

Organic supplements for in vitro growth and ex vitro establishment of native Cerrado orchids¹

Suplementos orgânicos no cultivo in vitro e no estabelecimento ex vitro de orquídeas nativas do Cerrado

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HIGHLIGHTS:

Culture media with organic compounds promoted in vitro growth of three Cerrado orchids.
The formulation of the culture media differently influenced ex vitro performance among the species.
Permeable flask closure improved seedling conditioning for ex vitro establishment.

ABSTRACT: Indiscriminate collection of native Cerrado orchids highlights the need for methodologies for conservation and sustainable production. Thus, this study evaluated the in vitro growth and ex vitro establishment of *Cattleya nobilior* Rchb.f., *Cattleya walkeriana* Gardner, and *Schomburgkia crispa* Lindl. cultivated in media supplemented with organic compounds in a micropropagation system with gas exchange, aiming to contribute to conservation and sustainable production strategies for these species. A completely randomized design with six treatments and five replicates was used. Seedlings were cultivated in vitro in Murashige and Skoog (MS) medium; MS + banana pulp (BP); MS + coconut water (CW); MS + banana flour (BF); MS + BP + CW or MS + BF + CW. After evaluation, the plants were transferred to an ex vitro environment and, after 180 days, evaluated for survival and the same initial characteristics. For *C. nobilior*, the MS + BP + CW medium promoted in vitro growth and favored the acclimatization phase. For *C. walkeriana*, the MS + BF + CW medium produced the best in vitro biometric performance; however, only the MS + CW medium ensured ex vitro survival (54.54%), making it strictly required for the complete propagation protocol. For *S. crispa*, in vitro cultivation in MS + BP medium favored both in vitro growth and plant acclimatization. It is concluded that the use of organic supplements and micropropagation systems with gas exchange favor growth and acclimatization, being recommended for the in vitro and ex vitro cultivation of these species.

Key words: Orchidaceae, *Cattleya nobilior*, *Cattleya walkeriana*, *Schomburgkia crispa*, micropropagation

RESUMO: A coleta indiscriminada de orquídeas nativas do Cerrado destaca a necessidade de metodologias para conservação e produção sustentável. Assim, este estudo avaliou o crescimento in vitro e o estabelecimento ex vitro de *Cattleya nobilior* Rchb.f., *Cattleya walkeriana* Gardner, e *Schomburgkia crispa* Lindl., cultivadas em meios suplementados com compostos orgânicos em sistema de micropropagação com trocas gasosas, visando contribuir para estratégias de conservação e produção sustentável dessas espécies. Foi utilizado delineamento inteiramente casualizado, com seis tratamentos e cinco repetições. As plântulas foram cultivadas in vitro em meio Murashige e Skoog (MS); MS + polpa de banana (PB); MS + água de coco (AC); MS + farinha de banana (FB); MS + PB + AC ou MS + FB + AC. Após a avaliação, as plantas foram transferidas para ambiente ex vitro e, após 180 dias, avaliadas quanto à sobrevivência e as mesmas características iniciais. Para *C. nobilior*, o meio MS + PB + AC promoveu o crescimento in vitro e favoreceu a fase de aclimatização. Para *C. walkeriana*, o meio MS + FB + AC apresentou o melhor desempenho biométrico in vitro, entretanto, apenas o meio MS + AC garantiu a sobrevivência ex vitro (54,54%), tornando-o estritamente necessário para o protocolo completo de propagação. Para *S. crispa*, o cultivo in vitro em meio MS + PB favoreceu tanto o crescimento in vitro quanto a aclimatização das plantas. Conclui-se que o uso de suplementos orgânicos e sistemas de micropropagação com troca gasosa favorecem o crescimento e a aclimatização, sendo recomendado para o cultivo in vitro e ex vitro dessas espécies.

Palavras-chave: Orchidaceae, *Cattleya nobilior*, *Cattleya walkeriana*, *Schomburgkia crispa*, micropropagação

INTRODUCTION

Orchids constitute one of the botanical groups with the highest ornamental value, appreciated for their morphological diversity, exuberant coloration, and high post-harvest longevity (Tiruwa et al., 2024). Brazil boasts approximately 2,675 species distributed across 247 genera, with the Cerrado being one of the phytogeographic domains richest in Orchidaceae species, hosting 652 registered species (Flora e Funga do Brasil, 2025a).

