Nativa, Sinop, v. 11, n. 1, p. 01-09, 2023.
Pesquisas Agrárias e Ambientais
DOI: https://doi.org/10.31413/nativa.v11i1.14394
ISSN: 2318-7670

Clonal microplant production, morphological evaluation and genetic stability of *Dendrocalamus asper* (Schult. & Schult.) Backer ex. K. Heyneke

Douglas Santos GONÇALVES[1], Denys Matheus Santana Costa SOUZA[1], Letícia Vaz MOLINARI[1], Maria Lopes Martins AVELAR[1], Dulcinéia de CARVALHO[1], Gustavo Leal TEIXEIRA[2], Gilvano Ebling BRONDANI[1]*

[1]Laboratory of *In Vitro* Culture of Forest Species, Department of Forestry Sciences, Federal University of Lavras, Lavras, MG, Brazil.
[2]Institute of Agricultural Science, Federal University of Minas Gerais, Montes Claros, MG, Brazil.
*E-mail: gilvano.brondani@ufla.br

Submitted on 09/20/2022; Accepted on 01/16/2023; Published on 02/07/2023.

ABSTRACT: Bamboo species have many commercial applications, considering that homogeneous plantations (formed from clonal plants) are essential to high sustainable biomass production. The cloning of selected plants on an industrial scale through *in vitro* cultivation has many advantages, being important for the supply of plants in sufficient quantity and quality to meet commercial demand. The control of the cloning is the basis for an industrial scale, and its knowledge can optimize the process. This work aimed to evaluate the cloning of *Dendrocalamus asper* selected plant through micropropagation. Morphological features by scanning electron microscopy and genetic stability with ISSR molecular markers were evaluated. Four times of immersion in sodium hypochlorite (NaOCl) on *in vitro* establishment of nodal segments were evaluated. The established explants were transferred to a culture medium that was supplemented with three concentrations of 6-benzylaminopurine (BAP). Three concentrations of indole-3-butyric acid (IBA) to the *in vitro* adventitious rooting were evaluated. NaOCl application for 10 min resulted in 71.4 % of establishment in 30 d. Supplementation of the culture medium with 2.0 and 3.0 mg L^{-1} BAP de resulted in the highest averages for multiplication and elongation stages. The formation of adventitious roots occurred with 4.0 mg L^{-1} IBA of supplementation. Micropropagated plants showed normal morphological features and genetic stability, confirming the cloning of selected plant.
Keywords: bamboo; micropropagation; vegetative propagation; *In vitro* culture; ISSR; plant growth regulator.

Produção de microplantas clonais, avaliação morfofisiológica e estabilidade genética de *Dendrocalamus asper* (Schult. & Schult.) Backer ex. K. Heyneke

RESUMO: Espécies de bambus apresentam diversas aplicações comerciais, visto que os plantios homogêneos (formados a partir de plantas clonais) são essenciais para a alta produção de biomassa sustentável. A clonagem de plantas selecionadas em escala industrial por meio do cultivo *in vitro* apresenta muitas vantagens, sendo uma importante ferramenta para o fornecimento de plantas em quantidade e qualidade suficientes para atender a demanda comercial. O controle da clonagem é a base para escala industrial, e seu conhecimento pode otimizar os processos. O trabalho teve como objetivo avaliar a clonagem de planta selecionada de *Dendrocalamus asper* por meio da técnica de micropropagação. Foram avaliadas as características morfológicas por microscopia eletrônica de varredura e estabilidade genética por meio de marcadores moleculares ISSR. Além disso, foram avaliados quatro tempos de imersão em hipoclorito de sódio (NaOCl) no estabelecimento *in vitro* de segmentos nodais. Os explantes estabelecidos foram transferidos para um meio de cultura que foi suplementado com três concentrações de benzilaminopurina (BAP). Por fim, foram avaliadas três concentrações de ácido indolbutírico (AIB) durante o enraizamento adventício *in vitro*. A adição de NaOCl por 10 min resultou em 71,4 % de estabelecimento em 30 d. A suplementação do meio de cultura com 2,0 e 3,0 mg L^{-1} BAP resultou nas maiores médias para as fases de multiplicação e alongamento. A formação de raízes adventícias ocorreu com a suplementação de 4,0 mg L^{-1} de AIB. Plantas micropropagadas apresentaram características morfológicas normais e estabilidade genética, confirmando a clonagem da planta selecionada.
Palavras-chave: bambu; micropropagação; propagação vegetativa; cultivo *in vitro*; ISSR; regulador de crescimento vegetal.

1. INTRODUCTION

Bamboo species occur naturally in tropical, subtropical, and temperate regions all over the world (SINGH et al., 2013a; CANAVAN et al., 2017; RAMAKRISHNAN et al., 2020; MUSTAFA et al., 2021). More than 4,000 traditional uses and 1,500 commercial applications with economic viability for bamboos have been described, besides its ecological and social usages - mainly, due to its morphological and silvicultural features (LIESE; KOHL, 2015; ZHAO et al., 2017; SAWARKAR et al., 2021; TEIXEIRA et al., 2021).

In Brazil the production of bamboo is still on a small scale, despite its great capacity for silvicultural applications

(INBAR, 2015; ROSA et al., 2016). To encourage the cultivation of bamboo species in Brazil, on 8 September 2011, the Law Number 12,484 was enacted (PNMCB, 2011), and it institutes a National Policy for Encouragement, Sustainable Management, and Bamboo Cultivation and aims the development of the culture through government actions and private corporations.

Dendrocalamus asper (Schult. & Schult.) Backer ex K. Heyneke, known as "giant bamboo" or "bucket bamboo", stands out from other species. According to Benton et al. (2015), its fibers produce a resistant raw material that is flexible and fast-growing, and is used for house construction in many rural areas and handicrafts. It is suitable for the production of cellulose and papermaking; the manufacture of household utensils and furniture; as food - for its edible sprouts; fiber; biofuel; for construction and the manufacture of musical instruments (INBAR, 2015; SINGH et al., 2012). Homogeneous plantings of the species can provide a rapid source of renewable biomass (MUSTAFA et al., 2021; KONZEN et al., 2021a), and the cloning of selected plants on an industrial scale (e.g., in biofactories) can provide plants in desirable quality and quantity.

Bamboos can be multiplied sexually or asexually; however, flowering cycles varies between species (some can reach up to 120 years), and in many cases plants die after flowering, and seeds have low viability (BANIK, 2015; BENTON, 2015; LIN et al., 2019; ZHAO et al., 2017). Thus, to enhance the value of superior genotypes, the best option is vegetative propagation. Separating clumps and planting stalk pieces are laborious techniques and low-efficiency (COSTA et al., 2017; KONZEN et al., 2021a). Despite the difficulties and considering some alternatives for the propagation of bamboo species, *in vitro* cultivation can be a viable tool for the production of a large number of clonal plants (RIBEIRO et al., 2016; FURLAN et al., 2018; HOSSAIN et al., 2018; RIBEIRO et al., 2020; TEIXEIRA et al., 2021).

