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Original Article

Cattleya walkeriana Gardner (Orchidaceae) propagation: culture medium, sealing system and irradiance

Propagação de *Cattleya walkeriana* Gardner (Orchidaceae): meio de cultura, sistema de vedação e irradiância

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Abstract

This study aimed to evaluate the influence of culture media, irradiance, and sealing system on the *in vitro* and *ex vitro* growth of *Cattleya walkeriana* Gardner. We used MS medium as culture medium, supplemented with 30 g L^{-1} of sucrose and solidified with 7.0 g L^{-1} of bacteriological agar. This medium served as a control, while for the other treatments we supplemented the media as follows: 2) MS with 150 g L^{-1} of banana pulp = P150; 3) MS with 300 g L^{-1} of banana pulp = P300; 4) MS with 150 g L^{-1} of banana peel = PE150; and 5) MS with 300 g L^{-1} of banana peel = PE300. The irradiances were provided by 3000K LED lamps: 86 µmol m^{-2} s^{-1} (Irradiance-1) and 128 µmol m^{-2} s^{-1} (Irradiance-2) and the conventional sealing (CSS) and sealing systems that allow gas exchange (GESS). After 120 (*in vitro*) and 180 days (*ex vitro*) of cultivation, we evaluated them for pseudobulb (PN), leaf (LN) and root number (RN), plant height (PH), pseudobulb diameter (PD), longest leaf (LL) and root length (RL), fresh mass (TFM) and survival (%SURV). There was a significant interaction for all the variables analyzed. The CM x SS double interaction was significant for PH, LL, and RL. The CM x I x SS interaction was significant for PN, LN, RN, PD, TFM, and %SURV traits of *C. walkeriana* grown *in vitro*. There was a significant interaction between CM x I x SS for all *C. walkeriana* traits evaluated in *ex vitro* culture. Using the medium with up to 150 g L^{-1} of banana pulp combined with Irradiance-2 and CSS provided the highest values for *in vitro* plant growth. However, prior cultivation in MS medium, Irradiance-1, and CSS provided the greatest survival and establishment of this species plants in *ex vitro* culture.

Keywords: Orchidaceae, native species, *in vitro*, *ex vitro*, banana pulp.

Resumo

Este estudo teve como objetivo avaliar a influência do meio de cultura, irradiância e sistema de vedação no crescimento *in vitro* e *ex vitro* de *Cattleya walkeriana* Gardner. Utilizamos como meio de cultura o meio MS, suplementado com 30 g L^{-1} de sacarose e solidificado com 7,0 g L^{-1} de ágar bacteriológico. Este meio serviu de controle, enquanto nos demais tratamentos o meio foi complementado da seguinte forma: 2) MS com 150 g L^{-1} de polpa de banana = P150; 3) MS com 300 g L^{-1} de polpa de banana = P300; 4) MS com 150 g L^{-1} de casca de banana = PE150; e 5) MS com 300 g L^{-1} de casca de banana = PE300. As irradiâncias foram fornecidas por lâmpadas LED de 3000K: 86 µmol m^{-2} s^{1} (Irradiância-1) e 128 µmol m^{-2} s^{-1} (Irradiância-2) e a vedação convencional (CSS) e sistema de vedação que permite troca gasosa (GESS). Aos 120 *(in vitro)* e 180 dias (*ex vitro*) de cultivo, avaliamos o número de pseudobulbos (PN), de folhas (LN) e raízes (RN), altura da planta (PH), diâmetro do pseudobulbo (PD), comprimento da maior folha (LL) e raiz (RL), massa fresca (TFM) e sobrevivência (%SURV). Houve interação significativa para todas as características analisadas. A dupla interação CM x SS foi significativa para PH, LL e RL. A interação CM x I x SS foi significativa para as características PN, LN, RN, PD, TFM e %SURV de *C. walkeriana* cultivada *in vitro*. Houve interação significativa entre CM x I x SS para todas as características de *C. walkeriana* avaliadas em cultivo *ex vitro*. A utilização do meio com até 150 g L^{-1} de polpa de banana combinada com Irradiância-2 e CSS proporcionou os maiores valores para o crescimento *in vitro* das plantas. No entanto, o cultivo prévio em meio MS, Irradiância-1 e CSS proporcionou maior sobrevivência e estabelecimento de plantas dessa espécie em cultivo *ex vitro*.

Palavras-chave: Orchidaceae, espécie nativa, *in vitro*, *ex vitro*, polpa de banana.

1. Introduction

The Orchidaceae family is the largest and most diverse family within the angiosperms. It covers a variety of species, highlighted both by their ornamental potential and their significant nutritional and pharmacological value (IUCN, 2022; Soares et al. 2023; WFO, 2024). The beauty and diversity of the flowers boosts the flower and ornamental

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plants sector, making it a promising segment of Brazilian agribusiness (Junqueira and Peetz, 2018; Ibraflor, 2022). Orchids, in particular, enjoy great popularity among growers, collectors and orchid lovers, although they are often illegally removed from the wild, leading many species to become threatened with extinction (Nongdam et al. 2023; Vendrame et al. 2023).

Since *Cattleya walkeriana* Gardner is an orchid of high ornamental potential and economic value, it undergoes much exploitation and is one of the most targeted. It is native to the Brazilian Cerrado and can also be found in the Atlantic Forest (Flora and Funga of Brazil, 2024). According to data from the National Institute for Space Research (INPE), deforestation in the Cerrado increased by 3% between August 2022 and July 2023, totaling 11,011.7 km^2 (Brasil, 2023). Furthermore, the areas under deforestation alert increased by 43.7% in 2023, and infractions related to crimes against flora, fined by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA), increased by 45% in comparison with the last 4 years (Brasil, 2024). According to the Red List's degree of threat proposed by the National Center for Flora Conservation, *C. walkeriana* is vulnerable because its population has decreased by about 30% since 2003 (CNCFlora, 2013).

