

Innovations in micropropagation of the orchid *Cattleya nobilior*: the effect of bioreactor and enriched culture media

Geisianny Pereira Nunes¹  José Carlos Sorgato^{1*}  Jéssica Celeste Mônico Ramos¹ 
Jackeline Schultz Soares¹  Rodrigo Kelson Silva Rezende¹  Rayner Bueno Peinado¹ 

¹Faculdade de Ciência Agrárias (FCA), Universidade Federal da Grande Dourados (UFGD), 79804-970, Dourados, MS, Brasil. E-mail: josesorgato@ufgd.edu.br. *Corresponding author.

ABSTRACT: *Cattleya nobilior* Rchb.f. is an epiphytic species of ornamental value, facing risks due to environmental threats, indiscriminate collection, and habitat loss. In this context, *in vitro* cultivation presents a viable alternative for its commercial production and conservation efforts. This pioneering study evaluated the initial *in vitro* growth and *ex vitro* establishment of *C. nobilior*, using supplemented culture media and comparing conventional micropropagation with temporary immersion bioreactors. The experimental design was completely randomized, with treatments in a 4x2 factorial scheme, involving four culture media and two micropropagation systems. After 180 days of *in vitro* cultivation, the plants were assessed for the number of leaves, pseudobulbs, and roots, length of the largest leaf and root, plant height, pseudobulb diameter, and total fresh mass. Subsequently, they were transplanted into containers with mixed substrate and transferred to an *ex vitro* environment. After another 180 days, survival was recorded, and the same characteristics initially evaluated were measured again to calculate their increments. The results showed that, in *in vitro* cultivation, plants grown by the conventional method exhibited tillering, resulting in an increase in the number of leaves, shoots, and roots. However, those cultivated in a bioreactor showed an increase in leaf and root length, pseudobulb diameter, and plant height, especially when grown in medium enriched with banana pulp. During the *ex vitro* phase, plants originating from bioreactors continued to show the highest results, especially those cultivated in medium with the addition of banana. This study is the first to investigate the use of temporary immersion bioreactors in the micropropagation of *C. nobilior*, demonstrating that the combination of bioreactor and culture media supplemented with banana pulp provides optimal conditions for the robust growth of plants, both *in vitro* and *ex vitro*, offering new perspectives for the commercial production and conservation of the species.

Key words: Orchidaceae, culture medium supplementation, *ex vitro* establishment, conservation, native species.

Inovações na micropropagação da orquídea *Cattleya nobilior*: efeito de biorreator e meios de cultura enriquecidos

RESUMO: *Cattleya nobilior* Rchb.f. é uma espécie epífita de valor ornamental, enfrenta riscos devido a ameaças ambientais, coleta indiscriminada e perda de habitat. Nesse contexto, o cultivo *in vitro* é uma alternativa viável para sua produção comercial e esforços de conservação. Este estudo pioneiro avaliou o crescimento inicial *in vitro* e o estabelecimento *ex vitro* de *C. nobilior*, utilizando meios de cultura suplementados e comparando a micropropagação convencional com biorreatores de imersão temporária. O delineamento experimental adotado foi inteiramente casualizado, com tratamentos em esquema fatorial 4x2, envolvendo quatro meios de cultivo e dois sistemas de micropropagação. Após 180 dias de cultivo *in vitro*, as plantas foram avaliadas quanto ao número de folhas, pseudobulbos e raízes, comprimento da maior folha e raiz, altura de planta, diâmetro de pseudobulbo e massa fresca total. Em seguida, foram transplantadas para recipientes contendo substrato misto e transferidas para ambiente *ex vitro*. Após mais 180 dias, a sobrevivência foi registrada, e as mesmas características avaliadas inicialmente foram novamente mensuradas para calcular seus incrementos. Os resultados mostraram que, no cultivo *in vitro*, as plantas cultivadas pelo método convencional, apresentaram perfilhamento, resultando em um aumento no número de folhas, brotos e raízes. Contudo, aquelas cultivadas em biorreator apresentaram um incremento em comprimento de folha e raiz, diâmetro de pseudobulbo e altura de planta, principalmente quando cultivadas em meio enriquecido com polpa de banana. Durante a fase *ex vitro*, as plantas originárias de biorreatores continuaram apresentando os maiores resultados, principalmente aquelas cultivadas em meio com adição de banana. Este estudo é o primeiro a investigar o uso de biorreatores de imersão temporária na micropropagação *C. nobilior*, demonstrando que a combinação de biorreator e meio de cultura suplementado com polpa de banana proporciona condições ótimas para o maior crescimento robusto das plantas, tanto *in vitro* quanto *ex vitro*, oferecendo novas perspectivas para a produção comercial e conservação da espécie.

