90%). Para a fase de multiplicação e alongamento *in vitro*, o uso do meio de cultivo líquido a partir do cultivo em biorreator de imersão temporária foi o mais adequado para as características número de gemas por explante (2,55 gemas), vigor (1,1), oxidação (1,3) e senescência (1,3) de acordo à escala de notas. Independentemente do método de cultivo *in vitro*, a porcentagem de enraizamento e aclimatização foram satisfatórios, sendo possível obter plantas completas em 190 dias.
Keywords: propagação *in vitro*; Campo Rupestre Ferruginoso; espécie endêmica.

1. INTRODUCTION

Mimosa calodendron Mart. ex Benth. is species endemic to Brazil that belongs to the family Leguminosae. It has a restricted geographic distribution in southeastern Minas Gerais associated with the Cerrado domain over rocky outcrops in the Iron Quadrangle (altitudes between 1,300 and 1,700 m) and the formation of the ferruginous rupestrian grassland (DUTRA; GARCIA, 2014). Its natural population has shrunk (DAYRELL et al., 2015) due to mining activities, parasitism, and seed predation, in addition to difficulties in seedling production due to physical dormancy and slow germination (DUTRA et al., 2022).

Given these problems, the micropropagation technique has numerous advantages, such as the possibility of mass propagation from a single propagule, the fixation of genetic gains in clonal populations and the propagation of high-quality plants in a small physical space in a short time, independent of climatic factors limiting seed production (ABIRI et al., 2020). However, the *in vitro* culture of *Mimosa calodendron* is still a challenge because there is a need for specific management in the stages of seed dormancy breaking, germination, establishment, multiplication, elongation, and rooting to obtain a complete plant.

Several technologies have been proposed to automate the micropropagation, such as adjustments to germination and multiplication protocols, as well as breaking physical dormancy through mechanical scarification, chemical sterilization to reduce contamination, and adoption of

protocols in culture systems and routine procedures using a temporary immersion bioreactor (TIB) (SOUZA et al., 2020a; MOLINARI et al., 2021; RAMÍREZ-MOSQUEDA; BELLO-BELLO, 2021).

The physical dormancy of *Mimosa calodendron* seeds is one of the greatest difficulties hindering its germination (DAYRELL et al., 2015). Many species that present dormancy do not germinate under adequate conditions, preventing their propagation and thus the production of seedlings for use in genetic recovery and conservation projects (ARAÚJO et al., 2020). Among the methods for breaking dormancy, mechanical scarification and thermal shock are often used to reduce physical impediments, which usually takes the form of a rigid tegument that prevents the imbibition process (ARAÚJO et al., 2020).

Aseptic control is also essential for the *in vitro* introduction (SOUZA et al., 2020a; MOLINARI et al., 2021), and it is important to sterilize the culture medium of microorganisms that hinder the development and growth of tissues (MEDJEMEM et al., 2016). Recent studies on the adequate exposure time of tissues to sterilizing agents have suggested ways to reduce contamination (MOLINARI et al., 2021), but such chemical agents added to the nutrient medium can cause phytotoxicity due to tissue oxidation and growth inhibition (TEIXEIRA et al., 2021), so it is important to determine the time of exposure to the chemical agent and its concentration (MOLINARI et al., 2021).

The multiplication, elongation, and rooting phases can be optimized using TIB technology. A TIB is an automated system that can improve nutrient supply and gas transfer, supporting fuller development of micropropagated cultures (CARVALHO et al., 2019). TIBs yield biomass gains, reducing the time required for propagation (COSTA et al., 2021) and increasing plant production per unit area (RIBEIRO et al., 2016; ALVES et al., 2021).

The present study aimed to assess the dormancy breaking, *in vitro* establishment, multiplication, elongation, rooting, and acclimatization of *Mimosa calodendron* from culture in a TIB system.

2. MATERIAL AND METHODS

2.1. Source of seeds

Seeds from adult plants of *Mimosa calodendron* Mart. ex Benth. in the natural environment of Ferruginous Rupestrian Grassland were collected in March 2017, Research and Innovation Unit belonging to Gerdau (GERDAU Açominas S.A.), located in Ouro Branco, Minas Gerais, Brazil (20°31'17.43"S, 43°44'18.89"W). In total, 2,500 seeds were collected from 50 plants of the species. Mean annual rainfall is 2,056 mm and mean temperature is 25.5°C. The climate of the region is classified as Aw (tropical) according to Köppen-Geiger, with the rainy season from November to March and dry winters.

2.2. *In vitro* establishment

Seeds were washed under running water and immersed in an antifungal solution containing 2.4 mg L^{-1} of orthocide 500® (50% of captan as the active ingredient) for 15 minutes. Then, the seeds were washed five times in autoclaved deionized water and immersed in sodium hypochlorite solution (NaOCl, 2.0-2.5% of active chlorine, Clarix®) – according to asepsis treatment (exposure time) – under constant agitation inside a horizontal laminar flow hood. Finally, the seeds were washed in deionized water and autoclaved five times. One seed was inoculated in each test tube (25 × 150 mm) containing 10 mL of culture medium (Figure 1A).

The basic culture medium used in the experiment was MS medium (MURASHIGE; SKOOG, 1962) supplemented with 30 g L^{-1} of sucrose (Synth Ltda) and 6 g L^{-1} of agar (Merck SA). The pH of the culture medium was adjusted to 5.8 (± 0.05) before the addition of agar. The culture medium was autoclaved at a temperature of 121°C and pressure of approximately 1.0 kgf cm^{-2} for 20 minutes. The seeds were kept for 30 days in a growroom at a temperature of 24°C (± 1°C) under a 16-hour photoperiod and 40 µmol m^{-2} s^{-1} of irradiance (quantified by radiometer, LI -COR®, LI-250A Light Metre) emitted by a cold-white fluorescent lamp.