Due to the growing demand for plants from the Orchidaceae family and the growing impact suffered by natural populations due to the degradation of their habitats, there is currently an effort to seek sustainable production, using advanced and economically viable techniques (Teixeira da Silva et al., 2017; Nongdam et al., 2023; Vendrame et al., 2023; Vitt et al., 2023). This fact implies the need for adjustments to *in vitro* cultivation protocols, especially for native species (Soares et al., 2023), agreeing with the Sustainable Development Goals of the UN Agenda 2030, which in item 15.5, emphasize the urgency of taking significant measures to reduce the degradation of natural habitats, halt the loss of biodiversity, and protect and prevent the extinction of threatened species. In this context, adaptations in micropropagation systems, light conditions in the growth room and culture media formulations can result in the development of desirable characteristics in propagated plants (Miranda et al., 2020; Santos et al., 2020; Nongdam et al., 2023).

The formulation of the cultivation medium is fundamental for the development of micropropagated plants, with different formulations, including the addition of banana, according to the species studied (Soares et al., 2020; Freitas et al., 2021). Light also acts on several metabolic processes in plants, and the effects of spectral quality and irradiance levels are still little studied, especially for native species (Sorgato et al., 2021). Another factor that influences production is gas exchange (mixotrophic cultivation), as they act directly in the *in vitro* cultivation and *ex vitro* establishment of orchids, influencing their physiology and final performance (Teixeira da Silva et al., 2017).

The use of different culture media, wavelengths, and micropropagation systems in the *in vitro* cultivation of *C. walkeriana* were related from Dignart et al. (2009), Moreira et al. (2013), Silva et al. (2014), Nardelli et al. (2020) and Nadal et al. (2023). However, this is the first study that investigates the *in vitro* cultivation of *C. walkeriana* under the influence of banana 'nanica' pulp and peel as an organic additive in the culture medium and different irradiances provided by LED lamps (3000K) in conjunction with the sealing systems.

The efficiency of *in vitro* cultivation of orchids is undeniable, but protocols need to be improved for commercial use. With efforts underway towards effective, reliable and more affordable solutions, mass production of orchids for commercial and conservation purposes is close to becoming a reality (Nongdam et al., 2023). Thus, this study hypothesizes that when the culture medium is supplemented with banana, combined with a seal that allows gas exchange and a light condition with higher irradiance, it can benefit this species' propagation.

Therefore, this study aimed to evaluate the effect of culture medium formulation, flask sealing system, and irradiance provided by LED lamps on the *in vitro* growth and the *ex vitro* survival and establishment of *C. walkeriana*.

2. Materials and Methods

This study was conducted at the Laboratory of *In Vitro* Culture of Flowers and Ornamental Plants, at the School of Agricultural Sciences (FCA) of the Universidade Federal da Grande Dourados (UFGD), using mature fruits of *C. walkeriana* donated by the researcher Dr. Renato F. Galdiano Júnior.

In order to prepare the plant material, the fruits were opened in an aseptic environment and the seeds were conditioned in a desiccator with silica gel (25±2 °C; 75% RH) for 14 days. Then, a 0.005 g sample of seeds was evaluated for viability using the tetrazolium test (Soares et al., 2014). The sample was discarded after viability confirmation.

After viability confirmation, another 0.005 g sample of seeds was taken to an aseptic environment and disinfected following the methodology described by Soares et al. (2020) to obtain the seed solution. Each culture flask was inoculated with 1.0 mL of the disinfected seed suspension for *in vitro* sowing. Sixty mL of culture medium was used per flask with a capacity of 600 mL. The medium used was Murashige and Skoog (MS) (1962) with half the concentration of all components of the original formulation (MS ½), solidified with 7.0 g L^{-1} of agar, supplemented with 30 g L^{-1} of sucrose, and pH adjusted to 5.8. Then the flasks were sealed under a conventional sealing system and the cultures were conditioned in a growth room with controlled temperature and photoperiod (25±2 °C; 16 h), and 22 µmol m^{-2} s^{-1} irradiance provided by white fluorescent lamps (6,500K). The cultures remained under these conditions for up to 360 days, and three subcultures were performed.

2.1. In vitro growth - 120 days

For both *in vitro* and *ex vitro* culture, the experimental design used was entirely randomized in a 5x2x2 factorial scheme (five culture media; two irradiances; two sealing systems), with five repetitions (flask) where each experimental unit comprised a flask containing four seedlings.

The seedlings were standardized for size (1,5 cm) and subcultured for the beginning of the experimental period.

MS medium (Murashige and Skoog, 1962) in original formulation, supplemented with 30 g L^{-1} sucrose and solidified with 7.0 g L^{-1} of bacteriological agar (Himedia®, India) was used as culture medium. This medium was used as control = MS (1) and for the other treatments the media was supplemented as follows: 2) MS supplemented with 150 g L^{-1} of banana 'nanica' pulp = P150; 3) MS with 300 g L^{-1} of banana 'nanica' pulp = P300; 4) MS with 150 g L^{-1} of banana 'nanica' peel = PE150; and 5) MS with 300 g L^{-1} of banana 'nanica' peel = PE300, using senescent fruits. The medium's pH was adjusted to 5.8 using KOH (0.1M) before sterilization in an autoclave (121 °C and 1.1 atm pressure) for 20 minutes, and 60 mL of the medium were distributed in each 600 mL^{-1} capacity flask, with four seedlings inoculated in an aseptic environment per culture flask. Then, half of the flasks was hermetically sealed with polyvinyl chloride (PVC) film (conventional sealing system - CSS) and the other half with PVC with a cotton filter (sealing system that allows gas exchange - GESS).

After that, the cultures were conditioned in a growth room with controlled temperature and photoperiod (25±2°C; 16h), under two irradiances (I) provided by 3000K LED lamps: 86 µmol m^{-2} s^{-1} (Irradiance - 1) or 128 µmol m^{-2} s^{-1} (Irradiance - 2) (Figure 1). Spectral distribution measurements were taken using an Ocean Optics portable spectrometer (Model MMO with fiber optics), at room temperature with a 10 ms integration time.