Micropropagation is important technique for cloning and improving selected phenotypes (BHADRAWALE et al., 2018; TAMBARUSSI et al., 2017; SILVEIRA et al., 2020; KONZEN et al., 2021a), as it can generate plants with the same genetic feature of the parent plant (i.e., selected plant in adult stage) on industrial scale (HARTMANN et al., 2011; BRONDANI et al., 2017; KONZEN et al., 2021b). However, in some cases, this technology can induce genetic and morphological changes, known as somaclonal variations (LARKIN; SCOWCROFT, 1981; KONZEN et al., 2021b). This variation is considered a disadvantage when the aim is cloning, and for this reason, the development of further studies on genetic stability through *in vitro* cultivation is important for understanding the factors involved in the clonal production of bamboo species (KONZEN et al., 2017; RAMAKRISHNAN et al., 2020; KONZEN et al., 2021b).

This work aimed to evaluate the cloning of *Dendrocalamus asper* through micropropagation technique, considering the morphological features and genetic stability of micropropagated plants.

2. MATERIAL AND METHODS

2.1. *In vitro* establishment

Seedling (i.e., selected plant) of *Dendrocalamus asper* (Schult. & Schult.) Backer ex K. Heyneke was transferred to four-liter-recipient containing mixture of washed sand and sifted subsoil - 3 mm mesh (1/1, v/v) and suspended at 1 m of height. The cultivation was carried out in a greenhouse without temperature and relative humidity control.

The selected plant was fertigated weekly with a nutrient solution developed for the growth and shoot induction (MOLINARI et al., 2020). Weed control was carried out by manual plucking. Irrigation was carried out once a day, directly on the substrate, avoiding the contact of water with the aerial parts of the plant.

Shoots from selected plant cultivated by one-year-old were collected and transported to the laboratory. Subsequently, the leaf sheaths were removed, and their remains were carefully scraped with a stylus for yolk exposition, facilitating asepsis (RIBEIRO et al., 2020; TEIXEIRA et al., 2021). The tissues were then washed with deionized and autoclaved water and neutral detergent. Shoots were reduced to 1 cm long nodal explants and immersed for 30 s in a 70 % hydroalcoholic solution followed by deionized and sterilized water. Subsequently, they were immersed in a sodium hypochlorite (NaOCl) solution (1.00 - 1.25 % of active chlorine), and four times of exposure to chemical disinfectant were tested (5 - control, 10, 15, and 20 min). After this immersion, the explants were transported to a laminar flow chamber, where they were subjected to four washes with deionized and sterilized water to eliminate surface residues of sodium hypochlorite.

The explants were inoculated vertically in test tubes (20 × 150 mm) containing 10 mL of MS culture medium (MURASHIGE; SKOOG, 1962), which were sealed with plastic film made with poly(vinyl chloride) (PVC). The experiment was conducted in a randomized design with four different times of NaOCl immersion, and 40 replications (one explant per replication). Percentage of total contamination [i.e., ∑(fungal + bacterial contamination)], tissue oxidation, establishment and shoot induction (i.e., explant with shoot) were evaluated at 30 d. Explants free of contamination and tissue oxidation were considered established.

2.2. *In vitro* multiplication and elongation

Established explants were transferred to glass flasks (72 × 72 × 100 mm) containing 50 mL of MS culture medium supplemented with three concentrations of 6-benzylaminopurine (BAP) (1.0 - control, 2.0, and 3.0 mg L^{-1}). Culture medium of all treatments was supplemented with 0.5 mg L^{-1} of α-naphthalene acetic acid (NAA). Three subcultures were performed out every 30 d (Sub 1 - 30 d, Sub 2 - 60 d, and Sub 3 - 90 d). The experiment was conducted in a completely randomized design in factorial arrangement with three concentrations of BAP and three subcultures, and 15 replications (one explant per replication). Percentage of survival, shoot induction, number of shoots per explant, and shoot length per explant (cm) were evaluated in each subculture.

2.3. *In vitro* adventitious rooting

Explants with 3-cm-long (from the *in vitro* multiplication and elongation stages) were inoculated in glass flasks (72 × 72 × 100 mm) containing 50 mL of MS culture medium supplemented with three concentrations of indole-3-butyric acid (IBA) (2.0 - control, 4.0, and 6.0 mg L^{-1}). The culture medium was supplemented with 1.0 mg L^{-1} NAA and 0.5 mg L^{-1} BAP in all treatments. The experiment was conducted in

a randomized design with three concentrations of IBA, and 15 replications (one explant per replication). Percentage of adventitious rooting was evaluated at 30 d. Leaf samples of rooted plants were collected for scanning electron microscopy (SEM) and genetic stability analysis.

2.4. Preparation of the culture medium and incubation conditions

Culture medium was prepared with deionized water, 6 g L^{-1} of agar and 30 g L^{-1} of sucrose. The pH of the solution was adjusted to 5.8 with HCl (0.1 M) and/or NaOH (0.1 M) before adding the agar to the culture medium, and then autoclaved at 127 °C (1.5 kgf cm^{-2}) for 20 min. BAP, NAA and IBA were added to the culture medium before autoclaving. Explants were cultivated in a grow-room with a temperature of 24 °C (±1 °C), irradiation of 40 μmol m^{-2} s^{-1} (cold white tube lamp), and 16 h of photoperiod.

2.5. Scanning electron microscopy

Leaf samples from *in vitro* rooted microplants were collected and sectioned in 0.5 cm^2 sizes, and placed in microtubes (1.5 mL) containing Karnovsky (1965) fixative (2.5 % of glutaraldehyde, 2.0 % of paraformaldehyde, 0.05 M of cacodylate buffer at pH 7.2, and 0.001 M of $CaCl_2$) for 72 h in a refrigerator (4 °C). Subsequently, the samples were washed three times in cacodylate buffer (0.05 M) for 10 min. The dehydration step was carried out with a graduated series of acetone (25, 50, 75, 90, and 100 %) for 10 min, with 100 % concentration transferred three times. Then, the samples were placed in porous support containing acetone and sent to the drying stage at the critical point, where the acetone was volatized and replaced by carbon dioxide (CO_2). The supporters were wrapped in aluminum foil and fixed in double-sided carbon tape for the assembly of leaf samples in the blocks. Finally, the gold metallization stage was carried out. The observation of images was made in SEM (LEO EVO-40).

2.6. Genetic stability

Young leaves were collected from the *in vitro* rooted microplants and selected plant for the evaluation of genetic stability. DNA extractions were performed according to an adapted protocol by Ferreira and Grattapaglia (1998). Eighteen primers were used (Chris, John, UBC809, UBC811, UBC814, UBC825, UBC827, UBC834, UBC835, UBC840, UBC841, UBC842, UBC844, UBC848, UBC857, UBC880, UBC898, and UBC901). ISSR reactions were prepared in microplates (PCR-96-Axygen Scientific), with 3 μL DNA (standardized at 20 ng μL^{-1} for all samples) and 10 μL of reaction mix [1.5 mM PCR buffer Phoneutria®, 1.5 mM dNTP, 1 U of Taq polymerase Phoneutria® (5.0 U μL^{-1}), Diluent Taq (based on BSA and Tris-HCl), and 0.2 mM of each primer, completing the final volume with ultrapure water (4.2 μL)].