Within the panorama of Brazilian flora, several native orchid species stand out for their high ornamental and commercial value. Among them are *Cattleya nobilior* Rchb.f. (popularly known as “Rainha do Cerrado” – Queen of the Cerrado) and *Cattleya walkeriana* Gardner, which is endemic to Brazil. Both are listed in the Red Book of Brazilian Flora, classified as “near threatened” and “vulnerable,” respectively, due to a significant reduction in their natural populations. These species can be found in different environments within the Cerrado biome (CNCFLORA, 2013; Flora e Funga do Brasil, 2025b; WFO, 2025).

Furthermore, *Schomburgkia crispa* Lindl. is another native species of relevant ornamental potential, occurring in Cerrado areas (Sorgato et al., 2021; Flora e Funga do Brasil, 2025c). *S. crispa*, like all Orchidaceae, is listed in Appendix II of CITES (CITES, 2017), which requires trade control to prevent overexploitation.

Predatory collection and degradation of natural habitats have contributed significantly to the population decline of various species, jeopardizing their in situ conservation and highlighting the need for sustainable production and conservation strategies. Facing these challenges, ex situ propagation strategies, particularly through in vitro cultivation, represent viable and sustainable alternatives for the conservation of these species (Chokheli et al., 2020; Santos et al., 2021).

Various culture media can be used in the in vitro propagation of orchids, with the MS medium, proposed by Murashige & Skoog (1962), being widely employed for providing essential nutrients for the initial development of plantlets (Handayani et al., 2023). However, the use of alternative media supplemented with organic compounds, such as banana pulp and coconut water, has proven to be a viable strategy for cultivating native orchids, as these materials contain vitamins, minerals, and growth regulators, in addition to having the potential to reduce costs and optimize micropropagation protocols (Machado & Zamarian, 2020; Freitas et al., 2021; Tuyekar et al., 2021; Ramos et al., 2024; Nunes et al., 2025).

In addition to the nutrient medium composition, the sealing system used during in vitro cultivation directly influences plantlet development. Hermetically sealed flasks limit gas exchange and maintain plantlets under predominantly heterotrophic conditions, which can restrict their growth and hinder the transition to the ex vitro environment. To overcome this limitation, systems that allow gas exchange between the internal and external environments of the flask, using materials such as cotton or permeable

filters, have been employed with the aim of reducing ethylene accumulation and stimulating plantlet development during in vitro cultivation (Freitas et al., 2021; Souza et al., 2023; Mawardi et al., 2024).

Therefore, the association between cultivation systems that favor gas exchange and medium supplementation with organic compounds can represent an efficient strategy for the in vitro growth and subsequent ex vitro establishment of native Cerrado orchids. Thus, this study aimed to evaluate the in vitro growth and ex vitro establishment of *C. nobilior*, *C. walkeriana*, and *S. crispa*, cultivated in media supplemented with organic compounds in a micropropagation system with gas exchange, aiming to contribute to conservation and sustainable production strategies for these species.

MATERIAL AND METHODS

The experiment was conducted at the Laboratory of in vitro Cultivation of Flowers and Ornamental Plants and in the nursery within the Horticulture area of the Faculty of Agrarian Sciences of the Federal University of Grande Dourados (UFGD, 22° 11' 53.2" S; 54° 56' 02.3" W, with altitude of 450 m), Dourados – MS, Brazil.

To produce the experimental material, seeds of *Cattleya nobilior* Rchb.f., *Cattleya walkeriana* Gardner, and *Schomburgkia crispa* Lindl., sourced from mature fruits of mother plants cultivated in the Orchidarium of the Faculty of Agrarian Sciences of UFGD, were subjected to asymbiotic germination, as described below. Initially, the seeds were subjected to the tetrazolium test. After confirming viability, 5 mg seed samples of each species were transferred to an aseptic environment and disinfested with 15 mL of 0.8% sodium hypochlorite solution for 5 min. Subsequently, they underwent a triple wash with distilled water. Immediately after, the suspension volume was made up to 50 mL with sterile distilled water for in vitro sowing.