2.3. Dormancy breaking

The experiment was arranged in a completely randomized design (CRD) with 30 replicates of each dormancy-breaking treatment (mechanical scarification and thermal shock) and the control (no dormancy breaking) and one seed per replicate. In the mechanical process, the seeds were scarified in the region opposite the hilum using sandpaper (water sandpaper, 225 mm × 275 mm). The thermal shock method was performed in hot water for 1 minute (temperature of 60°C) and then immersed in water at room temperature at 24°C, both controlled by a digital thermometer. Data on the percentage of *in vitro* germination were collected at 30 days.

2.4. Asepsis

Based on the best results of the dormancy breaking experiment, an experiment on asepsis was conducted. The experiment was arranged in a CRD to test three immersion times in NaOCl solution (2.0-2.5% of active chlorine, Clarix®): 5 (control), 10, and 15 minutes. Each treatment had 30 replicates, consisting of plots containing one seed. Data on the percentage of contamination, oxidation, unresponsive seeds, and *in vitro* germination (Figure 1B-C) were collected at 30 days.

2.5. *In vitro* multiplication and elongation

After seed germination and *in vitro* establishment (Figure 1D) at 30 days, three shoots were standardized to 0.5 cm in length and grown by two methods: 1) in semisolid culture medium in a 250-mL glass flask with 50 mL of MS culture medium, supplemented with 30 g L^{-1} of sucrose, 6 g L^{-1} of agar, 0.5 mg L^{-1} of 6-benzylaminopurine (BAP, Sigma®), and 0.05 mg L^{-1} of α-naphthalene acetic acid (NAA, Sigma®); and 2) in liquid culture medium in the TIB (250-mL glass flask containing 50 mL of MS culture medium, supplemented with 30 g L^{-1} of sucrose, 0.5 mg L^{-1} BAP, and 0.05 mg L^{-1} NAA) (Figure 1E).

Over the 90 days of culture, tissue immersion in the bioreactor occurred for 30 seconds at 3-hour intervals. Subculturing with renewal of the culture medium was performed every 30 days. The semisolid and liquid culture media were made with deionized water, and the pH was adjusted to 5.8 (± 0.05) with NaOH (0.1 M) and/or HCl (0.1 M) before autoclaving. Autoclaving of the culture medium and the bioreactor equipment was performed at a temperature of 121°C and pressure of approximately 1.0 kgf cm^{-2} for 20 minutes.

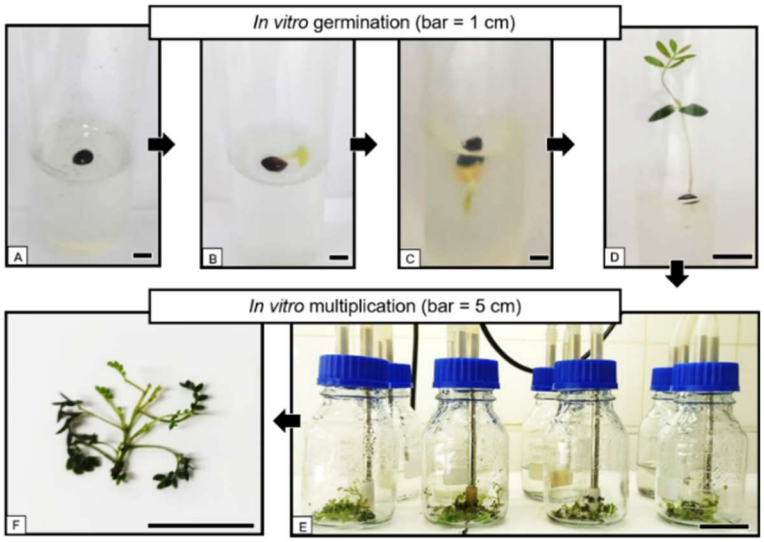

Figure 1. *In vitro* germination and multiplication of *Mimosa calodendron*. (A) Detail of *in vitro* inoculated seed. (B) Seed germinated with shoot-tip initiation. (C) Seed germinated with radicle initiation. (D) Explant considered established. (E) *In vitro* multiplication in the TIB. (F) Explant multiplied and elongated in the TIB system for 90 days. Bar = 1.0 cm (Figure A - D) or 5.0 cm (Figure F and E).
Figura 1. Germinação e multiplicação *in vitro* de *Mimosa calodendron*. (A) Detalhe da semente inoculada *in vitro*. (B) Semente germinada com iniciação da brotação apical. (C) Semente germinada com iniciação da radícula. (D) Explante considerado estabelecido. (E) Multiplicação *in vitro* em biorreator de imersão temporária (BIT). (F) Explante multiplicado e alongado em BIT aos 90 dias. Barra = 1.0 cm (Figura A-D) ou 5.0 cm (Figura F e E).

At 90 days, the mean number of shoots per explant (> 0.5 cm), shoot length (> 0.5 cm), vigor (Figure 2A-C), oxidation (Figure 2D-F), and senescence (Figure 2G-I) were determined according to the scale proposed by Souza et al. (2020b). The experiment was arranged in a CRD, with 20 replicates composed of five explants each.

2.6. *In vitro* adventitious rooting and acclimatization

Shoots produced in the multiplication and elongation phases were standardized by isolating four shoots to 3.0 cm in length with adequate vegetative vigor and inoculating them in test tubes (25 × 150 mm) containing 10 mL of MS culture medium supplemented with 30 g L^{-1} of sucrose, 6 g L^{-1} of agar, 0.5 mg L^{-1} NAA, and 0.05 mg L^{-1} BAP. The experiment was arranged in a CRD with 20 replicates composed of four explants each. At 30 days, the adventitious rooting percentage was assessed.