After 120 days of culture, the flasks were removed from the growth room, opened, and the seedlings were removed and washed in running tap water until total removal of the culture medium. Then, they were evaluated using a digital pachymeter and a precision scale for the following variables: survival (%SURV), number of pseudobulbs (NP), number of leaves (NL), number of roots (NR), plant height (PH), longest leaf length (LL) (mm), longest root length (RL) (mm), pseudobulb diameter (PD), and total fresh mass (TFM) (g). After the evaluations, the treatments were photographed using a camera attached to a mini photo studio.

2.2. Ex vitro growth - 180 days

After initial evaluations, the plants were transferred to 1,000 mL transparent polypropylene disposable containers (20 x 10 x 5 cm) with holes in the lid for gas exchange and in the base for substrate drainage, and ⅓ of its volume was filled with pink sphagnum moss (Agrolink, Holambra-SP) + coconut fiber (Golden-Mix Chips, Amafibra) (1:1, $v:v^{-1}$). After transplanting, the containers were placed in a shaded nursery, remaining for 180 days in a covered nursery with two 50% shading screens, which provided 235 µmol m^{-2} s^{-1} irradiance and average temperature and relative humidity conditions of 22.6±5 ºC and 73.9 ± 10%, respectively. For the first 15 days the containers remained with the lids closed to minimize the stress provided by the environment change (from *in vitro* to *ex vitro*) (Ramos et al., 2023). After that, the containers were opened. Micro-sprinklers were positioned one meter above the plants, providing a 1 mm water blade per day^{-1}.

Fertilizations were carried out every 15 days through foliar application of 2.0 mL L^{-1} of NPK 10-10-10, plus the following micronutrients: 0.025% magnesium, 0.02% boron, 0.05% copper, 0.10% iron, 0.05% manganese, 0.0005% molybdenum, and 0.05% zinc (Peters®). The plants were preventively disinfested with O-S-dimethyl-N-acetyl-N-phosphoramidothioate (4 mg L^{-1}) and Mancozeb (4 mg L^{-1}) at 0, 30, and 60 days. A 5 L capacity knapsack sprayer was used for both foliar fertilization and disinfestation.

The plants were removed from the containers and washed under running water until complete substrate removal. Finally, they were evaluated for the same initial variables (SURV, NP, NL, NR, PH, LL, RL, PD, and TFM) for the *ex vitro* growth evaluation.

Assuming the hypothesis of increased plant growth during the *ex vitro* phase, according to the treatments to which they were initially exposed, their increments (I) regarding the initial values were calculated through the expression I = (VF - VI). Where VI is the variable value before the plant was acclimatized and VF is the same variable value after the *ex vitro* period. Their values were expressed as percentages and submitted to analysis of variance, according to Ribeiro et al. (2019).

The results were submitted to analysis of variance and irradiances and seals were compared using the F test, while culture media were compared using Tukey's Test ($p<0.05$), using the SISVAR software (Programa de Análises Estatísticas v.5.3 Universidade Federal de Lavras, MG) (Ferreira, 2011).

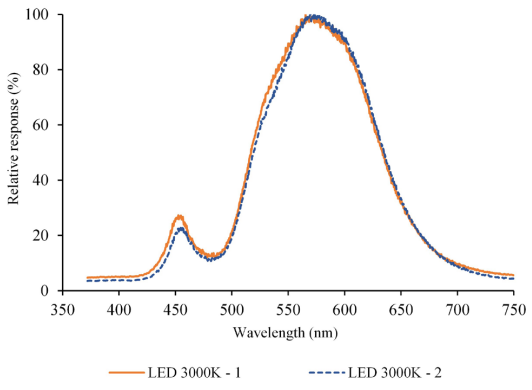

Figure 1. Spectral energy distribution of the LEDs.

3. Results and Discussion

3.1. In vitro culture

There was a significant interaction between treatments for all analyzed variables. The CM x SS double interaction was significant for PH, LL, and RL. The CM x I x SS triple interaction was significant for the NP, NL, NR, PD, TFM, and %SURV variables of *C. walkeriana*.

Regarding the interaction between culture media and sealing systems, greater values for PH (24.94 mm) and RL (37.87 mm) were found when *C. walkeriana* plants were subjected to MS + CSS, although with no significant difference for P150 + CSS (23.81 mm and 31.51 mm respectively). For LL, the highest values were found when using the PE150 + GESS medium (17.88 mm), not

significantly different from P300 + GESS (16.91 mm) and MS + GESS (16.56 mm) (Figure 2).

Regarding the CM x I x SS interaction, using the MS + CSS culture medium under I - 2 (128 µmol m^{-2} s^{-1}) provided the highest results for NL (22.93), although with no significant difference for P150 + Irradiance - 2 + CSS. For NP and NR, the highest values were found in MS + 128 µmol m^{-2} s^{-1} + CSS, with averages of 13.02 pseudobulbs and 25.95 roots, respectively (Table 1).

For PD, the highest results were observed when using the P150 + Irradiance - 1 + CSS medium (3.12 mm), not significantly different from the results found for MS + Irradiance - 1 + CSS. Regarding the plants' TFM, the highest values were observed when cultivated in the MS + Irradiance - 2 + CSS medium (3.83 g), compared to all the treatments studied. The highest %SURV was observed in plants cultivated on the P150 medium (100%) regardless of the irradiance and sealing system used. The highest values were also observed in the PE150 + GESS + Irradiance - 1 (100%), P300 + GESS + Irradiance - 2 (100%), and MS + GESS + Irradiance - 1 (100%) treatments (Table 1).

Overall, the highest values were found when MS or P150 + Irradiance-2 + CSS media were used. The results observed when adding banana pulp, regardless of the sealing system or irradiance used, support Silva et al. (2016), who studied the orchid species *Epidendrum nocturnum* Jacq. and found that the medium supplemented with 100 g of banana provided a higher survival percentage in this species' *in vitro* culture. A possible explanation for this fact may derive from the banana pulp composition, which is rich in auxins, cytokinins, gibberellins, potassium, vitamins, and amino acids (Dolce et al., 2020). Thus, supplying these components may have contributed to the plants' survival in the *in vitro* system. On the other hand, in the MS medium already supplemented with 30 g L^{-1} of sucrose, the increased in concentrations may have provided an overload of carbon sources, which may explain the lower survival rates in media supplemented with more than 150 g L^{-1} of banana cultivated under CSS.