In the first stage, for seed germination, half-strength MS (Murashige & Skoog, 1962) was used as the culture medium. The pH of the medium was measured and adjusted to 5.8, and 60 mL of the medium was dispensed into each 600 mL capacity culture flask before sterilization in an autoclave, performed at 121 °C for 20 min. Sowing was carried out using an automatic pipettor, inoculating 1.0 mL of the seed suspension (equivalent to 0.1 mg of seeds) per flask. The material was then kept in a growth room under controlled temperature, irradiance provided by 3000K LED lamps, and photoperiod (25 ± 2 °C; $86 \mu\text{mol m}^{-2} \text{s}^{-1}$; 16 hours, respectively) for 180 days, with a single subculture at 90 days into half-strength MS medium.

After 180 days, uniform plants approximately 2.0 ± 0.5 cm in height, with at least two roots and two leaves, and free of contamination, were selected and transferred under aseptic conditions to the following culture media: 1) Murashige & Skoog (MS); 2) MS + banana pulp (BP, 100 g L⁻¹ apple banana pulp, *Musa* spp.); 3) MS + fresh coconut water (CW, 100 mL L⁻¹, extracted from green fruits approximately 6-8 months old); 4) MS + banana flour (BF, 25 g L⁻¹, Natural Life®, Brazil);

5) MS + BP (100 g L⁻¹) + CW (100 mL L⁻¹); and 6) MS + BF (25 g L⁻¹) + CW (100 mL L⁻¹). The concentrations of organic supplements were adapted based on previous protocols established by Castillo-Pérez et al. (2021) and Freitas et al. (2021).

The apple banana pulp used showed the following chemical composition per kilogram: 0.90 g N, 2.94 g P, 1.33 g K, 0.23 g Ca, 1.09 g Mg, 0.26 g S, 109.86 mg Fe, 14.19 mg Mn, 1.99 mg Cu, and 0.03 mg Zn, as determined by chemical analysis performed at the Soil Laboratory of the Faculty of Agrarian Sciences - UFGD. Additionally, bromatological analysis of the pulp revealed the following composition (%): moisture (74.43 ± 0.19), ash (0.57 ± 0.10), lipids (0.61 ± 0.09), proteins (1.37 ± 0.19), crude fiber (0.48 ± 0.03), starch (10.82 ± 3.18), and total reducing sugars (16.22 ± 2.84), determined according to official analytical methods (AOAC, 2005; Zenebon et al., 2008). The physicochemical characteristics of the green coconut water included (per 100 mL): 152.00 mg K, 31.95 mg Ca, 13.93 mg Mg, 7.67 mg Na, 3.31 mg P, 0.19 mg Mn, and 0.14 mg Fe, with an electrical conductivity of 4.82 mS cm⁻¹ (Silva et al., 2009). Additionally, according to the manufacturer's nutritional information, the commercial banana flour (Natural Life®, Brazil) contained (per 100 g): 50 g of carbohydrates, 5.0 g of proteins, 34 g of dietary fiber, and 1353 mg of potassium, being free of sodium and fats. The experimental design was completely randomized, consisting of the six culture media treatments described above, with five replications of one flask each.

All media were prepared without sucrose addition, as the organic supplements used provide sufficient carbohydrate sources and the gas exchange system stimulates photoautotrophic metabolism, reducing dependence on exogenous carbon (Soares et al., 2023). The media were solidified with 6.0 g L⁻¹ of bacteriological agar (Himedia®, India). The pH of the medium was measured and adjusted using KOH (0.1 M) to reach 5.8, and then 60 mL of the tested medium were dispensed into each 600 mL flask. These flasks were sterilized in an autoclave at 121 °C (1.1 atm pressure) for 20 min. After the media reached room temperature, the flasks were transferred to a sterile environment.

For each species studied, six plants were inoculated per culture flask. Subsequently, the flasks were sealed with polyvinyl chloride (PVC) film provided with a cotton filter (gas exchange micropropagation system). This system was adopted as a fixed cultivation condition for all treatments, rather than an experimental variable, based on previous studies demonstrating its superiority for orchid micropropagation (Soares et al., 2023). The material was then placed in a growth room with controlled photoperiod and temperature (16 hours; 25 ± 2 °C, respectively) and irradiance supplied by a 3000K LED lamp (86 µmol m⁻² s⁻¹), remaining under these conditions for 180 days without subculturing.

Next, the plants were removed from the culture flasks and washed under running water to completely remove the medium. Biometric evaluations were performed as follows: leaf number (LN), root number (RN), and shoot number (SN) were determined by manual counting; and the length of the longest leaf (LL), length of the longest root (LR), plant

height (PH), and pseudobulb diameter (PD) (except for *S. crispera*) were measured using a digital caliper (mm). Total fresh mass (FM) was obtained using an analytical balance with 0.0001 g precision. After evaluations, the treatments were photographed with a camera coupled to a mini photographic studio.