In vitro–rooted plants 5 cm in length and three fully expanded leaves were subjected to the acclimatization. Seedlings were transferred to a plastic container containing 50 mL of commercial substrate based on decomposed pine bark and vermiculite, with moisture controlled daily. The containers were isolated with plastic film for 5 days with gradual opening. The efficiency of acclimatization was verified through survival at 40 days.

2.7. Data analysis

The variables that did not have a normal distribution according to the Shapiro-Wilk test ($p > 0.05$) were arcsin-transformed. Hartley test ($p > 0.05$) was used to verify the homogeneity of variances. The groups were compared by analysis of variance ($p < 0.05$), and the means were compared by Tukey's test ($p < 0.05$). The analyses were processed in the software R version 3.0.3 (R CORE TEAM, 2018).

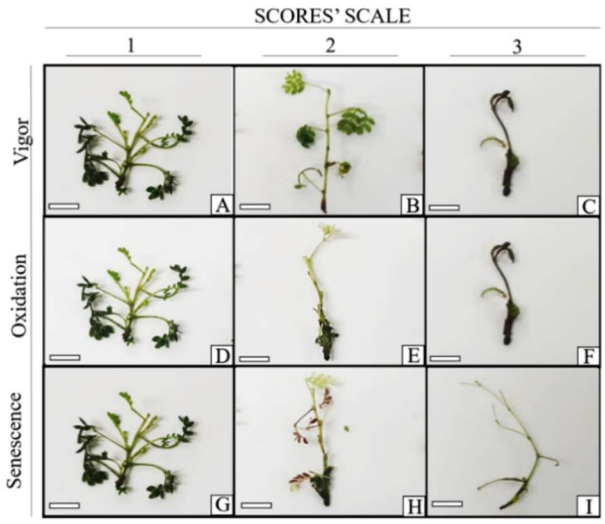

Figure 2. Assessments of vigor, oxidation, and senescence according to the scores scale of *Mimosa calodendron* explant. (A-C) Vigor of shoots (1 = Excellent: emission of shoots with active growth, without apparent nutritional deficiency; 2 = Good: emission of shoots, but with reduced leaves; 3 = Low: no emission of shoots and/or senescence and death). (D-F) Oxidation of shoots (1 = Null: no oxidation; 2 = Medium: reduced oxidation of explants; 3 = High: complete oxidation of explants). (G-I) Senescence of shoots (1 = Null: no leaf senescence; 2 = Medium: reduced leaf senescence of the explants; 3 = High: complete leaf senescence of the explants). Bar = 1 cm.
Figura 2. Avaliações de vigor, oxidação e senescência de acordo com a escala de notas. (A-C) Vigor das brotações (1 = Ótimo: emissão de brotações com crescimento ativo, sem deficiência nutricional aparente; 2 = Bom: emissão de brotações, porém com folhas de tamanho reduzido; 3 = Baixo: ausência de emissão de brotações e, ou, senescência e morte). (D-F) Oxidação das brotações (1 = Nula: sem oxidação; 2 = Média: reduzida oxidação dos explantes; 3 = Alta: oxidação completa dos explantes). (G-I) Senescência das brotações (1= Nula: sem senescência foliar; 2 = Média: reduzida senescência foliar dos explantes; 3 = Alta: senescência foliar completa dos explantes. Barra = 1 cm.

3. RESULTS

3.1. *In vitro* establishment

There was a significant difference between the treatments tested for breaking dormancy. Mechanical scarification resulted in 80% of germination, thermal shock only 40% (Figure 3A). There was no seedling germination in the control treatment without dormancy breaking (Figure 3A).

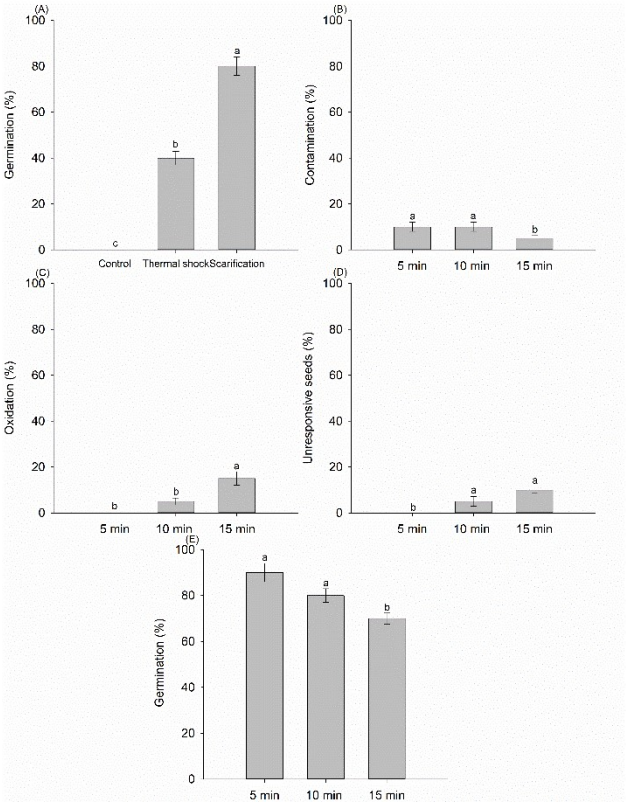

Figure 3. Characteristics assessed during the *in vitro* establishment phase of *Mimosa calodendron* according to the dormancy breaking method and exposure time to the chemical agent. (A) Percentage of *in vitro* germination according to dormancy breaking method. (B) *In vitro* contamination according to exposure time to the chemical agent. (C) Tissue oxidation according to exposure time to the chemical agent. (D) Unresponsive seeds according to exposure time to the chemical agent. (E) Percentage of *in vitro* germination according to exposure time to the chemical agent. *Mean values followed by the same letters do not differ significantly according to the Tukey's test ($p < 0.05$). Bars represent the standard deviation relative to the mean value.