2.7. Statistical analysis

Data analysis was performed with the R Core Team Software (2018), ExpDes package version 1.1.2 (Ferreira et al. 2013). Collected data were analyzed for Hartley ($p > 0.05$) and Shapiro-Wilk ($p > 0.05$) tests. According to the results, the data were transformed using the Box-Cox test. After, the data were submitted to analysis of variance (ANOVA, $p < 0.05$) and the means were compared using the Tukey's test ($p < 0.05$).

3. RESULTS

3.1. *In vitro* establishment

NaOCl application as a disinfectant chemical agent, significantly influenced the *in vitro* establishment of *Dendrocalamus asper* explants (Figure 1). High percentage of fungal (93.3 %) and bacterial (6.7 %) contamination in 5 min of exposure to NaOCl denotes the importance of controlling *in vitro* establishment conditions (Figure 1A), because there was total loss of explants. Increasing the exposure time from 10 to 20 min resulted in low contamination (24.4 to 28.5 %) (Figure 1A), being considered efficient for asepsis. However, the *in vitro* establishment and morphological features were affected (Figure 1B-D).

Percentage of tissue oxidation varied according to time of exposure to the chemical agent (Figure 1B), and it is possible to observe that the longest exposure time (i.e., 15 to 20 min) there was higher tissue oxidation (17.0 to 40.0 %). There was no tissue oxidation in 10 min of exposure to NaOCl (Figure 1B), furthermore, the highest percentage of *in vitro* establishment (71.4 %) (Figure 1C) and explants that emitted shoots (62.9 %) (Figure 1D) were observed in this time.

3.2. *In vitro* multiplication and elongation

No significant difference was observed between the BAP supplementations for explant survival in 90 d of *in vitro* culture, with values ranging from 75.0 to 100 % (Table 1). Thus, it can be considered that the protocol for the *in vitro* multiplication and elongation of *Dendrocalamus asper* was efficient, according to this parameter, due to the low percentage of explant mortality and adequate shoot induction (average of 87.5 % of explant with shoot in 90 d).

Multiplication and elongation stages occurred simultaneously for *Dendrocalamus asper*, representing an important phenomenon when considering *in vitro* culture to obtain large-scale plants in a reduced time. There was a significant difference for the number of shoots, in which the highest values with the use of 3.0 mg L^{-1} BAP (2.2 shoots per explant, Figure 2A) and in the third subculture at 90 d (2.1 shoots per explant, Figure 2B) were observed.

Highest shoot length per explant was observed in 2.0 and 3.0 mg L^{-1} BAP supplementation to the culture medium, resulting in shoots with an average length of 4.4 and 5.0 cm, respectively (Figure 2C). Considering the number of subcultures (performed every 30 d), the highest mean of shoot length was found in the second (4.5 cm) and third (5.1 cm) subcultures (Figure 2D).

Table 1. Percentage of *in vitro* survival of *Dendrocalamus asper* explants according to BAP supplementation and subcultures.
Tabela 1. Porcentagem de sobrevivência *in vitro* de explantes de *Dendrocalamus asper* em relação à suplementação de benzilaminopurina (BAP) e subcultivo.

BAP (mg L^{-1})	Sub 1 (%)	Sub 2 (%)	Sub 3 (%)
1.0 (control)	75.0^a (±15.0)	75.0^a (±15.0)	75.0^a (±15.0)
2.0	100.0^a (±0.0)	87.0^a (±10.0)	87.0^a (±10.0)
3.0	100.0^a (±0.0)	75.0^a (±15.0)	75.0^a (±15.0)

Means with the same letters do not differ significantly by the Tukey's test ($p < 0.05$). Data presented as mean ± standard error. Sub = subculture (Sub 1 - 30 d, Sub 2 - 60 d, and Sub 3 - 90 d).

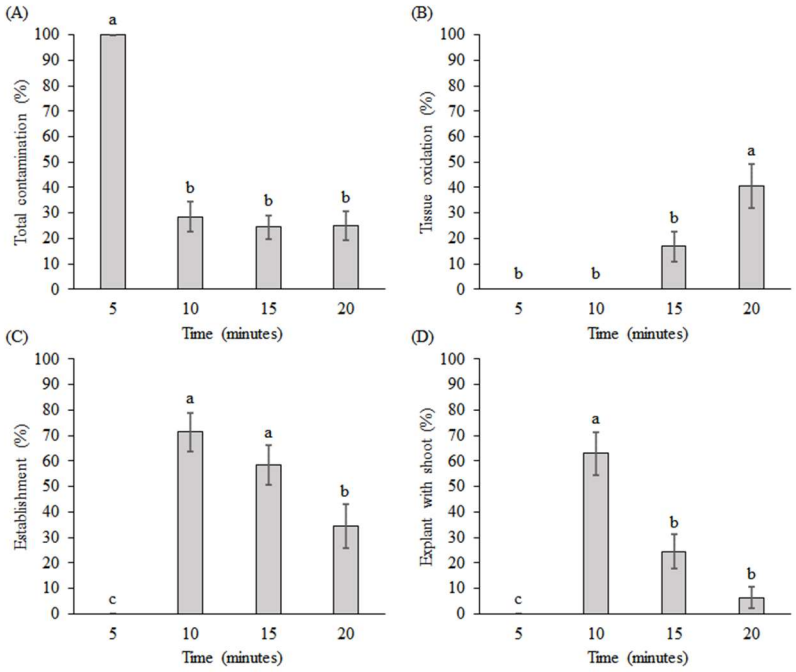

Figure 1. Variables evaluated during *in vitro* establishment of *Dendrocalamus asper* explants, at 30 d. (A) Percentage of total contamination [∑(fungal + bacterial contamination)]; (B) Percentage of tissue oxidation; (C) Percentage of *in vitro* establishment; and (D) Percentage of explants with shoot (i.e., shoot induction). Means with the same letters do not differ significantly by the Tukey's test ($p < 0.05$). Data presented as mean ± standard error.

Figura 1. Variáveis avaliadas durante do estabelecimento *in vitro* de explantes de *Dendrocalamus asper*, aos 30 d. (A) Porcentagem de contaminação total [∑(fúngica + bacteriana)]; (B) Porcentagem de oxidação tecidual; (C) Porcentagem de estabelecimento *in vitro*; e (D) Porcentagem de explantes apresentando broto (indução de broto). Médias com a mesma letra não diferem significativamente pelo teste de Tukey ($p < 0.05$). Dados apresentados como média ± erro padrão.