Palavras-chave: Orchidaceae, suplementação de meio de cultura, estabelecimento *ex vitro*, conservação, espécie nativa.

INTRODUCTION

The Orchidaceae family is recognized as the largest and most diverse among the Angiosperms. In Brazil, approximately 2,679 orchid species are

recorded, of which 1,487 are endemic. The genus *Cattleya*, one of the most commercialized in the country, has about 48 species distributed throughout tropical America. This region is characterized by habitats that provide abundant light and high relative

humidity, essential conditions for the development of these plants (FLORA & FUNGA OF BRAZIL, 2024; JUNQUEIRA & PEETZ, 2017).

Among the species of the genus, the native orchid *Cattleya nobilior* Rchb.f. stands out for its economic importance due to its ornamental characteristics, such as large, exuberant flowers with pink-purple coloration (ROMAHN, 2020; FLORA & FUNGA OF BRAZIL, 2024). These qualities, combined with the constant threats to its natural habitat and indiscriminate collection, place this species in a vulnerable position, making *in vitro* cultivation a promising strategy for both commercial seedling production and conservation efforts (STEFANO et al., 2022; VENDRAME et al., 2023).

In this scenario, the exploration of innovative and efficient cultivation systems becomes fundamental to overcome the limitations of conventional cultivation, especially regarding commercial viability. Cultivation techniques that use temporary immersion bioreactors are indicated as a viable alternative, standing out for their ability to optimize the control of essential physical parameters for plant development, in addition to allowing temporary and efficient contact of the plants with the cultivation medium, thus facilitating the uniform absorption of essential nutrients. Temporary immersion bioreactors have been used to propagate some orchid genera, such as *Cattleya*, *Brassavola*, *Vanilla*, *Bletilla*, *Dendrobium* and *Phalaenopsis*, using different media, growth regulators, and explants (VENDRAME et al., 2023; NONGDAM et al., 2023).

The composition of the cultivation medium, enriched with organic supplements such as coconut water and banana pulp, plays a fundamental role, acting as a rich source of vitamins, amino acids, fatty acids, carbohydrates, peptides, and growth factors. The addition of these compounds, whether in isolation or in combination, can enhance the germination of seeds and the development of seedlings, varying according to the species and genotype in question. (UTAMI & HARIYANTO, 2020; STEFANO et al., 2022).

Additionally, the acclimatization phase represents a challenge for the success of this type of propagation, requiring careful adaptation of the plants to a new environment (*ex vitro*). This process is fundamental to ensure the survival of micropropagated plants when they are transferred to heterotrophic conditions, highlighting the importance of well-developed multiplication protocols (STEFANO et al., 2022; SOARES et al., 2023).

Considering that there are no previous studies using temporary immersion bioreactors in

the *in vitro* cultivation of the *C. nobilior* species (NONGDAM et al., 2023), this pioneering research investigated the use of this technology in the propagation of this species. Thus, with the objective of investigating the hypothesis that the use of bioreactors and the supplementation of the medium with banana pulp and coconut water have a positive influence both in the *in vitro* cultivation and in the acclimatization of the studied species, the present study evaluated the *in vitro* growth and *ex vitro* establishment of *C. nobilior* using different supplemented culture media and comparing them with conventional micropropagation systems and the use of temporary immersion bioreactor. This research contributed to the advancement of scientific knowledge but also provides valuable information for the sustainable production and conservation of threatened orchid species.

MATERIALS AND METHODS

The study material consisted of ripe fruits of *Cattleya nobilior* Rchb.f., obtained from manual pollination of matrices over fifteen years old, grown in a greenhouse screen under two overlapping layers of 50% shade cloth, providing an irradiance of $235 \mu\text{mol m}^{-2} \text{s}^{-1}$. The average conditions of temperature and relative humidity were maintained at $22.6 \pm 5^\circ\text{C}$ and $73.9 \pm 10\%$, respectively, with irrigation performed by ballerina-type micro sprinklers located one meter above the plants, resulting in a water depth of 1 mm day^{-1} .