Figura 3. Características avaliadas durante a fase de estabelecimento *in vitro* de *Mimosa calodendron* conforme o método de quebra de dormência e tempo de exposição ao agente quimico. (A) Porcentagem de germinação *in vitro* de acordo com o método de quebra de dormência. (B) Contaminação *in vitro* de acordo com o tempo de exposição ao agente químico. (C) Oxidação dos tecidos de acordo com o tempo de exposição ao agente químico. (D) Sementes não responsivas de acordo com o tempo de exposição ao agente químico. (E) Porcentagem de germinação *in vitro* de acordo com o tempo de exposição ao agente químico. *Médias seguidas por letras iguais não diferem entre si, pelo teste de Tukey ($p < 0.05$). Barras representam o desvio padrão em relação ao valor médio.

In vitro establishment significantly differed between treatments for all assessed traits (Figure 3B-E). The treatments of 5 and 10 minutes resulted in the highest contamination averages (10%), differing from the immersion time in NaOCl for 15 minutes (5%), which showed lower *in vitro* contamination of *Mimosa calodendron* seeds (Figure 3B). In contrast, the percentage of phenolic oxidation of tissues was significantly higher under the 15-minute treatment (15%) than under the 5-minute (0%) and 10-minute treatments (5%) (Figure 3C).

Unresponsive seeds were lowest with the 5-minute treatment (0%), differing significantly from the 10-minute (5%) and 15-minute treatments (10%) (Figure 3D). The best results of germination percentage were also observed in the 5-minute group (90%), followed by the 10-minute (80%) and 15-minute immersion groups (70%) (Figure 3E).

3.2. *In vitro* multiplication and elongation

At 90 days of *in vitro* cultivation of *Mimosa calodendron* explants in the semisolid and TIB cultivation systems, the number of buds per explant, shoot length, vigor, oxidation, and senescence were assessed (Figure 4A-E). There was a significant difference between treatments (semisolid and liquid culture systems in the TIB) for all morphological characteristics assessed except for shoot length (Figure 4B).

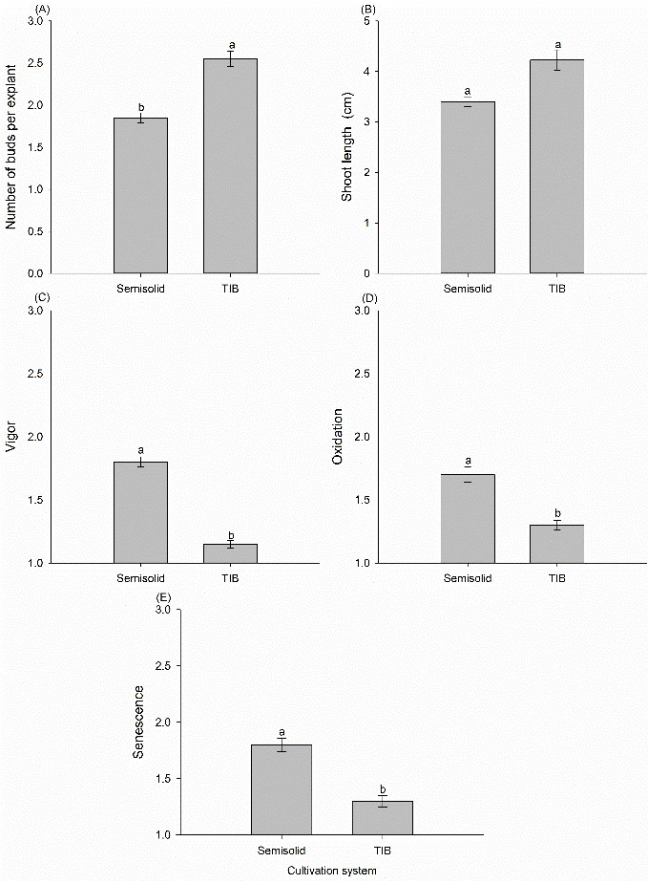

Figure 4. Characteristics assessed during the *in vitro* multiplication and elongation phases of *Mimosa calodendron* in semisolid and TIB cultivation systems. (A) Number of buds per explant. (B) Shoot length (cm). (C) Vigor. (D) Tissue oxidation. (E) Tissue senescence. *Mean values followed by the same letters do not differ significantly according to the Tukey's test ($p < 0.05$). Bars represent the standard deviation relative to the mean value.

Figura 4. Características avaliadas durante as fases de multiplicação e alongamento *in vitro* de *Mimosa calodendron* em sistemas de cultivo semisólido e BIT. (A) Número de gemas por explante. (B) Comprimento de brotos (cm). (C) Vigor. (D) Oxidação dos tecidos. (E) Senescência dos tecidos. *Médias seguidas por letras iguais não diferem entre si, pelo teste de Tukey ($p < 0.05$). Barras representam o desvio padrão em relação ao valor médio.

Considering the number of buds per explant (2.55 buds per explant, Figure 4A), vigor (1.1, Figure 4C), oxidation (1.3, Figure 4D), and senescence (1.3, Figure 4E), according to a

grading scale (Figure 2), the best results were observed with the culture in TIB liquid medium, differing significantly from the culture in semisolid culture medium (1.85 buds per explant, Figure 4A; 1.8 of vigor, Figure 4C; 1.7 oxidation, Figure 4D; 1.8 senescence, Figure 4E).