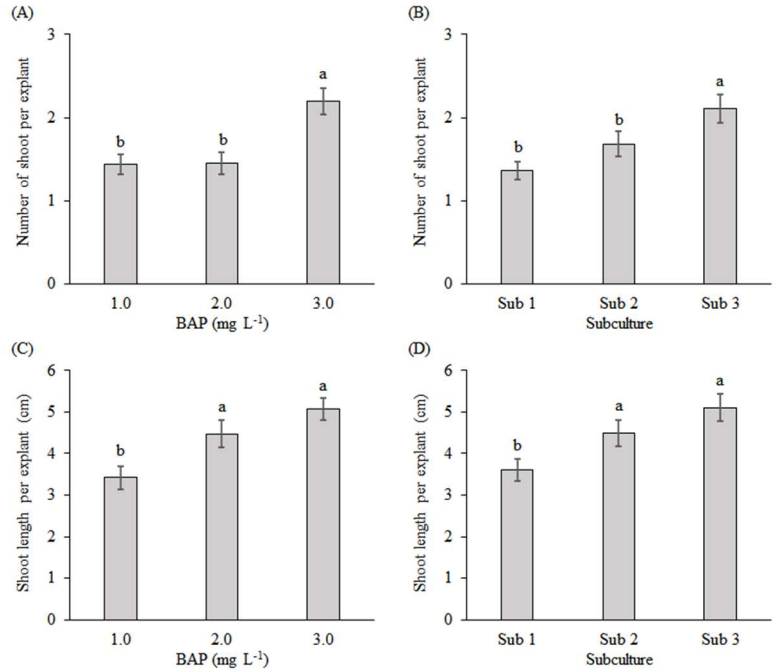

Figure 2. Variables evaluated on *in vitro* multiplication and elongation stages of *Dendrocalamus asper* explants. (A) Number of shoots per explant according to BAP supplementation; (B) Number of shoots per explant according to subculture; (C) Shoot length per explant (cm) according to BAP supplementation; and (D) Shoot length per explant (cm) according to subculture. Means with the same letters do not differ significantly by the Tukey's test ($p < 0.05$). Data presented as mean ± standard error. Sub = subculture (Sub 1 - 30 d, Sub 2 - 60 d, and Sub 3 - 90 d).

Figura 2. Variáveis avaliadas durante a multiplicação e alongamento *in vitro* of explantes de *Dendrocalamus asper*. (A) Número de brotações por explante de acordo com a suplementação de BAP; (B) Número de brotações por explante de acordo com o subcultivo; (C) Comprimento de broto por explante (cm) de acordo com a suplementação de BAP; e (D) Comprimento de broto por explante (cm) de acordo com o subcultivo. Médias com a mesma letra não diferem significativamente pelo teste de Tukey ($p < 0.05$). Dados apresentados como média ± erro padrão. Sub = subcultivo (Sub 1 - 30 d, Sub 2 - 60 d, e Sub 3 - 90 d).

3.3. *In vitro* adventitious rooting

Adventitious rooting was only observed when the culture medium was supplemented with 4.0 mg L^{-1} IBA, resulting in 60.0 % at 30 d of *in vitro* culture (Table 2).

Table 2. Percentage of *in vitro* adventitious rooting of *Dendrocalamus asper* explants according to IBA supplementation in 30 days.
Tabela 2. Porcentagem de enraizamento adventício *in vitro* de explantes de *Dendrocalamus asper* de acordo com a suplementação de ácido indolbutírico (IBA), aos 30 dias.

IBA (mg L^{-1})	Adventitious rooting (%)
2.0 (control)	0.0^{b} (±0.0)
4.0	60.0^{a} (±18.0)
6.0	0.0^{b} (±0.0)

Means with the same letters do not differ significantly by Tukey's test ($p < 0.05$). Data presented as mean ± standard error.

3.4. Scanning electron microscopy

Morphological features of leaf surface of *Dendrocalamus asper* microplants *in vitro* grown were performed (Figure 3A-D). The adaxial surface of the leaf blade has normal stomata, spines, single-celled trichomes, and microtrichomes, both in small quantities (Figure 3A), and the stomata surrounded by epidermal papillae and microtrichomes (Figure 3B).

On the abaxial surface (Figure 3C), hook-shaped trichomes, long single-celled trichomes, stomata arranged in rows close to the trichomes, and a high papillae density was observed. Papillae (Figure 3D) is often associated with stomata, hook-shaped trichomes, microtrichome, spine, and the distribution pattern of the stomata.

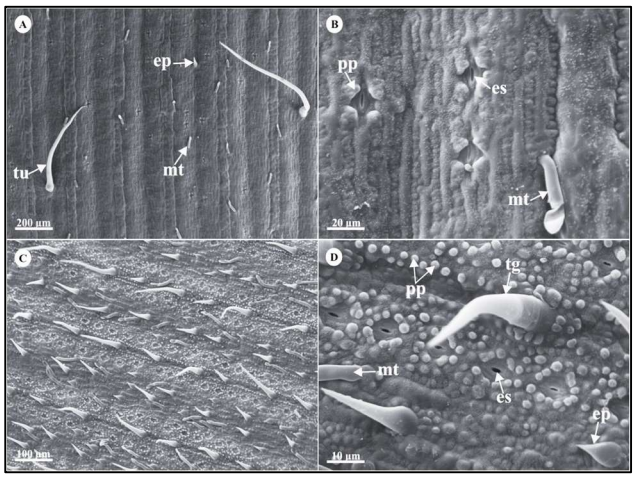

Figure 3. Morphological features of the leaf surface of *Dendrocalamus asper* microplant *in vitro* grown. (A) Paradermal section of the adaxial face with the presence of unicellular trichomes, stomata, spines and microtrichomes; (B) details of stomata and presence of microtrichome; (C) abaxial face, microtrichomes, spines, papillae, stomata and trichomes; and (D) detail of stomata with papillae and hook-shaped trichomes. (ep) Thorn; (es) Stomata; (mt) Microtrichome; (pp) Papilla; (tg) Hook-shaped trichome; and (tu) Unicellular trichome.
Figura 3. Características morfológicas da superfície foliar de microplanta de *Dendrocalamus asper* cultivada *in vitro*. (A) Corte paradérmico da face adaxial com presença de tricomas unicelulares, estômatos, espinhos e microtricomas; (B) Detalhes dos estômatos e presença de microtricomas; (C) Face abaxial, microtricomas, espinhos, papilas, estômatos e tricomas; e (D) Detalhe dos estômatos com papilas e tricomas em forma de gancho. (ep) Espinho; (es) Estômato; (mt) Microtricoma; (pp) Papila; (tg) Tricoma em forma de gancho; e (tu) Tricoma unicelular.

3.5. Genetic stability

The microplants were identical clones to the parent plant of *Dendrocalamus asper* (i.e., selected plant). All tested primers showed adequate amplification and discernible bands in the evaluation of genetic stability. There was no polymorphism, that is, the bands exhibited the same profile for all microplants (Table 3). The presence of monomorphism confirms that there was no somaclonal variation during the *in vitro* subculture in 90 d.

Table 3. Primer amplification result of *Dendrocalamus asper* micropagated clonal plants at 90 d.
Tabela 3. Resultado da amplificação do primer de plantas clonais micropagadas de *Dendrocalamus asper*, aos 90 d.