After these evaluations, all plants were transferred to an ex vitro environment. Transparent polypropylene containers with a capacity of 1 dm³ (20 × 10 × 5 cm) were used, equipped with holes at the base for substrate drainage and on the lid for gas exchange. One-third of their volume was filled with pink sphagnum (Agrolink, Holambra-SP) + coconut fiber (Golden-Mix Chips, Amafibra) (1/2, v:v).

After planting, the plants were allocated to an orchidarium in a nursery covered by two layers of 50% shade netting, providing an irradiance of 235 µmol m⁻² s⁻¹, under average temperature and relative humidity conditions of 22.6 ± 5 °C and 73.9 ± 10%, respectively. Irrigation was performed by micro-sprinklers positioned one meter above the plants, totaling a water depth of 1 mm per day, remaining under these conditions for 180 days. The containers remained covered during the first 15 days of the acclimatization period, for pre-acclimatization, and were then opened.

After 180 days of acclimatization, the plants were removed from the containers and washed under running water to completely remove the substrate. Subsequently, they were evaluated for survival (SURV) and the increment of the same initial characteristics (LN, RN, LL, LR, PH, PD, SN, and FM) was calculated. To investigate the hypothesis of increased plant growth during the ex vitro phase, according to the treatments they were exposed to in the in vitro phase, their increments (I) were calculated relative to the initial values using the expression described by Ribeiro et al. (2019), $I = (VF - VI)$, where VI is the value of the characteristic before the plant was acclimatized and VF is the value of the same characteristic after the ex vitro period. Their values were expressed as percentages and subjected to analysis of variance.

For each species studied, a separate statistical analysis was performed due to their distinct morphological characteristics and specific growth rates. The biometric evaluations were recorded individually per plant, the average of the six observational units (plants) was calculated to represent the single value of the respective flask (experimental unit) for the statistical analysis. For this analysis, continuous variables (LL, LR, PH, PD, and FM) were subjected to analysis of variance without any transformation. Because discrete count variables (LN, RN, and SN) typically show non-normal distribution and high coefficients of variation in micropropagation studies, data for these specific variables were transformed to $\sqrt{(x+1)}$ prior to the ANOVA to meet the assumptions of normality and homoscedasticity. All analyses were performed using the SISVAR software (Ferreira, 2011). When significant, means were compared by the Scott-Knott test ($p \leq 0.05$). To facilitate biological interpretation, the mean values presented in all tables correspond to the original non-transformed data, followed by the statistical letters derived from the transformed data analysis.

RESULTS AND DISCUSSION

A significant effect of culture media ($p \leq 0.05$) was observed for all evaluated characteristics in the three species studied. *C. nobilior* plants showed higher LN (15.13) and RN (14.43) when cultivated in MS + BP + CW medium. For LL, the highest values were obtained with MS + BP medium, not differing statistically from MS + BP + CW and MS + BF + CW media (30.79, 28.57, and 28.88 mm, respectively). Regarding LR and PH, MS + BP, MS + BF, and MS + BP + CW media showed the highest means compared to the other evaluated media and were statistically similar (46.45, 53.34, and 51.44 mm, and 81.47, 83.74, and 81.01 mm, respectively). For PD, the highest mean occurred for plants cultivated in MS + BP + CW medium (3.29 mm), but without significant difference from MS + BF medium (3.00 mm). On the other hand, when MS + BP + CW medium was used, the highest values for SN (5.00) and FM (1.67 g) were obtained (Table 1).

In *C. walkeriana*, higher means for LN (14.25, 17.00, and 15.66), PD (3.05, 3.16, and 3.70 mm), SN (3.75, 3.80, and 4.66), and FM (1.21, 1.41, and 1.63 g) were observed when plants were cultivated in MS + BP, MS + CW, and MS + BF + CW media, respectively, with all these media being statistically similar to each other. For the LL variable, the highest mean was obtained for the MS + BP culture medium (37.53 mm), but without significant difference from the MS + BP + CW medium (37.30 mm). For RN, the highest means

were observed for MS + CW and MS + BF + CW media (10.00 and 8.33, respectively). As for LR and PH, the highest values were observed when plants were cultivated in MS, MS + BP, MS + BP + CW and MS + BF + CW media with no statistical difference (38.84; 36.60; 43.48 and 35.97 mm for root length) and (63.32, 77.65, 80.18, and 66.25 mm for height, Table 2).