In this study, the NL, NP, NR, and TFM variables were positively influenced by using MS or P150 + Irradiance - 2 + CSS. Only the PD variable showed the highest results with the same media and sealing system, although with Irradiance - 1. Freitas et al. (2021) observed that using 100 g L^{-1} of banana pulp resulted in the highest leaf production in *Cattleya nobilior* Rchb.f. plants, which supports this study's results. Culture media formulation can directly influence the growth and development of *in vitro*-cultivated plants. For *C. walkeriana* seedlings, the MS medium formulation supplemented or not with 150 g L^{-1} of banana pulp provided *in vitro* growth.

Regarding the sealing system, in this study the conventional sealing provided the highest values for the NL, NP, NR, PD, and TFM variables in *C. walkeriana* plants cultivated *in vitro*. These results support Ribeiro et al. (2019), who when evaluating the influence of the sealing system on the growth and development of *Dendrobium bigibbum* Lindl. (Orchidaceae), also observed higher number of leaves and pseudobulbs using the conventional sealing system. These higher values may be related to the flasks' internal environment since the total sealing of the culture flasks provides an increase in the air relative humidity, which results in a greater water accumulation in plant tissues (Freitas et al., 2021; Soares et al., 2023). Furthermore, the sealing system used interferes with gas exchange, since the hermetic sealing of the bottles increases ethylene gas concentrations and decreases CO_2 concentrations, which can result in morpho-physiological changes in plants, particularly stomatal malfunction, increased water loss through leaf tissue and potential decrease in the survival

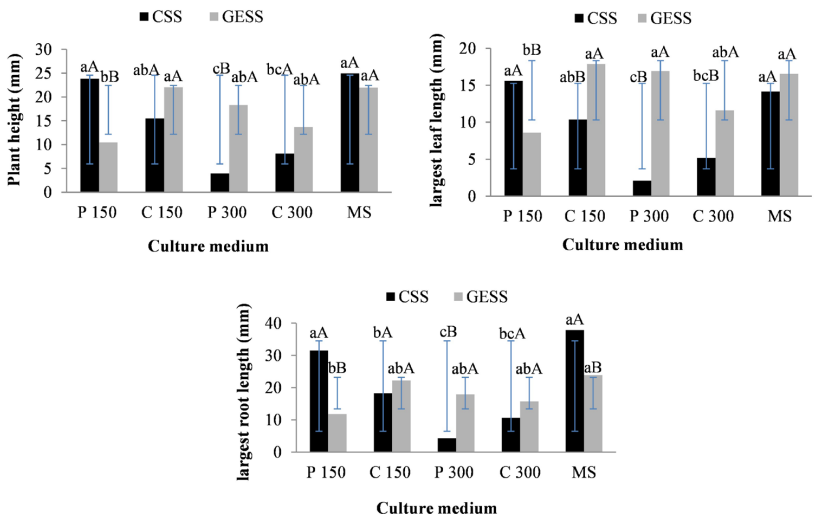

Figure 2. Plant height (mm) (A), largest leaf length (mm) (B), and largest root length (mm) (C) of *Cattleya walkeriana* as a function of the culture medium and sealing system studied. P150 = 150 g L^{-1} of banana pulp; P300 = 300 g L^{-1} of banana pulp; PE150 = 150 g L^{-1} of banana peel; PE300 = 300 g L^{-1} of banana peel; MS = Murashige and Skoog (1962); GESS = sealing system that allows gas exchange; CSS = conventional sealing system. Lower case letters compare the same sealing system on different culture media. Upper case letters compare different sealing systems on the same culture medium. Equal letters do not differ by Tukey's test and T test, respectively ($p < 0.05$).

Table 1. Number of pseudobulbs (NP), number of leaves (NL), number of roots (NR), pseudobulb diameter (PD), total fresh mass (TFM) and survival percentage (%SURV) of *Cattleya walkeriana* as a function of the culture medium, sealing system, and irradiance studied.

Media	NP				NL				NR			
	CSS		GESS		CSS		GESS		CSS		GESS	
	I - 1	I - 2	I - 1	I -2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2
P150	7.75 aA*a*	5.88 bA*a*	1.15 aA*b*	3.38 aA*a*	21.85 aA*a*	22.93 abA*a*	3.94 aB*a*	13.74 aA*a*	14.95 aA*a*	12.29 bA*a*	4.70 aB*b*	12.78 aA*a*
PE150	0.79 bB*a*	4.96 bA*a*	1.52 aA*a*	1.25 aA*b*	2.65 bB*a*	17.60 bcA*a*	5.04 aA*a*	5.24 bA*a*	2.01 bA*a*	6.43 bcA*a*	5.89 aA*a*	4.80 bA*a*
P300	0.47 bB*a*	2.60 bA*a*	1.15 aA*a*	1.69 aA*a*	1.73 bA*a*	6.11 cA*a*	3.14 aA*a*	4.95 bA*a*	1.21 bA*a*	1.23 cA*a*	3.50 aA*a*	4.87 bA*a*
PE300	0.81 bB*a*	5.20 bA*a*	1.24 aA*a*	2.15 aA*a*	2.32 bB*a*	13.20 bcA*a*	3.57 aA*a*	5.25 bA*b*	1.85 bB*a*	8.60 bA*a*	3.89 aA*a*	7.20 abA*a*
MS	2.68 bB*a*	13.02 aA*a*	1.69 aA*a*	2.00 aA*b*	7.29 bB*a*	32.01 aA*a*	5.55 aA*a*	6.79 bA*b*	6.43 bB*a*	25.95 aA*a*	3.87 aA*a*	5.05 bA*b*
OA	2.50	6.33	1.35	2.09	7.17	18.37	4.25	7.19	5.29	10.90	4.37	6.94
CV (%)	30.04				35.01				26.93			
Media	**PD**				**TFM**				**%SURV**			
	CSS		GESS		CSS		GESS		CSS		GESS	
	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2
P150	3.12 aA*a*	1.50 abB*a*	1.70 abA*b*	1.27 bcA*a*	2.06 aA*a*	1.69 bA*a*	0.17 aA*b*	0.56 aA*b*	100.00 aA*a*	100.00 aA*a*	100.00 aA*a*	100.00 aA*a*
PE150	0.63 bB*b*	1.66 abA*a*	2.56 aA*a*	0.76 cB*a*	0.20 bA*a*	0.78 bcA*a*	0.45 aA*a*	0.32 aA*a*	38.33 bB*b*	88.17 abA*a*	100.00 aA*a*	80.00 aA*a*
P300	0.34 bA*b*	0.53 bA*b*	2.07 abA*a*	2.16 abA*a*	0.08 bA*a*	0.15 cA*a*	0.24 aA*a*	0.21 aA*a*	20.00 bA*b*	20.00 cA*b*	80.00 aA*a*	100.00 aA*a*
PE300	0.87 bA*a*	1.02 bA*b*	1.10 bB*a*	2.17 abA*a*	0.08 bB*a*	1.02 bcA*a*	0.27 aA*a*	0.46 aA*a*	40.00 bA*a*	40.00 bcA*b*	60.00 aA*a*	95.00 aA*a*
MS	2.84 aA*a*	2.46 aA*a*	2.14 abA*a*	2.69 aA*a*	0.57 bB*a*	3.83 aA*a*	0.27 aA*a*	0.49 aA*b*	100.00 aA*a*	85.00 abA*a*	97.28 aA*a*	96.00 aA*a*
OA	1.56	1.43	1.91	1.81	0.60	1.49	0.28	0.41	59.67	66.63	87.46	94.20
CV (%)	19.02				17.87				33.09			