Shoot length was similar between the liquid medium treatments in the TIB and the semi-solid treatment with no significant difference (Figure 4B).

3.3. *In vitro* adventitious rooting and acclimatization

The percentage of *in vitro* rooting was similar between the semisolid (85% of rooting) and TIB (90% of rooting) systems at 30 days of cultivation (Figure 5). The rooted plants from the TIB and semisolid systems showed no significant difference in acclimatization, which had an overall mean of 77.8% at 40 days.

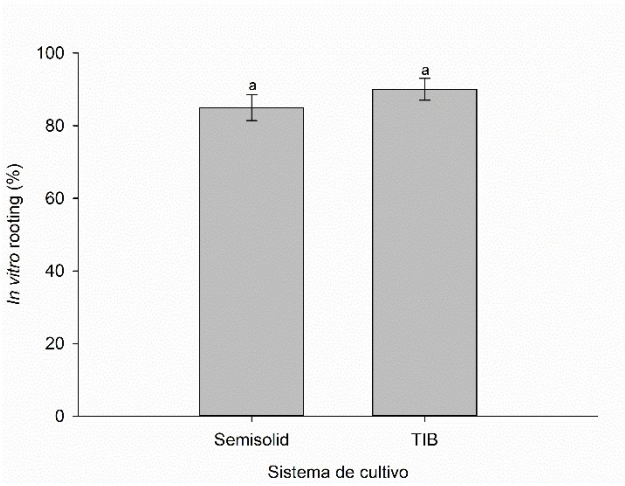

Figure 5. Percentage of *in vitro* adventitious rooting of *Mimosa calodendron* in semisolid and TIB cultivation systems. *Means followed by the same letters do not differ according to the Tukey's test ($p < 0.05$). Bars represent the standard deviation relative to the mean value.

Figura 5. Porcentagem de enraizamento adventício *in vitro* de *Mimosa calodendron* em sistema de cultivo semissólido e BIT. *Médias seguidas por letras iguais não diferem entre si, pelo teste de Tukey ($p < 0.05$). Barras representam o desvio padrão em relação ao valor médio.

4. DISCUSSION

4.1. *In vitro* establishment

Seed dormancy and slow germination are factors that hinder the production of *Mimosa calodendron* seedlings, and studies that investigate dormancy-breaking and germination mechanisms are important to provide support for the propagation of the species (DAYRELL et al., 2015). This *in vitro* germination experiment comparing two treatments of breaking dormancy, mechanical scarification and thermal shock, showed promising results for obtaining seedlings through *in vitro* cultivation.

Methods for breaking dormancy by mechanical scarification in *Mimosa calodendron* seeds showed efficiency, with 80% of germination (Figure 3A). The seeds that did not germinate in the control treatment showed that the species has physical dormancy. In a study by Dayrell et al. (2015), assessing the effect of mechanical scarification, lighting, and different incubation temperatures on seed germination showed the need for pre-treatment to break physical dormancy, and scarification was a highly effective method. Other studies have shown a positive effect of scarification treatment on germination in other species of *Mimosa* (OROZCO-ALMANZA et al., 2003; CHAUHAN; JOHNSON, 2009; ROSA et al., 2012).

Mechanical scarification is a simple, low-cost technique that is highly efficient at breaking tegumentary or physical dormancy, promoting rapid and uniform germination (SANTOS et al., 2004). This type of dormancy results from the impermeability of the integument, which may arise due to the presence of a cuticle and a developed layer of cells in the palisade, which prevents water absorption and gas exchange and imposes a mechanical restriction on the growth of the embryo, delaying the germination process (SANTOS et al., 2004).

For the asepsis experiment performed with the germinated material, the best results for most of the assessed characteristics were observed with immersion times of 5 minutes and 10 minutes in NaOCl solution at 2.0-2.5% of active chlorine. Although the time of 15 minutes reduced the percentage of contamination in the culture medium, this treatment also promoted the highest percentage of oxidation, unresponsive seeds, and lower germination. Prolonged exposure to NaOCl for disinfection can hinder seed germination due to long exposure of cellular tissues, increased permeability of the tegument, and leaching of plant hormones that are needed for germination (SILVA et al., 2019).

Therefore, the determination of the exposure time to the NaOCl disinfectant is important because it helps reduce the cytotoxicity and genotoxicity of tissues (SANTOS et al., 2020). In seedlings of *Melanoxylon brauna*, the use of NaOCl at 2.5% of active chlorine reduced contamination and thus favoured a higher percentage of healthy seedlings under a maximum exposure time of 25 minutes (SILVA et al., 2019). Seeds of *Dalbergia nigra*, after more than 14 minutes of exposure to NaOCl (2.0-2.5%), showed the development of seedlings with physiological and genetic disorders, attributed to phytotoxic, cytotoxic, and genotoxic effects (SANTOS et al., 2020). NaOCl solution at 5% of active chlorine allowed lower contamination (63%) than the concentration of 2.5% of active chlorine (83% of contamination) for explant of *Lychnophora pohlii*. However, immersion time (5, 10, 15, 20, or 25 minutes) did not influenced the contamination (GONZAGA et al., 2021).

Given the above, as the time of immersion in NaOCl increases, more residues can be adsorbed by the seed, reacting with the amino acids and generating a high concentration of ammonium chloride (NH_4Cl) and carbon dioxide (CO_2) in the test tube (SANTOS et al., 2020). In addition, the hydrolysis of NaOCl produces hypochlorous acid (HClO), a toxic compound that causes cellular and photosynthetic changes, for example, which negatively affect growth and cause abnormalities in seedlings (GAMAGE et al., 2018; SILVA et al., 2019; SANTOS et al., 2020).