A sample of 0.005 g of seeds was weighed and subjected to the tetrazolium test, following the methodology of RIBEIRO et al. (2021), to confirm their viability. After confirmation, another sample of 0.005 g of seeds was taken to an aseptic environment and disinfected according to the methodology of SOARES et al. (2020), aiming to obtain the seed solution. Each culture flask was inoculated with 1 mL of this disinfected seed suspension for *in vitro* sowing. A total of 60 mL of Murashige & Skoog medium with half salt concentration ($\text{MS } \frac{1}{2}$) was used per flask with a capacity of 600 mL. Subsequently, the cultures were placed in a growth room with controlled temperature and photoperiod ($25 \pm 2^\circ\text{C}$; 16 h) and irradiance of $22 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided by two white fluorescent lamps ($18.9 \mu\text{mol m}^{-2} \text{s}^{-1}$). The cultures remained under these conditions for 180 days, with one subculture performed during this period.

At the beginning of the experimental period, the plants, 180 days after *in vitro* sowing, were standardized in size ($1.5 \pm 0.3 \text{ cm}$) and transferred,

in an aseptic environment, to the following culture media: 1- MS; 2- MS + apple banana pulp (BP) (100 g L⁻¹ of apple banana pulp); 3- MS + coconut water (CW) (100 mL L⁻¹); 4- MS + BP (100 g L⁻¹) + CW (100 mL L⁻¹). Subsequently, they were allocated to two micropropagation systems: 1) conventional system (CS); 2) temporary immersion system (TIS).

In the conventional cultivation, 40 mL of the medium solidified with 6.0 g L⁻¹ of bacteriological agar (Himedia®, India) and 30 g L⁻¹ of sucrose were added to each flask. For the bioreactor, 200 mL of the same medium was used, but without a gelling agent, and the bioreactor was programmed with aeration cycles of 3 minutes every 5 hours and 57 minutes. The pH of all media was adjusted to 5.8 before autoclaving, carried out at 121 °C and a pressure of 1.1 atm for 20 minutes. The inoculated material (25 plants per 200 mL of medium in each micropropagation system) was maintained under controlled conditions of photoperiod (16 hours), temperature (25±2 °C), and irradiance (18.9 μmol m⁻² s⁻¹), under LED lighting composed of 50% white (6500K), 25% blue, and 25% red, for 180 days.

After this period, the plants were removed from the flasks, washed with water until the complete removal of the culture medium, and evaluated for the number of leaves (NL), number of roots (NR), number of shoots (NS), greatest leaf length (LL) (mm), greatest root length (RL) (mm), plant height (PHE) (mm), pseudobulb diameter (PD) (mm), and fresh mass (FM) (g). After the evaluations, the treatments were photographed using a camera attached to a mini photo studio.

Subsequently, the plants were transferred to transparent polypropylene containers (20 x 10 x 5 cm) with a capacity of 1000 mL with holes in the base and the lid, for substrate drainage and gas exchanges, respectively. The containers were filled with a substrate composed of 1/3 of its volume of pink sphagnum (Agrolink, Holambra - SP) and coconut fiber (Golden-Mix Chips, Amafibra) in a 1:2 (v:v) ratio. After planting, the containers were placed in a shaded greenhouse, under the same conditions as the parent plants. These containers remained covered during the first 15 days of acclimatization (pre-acclimatization) to minimize the initial effects of the transfer from the *in vitro* to the *ex vitro* environment, and then they were opened.

After 180 days of acclimatization, the plants were evaluated for survival and the same initial characteristics (NL, NR, NS, LL, RL, PHE, PD, and FM). To investigate the hypothesis of increased plant growth during the *ex vitro* phase, according to the

treatments to which they were exposed in the *in vitro* phase, their increments (I) in relation to the initial values were calculated, using the expression proposed by RIBEIRO et al. (2019), $I = (VF - VI)$, where VI is the value of the variable before acclimatization and VF is the value after the *ex vitro* period. The values were expressed in percentage and submitted to analysis of variance.