Primer	Sequence	Total	Monomorphic	Polymorphic
Chris	$(CA)_7$-YG	3	3	0
John	$(AG)_7$-YC	6	6	0
UBC809	$(AG)_8$-G	6	6	0
UBC811	$(GA)_8$-C	6	6	0
UBC814	$(CT)_8$-T	6	6	0
UBC825	$(AC)_8$-T	5	5	0
UBC827	$(AC)_8$-G	4	4	0
UBC834	$(AG)_8$-YT	6	6	0
UBC835	$(AG)_8$-YC	4	4	0
UBC840	$(GA)_8$-YT	6	6	0
UBC841	$(GA)_8$-YC	5	5	0
UBC842	$(GA)_8$-YG	3	3	0
UBC844	$(CT)_8$-RC	4	4	0
UBC848	$(CA)_8$-RG	5	5	0
UBC857	$(AC)_8$-YG	6	6	0
UBC880	$(GGAGA)_3$	6	6	0
UBC898	$(CA)_6$-RY	6	6	0
UBC901	$(GT)_6$-YR	3	3	0

Note: R = purine (A or G) and Y = pyrimidine (C or T).

4. DISCUSSION

4.1. *In vitro* establishment

Biotechnology involves effective tools for bamboos propagation on a large scale (SINGH et al., 2013a; ORNELLAS et al., 2017). However, contamination by phytopathogenic organisms is one significant obstacle to achieving success in the *in vitro* establishment (RIBEIRO et al., 2016; TORRES et al., 2016; FURLAN et al., 2018; RIBEIRO et al., 2020). Microorganism *in vitro* contamination has been reported for several bamboo species, such as *Bambusa ventricosa* (WEI et al., 2015), *Bambusa vulgaris* (FURLAN et al., 2018; RIBEIRO et al., 2020; TEIXEIRA et al., 2021), *Dendrocalamus asper* (SINGH et al., 2012) and *Dendrocalamus strictus* (PANDEY; SINGH, 2012), being an important event to be controlled during the tissue *in vitro* introduction. Therefore, defining asepsis protocols with antimicrobial agents is essential to minimize the contamination (BRONDANI et al., 2017; ORNELLAS et al., 2017).

Sodium hypochlorite is an alternative in the micropropagation of *Dendrocalamus asper*, as it is considered a low cost and effective product for tissue disinfestation of forest species (BRONDANI et al., 2013; BRONDANI et al., 2017). In the present study, the use of this chemical agent was effective for the control of fungal and/or bacterial contamination (Figure 1A). However, the exposure time to

NaOCl can increase tissue oxidation (Figure 1B), and it is important to define the better exposure time.

The percentage of tissue oxidation increased according to use of sodium hypochlorite, and the longer exposure times resulted in greater oxidation (15 at 20 min), denoting that the species' tissues may be highly susceptible when exposed to chemical treatment for longer period of time. Oxidation events are most often related to tissue healing mechanisms, which cause exudation of phenolic compounds in response to lesions in explants during preparation and *in vitro* inoculation (MUDOI et al., 2013; KONZEN et al., 2021a), which can make explants unviable, affecting the next stages of cultivation (BRONDANI et al., 2017; BHADRAWALE et al., 2018; RIBEIRO et al., 2020).

High percentage of *in vitro* establishment (Figure 1C) and shoot induction (Figure 1D) at 30 d were observed in 10 min of exposure to NaOCl. The value for the establishment can be considered adequate, denoting the importance of high percentages of shoot induction to be used for the other stages (e.g., multiplication and elongation). Satisfactory values for *in vitro* establishment were observed for *Dendrocalamus asper* (SINGH et al., 2012) and *Dendrocalamus strictus* (PANDEY; SINGH, 2012), using active chlorine as a disinfectant - what corroborates with the results of the present study.

In addition, with the increased exposure time to the disinfesting agent, the shoot induction was lower. This factor may be related to the toxicity that the active chlorine can cause to the explant tissues. According to Brondani et al. (2013), multiple factors can interfere in the *in vitro* establishment, such as genetic material, type and origin of the explant, ontogenetic and physiological conditions of the selected plant, asepsis method, and phytotoxicity caused by the disinfecting agents.

4.2. *In vitro* multiplication and elongation

A direct relationship between BAP concentration and the number and length of shoots per explant was observed (Figure 2A-D), considering that the highest percentage of multiplication and elongation was observed in higher concentrations of the plant growth regulator. The addition of cytokinin and auxin in the culture medium is used to induce multiplication and shoot elongation (HARTMANN et al., 2011). Plant growth regulators are applied exogenously and can be used singly or combined, depending of the objective, species, and the endogenous levels found in the tissues (SINGH et al., 2004; BANIK, 2015). Higher concentrations of cytokinin in the multiplication and elongation were reported for bamboos (NEGI; SAXENA, 2011; PANDEY; SINGH, 2012), corroborating with the findings in this study. BAP supplementation in culture medium is reported to get high multiplication in *Dendrocalamus asper* (SINGH et al., 2012; SINGH et al., 2013b; ORNELLAS et al., 2017) and *Drepanostachyum luodianense* (LIN et al., 2019).

Highest values for the number of shoots per explant occurred in the third subculture (Figure 2B); and shoot length in the second and third subcultures (Figure 2D), suggesting that the increase in the number of subcultures favors the multiplication and elongation of *Dendrocalamus asper* simultaneously, showing a high apical dominance. Nevertheless, the increase in BAP concentration in the culture medium did not favor growth in length of *Dendrocalamus asper* (SINGH et al., 2012), showing that lower concentrations indicated better results under certain *in vitro* culture conditions and should be considered.

According to Santos et al. (2016), defining the ideal number of subcultures is of great relevance for adapting explants to the newly established conditions, favoring the absorption of the exogenous source of cytokinin and auxin, for increasing the response to the morphogenic stimulus. Numerous subcultures may be necessary in multiplication until reaching the desired number of microplants, with the identical genetic composition of the parent plant (NOGUEIRA et al., 2017).

4.3. *In vitro* adventitious rooting

There was root formation only in the treatment with 4.0 mg L^{-1} IBA (60.0 % of adventitious rooting, Table 2). Rooting stage is recommended for radial system obtention with standard and functional structure, which may favor plants' survival and *ex vitro* growth, avoiding possible losses during acclimatization (NOGUEIRA et al., 2017; KONZEN et al., 2021a; TEIXEIRA et al., 2021). However, this stage of micropropagation is one of the major obstacles during *in vitro* cultivation in bamboo species (SINGH et al., 2012).

Adventitious rooting is a fundamental step in any micropropagation system (RIBEIRO et al., 2016; FURLAN et al., 2018; HOSSAIN et al., 2018; SILVEIRA et al., 2020). Commonly, bamboo species do not root easily, and it is common observing a low number of responsive explants (MUDOI et al., 2013; SANDHU et al., 2018). However, Ramanayake et al. (2008) evaluated different IBA concentrations in the rooting of *Dendrocalamus giganteus*, and verified up to 100 % of rooting. Nogueira et al. (2019) found high rooting in *Guadua magna* and *Guada angustifolia* when used 3.0 mg L^{-1} IBA. These observations reinforce the need to supplement the culture medium with IBA to induce adventitious root in *Dendrocalamus asper*.