Lowercase letters in the column compare culture media in the same seal and in the same irradiance. Capital letters, on the line, compare the irradiance within the same culture medium and type of seal. Letters in italics, on the line, compare the type of seal in the same media culture and irradiance (F and Tukey test $p<0.05$). P150 = 150 g L^{-1} of banana pulp; P300 = 300 g L^{-1} of banana pulp; PE150 = 150 g L^{-1} of banana peel; PE300 = 300 g L^{-1} of banana peel; MS = Murashige and Skoog (1962); GESS = sealing system that allows gas exchange; CSS = conventional sealing system; I - 1 (86 µmol m^{-2} s^{-1}); I - 2 (128 µmol m^{-2} s^{-1}); GM = General Media.

of orchid seedlings during the *ex vitro* acclimatization process (Teixeira da Silva et al., 2017; Miranda et al., 2020; Fritsche et al., 2022; Soares et al., 2023).

Regarding light, Irradiance - 2 positively influenced growth in *in vitro*-cultivated plants, providing the highest values for the NL, NR, NP, and TFM variables. Growth rooms generally use fluorescent lamps for *in vitro* culture. They are gradually being replaced by LED lamps that have advantages when compared to fluorescent lamps, such as: fewer physiological and morphological variations in embryos, high efficiency in the light generation process and low heat emission, requiring less energy in the growth room and for the cooling system, and a long service life (Gupta and Agarwal, 2017; Hanus-Fajerska and Wojciechowska, 2017; Fritsche et al., 2022). In this study, using a LED 3000K lamp with 128 µmol m^{-2} s^{-1} irradiance (Irradiance 2), even with wavelength similarity (Figure 1), showed greater results than the LED 3000K with 86 µmol m^{-2} s^{-1} irradiance (Irradiance 1). This fact shows that using the higher irradiance contributed to these seedlings' development. Taiz et al. (2017) described that the quality and quantity of light made available to the propagated material are important for regulating several biochemical pathways that control everything from growth to morphogenesis, a fact which was also observed in this study. Moreover, the light condition used in the *in vitro* culture can also promote leaf growth, carbohydrate accumulation, and anatomical changes (Hung et al., 2016; Fritsche et al., 2022). Thus, these factors may have directly contributed to the fresh mass increase in *C. walkeriana* plants.

This study's results may also be related to the tillering of these plants when cultivated under the hermetic sealing of the flasks (Figure 3), which occurred with *C. nobilior*, *D. bigibbum* Lindl. and *Brassavola tuberculata* Hook. in Freitas et al. (2021), Ribeiro et al. (2019) and Soares et al. (2023) respectively. According to these authors, this sealing system provides the presence of ethylene gas and low CO_2 concentrations in the hermetic environment, it can reduce plant height and photosynthetic pigment content, thereby influencing the tillering of in vitro-grown plants, which may have contributed to the increase to plant structures in the number and, consequently, the fresh mass in this study. In Figure 3, it can be visually observed that the plants underwent tillering when cultivated under 128 µmol m^{-2} s^{-1} irradiance in a conventional system.

Plants cultivated in a system that allows gas exchange present structures with greater length and diameter, which seem to have adequate characteristics for *ex vitro* culture, such as more developed pseudobulbs, leaves, and roots. Epiphytic orchids exhibit thick organs in roots, leaves, or pseudobulbs, enabling their adaptation in adverse climates. Pseudobulbs play a crucial role in plant survival under water or nutrient stress, while roots store water and nutrients and contribute to photosynthesis and plant