The experimental design was completely randomized, and the treatments were arranged in a 4 x 2 factorial scheme (four culture media and two micropropagation systems), with five replicates of five plants each. The results were transformed to $\sqrt{x+1}$ and subsequently subjected to analysis of variance. Differences between the micropropagation systems were verified by the Bonferroni t-test, while the Tukey test was used at a 5% probability level to check differences between the culture media, using the SISVAR software (Statistical Analysis Program v.5.3, Universidade Federal de Lavras (UFLA), MG).

RESULTS

In vitro growth

The analysis of variance showed a significant effect ($P < 0.05$) of the interaction between the culture media and the micropropagation systems on all evaluated variables in *Cattleya nobilior*. For the variables leaf number (NL), root number (NR), and shoot number (NS), the system x medium (S x M) interaction indicated that the highest values were observed in the conventional cultivation system (CS) combined with MS medium (52.40; 26.40; 11.80, respectively) (Table 1).

For the length of the longest leaf (LL), the highest averages were observed when the plants were grown in the temporary immersion system (TIS) with the MS + BP medium, reaching 36.22 mm. Similarly, the highest values for the length of the longest root (RL) were obtained in plants grown in a bioreactor with MS medium, with no significant statistical differences compared to the MS + BP medium (65.30; 57.55 mm, respectively) (Table 1).

C. nobilior showed the highest plant height values (PHE) when grown in MS + BP medium, although it was not statistically different from those grown in the original MS medium formulation (105.67; 94.27 mm, respectively) and the largest pseudobulb diameter (PD) was observed in MS + BP medium (2.44 mm), both using TIS. Conversely, the highest fresh mass values (1.78 g) were achieved when the CS combined with MS medium was used (Table 1).

Table 1 - Number of leaves (NL), number of roots (NR), and number of shoots (NS), longest leaf length (LL), longest root length (RL), plant height (PHE), pseudobulb diameter (PD), and fresh mass (FM) of *Cattleya nobilior* Rchb.f. according to the culture media [MS; MS + apple banana pulp (BP); MS + coconut water (CW); MS + BP + CW] and micropropagation system (CS = conventional system; TIS = temporary immersion system) 180 days after *in vitro* cultivation.

Culture Media	-----NL-----		-----NR-----		-----NS-----		-----LL (mm)-----	
	CS	TIS	CS	TIS	CS	TIS	CS	TIS
MS	52.40 Aa	11.40 Ba	26.40 Aa	6.40 Ba	11.80 Aa	2.40 Ba	13.04 Ba	20.62 Ab
MS + BP	7.60 Ab	7.60 Aa	7.00 Ab	9.40 Aa	2.00 Ab	2.00 Aa	15.65 Ba	36.22 Aa
MS + CW	18.20 Ab	9.00 Aa	8.80 Ab	11.80 Aa	3.40 Ab	2.40 Aa	11.56 Ba	21.12 Ab
MS + BP + CW	8.50 Ab	5.16 Aa	7.50 Ab	6.33 Aa	2.00 Ab	1.33 Aa	18.84 Aa	13.34 Ab
Average	21.67	8.29	12.42	8.48	4.80	2.03	14.77	22.82
CV (%)	-----22.47-----		-----19.74-----		-----19.79-----		-----18.48-----	
Culture Media	-----RL (mm)-----		-----PHE (mm)-----		-----PD (mm)-----		-----FM (g)-----	
	CS	TIS	CS	TIS	CS	TIS	CS	TIS
MS	30.95 Bb	65.30 Aa	52.60 Ba	94.27 Aab	1.97 Aa	1.39 Ab	1.78 Aa	0.73 Bb
MS + BP	58.84 Aa	57.55 Aa	79.64 Ba	105.67 Aa	1.72 Ba	2.44 Aa	1.06 Bb	1.63 Aa
MS + CW	39.95 Ab	39.49 Ab	61.30 Aa	67.82 Abc	2.11 Aa	1.46 Bb	0.73 Ab	0.80 Ab
MS + BP + CW	35.50 Ab	25.88 Ab	65.57 Aa	44.36 Ac	2.01 Aa	1.34 Bb	0.77 Ab	0.49 Ab
Average	41.31	47.05	64.77	78.03	1.95	1.65	1.08	0.91
CV (%)	-----18.23-----		-----19.02-----		-----9.92-----		-----6.40-----	

Means followed by the same uppercase letter in the row do not statistically differ from each other by the Bonferroni t-test ($P \geq 0.05$), and means followed by the same lowercase letter in the column do not statistically differ from each other by the Tukey test ($P \geq 0.05$) within the variables.