Nevertheless, it is important that the selected plants (i.e., stock plant) utilized for tissue *in vitro* inoculation have seminal origin, which indicates a low ontogenetic age. This feature can influence cellular ability response to plant growth regulator stimuli, inducing more adventitious rooting capacity due to tissue juvenility (HARTMANN et al., 2011; WENDLING et al., 2014; KUMAR et al., 2022).

4.4. Scanning electron microscopy

Ultrastructural morphological knowledge is important in several investigations, mainly for species identification (MONTIEL; SÁNCHEZ, 2006a). Leaf surface morphology of *Dendrocalamus asper* microplants *in vitro* grown (Figure 3A-D) were compatible with the characterization performed by Montiel and Sánchez (2006a; 2006b). According to these authors, the leaf blade surfaces of the clones have broad trichomes and spines, corroborating with the description for *Dendrocalamus asper*, denoting absence of morphological variation.

On the abaxial surface, a large density of papillae was found (Figure 3C). Those are important structures in the taxonomy of bamboo species (OLIVEIRA et al., 2008). Papillae in greater abundance on the abaxial face in *Dendrocalamus asper* were observed, but it could also be found on the adaxial face. It was also observed that there was a greater presence of stomata, trichomes and papillae cells on the abaxial face when compared to the adaxial face (Figure 3A-D).

4.5. Genetic stability

Dendrocalamus asper microplants are clones from selected plant, according to observed results. Even though somaclonal variation was not observed in the present study, with the increase in subcultures, this phenomenon may occur due to several factors, including the addition of high concentrations of plant growth regulators to the culture media (VENKATACHALAM et al., 2007; KONZEN et al., 2017; RAMAKRISHNAN et al., 2020; KONZEN et al., 2021b). It can also happen due to cell cycle disturbances caused by exogenous application of plant growth regulators (PESCHKE; PHILLIPS, 1992; HARTMANN et al., 2011). According to Singh et al. (2013b), verifying the genetic stability of micropropagated plants in an early stage can contribute to the definition of reliable protocols, avoiding future problems with the plants in the field after planting.

Several types of research have been carried out with bamboo species to verify the genetic stability of clones when compared to the parent plants, using ISSR markers, such as in *Dendrocalamus asper* (SINGH et al., 2012), *Bambusa bamboo* (ANAND et al., 2013), *Guadua magna* and *Guada angustifolia* (NOGUEIRA et al., 2019). In this study, no genetic variation of microplant *in vitro* grown was observed even after 6 mth of *in vitro* cultivation using plant growth regulators. Thus, it is possible to suggest that the methodology was efficient for the clonal microplant production of *Dendrocalamus asper*, and can be used for applications in biofactories aiming at the formation of homogeneous forests.

5. CONCLUSIONS

Sodium hypochlorite (NaOCl) in a concentration of 1.00 - 1.25 % of active chlorine for 10 min favored the *in vitro* establishment and shoot induction.

Culture medium supplemented with 2.0 - 3.0 mg L^{-1} BAP favored the multiplication and elongation stages simultaneously, showing a high apical dominance.

Adventitious root formation was confirmed only in 4.0 mg L^{-1} IBA supplemented at culture medium.

Micropropagated plants have normal leaf morphology and genetic stability to selected plant, which could favor the formation of clonal plantations of the species.

6. REFERENCES

ANAND, M.; BRAR, J.; SOOD, A. *In vitro* propagation of an edible bamboo *Bambusa bamboos* and assessment of clonal fidelity through molecular markers. **Journal of Medical and Bioengineering**, v. 2, n. 4, p. 257-261, 2013. https://doi.org/10.12720/jomb.2.4.257-261

BANIK, R. L. Bamboo silviculture. In: LIESE, W.; KÖHL, M. (Eds.). **Bamboo**: tropical forestry. vol. 10. Springer, Cham, 2015. p. 113-174.

BENTON, A. Priority species of bamboo. In: LIESE, W., KÖHL, M. (Eds.). **Bamboo**: tropical forestry. vol. 10. Springer, Cham, 2015. p. 31-41.

BHADRAWALE, D.; MISHRA, J. P.; MISHRA, Y. An improvised *in vitro* vegetative propagation technique for *Bambusa tulda*: influence of season, sterilization and hormones. **Journal of Forestry Research**, v. 29, p. 1069-1074, 2018. https://doi.org/10.1007/s11676-017-0569-2

BRONDANI, G. E.; OLIVEIRA, L. S.; BERGONCI, T.; BRONDANI, A. E.; FRANÇA, F. A. M.; SILVA, A. L. L.; GONÇALVES, A. N. Chemical sterilization of culture medium: a low cost alternative to *in vitro* establishment of plants. **Scientia Forestalis**, v. 41, n. 98, p. 257-264, 2013.

BRONDANI, G. E.; OLIVEIRA, L. S.; FURLAN, F. C.; RIBEIRO, A. S. Estabelecimento *in vitro* de *Bambusa vulgaris* Schrad. ex JC Wendl e *Dendrocalamus asper* (Schult. et Schult. F.) Backer ex K. Heyne. In: DRUMOND, P. M.; WIEDMAN, G. (Orgs.). **Bambus no Brasil**: da biologia à tecnologia. Rio de Janeiro, Brasil: ICH, 2017. p. 86-102.

CANAVAN, S.; RICHARDSON, D. M.; VISSER, V.; ROUX, J. J. L.; VORONTSOVA, M. S.; WILSON, J. R. U. The global distribution of bamboos: assessing correlates of introduction and invasion. **AoB PLANTS**, v. 9, n. 1, plw078, 2017. https://doi.org/10.1093/aobpla/plw078

COSTA, F. A.; MARQUES, A. A.; RONDON, J. N.; CEREDA, M. P. Protocolo para micropropagação de duas espécies de *Guadua*. In: DRUMOND, P. M.; WIEDMAN, G. (Orgs.). **Bambus no Brasil**: da biologia à tecnologia. Rio de Janeiro, Brasil: ICH, 2017. p. 71-85.

FERREIRA, E. B.; CAVALCANTI, P. P.; NOGUEIRA, D. A. **ExpDes**: Experimental designs package. R package version 1.1.2, 2013.

FERREIRA, M. E.; GRATTAPAGLIA, D. **Introdução ao uso de marcadores moleculares em análise genética**. 2 ed. Brasília, DF: Embrapa-Cenargen, 1998. 220 p.

FURLAN, F. C.; GAVILAN, N. H.; ZORZ, A. Z.; OLIVEIRA, L. S.; KONZEN, E. R.; BRONDANI, G. E. Active chlorine and charcoal affect the *in vitro* culture of *Bambusa vulgaris*. **Bosque**, v. 39, n. 1, p. 61-70, 2018. http://dx.doi.org/10.4067/S0717-92002018000100061

HARTMANN, H. T.; KESTER, *D. E.*; DAVIES Jr., F. T.; GENEVE, R. L. **Plant propagation**: principles and practices. 8ª ed. São Paulo: Prentice-Hall, 2011. 915 p.