Optimal exposure time to the disinfecting agent NaOCl should be assessed individually and carefully for each species. Our data showed that *Mimosa calodendron* showed high sensitivity to immersion time in NaOCl for almost all assessed traits, so 15-minute immersion is not recommended. Five and 10-minutes treatments are the most promising for the asepsis of *Mimosa calodendron*, as they provide a lower percentage of oxidation, more responsive seeds, and thus better germination.

4.2. *In vitro* multiplication and elongation

The improvement of protocols for vegetative propagation that supports plant development was studied in an attempt to establish the most appropriate cultivation system to maximize the micropropagation of *Mimosa calodendron*. The results of the morphological characteristics indicate the optimization of the multiplication and *in vitro* elongation in TIB systems.

Genetic material used in this study presented different responses depending on the crop system used. The TIB led to better results for all assessed traits (number of buds per explant, shoot length, vigor, oxidation, and senescence) than the semisolid cultivation system. As in the present study, the use of TIB has also been more efficient in the multiplication of buds and elongation of shoots in *Bambusa vulgaris* (RIBEIRO et al., 2016), *Corema album* (ALVES et al., 2021), and *Agave guiengola* (CHÁVEZ-ORTIZ et al., 2021).

According to Nogueira et al. (2017), when there is greater contact between the surface of the explants and the culture medium, the growth rate and vigor are higher due to greater absorption of water and nutrients. This relationship was observed in the present study, as the highest mean number of shoots per explant, length, and vigor of shoots were observed in explants subjected to TIB. Therefore, the number of buds per explant, length, and vigor of shoots are some of the best characteristics to assess the efficiency of the *in vitro* multiplication and elongation (SILVA et al., 2016; SOUZA et al., 2020b).

Phenolic oxidation and senescence of explants are problems associated with micropropagation that may influence crop development. These results may be related to cultivation factors, such as the use of semisolid medium in flasks with lower gas exchange, which tend to have low concentrations of carbon dioxide and high ethylene concentrations (CHÁVEZ-ORTIZ et al., 2021). In this context, TIBs can improve the supply of nutrients and the transfer of gases, making it possible to minimize physiological disturbances, which will result in greater development of micropropagated cultures (CARVALHO et al., 2019). Thus, methods aimed at breaking or reducing phenolic oxidation and senescence in tissues and organs are important strategies to be adopted in propagation systems, as observed during the use of TIB.

Given the above, it is of utmost importance to consider the physical state of the culture medium during *in vitro* culture, which can be semisolid or liquid and can directly interfere with the development of explants due to the different forms of contact of the plant with the culture medium (SOUZA et al., 2020a). Therefore, it is necessary to define the composition and type of culture medium most suitable for the growth and development of cultured tissues (SOTA et al., 2021), as this is a factor that exerts a great influence on *in vitro* culture.

4.3. *In vitro* adventitious rooting and acclimatization

In the process of adventitious rooting and acclimatization, several factors underlie the ability to form roots in microplants. Among these factors are plant hormones, cultivation environment (light, temperature, and gas exchange), and cultivation system, all of which play a predominant role in rhizogenesis (LIMA et al., 2022). The difficulty of propagation through adventitious rooting of microplants *in vitro* is one of the main problems encountered in the production of clonal plants of many native and woody species (ABIRI et al., 2020).

In the present study, regardless of the cultivation system, the percentages of rooting (90%) and acclimatization (77.8% survival) were satisfactory, and it was possible to obtain complete plants in 190 days. Although adventitious rooting and acclimatization through different cultivation systems are important factors in micropropagation, there are still few studies of their effects on *Mimosa calodendron*. Knowledge about the most appropriate cultivation system for the growth and development of plantlets would provide a basis for making protocols more efficient, thus allowing large-scale planning and propagation regardless of the season.

5. CONCLUSIONS

Breaking the dormancy of *Mimosa calodendron* seeds by mechanical scarification resulted in the highest percentage of *in vitro* germination.

Immersion time of 5 minutes in NaOCl (2.0-2.5% of active chlorine) resulted in better asepsis in seeds.

Liquid culture system with the use of a TIB proved to be the most appropriate technology for *in vitro* multiplication and elongation.

Adventitious rooting and acclimatization were satisfactory, and it was possible to obtain complete plants in 190 days.

6. AKNOWLEDGEMENTS

We thank the National Council for Scientific and Technological Development, Brazil ('Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq'), Coordination for Improvement of Higher Education Personnel, Brazil ('Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES – Código de Financiamento 001'), and Foundation for Research of the State of Minas Gerais, Brazil ('Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG'). We also thank GERDAU Açominas S.A.