Figure 1 illustrates the morphological variation of the plants as a function of the culture media and micropropagation systems. Notably, *C. nobilior* plants grown in CS showed tillering, resulting in an increase in the number of leaves, shoots, and roots, to the detriment of growth. However, it was visually observed that, in the TIS system, the plant organs increased in size, diameter, and length, especially when grown in MS+BP medium.

Ex vitro growth

After 180 days of acclimatization, the analysis of variance showed a significant effect ($P < 0.05$) of the interaction between the culture media and the micropropagation systems on all evaluated characteristics in the *ex vitro* cultivation of *C. nobilior*. At the end of the experimental period, the highest survival rate (%SURV) was observed in plants originating from *in vitro* cultivation in TIS with the MS + BP and MS + CW media, both achieving an average of 100% live plants (Figure 2).

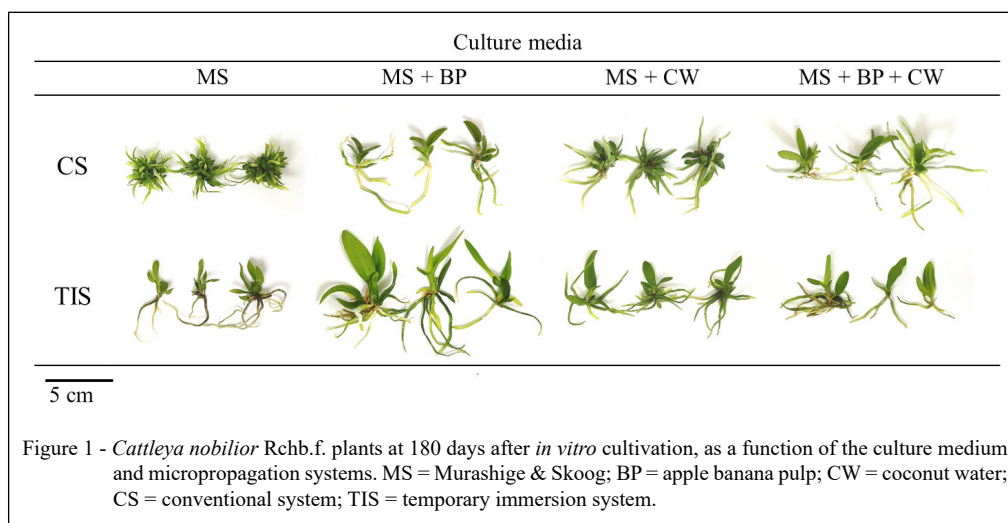
Regarding the increases in NL and NR, the S x M interaction showed that the greatest increases were observed in plants from *in vitro* cultivation in MS + CW (27.40%) and MS (26.60%), both using TIS. For the increase in the number of shoots (NB),

the greatest increase was observed in plants cultivated in the conventional system combined with MS + BP medium (19.80%) (Table 2).

In terms of the increase in LL, the greatest increase was observed in plants from *in vitro* cultivation in CS using MS medium in its original formulation (33.96%), and for the increase in RL (Length of the Longest Root), in plants obtained through cultivation in TIS combined with MS + BP medium (9.75%) (Table 2).

Additionally, in the S x M interaction, the greatest increase in PHE (8.92%) was observed in plants cultivated in TIS using MS + BP medium. For the other variables, the greatest increase occurred in plants from *in vitro* cultivation in CS combined with MS + BP medium for the PD variable and MS + BP + CW for FM (Fresh Mass) (55.18; 26.57%, respectively) (Table 2).

Figure 3 shows that the *in vitro* cultivation conditions had a significant influence on plant growth during the *ex vitro* period. In general, as visually observed, plants cultivated in TIS presented the best results, especially in MS + BP medium. This cultivation system, associated with the culture medium supplemented with banana pulp, provided conditions for robust growth of *C. nobilior* both *in vitro* (Figure 1) and *ex vitro* (Figure 3).