For *S. crispa*, the analyzed results demonstrated that for LN, the highest means were obtained in MS + BP, MS + BP + CW, and MS + BF + CW media (37.00, 28.88, and 23.96, respectively). Regarding LL, LR, PH, and FM, the highest values were observed in MS + BP and MS + BP + CW media (29.33 and 20.63 mm, 28.20 and 28.84 mm, 63.31 and 55.47 mm, and 1.04 and 0.90 g, respectively). On the other hand, for RN, the highest values were observed in MS, MS + BP, and MS + BP + CW media (10.96, 13.78, and 12.92, respectively), and for SN, MS + BP, MS + BF, and MS + BP + CW culture media promoted the highest means (6.17, 4.14, and 5.36, respectively), being statistically similar (Table 3).

Overall, it was observed that the organic supplements added to the media were beneficial for the in vitro cultivation of the three orchid species studied. For *C. nobilior*, the MS + BP + CW medium stood out, while for *C. walkeriana*, the MS + BP and MS + BF + CW media were prominent, and for *S. crispa*, the MS + BP medium was outstanding. Considering the results obtained regarding the effect of the media on the cultivation of different species of the Orchidaceae family, it can

Table 1. Leaf number (LN), length of the longest leaf (LL) (mm), root number (RN), length of the longest root (LR) (mm), plant height (PH) (mm), pseudobulb diameter (PD) (mm), shoot number (SN) and fresh mass (FM) (g) of *Cattleya nobilior* Rchb.f., as a function of different culture media (MS; MS + banana pulp (BP); MS + coconut water (CW); MS + banana flour (BF); MS + BP + CW and MS + BF + CW) after 180 days of in vitro cultivation

Treatments	Characteristics							
	LN	LL	RN	LR	PH	PD	SN	FM
MS	7.11 c	20.10 c	7.84 c	43.31 b	63.63 b	1.12 c	1.47 d	0.64 c
MS + BP	6.20 c	30.79 a	7.44 c	46.45 a	81.47 a	1.64 b	1.56 d	0.92 b
MS + CW	10.55 b	20.86 c	8.24 c	34.17 b	56.84 b	1.36 c	2.16 c	0.64 c
MS + BF	9.89 b	24.16 b	9.61 b	53.34 a	83.74 a	3.00 a	3.24 b	1.00 b
MS + BP + CW	15.13 a	28.57 a	14.43 a	51.44 a	81.01 a	3.29 a	5.00 a	1.67 a
MS + BF + CW	7.52 c	28.88 a	6.52 c	39.25 c	69.85 b	1.71 b	1.80 d	0.84 b
CV%	18.99	11.70	15.25	18.97	13.72	9.75	12.67	9.52

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test ($p \leq 0.05$); CV - Coefficient of variation

Table 2. Leaf number (LN), length of the longest leaf (LL, in mm), root number (RN), length of the longest root (LR, in mm), plant height (PH, in mm), pseudobulb diameter (PD, in mm), shoot number (SN) and fresh mass (FM, in g) of *Cattleya walkeriana* Gardner, as a function of different culture media (MS; MS + banana pulp (BP); MS + coconut water (CW); MS + banana flour (BF); MS + BP + CW, and MS + BF + CW) after 180 days of in vitro cultivation

Media	Characteristics							
	LN	LL	RN	LR	PH	PD	SN	FM
MS	7.33 b	19.97 b	6.33 b	38.84 a	63.32 a	2.65 b	2.66 b	0.81 b
MS + BP	14.25 a	37.53 a	6.50 b	36.60 a	77.65 a	3.05 a	3.75 a	1.21 a
MS + CW	17.00 a	23.03 b	10.00 a	27.35 b	53.67 b	3.16 a	3.80 a	1.41 a
MS + BF	7.80 b	18.06 b	4.60 b	20.98 b	38.47 b	2.12 b	1.40 b	0.56 b
MS + BP + CW	9.20 b	37.30 a	5.20 b	43.48 a	80.18 a	1.84 b	2.00 b	0.95 b
MS + BF + CW	15.66 a	25.57 b	8.33 a	35.97 a	66.25 a	3.70 a	4.66 a	1.63 a
CV%	16.36	13.75	10.96	12.92	11.41	7.72	11.56	9.74

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test ($p \leq 0.05$); CV - Coefficient of variation

be inferred that each genotype responds differently. Therefore, in addition to the culture medium formulation, the nutritional and endogenous hormonal status of each study material must also be considered, highlighting the need for specific studies in relation to the genotype of interest (Costa et al., 2024).