7. REFERENCES

ABIRI, R.; ATABAKI, N.; HAMID, H. A.; SANUSI, R.; SHUKOR, N. A. A.; SHAHARUDDIN, N. A.; AHMAD, S. A.; MALIK, S. The prospect of physiological events associated with the micropropagation of *Eucalyptus* sp. **Forests**, v. 11, n. 11, 1211, 2020. DOI: 10.3390/f11111211

ALVES, V.; PINTO, R.; DEBIASI, C.; SANTOS, M. C.; GONÇALVES, J. C.; DOMINGUES, J. Micropropagation of *Corema album* from adult plants in semisolid medium and temporary immersion bioreactor. **Plant Cell, Tissue and Organ Culture**, v. 145, p. 641-648, 2021. DOI: 10.1007/s11240-021-02034-1

ARAÚJO, F. S.; FELIX, F. C.; SOUSA, N. A.; ALVES, E. C.; BRUNO, R. L. A.; ALVES, E. U.; NASCIMENTO, L. C. Otimizando a superação da dormência em sementes de *Acacia mangium* Willd. **Agronomic Crop Journal**, v. 29, n. 3, p. 313-325, 2020. DOI: 10.32929/2446-8355.2020v29n3p313-325

CARVALHO, L. S. O.; OZUDOGRU, A.; LAMBARDI, M.; PAIVA, L. V. Temporary immersion system for micropropagation of tree species: a bibliographic and systematic review. **Notulae Botanicae Horti**

Agrobotanici Cluj-Napoca, v. 47, n. 2, p. 269-277, 2019. DOI: 10.15835/nbha47111305

CHAUHAN, B. S.; JOHNSON, D. E. Germination, emergence, and dormancy of *Mimosa pudica*. **Weed Biology and Management**, v. 9, n. 1, p. 38-45, 2009. DOI: 10.1111/j.1445-6664.2008.00316.x

CHÁVEZ-ORTIZ, L. I.; MORALES-DOMÍNGUEZ, J. F.; RODRÍGUEZ-SAHAGÚN, A.; PÉREZ-MOLPHE-BALCH, E. *In vitro* propagation of *Agave guiengola* gentry using semisolid medium and temporary immersion bioreactors. **Phyton-International Journal of Experimental Botany**, v. 90, n. 3, p. 1003-1013, 2021. DOI: 10.32604/phyton.2021.012862

COSTA, B. N. S.; NETO, A. R.; CHAGAS, E. A.; CHAGAS, P. C.; PASQUAL, M.; VENDRAME, W. A. Influence of silicon and *in vitro* culture systems on the micropropagation and acclimatization of "Dwarf Cavendish" banana. **Acta Scientiarum. Agronomy**, v. 43, e47490, 2021. DOI: 10.4025/actasciagron.v43i1.47490

DAYRELL, R. L. C.; GONÇALVES-ALVIM, S. J.; NEGREIROS, D.; FERNANDES, W. G.; SILVEIRA, F. A. O. Environmental control of seed dormancy and germination of *Mimosa calodendron* (Fabaceae): implications for ecological restoration of a highly threatened environment. **Brazilian Journal of Botany**, v. 38, p. 395-399, 2015. DOI: 10.1007/s40415-015-0145-y

DUTRA, V. F.; GARCIA, F. C. P. Three new species of *Mimosa* sect. *Mimosa* (Leguminosae, Mimosoideae) from the Campos Rupestres of Minas Gerais, Brazil. **Brittonia**, v. 66, p. 33-41, 2014. DOI: 10.1007/s12228-013-9304-2

DUTRA, V. F.; MORALES, M.; JORDÃO, L. S. B.; BORGES, L. M.; SILVEIRA, F. S.; SIMON, M. F.; SANTOS-SILVA, J.; NASCIMENTO, J. G. A.; RIBAS, O. D. S. Mimosa. In: **Flora do Brasil 2020**. Jardim Botânico do Rio de Janeiro. Disponível em: <http://reflora.jbrj.gov.br/reflora/floradobrasil/FB31093>. Acesso em: 21 jan. 2022.

GAMAGE, D.; THOMPSON, M.; SUTHERLAND, M.; HIROTSU, N.; MAKINO, A.; SENEWEERA, S. New insights into the cellular mechanisms of plant growth at elevated atmospheric carbon dioxide concentrations. **Plant, Cell & Environment**, v. 41, n. 6, p. 1233-1246, 2018. DOI: 10.1111/pce.13206

GONZAGA, A. P. D.; MIRANDA, N. A.; TITON, M.; MACHADO, E. L. M.; ALMEIDA, H. S.; LEÃO, B. M. Desinfestação e germinação *in vitro* de *Lychnophora pohlii*. **Advances in Forestry Science**, v. 8, n. 1, p. 1253-1259, 2021. DOI: 10.34062/afs.v8i1.9343

LIMA, M. S.; ARAUJO, M. M.; BERGHETTI, A. L. P.; AIMI, S. C.; GRIEBELER, A. M.; SOMAVILLA, L. M.; SANTOS, O. P.; VALENTE, B. M. R. T. Mini-cutting technique application in *Corymbia* and *Eucalyptus*: effects of mini-tunnel use across seasons of the year. **New Forests**, v. 53, p. 161-179, 2022. DOI: 10.1007/s11056-021-09851-4

MEDJEMEM, N.; HARABI, A.; BOUZERARA, F.; FOUGHALI, F.; BOUDAIRA, B.; GUECHI, A.; BRIHI, N. Elaboration and characterization of low-cost ceramics microfiltration membranes applied to the sterilization of plant tissue culture media. **Journal of the Taiwan Institute of Chemical Engineers**, v. 59, p. 79-85, 2016. DOI: 10.1016/j.jtice.2015.07.032

MOLINARI, L. V.; SOUZA, D. M. S. C.; AVELAR, M. L. M.; FERNANDES, S. B.; GONÇALVES, D. S.; FARIA, J. C. T.; CARVALHO, D.; BRONDANI, G. E. Effects of chemical sterilization of the culture media, porous membranes and luminosity on *in vitro* culture of *Eucalyptus grandis* × *Eucalyptus urophylla*. **Journal Forestry Research**, v. 32, p. 1587-1598, 2021. DOI: 10.1007/s11676-020-01240-5

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum**, v. 15, n. 3, p. 473-497, 1962. DOI: 10.1111/j.1399-3054.1962.tb08052.x

NOGUEIRA, J. S.; COSTA, F. H. S.; VALE, P. A. A.; LUIS, Z. G.; SCHERWINSKI-PEREIRA, J. E. Micropropagação de bambu em larga-escala: princípios, estratégias e desafios. In: DRUMOND, P. M.; WIEDEMAN, G. (Org.) **Bambus no Brasil**: da biologia à tecnologia. Rio de Janeiro, Brasil: ICH. 1 ed. 2017. p. 103-129.