DISCUSSION

In vitro growth

Plants grown in a conventional cultivation system, using hermetically sealed flasks and MS medium, showed higher values of NL, NR, and NS. This fact can be attributed to physiological disturbances and changes in tissue development, as the conventional cultivation system prevents gas exchanges between the internal microenvironment of the flask and the external environment. Such restriction results in reduced CO₂ concentrations and

accumulation of ethylene (MIRANDA et al., 2020; FRITSCHÉ et al., 2022), conditions that can induce tillering in plants grown under these circumstances.

Conversely, *C. nobilior* plants grown in TIS showed increases in leaf length and root system, as well as greater height and pseudobulb diameter. Temporary immersion bioreactors offer a promising alternative, combining the benefits of liquid media with low operational costs, enhancing plant growth (EKMEKÇİGİL et al., 2019; NONGDAM et al., 2023). Temporary immersion provides greater contact of the plants with the culture medium,

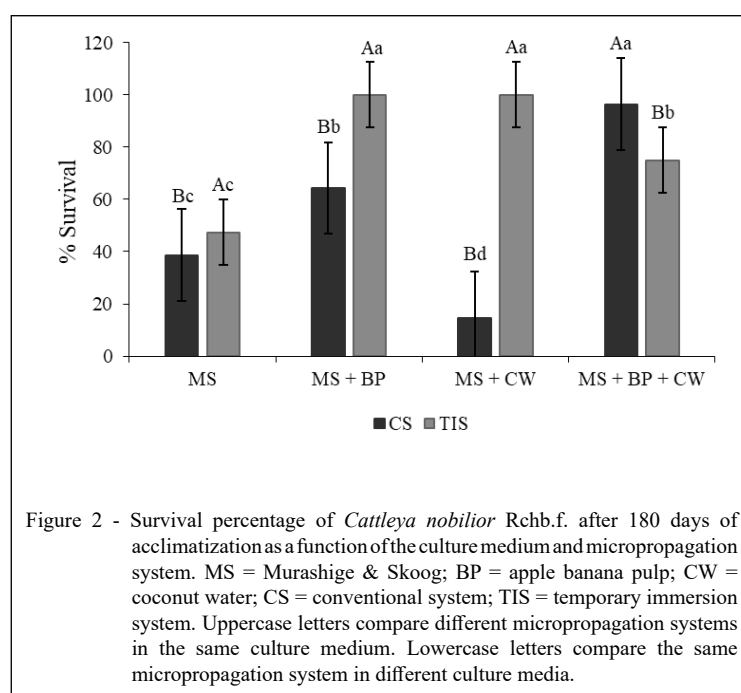


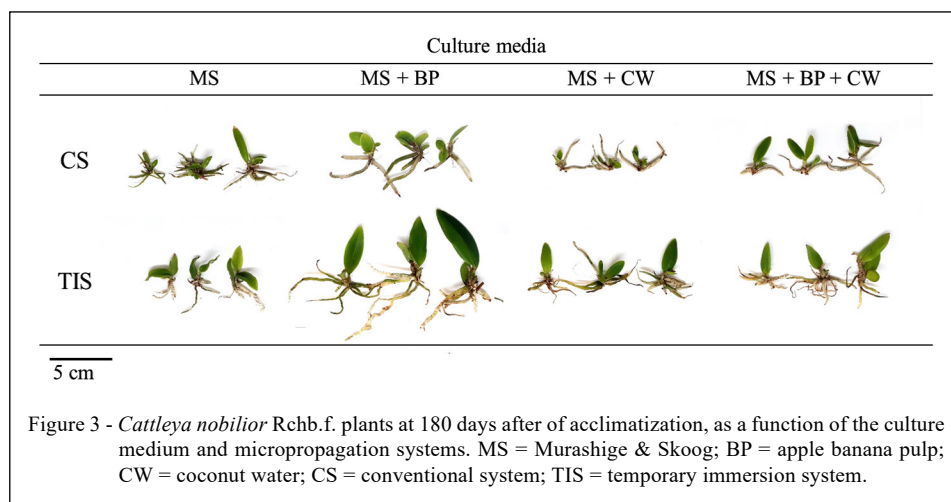
Table 2 - Number of leaves (NL), number of roots (NR), and number of shoots (NS), longest leaf length (LL), longest root length (RL), plant height (PHE) (mm), pseudobulb diameter (PD) (mm), and fresh mass (FM) (g) of *Cattleya nobilior* Rchb.f. according to the culture media [MS; MS + apple banana pulp (BP); MS + coconut water (CW); MS + BP + CW] and micropropagation system (CS = conventional system; TIS = temporary immersion system) 180 days after of acclimatization.