The addition of organic supplements, such as banana pulp, to the culture medium has been shown to be beneficial for the in vitro growth of various orchid species, such as *Dendrobium nobile* Lindl. (Su et al., 2012), *Laeliocattleya* (*Hadrolaelia purpurata* Lindl. x *Cattleya intermedia* Graham ex Hook.) (Gonçalves et al., 2016), *Epidendrum nocturnum* Jacq. (Silva et al., 2016), *C. nobilior* Rchb.f. (Freitas et al., 2021; Nunes et al., 2025), and *C. walkeriana* Gardner (Ramos et al., 2024). Banana pulp contains sugars, potassium, vitamins, and compounds with cytokinin- and auxin-like activity, which collectively may stimulate in vitro plant development (Dolce et al., 2020; Stefano et al., 2022). The vegetative growth observed in media containing banana pulp and flour may be related to their characterized chemical profiles. The high carbohydrate content (50% in the flour, and approximately 27% in the pulp) likely acted as an essential carbon source for biomass production, while the potassium levels (1.33 g kg⁻¹ in the pulp and 1353 mg 100 g⁻¹ in the flour) are known to be fundamental for osmoregulation, enzyme activation, and cell expansion, which could explain the higher biometric values obtained. Consequently, this complex nutritional matrix results in plants with desirable characteristics for ex vitro cultivation, as evidenced in Figure 1 and corroborated by Freitas et al. (2021).

Specifically for *C. nobilior*, the combined supplementation of banana pulp and coconut water (MS + BP + CW) resulted in the highest values for leaf and root numbers (Table 1). This superior combined effect can be attributed to the diverse composition of these organic additives. Coconut water is a rich source of mineral salts, sugars, and growth regulators such as cytokinins, which favor plant development (Tuyekar et al., 2021), while banana pulp provides potassium, vitamins, and natural auxins that support vigorous vegetative growth (Dolce et al., 2020; Stefano et al., 2022). Regarding LL, media containing banana pulp (alone or combined) promoted greater elongation, corroborating Freitas et al. (2021). Additionally, this enhanced growth was reflected in the highest accumulation of FM and SN. The carbohydrates

in the banana pulp possibly served as an essential carbon source for biomass production, while the hormonal balance provided by the coconut water may have stimulated lateral bud break, favoring shoot proliferation (Tuyekar et al., 2021).

Complementing these results, organic supplementation also had a positive effect on *C. walkeriana*. The addition of banana pulp and its combination with coconut water promoted the development of vigorous plants, with higher means for height, root length, and fresh mass (Table 2). These results corroborate Ramos et al. (2024), who reported that enriched culture media significantly favored the in vitro growth of *C. walkeriana*. Additionally, the positive influence of coconut water observed in this study aligns with findings by Machado & Zamarian (2020), who reported that adding coconut water to the MS medium enhanced the growth of *Lycaste* sp. explants. The beneficial action of these supplements reinforces the importance of their complex nutritional matrix rich in carbohydrates, potassium, and growth-promoting substances for the establishment of seedlings (Tuyekar et al., 2021).

Regarding *S. crispa*, the addition of banana pulp alone proved to be the most effective and economical treatment, promoting the highest means for all biometric variables analyzed (statistically equivalent to MS + BP + CW, but without the need for coconut water) (Table 3). This species demonstrated a strong preference for the nutritional complex of banana pulp, which likely supplied the demand for carbohydrates and potassium required for its growth habit. The positive influence of banana pulp as a single supplement suggests that it effectively supports the nutritional demands of *S. crispa*, optimizing costs while acting as a potent growth promoter as observed in other epiphytic orchids (Dolce et al., 2020).