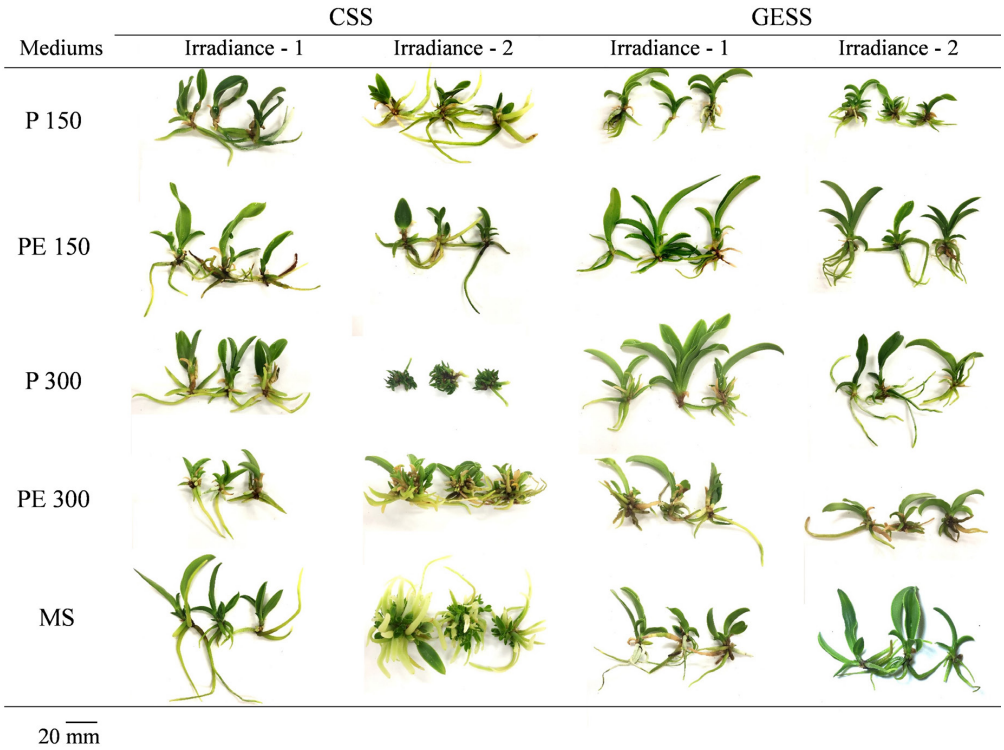

Figure 3. *Cattleya walkeriana* Gardner. plants at 180 days of *ex vitro* culture as a function of the culture medium, sealing system, and irradiance studied. P150 = 150 g L^{-1} of banana pulp; P300 = 300 g L^{-1} of banana pulp; PE150 = 150 g L^{-1} of banana peel; PE300 = 300 g L^{-1} of banana peel; MS = Murashige and Skoog (1962); GESS = sealing system that allows gas exchange; CSS = conventional sealing system; Irradiance - 1 (86 µmol m^{-2} s^{-1}); Irradiance - 2 (128 µmol m^{-2} s^{-1}).

fixation. Leaves reduce transpiration, retain moisture, and store water. Increased growth of these organs is directly linked to resistance and survival in harsh conditions, along with CAM metabolism, suggesting optimization of water use and carbon economy during stress periods (De and Biswas, 2022; Endres Júnior et al., 2024).

3.2. Ex vitro culture - 180 days

At 180 days after the *ex vitro* culture began, there was significant interaction of the *in vitro* culture for all the evaluated *ex vitro* traits. The CM x I x SS interaction of the *in vitro* culture was significant for all *C. walkeriana* traits evaluated.

At the end of the experimental period there was a higher survival percentage when plants were previously cultivated *in vitro* under CSS combined with MS medium + Irradiance - 1 (100%), or in the same sealing system with PE300 medium + Irradiance - 2 (100%). Regarding the increase percentage for NP (63.75%) and NL (54.91%), the highest values were observed when *C. walkeriana* plants were previously cultivated on MS medium combined with Irradiance-1 and CSS (Table 2).

Regarding the NR (21.74%), the best averages were found when *C. walkeriana* plants were previously cultivated on MS medium combined with Irradiance-1 and CSS. The PH and LL of plants cultivated in P300 + Irradiance - 1 + CSS showed the highest percentage increase (745.0% and 779.44% respectively) (Table 2).

For RL of plants cultivated in P300 + Irradiance - 1 + CSS showed the highest percentage increase (195.56%). Similarly, PD and TFM showed higher percentage increase when using the P300 + Irradiance - 1 + CSS culture medium (PD = 1128% and TFM = 888.62%). However, the percentage increase in fresh mass did not significantly differ from the P150 + Irradiance - 1 + CSS medium (743.96%) (Table 2).

Overall, in *ex vitro* culture, the highest survival values were found when *C. walkeriana* plants were cultivated *in vitro* using MS or PE300 + Irradiance-1 + CSS media. Some studies have reported that it is possible to stimulate the *in vitro* growth of several species by varying the light condition inside growth rooms. Thus, using different LEDs can directly influence the increase in plant survival during the acclimatization phase (Ferreira et al., 2017).

For most variables (NP, NL, NR, PH, LL, and RL), the highest values were observed when plants were cultivated on MS or P300 medium under the same irradiance and survival sealing system.

The results for PH are in agreement with those of Araújo et al. (2006), who reported that *Cattleya loddigesii 'Grande' x Cattleya 'Alba'* plants showed greater height when cultivated on Knudson medium supplemented with 100 g L^{-1} of banana pulp. According to these authors, this may be related to the banana's ability to influence the aerial part development in the orchid *in vitro* culture, and also the adventitious shoot emission, which can be observed in the variables evaluated in this study. Furthermore, banana can be used in the orchid *in vitro* culture because its formulation is rich in potassium, acting as a rooting stimulator, in the growth and/or thickening of the roots (Utami and Hariyanto, 2020; De Stefano et al., 2022), a fact that can be noted in the RL variable.

Corroborating this statement, Freitas et al. (2021) observed that the medium containing banana pulp showed better root development in *C. nobilior* Lindl. Also, banana pulp can increase the aerial part growth (number of structures, diameter, and height) of plants obtained from *in vitro* explants (Arditti and Ernst, 1993; Utami and Hariyanto, 2020). This fact may explain the higher percentage of increase in the plants' pseudobulb diameter.

Regarding the sealing system, the highest values were provided when using CSS. These results ratify those of Ribeiro et al. (2019), who observed greater increase on pseudobulb diameter and largest root length when *D. bigibbum* plants were subcultured in an hermetic environment. This increase can be explained by the plants' transfer to the *ex vitro* environment, which allows them to complete their autotrophism since the ability to manage water and respond to activity during and after *in vitro* culture are factors that determine the final performance of the propagated plant material (Soares et al., 2023).