Culture media	-----NL-----		-----NR-----		-----NS-----		-----LL-----	
	CS	TIS	CS	TIS	CS	TIS	CS	TIS
MS	0.00 Aa	0.00 Ab	12.40 Ba	26.60 Aa	0.00 Ab	0.00 Ab	33.96 Aa	15.78 Bb
MS + BP	0.00 Aa	0.00 Ab	0.00 Bb	4.40 Ab	19.80 Aa	0.00 Bb	22.49 Ab	7.90 Bc
MS + CW	0.00 Ba	27.40 Aa	0.00 Ab	0.00 Ab	0.00 Bb	19.60 Aa	14.31 Ac	11.10 Abc
MS + BP + CW	0.00 Aa	0.00 Ab	8.80 Aa	0.00 Bb	0.00 Ab	0.00 Ab	14.21 Bc	28.52 Aa
Average	0.00	6.85	5.30	7.75	4.95	4.90	21.24	15.82
CV (%)	-----12.58-----		-----14.00-----		-----13.38-----		-----7.41-----	
Culture media	-----LR-----		-----PHE (mm)-----		-----PD (mm)-----		-----FM (g)-----	
	CS	TIS	CS	TIS	CS	TIS	CS	TIS
MS	0.32 Aa	0.00 Ab	4.00 Aa	0.00 Bb	0.00 Bc	18.72 Aab	3.53 Ac	1.61 Ac
MS + BP	0.00 Ba	9.75 Aa	3.70 Bab	8.92 Aa	55.18 Aa	13.37 Bc	12.41 Ab	14.30 Aa
MS + CW	0.00 Aa	0.00 Ab	0.00 Ac	0.00 Ab	0.00 Bc	20.19 Aa	0.00 Bc	12.51 Aab
MS + BP + CW	0.00 Aa	0.00 Ab	1.96 Ab	0.00 Bb	6.71 Bb	15.26 Abc	26.57 Aa	8.60 Bb
Average	0.08	2.43	2.41	2.23	15.47	16.89	10.62	9.26
CV (%)	-----7.42-----		-----13.49-----		-----6.99-----		-----11.42-----	

Means followed by the same uppercase letter in the row do not statistically differ from each other by the Bonferroni t-test ($P \geq 0.05$), and means followed by the same lowercase letter in the column do not statistically differ from each other by the Tukey test ($P \geq 0.05$) within the variables.

favoring nutrient absorption and; consequently, optimized plant development (NONGDAM et al., 2023). This could be one of the explanations for the observed results in the increase in leaf and root length. Previous studies support these findings, reporting success in the cultivation of various orchid species using TIS, including *Cattleya walkeriana* Gardner (MOREIRA et al., 2013), *Brassavola nodosa* Lindl. (VENDRAME et al., 2023), *Vanilla planifolia* Jacks. Ex Andrews (SPINOSO-CASTILLO et al., 2017;

RAMÍREZ-MOSQUEDA et al., 2019; RAMÍREZ-MOSQUEDA & BELLO-BELLO, 2021), *Cattleya tigrina* (FRITSCHÉ et al., 2022), *Cymbidium sinense* (GAO et al., 2014), *Dendrobium candidum* Lindl. (CUI et al., 2014), *Dendrobium nobile* Lindl. (ZHANG et al., 2019), *Epidendrum fungens* Brongn. (FRITSCHÉ et al., 2022), *Mokara leuen* Berger (MINH, 2022), *Paphiopedilum rothschildianum* Rchb.f. (MASNODDIN et al., 2018), *Paphiopedilum rothschildianum* Rchb.f. (MASNODDIN et al.,



2016), *Phalaenopsis* (SHEN & SHU, 2018), and *Vanda tricolor* Lindl. (ESYANTI et al., 2016), as cited by NONGDAM et al. (2023) in their review.