Furthermore, the addition of organic supplements to the culture medium, associated with a sealing system that allows gas exchange, also positively influenced the in vitro cultivation of the studied species. These results may be related to the increased photochemical efficiency and photosynthetic carbon assimilation, which results in plants with a more efficient metabolism and greater robustness (Fritsche et al., 2022; Soares et al., 2023). Additionally, plants cultivated with gas exchange exhibit structures with greater length and diameter, such as more developed pseudobulbs, leaves, and roots, which are suitable characteristics for ex vitro cultivation. This growth is primarily influenced by the elimination of ethylene

Table 3. Leaf number (LN), length of the longest leaf (LL, in mm), root number (RN), length of the longest root (LR, in mm), plant height (PH, in mm), shoot number (SN) and fresh mass (FM, in g) of *Schomburgkia crispa* Lindl., as a function of different culture media (MS; MS + banana pulp (BP); MS + coconut water (CW); MS + banana flour (BF); MS + BP + CW, and MS + BF + CW) after 180 days of in vitro cultivation

Media	Characteristics						
	LN	LL	RN	LR	PH	SN	FM
MS	25.52 b	17.25 b	10.96 a	16.66 b	41.69 b	3.00 b	0.48 b
MS + BP	37.00 a	29.33 a	13.78 a	28.20 a	63.31 a	6.17 a	1.04 a
MS + CW	17.56 b	18.07 b	9.48 b	20.45 b	43.84 b	2.04 b	0.41 b
MS + BF	16.25 b	17.03 b	6.40 c	15.18 b	34.56 b	4.14 a	0.47 b
MS + BP + CW	28.88 a	20.63 a	12.92 a	28.84 a	55.47 a	5.36 a	0.90 a
MS + BF + CW	23.96 a	13.99 b	9.40 b	17.82 b	40.61 b	2.80 b	0.52 b
CV%	27.01	24.17	19.37	19.48	19.46	22.79	8.92

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test ($p \leq 0.05$); CV - Coefficient of variation

to the external environment of the flask, thus contributing to the in vitro growth of the species (Freitas et al., 2021; Endres Júnior et al., 2024).

As observed in this experiment, each species showed different responses to the treatments used. According to Costa et al. (2024), the response of orchid genotypes to culture media is significantly influenced by their unique genetic and hormonal profiles, and each genotype shows distinct nutrient requirements and hormonal balance, requiring personalized studies to optimize growth conditions, with this specificity being crucial for improving characteristics such as flower development, longevity, and overall health.

Therefore, the results obtained in this work allow us to infer that the different compositions of the nutritive medium influenced the growth and development of *C. nobilior*, *C. walkeriana*, and *S. crispa*, with each studied species showing different responses according to the medium used, as can be seen in Figure 1.

After 180 days of plant acclimatization, analysis of variance demonstrated a significant effect among the culture media for all evaluated characteristics in all species studied.

Regarding the %SURV of *C. nobilior* plants, the best result was observed when the plants were previously cultivated in MS

+ BP + CW medium, promoting 100% survival. For LN and RN, the highest values were found when MS + BP medium was used (12.38 and 5.00, respectively). For LL, a greater increase was observed in plants that originated from in vitro cultivation in MS medium (34.63%). Regarding the PH and FM variables, the use of MS + CW medium in the in vitro cultivation of *C. nobilior* led to greater increases after acclimatization (1.08 and 34.71%). For LR and SN, greater increments were observed in those obtained through previous cultivation in MS + BF + CW medium (57.55 and 40.00%). For PD, the highest mean was found when MS + BP medium was used (60.54%), but it did not differ statistically from MS medium (58.76%) (Table 4).

For *C. walkeriana*, the MS + CW medium was the only treatment that resulted in plant survival (54.54%) after acclimatization, whereas plants from all other media did not survive. Consequently, the highest increments for all biometric variables were recorded exclusively for plants originating from this medium: LN (2.50), LL (47.29 mm), RN (3.33), LR (0.10 mm), PH (0.48 mm), PD (0.96 mm), SN (6.66), and FM (6.81 g) (Table 5).