In this study, similarly to that of Ribeiro et al. (2019), when plants were cultivated *in vitro* in CSS, they increased their growth rate in the *ex vitro* phase. However, plants from the GESS, which had already started their rustification during the *in vitro* culture, only maintained this rate. This fact may have directly contributed to the *C. walkeriana* increment variables in this study.

Regarding light, Irradiance - 1 positively influenced all the evaluated variables except TFM, which obtained the highest values under the influence of Irradiance - 2. When observing Figure 1, it can be seen that even with similar wavelength light sources, pre-cultivation under lower irradiance (86 µmol m^{-2} s^{-1}) provided the best results for this species' *ex vitro* culture. It is worth noting that plant responses to light irradiance are strongly associated with the species (Hung et al., 2016; Taiz et al., 2017). As such, light quality, photoperiod, and photon flux density are factors directly related to plant morphogenesis development when cultivated *in vitro* (Taiz et al., 2017), favoring an adequate electron transport rate for less energy dissipation. Thus, the results found in this study suggest that the Irradiance - 1 used in the *in vitro* culture was the most appropriate for this species, since it may have contributed to the early rustification and better performance of *ex vitro* plants, as shown in Figure 4.

In orchid species propagation protocols, it is crucial to consider not only the number of micropropagated plants but also the morphophysiological quality of the resulting plants. Thus, the findings of this study, support the inference that gas exchange micropropagation systems and the Irradiance I promote the *ex vitro* establishment of plants when cultivated *in vitro*, making them recommended for the *in vitro* cultivation of *C. walkeriana*. Furthermore, by reducing dependence on traditional propagation methods, such as harvesting wild plants, the in vitro cultivation of this species contributes to the preservation of natural habitats and biological diversity, highlighting its fundamental role in promoting more sustainable practices and in the preservation of plant biodiversity.

Table 2. Increases (%) on *Cattleya walkeriana* survival (%SURV), on the number of pseudobulbs (NP), number of leaves (NL), of roots (NR), plant height (PH) and on the length of the largest leaf (LL), largest root (RL), pseudobulb diameter (PD) and total fresh mass (TFM) as a function of the culture medium, sealing system, and irradiance studied.

Media	%SURV				NP				NL			
	CSS		GESS		CSS		GESS		CSS		GESS	
	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2
P150	66.67 bA*b*	72.00 bA*a*	95.00 aA*a*	16.00 cB*b*	0.00 bB*b*	22.73 aA*a*	59.66 aA*a*	0.00 bB*b*	0.00 Bb*a*	0.00 bB*a*	0.00 bB*a*	0.00 bB*a*
PE150	20.00 cA*a*	28.0 cA*a*	10.00 cA*a*	10.00 cA*b*	3.13 bA*a*	0.00 bB*a*	0.00 cB*a*	11.43 bA*a*	0.00 bB*a*	0.00 bB*a*	0.00 bB*a*	0.00 bB*a*
P300	60.00 bA*a*	0.00 dB*b*	10.00 cA*b*	16.00 cA*a*	0.00 bA*a*	0.00 bA*a*	0.00 cA*a*	0.00 bA*a*	0.00 bB*a*	0.00 bB*a*	0.00 bB*a*	0.00 bB*a*
PE300	50.00 bB*a*	100.00 aA*a*	33.80 bA*b*	40.00 bA*b*	0.00 bA*a*	0.00 bA*b*	9.94 bB*a*	44.17 aA*a*	0.00 bB*a*	0.00 bB*a*	0.00 bB*a*	0.00 bB*a*
MS	100.0 aA*a*	13.89 cdB*b*	56.67 bB*b*	92.00 aA*a*	63.75 aA*a*	0.00 bB*b*	8.83 bA*b*	5.56 bA*a*	54.91 aA*a*	0.00 bB*a*	0.00 bB*b*	0.00 bB*a*
OA	59.33	42.78	39.33	34.80	14.18	4.55	15.69	12.23	11.00	0.00	0.00	0.00
CV (%)	14.77				48.04				34.33			
Media	NR				PH				LL			
	CSS		GESS		CSS		GESS		CSS		GESS	
	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2
P150	0.00 bA*a*	0.00 bA*a*	0.00 bA*a*	0.00 bA*a*	7.05 cB*b*	15.25 aA*a*	228.85 aA*a*	46.58 bB*a*	17.50 cA	0.00 bA	164.65 aA	59.93 bB
PE150	0.00 bA*a*	0.00 bA*a*	0.00 bA*a*	0.00 bA*a*	52.43 cB*a*	22.97 aA*a*	63.54 bA*a*	0.00 bB*a*	102.53 cA*a*	102.45 aA	57.72 abA*a*	0.00 bB
P300	0.00 bA*a*	0.00 bA*a*	0.00 bA*a*	0.00 bA*a*	745.0 aA*a*	0.00 aB*a*	0.00 bA*b*	17.03 bA*a*	779.44 aA	0.00 bB	0.00 bA	46.39 bA
PE300	0.00 bA*a*	0.00 bA*a*	0.00 bB*a*	0.00 bB*a*	178.90 bA*a*	46.66 aB*b*	31.30 bB*b*	199.13 aA*a*	329.84 bA	100.73 aB	27.44 bB	175.77 aA
MS	21.74 aA*a*	0.00 bB*b*	0.00 bB*b*	1.50 aA*a*	66.37 cA*a*	16.43 aA*a*	37.48 bA*a*	6.37 bA*a*	69.10 cA	39.26 abA	73.80 abA	26.33 bA
OA	4.35	0.00	0.00	0.30	209.95	20.26	72.23	53.82	259.68	48.50	59.23	61.68
CV (%)	2.96				27.61				33.15			
Media	RL				PD				TFM			
	CSS		GESS		CSS		GESS		CSS		GESS	
	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2	I - 1	I - 2
P150	0.00 cA*b*	0.00 bA*a*	71.14 aA*a*	0.00 bB*a*	0.00 cA*b*	23.15 aA*b*	139.67 aB*a*	265.85 aA*a*	743.96 aA*a*	0.00 aB*a*	277.48 aA*b*	0.00 aB*a*
PE150	0.00 cB*a*	18.33 abA*a*	0.00 bB*a*	29.05 aA*a*	137.90 aA*a*	0.00 aB*a*	0.00 bA*a*	0.00 cAa	30.77 bA*a*	0.00 aA*a*	0.00 cA*a*	86.74 aA*a*
P300	195.56 aA*a*	0.00 bB*a*	0.00 bA*a*	0.00 bAa	1128.85 aA*a*	0.00 aB*b*	0.00 bB*b*	96.73 bA*a*	888.62 aA*a*	0.00 aB*a*	0.00 cA*b*	26.45 aA*a*
PE300	76.37 bA*a*	30.13 aB*a*	11.85 bB*b*	39.48 aA*a*	55.48 bcA*a*	49.90 Aa*a*	63.61 abA*a*	25.44 bcA*a*	140.47 bA*a*	0.00 aA*a*	46.25 aA*a*	18.63 aA*a*
MS	15.55 cA*a*	0.00 bA*b*	0.00 bB*a*	42.90 aA*a*	42.00 cA*a*	36.58 aA*a*	15.91 bA*a*	20.54 bcA*a*	51.70 bA*a*	0.00 aA*a*	91.90 aA*a*	20.75 aA*a*
OA	57.50	19.38	14.23	22.29	272.85	21.93	43.84	81.71	371.10	0.00	83.13	30.51
CV (%)	30.51				21.26				47.13			