HOSSAIN, M. A.; KUMAR, S. M.; SECA, G.; MAHERAN, A. A.; NOR-AINI, A. S. Mass propagation of *Dendrocalamus asper* by branch cutting. **Journal of Tropical Forest Science**, v. 30, n. 1, p. 82-88, 2018. https://doi.org/10.26525/jtfs2018.30.1.8288

INBAR. International Network for Bamboo and Rattan. **Evaluation of bamboo resources in Latin America** [online], 2015. Available in: <http://www.inbar.int/#1>. Acessed on 15 Jan. 2020.

KARNOVSKY, M. J. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. **Journal of Cell Biology**, v. 27, p. 137-138, 1965.

KONZEN, E. R.; PERÓN, R.; ITO, M. A.; BRONDANI, G. E.; TSAI, S. M. Molecular identification of bamboo general and species based on RAPD-RFLP markers. **Silva Fennica**, v. 51, n. 4, e1691, 2017. https://doi.org/10.14214/sf.1691

KONZEN, E. R.; POZZOBON, L. C.; SOUZA, D. M. S. C.; FERNANDES, S. B.; CAMPOS, W. F.; BRONDANI, G. E.; CARVALHO, D.; TSAI, S. M. Molecular markers in bamboos: understanding reproductive biology, genetic structure, interspecies diversity, and clonal fidelity for conservation and breeding. In: AHMAD, Z.; DING, Y.; SHAHZAD, A. (Orgs.). **Biotechnological advances in bamboo**. 1. ed. vol. 1. Singapore, Springer Singapore, 2021b. p. 33-62. https://doi.org/10.1007/978-981-16-1310-4_2

KONZEN, E. R.; SOUZA, D. M. S. C.; FERNANDES, S. B.; BRONDANI, G. E.; CARVALHO, D.; CAMPOS, W. F. Management of bamboo genetic resources and

clonal production systems. In: AHMAD, Z.; DING, Y.; SHAHZAD, A. (Org.). **Biotechnological advances in bamboo**. 1. ed. vol. 1. Singapore, Springer Singapore, 2021a. p. 207-228. https://doi.org/10.1007/978-981-16-1310-4_9

KUMAR, P.; MISHRA, J. P.; SONKAR, M. K.; MISHRA, Y.; SHIRIN, F. Relationship of season and cuttings' diameter with rooting ability of culm-branch cuttings in *Bambusa tulda* and *Bambusa nutans*. **Journal of Plant Growth Regulation**, v. 41, p. 2491-2498, 2022. https://doi.org/10.1007/s00344-021-10461-9

LARKIN, P. J.; SCOWCROFT, W. R. Somaclonal variation – a novel source of variability from cell cultures for plant improvement. **Theoretical and Applied Genetics**, v. 60, p. 197-214, 1981. https://doi.org/10.1007/BF02342540

LIESE, W.; KOHL, M. **Bamboo**: the plant and its uses. Springer International Publishing, 2015. 356 p.

LIN, S.; LIU, G.; GUO, T.; ZHANG, L.; WANG, S.; DING, Y. Shoot proliferation and callus regeneration from nodular buds of *Drepanostachyum luodianense*. **Journal of Forestry Research**, v. 30, p. 1997-2005, 2019. https://doi.org/10.1007/s11676-018-0772-9

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 of Forestry Research**, v. 32, p. 1587-1598, 2020. https://doi.org/10.1007/s11676-020-01240-5

MONTIEL, M.; SÁNCHEZ, E. Ultraestructura de bambúes del género *Dendrocalamus* (Poaceae: Bambusoideae) cultivados en Costa Rica III: *Dendrocalamus giganteus*. **Revista de Biología Tropical**, v. 54, sup. 2, p. 59-63, 2006a.

MONTIEL, M.; SÁNCHEZ, E. Ultraestructura de bambúes del género *Dendrocalamus* (Poaceae: Bambusoideae) cultivados en Costa Rica IV: *Dendrocalamus asper*, clones Taiwán y Tailandia Mayra. **Revista de Biología Tropical**, v. 54, sup. 2, p. 65-75, 2006b.

MUDOI, K. D.; SAIKIA, S. P.; GOSWAMI, A.; BORA, D.; BORTHAKUR, M. Micropropagation of important bamboos: a review. **African Journal of Biotechnology**, v. 12, n. 20, p. 2770-2785, 2013.

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

MUSTAFA, A. A.; DERISE, M. R.; YONG, W. T. L.; RODRIGUES, K. F. A concise review of *Dendrocalamus asper* and related bamboos: germplasm conservation, propagation and molecular biology. **Plants**, v. 10, n. 9, id 1897, 2021. https://doi.org/10.3390/plants10091897

NEGI, D.; SAXENA, S. *In vitro* propagation of *Bambusa nutans* Wall. ex Munro through axillary shoot proliferation. **Plant Biotechnology Reports**, v. 5, p. 35-43, 2011. https://doi.org/10.1007/s11816-010-0154-z

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. (Orgs.). **Bambus no Brasil**: da biologia à tecnologia. 1 ed. Rio de Janeiro: ICH, 2017. p. 103-129.

NOGUEIRA, J. S.; GOMES, H. T.; SCHERWINSKI-PEREIRA, J. E. Micropropagation, plantlets production estimation and ISSR marker-based genetic fidelity analysis of *Guadua magna* and *G. angustifolia*. **Pesquisa Agropecuária Tropical**, v. 49, e53743, 2019. https://doi.org/10.1590/1983-40632019v4953743.

OLIVEIRA, R. P.; LONGHI-WAGNER, H. M.; LEITE, K. R. B. A contribuição da anatomia foliar para a taxonomia de *Raddia* Bertol. (Poaceae: Bambusoideae). **Acta Botanica Brasilica**, v. 22, n. 1, p. 1-19, 2008. https://doi.org/10.1590/S0102-33062008000100002

ORNELLAS, T. S.; WERNER, D.; HOLDERBAUM, D. F.; SCHERER, R. F.; GUERRA, M. P. Effects of Vitrofural, BAP and meta-Topolin in the *in vitro* culture of *Dendrocalamus asper*. **Acta Horticulturae**, e1155, p. 285-292, 2017. https://doi.org/10.17660/ActaHortic.2017.1155.41

PANDEY, B. N.; SINGH, N. B. Micropropagation of *Dendrocalamus strictus* Nees from mature nodal explants. **Journal of Applied and Natural Science**, v. 4, n. 1, p. 5-9, 2012. https://doi.org/10.31018/jans.v4i1.213

PESCHKE, V. M.; PHILLIPS, R. L. Genetic implications of somaclonal variation in plants. In: SCANDALIOS, J. G.; WRIGHT, T. R. F. (Eds.). **Advances in genetics**. vol. 30. Academic Press, 1992. p. 41-75. https://doi.org/10.1016/S0065-2660(08)60318-1

PNMCB. **Política Nacional de Incentivo ao Manejo Sustentado e ao Cultivo do Bambu**. Lei nº 12.484, de 8 de setembro de 2011. Diário Oficial da União, Brasília, DF, Brazil, 2011.