TIS is considered one of the most effective methods for *in vitro* micropropagation of plants on a commercial scale, offering advantages such as the reduction of subculture time and operational costs, which can be significantly lower, surpassing in some cases 40% of traditional costs. Moreover, it is crucial that the microenvironment provided by TIS is specifically idealized for the growth and development of plant tissues and organs, as these are specific requirements for large-scale plant micropropagation (NONGDAM et al., 2023).

The addition of organic supplements, such as banana pulp, to the culture media has proven beneficial for the *in vitro* growth of various orchid species, like *Dendrobium nobile* Lindl. (SU et al., 2012), *Laeliocattleya* (GONÇALVES et al., 2016; NONGDAM et al., 2023) and *C. nobilior* Rchb. f. (FREITAS et al., 2021). Compounds from the banana enrich the medium, providing essential vitamins, amino acids, and growth regulators, which are readily absorbed by the *in vitro* plants (DOLCE et al., 2020; STEFANO et al., 2022). This nutritional enrichment is reflected in accelerated growth and increased plant size, highly desirable characteristics for *ex vitro* cultivation, especially when the medium is supplemented with banana pulp, as evidenced in figure 3 and corroborated by FREITAS et al. (2021).

Ex vitro growth

Overall, the *ex vitro* establishment of *C. nobilior* revealed that the prior use of TIS with MS media enriched with banana pulp or coconut water provided a higher percentage of plant survival. Notably, the addition of BP not only favored survival but also promoted significant increases in plant size, both in height and root length, highly desirable characteristics during the acclimatization phase. The use of MS + BP medium combined with TIS resulted in good plant development, leading to better performance in *ex vitro* cultivation.

This fact can be attributed to the accumulation of carbohydrates in the culture medium, resulting from the supplementation with banana pulp. These carbohydrates serve as an energy source for the micropropagated seedlings, playing a fundamental role both in *in vitro* growth and development and in the acclimatization process (BADR et al., 2015). Banana pulp, a widely used organic additive in the *in vitro* propagation of orchids, acts as a rich

source of potassium and a rooting stimulator. This is due to the presence of mineral salts and natural growth regulators, such as auxins, cytokinins, and gibberellins, which are essential for plant growth and development (DOLCE et al., 2020; MACHADO & ZAMARIAN, 2020; STEFANO et al., 2022).

Furthermore, the increase in plant growth rate during the acclimatization phase can be associated with the *in vitro* cultivation system employed. TIS facilitates and allows gas exchanges, leading to a decrease in relative humidity and an increase in transpiration and absorption of water and nutrients by the seedlings. This system also promoted the functionality of the stomata and reduces the accumulation of ethylene and CO₂, which favors plant growth in *in vitro* cultivation and provides subsequent hardening when transferred to the *ex vitro* phase (VENDRAME et al., 2023; NONGDAM et al., 2023).

Thus, during the multiplication phase using bioreactors, as stated by VENDRAME et al. (2023), root development is fundamental for the success of the establishment and survival of the seedlings. These authors reported a 93% survival rate when using TIS in the propagation of the species *B. nodosa*. Moreover, it is crucial to adapt the immersion and aeration parameters in TIS, as the frequency and duration of these processes directly influence *in vitro* multiplication and plant development according to the species (VENDRAME et al., 2023; NONGDAM et al., 2023). Thus, this fact highlighted the influence of genotype on *in vitro* responses, indicating the need to evaluate and adjust such parameters according to the specific characteristics of each species.

Therefore, the results of this study confirmed that the combination of TIS with the supplementation of banana pulp in the culture medium provided favorable conditions for the development of *C. nobilior* plants, both *in vitro* and *ex vitro* cultivation, as illustrated in figures 1 and 3.

CONCLUSION

This study is pioneering in the use of the temporary immersion bioreactor system for the micropropagation of the native orchid *Cattleya nobilior* Rchb.f. To promote *in vitro* growth and *ex vitro* establishment of this species, we recommend *in vitro* cultivation using the temporary immersion system with MS medium supplemented with 100 g L⁻¹ of apple banana pulp. This approach is recommended for both commercial and conservationist applications.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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