Lowercase letters in the column compare culture media in the same seal and in the same irradiance. Capital letters, on the line, compare the irradiance within the same culture medium and type of seal. Letters in italics, on the line, compare the type of seal in the same media culture and irradiance (F and Tukey test $p<0.05$). P150 = 150 g L^{-1} of banana pulp; P300 = 300 g L^{-1} of banana pulp; PE150 = 150 g L^{-1} of banana peel; PE300 = 300 g L^{-1} of banana peel; MS = Murashige and Skoog (1962); GESS = sealing system that allows gas exchange; CSS = conventional sealing system; I - 1 (86 µmol m^{-2} s^{-1}); I - 2 (128 µmol m^{-2} s^{-1}); GM = General Media.

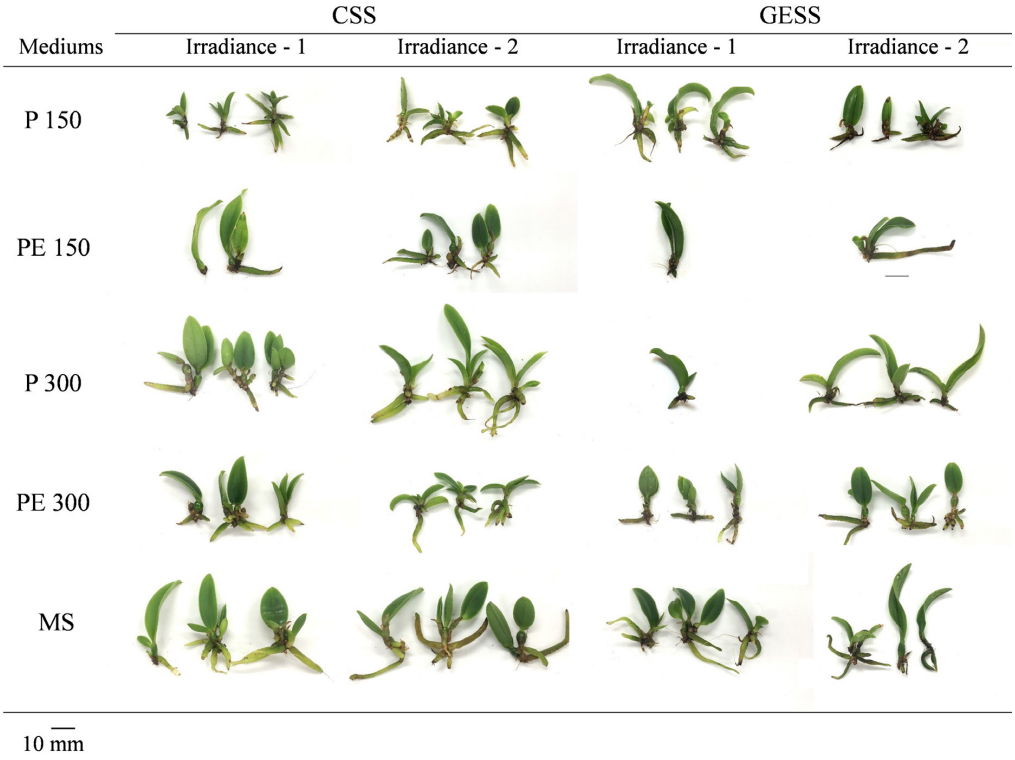

Figure 4. *Cattleya walkeriana* Gardner. plants at 180 days of *ex vitro* culture as a function of the culture medium, sealing system, and irradiance studied. P150 = 150 g L^{-1} of banana pulp; P300 = 300 g L^{-1} of banana pulp; PE150 = 150 g L^{-1} of banana peel; PE300 = 300 g L^{-1} of banana peel; MS = Murashige and Skoog (1962); GESS = sealing system that allows gas exchange; CSS = conventional sealing system; Irradiance - 1 (86 µmol m^{-2} s^{-1}); Irradiance - 2 (128 µmol m^{-2} s^{-1}).

4. Conclusion

The initial hypotheses were largely confirmed by the study. It was observed that the *in vitro* cultivation of *C. walkeriana* using up to 150 g L^{-1} of banana 'nanica' pulp, a micropropagation system that allows gas exchange and the use of irradiation of 86 µmol m^{-2} s^{-1} are recommended for the *in vitro* cultivation of *C. walkeriana*, promoting greater success in *ex vitro* establishment and contributing to more sustainable practices for propagating and preserving plant biodiversity.

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