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

RAMAKRISHNAN, M.; YRJÄLÄ, K.; VINOD, K. K.; SHARMA, A.; CHO, J.; SATHEESH, V.; ZHOU, M. Genetics and genomics of moso bamboo (*Phyllostachys edulis*): current status, future challenges, and biotechnological opportunities toward a sustainable bamboo industry. **Food and Energy Security**, v. 9, n. 4, e229, 2020. https://doi.org/10.1002/fes3.229

RAMANAYAKE, S. M. S. D.; MADDEGODA, K. M. M. N.; VITHARANA, M. C.; CHATURANI, G. D. C. Root induction in three species of bamboo with different rooting abilities. **Scientia Horticulturae**, v. 118, n. 3, p. 270-273, 2008. https://doi.org/10.1016/j.scienta.2008.06.004

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.

RIBEIRO, A. S.; FIGUEIREDO, A. J. R.; TORMEN, G. C. R.; SILVA, A. L. L.; CAMPOS, W. F.; BRONDANI, G. E. Clonal bamboo production based on *in vitro* culture. **Bioscience Journal**, v. 36, n. 4, p. 1261-1273, 2020. https://doi.org/10.14393/BJ-v36n4a2020-48169

ROSA, R. A.; PAES, J. B.; SEGUNDINHO, P. G. A.; VIDAURRE, G. B.; OLIVEIRA, A. K. F. Influência da espécie, tratamento preservativo e adesivo nas propriedades físicas do bambu laminado colado. **Ciência Florestal**, v. 26, n. 3, p. 913-924, 2016. https://doi.org/10.5902/1980509824220

SANDHU, M.; WANI, S. H.; JIMÉNEZ, V. M. *In vitro* propagation of bamboo species through axillary shoot proliferation: a review. **Plant Cell, Tissue and Organ Culture**, v. 132, p. 27-53, 2018. https://doi.org/10.1007/s11240-017-1325-1

SANTOS, E. O.; RODRIGUES, A. A. J.; SILVA, E. R.; CARVALHO, A. C. P. P. BAP concentration and subcultive number in torch ginger multiplication. **Ornamental Horticulture**, v. 22, n. 1, p. 88-93, 2016. https://doi.org/10.14295/oh.v22i1.826

SAWARKAR, A. D.; SHRIMANKAR, D. D.; KUMAR, M.; KUMAR, P.; KUMAR, S.; SINGH, L. Traditional system versus DNA barcoding in identification of bamboo species: a systematic review. **Molecular Biotechnology**, v. 63, p. 651-675, 2021. https://doi.org/10.1007/s12033-021-00337-4

SILVEIRA, A. A. C.; LOPES, F. J. F.; SIBOV, S. T. Micropropagation of *Bambusa oldhamii* Munro in heterotrophic, mixotrophic and photomixotrophic systems. **Plant Cell, Tissue and Organ Culture**, v. 141, p. 315-326, 2020. https://doi.org/10.1007/s11240-020-01788-4

SINGH, S. R.; DALAL, S.; SINGH, R.; DHAWAN, A. K.; KALIA, R. K. Micropropagation of *Dendrocalamus asper* {Schult. e Schult. F.} Backer ex K. Heyne): an exotic edible bamboo. **Journal of Plant Biochemistry and Biotechnology**, v. 21, p. 220-228, 2012. https://doi.org/10.1007/s13562-011-0095-9

SINGH, S. R.; DALAL, S.; SINGH, R.; DHAWAN, A. K.; KALIA, R. K. Evaluation of genetic fidelity of *in vitro* raised plants of *Dendrocalamus asper* (Schult. & Schult. F.) Backer ex K. Heyne using DNA-based markers. **Acta Physiologiae Plantarum**, v. 35, p. 419-430, 2013b. https://doi.org/10.1007/s11738-012-1084-x

SINGH, S. R.; KUMAR, P.; ANSARI, S. A. A simple method for large-scale propagation of *Dendrocalamus asper*. **Scientia Horticulturae**, v. 100, n. 1-4, p. 251-255, 2004. https://doi.org/10.1016/j.scienta.2003.08.006

SINGH, S. R.; SINGH, R.; KALIA, S.; DALAL, S.; DHAWAN, A. K.; KALIA, R. K. Limitations, progress and prospects of application of biotechnological tools in improvement of bamboo – a plant with extraordinary qualities. **Physiology and Molecular Biology of Plants**, v. 19, p. 21-41, 2013a. https://doi.org/10.1007/s12298-012-0147-1

TAMBARUSSI, E. V.; ROGALSKI, M.; GALEANO, E.; BRONDANI, G. E.; MARTIN, V. F.; SILVA, L. A.; CARRER, H. Efficient and new method for *Tectona grandis in vitro* regeneration. **Crop Breeding and Applied Biotechnology**, v. 17, n. 2, p. 124-132, 2017. https://doi.org/10.1590/1984-70332017v17n2a19

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, e-102979, 2021. https://www.doi.org/10.1590/01047760202127012979

TORRES, G. R. C.; HOULLOU, L. M.; SOUZA, R. A. Control of contaminants during introduction and establishment of *Bambusa vulgaris in vitro*. **Research in Biotechnology**, v. 7, p. 58-67, 2016. https://doi.org/10.19071/rib.2016.v7.3056

VENKATACHALAM, L.; SREEDHAR, R. V.; BHAGYALAKSHMI, N. Micropropagation in banana using high levels of cytokinins does not involve any genetic changes as revealed by RAPD and ISSR markers. **Plant Growth Regulation**, v. 51, p. 193-205, 2007. https://doi.org/10.1007/s10725-006-9154-y

WEI, Q.; CAO, J.; QIAN, W.; XU, M.; LI, Z.; DING, Y. Establishment of an efficient micropropagation and callus regeneration system from the axillary buds of *Bambusa ventricosa*. **Plant Cell, Tissue and Organ Culture**, v. 122, p. 1-8, 2015. https://doi.org/10.1007/s11240-015-0743-1

WENDLING, I.; TRUEMAN, S. J.; XAVIER, A. Maturation and related aspects in clonal forestry - part II: reinvigoration, rejuvenation and juvenility maintenance. **New Forests**, v. 45, p. 473-486, 2014. https://doi.org/10.1007/s11056-014-9415-y

ZHAO, H. et al. Announcing the genome atlas of bamboo and rattan (GABR) project: promoting research in evolution and in economically and ecologically beneficial plants. **GigaScience**, v. 6, n. 7, p. 1-7, 2017. https://doi.org/10.1093/gigascience/gix046

Acknowledgements

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").

Author Contributions

D.S.G. - conceptualization, methodology, investigation or data collection, statistical analysis and writing; D.M.S.C.S., L.V.M., M.L.M.A. - investigation or data collection, writing. D.C., G.L.T. validation, review; G.E.B. - conceptualization, acquisition of financing, methodology, supervision, validation, review. All authors read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable

Data Availability Statement

Study data can be obtained by request to the corresponding author, via e-mail.

Conflicts of Interest

The authors declare no conflict of interest. Supporting entities had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.