OROZCO-ALMANZA, M. S.; LEÓN-GARCÍA, L. P.; GRETHER, R.; GARCÍA-MOYA, E. Germination of four species of the genus *Mimosa* (Leguminosae) in a semi-arid zone of central Mexico. **Journal of Arid Environments**, v. 55, n. 1, p. 75-92, 2003. DOI: 10.1016/S0140-1963(02)00265-3

R CORE TEAM. **R**: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2018.

RAMÍREZ-MOSQUEDA, M. A.; BELLO-BELLO, J. J. SETIS™ bioreactor increases *in vitro* multiplication and shoot length in vanilla (*Vanilla planifolia* Jacks. Ex Andrews). **Acta Physiologiae Plantarum**, v. 43, 52, 2021. DOI: 10.1007/s11738-021-03227-z

RIBEIRO, A. S.; BRONDANI, G. E.; TORMEN, G. C. R.; FIGUEIREDO, A. J. R. Cultivo *in vitro* de bambu em diferentes sistemas de propagação. **Nativa**, v. 4, n. 1, p. 15-18, 2016. DOI: 10.31413/nativa.v4i1.3241

ROSA, F. C.; REINIGER, L. R. S.; GOLLE, D. P.; MUNIZ, M. F. B.; CURTI, A. R. Overcome dormancy and *in vitro* germination of seeds of *Mimosa scabrella* Bentham. **Semina: Ciências Agrárias**, v. 33, n. 3, p. 1021-1026, 2012. DOI: 10.5433/1679-0359.2012v33n3p1021

SANTOS, M. M.; CEZARIO, L. F. C.; SIMÕES, I. M.; BAPTISTA, J. O.; ARAUJO, C. P.; MELLO, T.; MAYARD, H.; GONÇALVES, E. O.; FONTES, M. M. P.; SCHMILDT, E. R.; LOPES, J. C.; CALDEIRA, M. V. W.; ALEXANDRE, R. S. Disinfection protocol and *in vitro* germination of seeds of *Dalbergia nigra*. **Cerne**, v. 26, n. 2, p. 238-246, 2020. DOI: 10.1590/01047760202026022714

SANTOS, T. O.; MORAIS, T. G. O.; MATOS, V. P. Escarificação mecânica em sementes de chichá (*Sterculia foetida* L.). **Revista Árvore**, v. 28, v. 1, p. 1-6, 2004. DOI: 10.1590/S0100-67622004000100001

SILVA, E. R.; SIMÕES, I. M.; BAPTISTA, J. O.; BIGHI, K. N.; FONTES, M. M. P.; SCHMILDT, E. R.; LOPES, J. C.; CALDEIRA, M. V. W.; ALEXANDRE, R. S. *In vitro* germination of *Melanoxylon brauna* Schott. and evaluation of the toxicity of disinfecting agents in the *Lactuca sativa* L. model plant. **Cerne**, v. 25, n. 4, p. 375-385, 2019. DOI: 10.1590/01047760201925042688

SILVA, I. M. C.; CUCHIARA, C. C.; WINHELMANN, M. C.; BIANCHI, V. J.; BRAGA, E. J. B.; DUTRA, L. F.; PETERS, J. A. *In vitro* multiplication of pear tree cultivar Cascatense. **Semina: Ciências Agrárias**, v. 37, n. 2, p. 581-594, 2016. DOI: 10.5433/1679-0359.2016v37n2p581

SOTA, V.; BENELLI, C.; ÇUKO, B.; PAPAKOSTA, E.; DEPAOLI, C.; LAMBARDI, M.; KONGJIKA, E. Evaluation of ElecTIS bioreactor for the micropropagation of *Malus sylvestris* (L.) Mill., an important autochthonous species of Albania. **Horticultural Science**, v. 48, p. 12-21, 2021. DOI: 10.17221/69/2020-HORTSCI

SOUZA, D. M. S. C.; AVELAR, M. L. M.; FERNANDES, S. B.; SILVA, E. O.; DUARTE, V. P.; MOLINARI, L. V.; BRONDANI, G. E. Spectral quality and temporary immersion bioreactor for *in vitro* multiplication of *Eucalytpus grandis* × *Eucalyptus urophylla*. **3 Biotech**, v. 10, 457, 2020a. DOI: 10.1007/s13205-020-02447-3

SOUZA, D. M. S. C.; XAVIER, A.; MIRANDA, N. A.; GALLO, R.; OTONI, W. C. Light quality, 6-benzyladenine and number of subcultives for *in vitro* multiplication of hybrid clones of *Corymbia*. **Scientia Forestalis**, v. 48, e3282, 2020b. DOI: 10.18671/scifor.v48n128.03

TEIXEIRA, G. C.; GONÇALVES, D. S.; MODESTO, A. C. B.; SOUZA, D. M. S. C.; CARVALHO, D.; MAGALHÃES, T. A.; OLIVEIRA, L. S.; TEIXEIRA, G. L.; BRONDANI, G. E. Clonal micro-garden formation of *Bambusa vulgaris*: effect of seasonality, culture environment, antibiotic and plant growth regulator on *in vitro* culture. **Cerne**, v. 27, e102979, 2021. DOI: 10.1590/01047760202127012979