Regarding the *S. crispa* species, the highest %SURV was observed when plants were previously cultivated in MS +

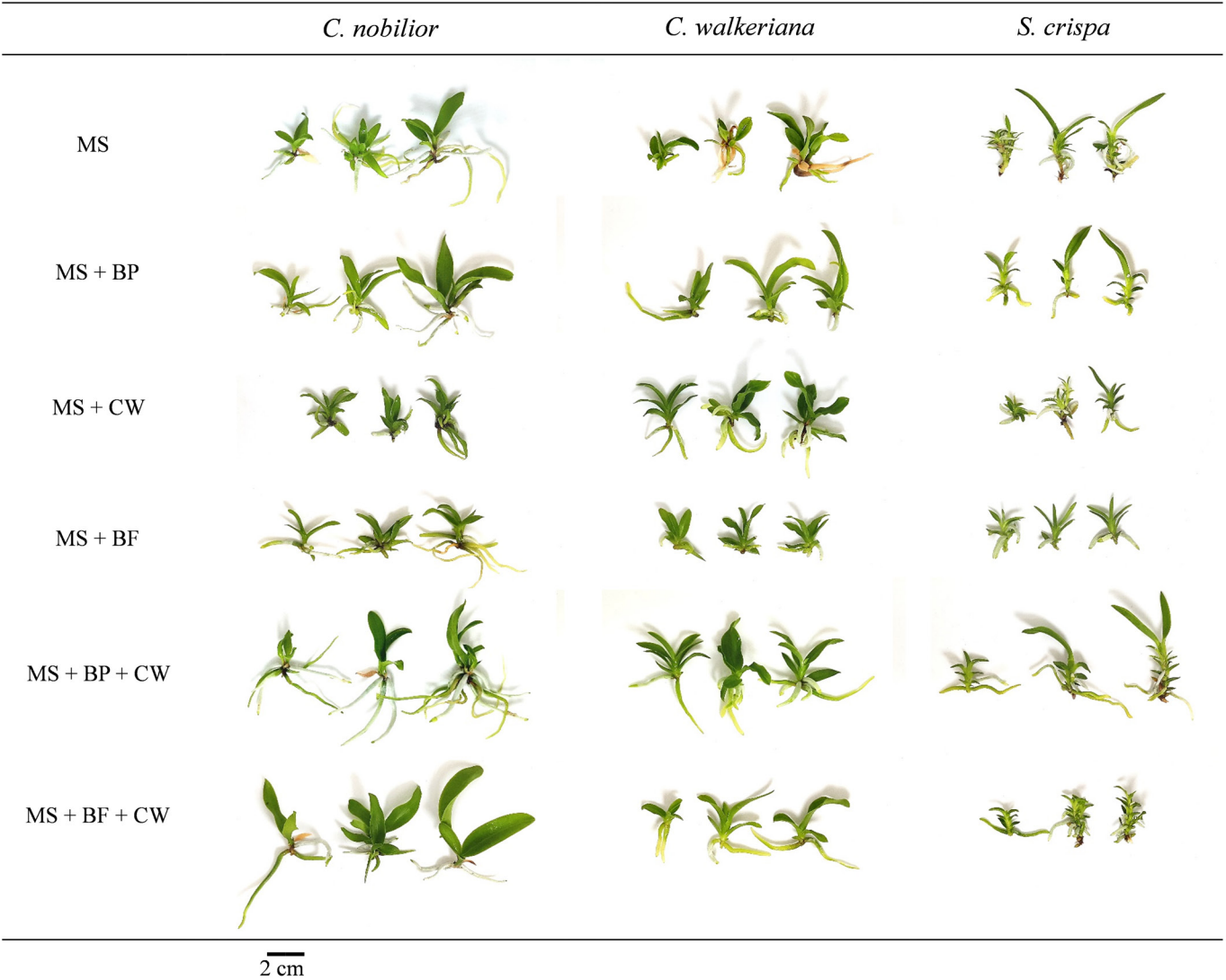


Figure 1. Plants of *Cattleya nobilior* Rchb.f., *Cattleya walkeriana* Gardner, and *Schomburgkia crispa* Lindl., as a function of different culture media (MS; MS + banana pulp (BP); MS + coconut water (CW); MS + banana flour (BF); MS + BP + CW and MS + BF + CW) after 180 days of in vitro cultivation

BP medium (57.89%). As for the other evaluated variables, the highest increment values were found when plants were cultivated in vitro in MS + BF + CW medium (Table 6).

Upon observing the results obtained in the acclimatization phase, it was possible to verify that, for *C. nobilior* and *S. crispa* species, plants that showed better results in previous in vitro cultivation did not increase their growth rate when transferred to the ex vitro environment. This may have occurred because the plants had already begun their hardening process during in vitro cultivation. These results corroborate those of Ribeiro et al. (2019), who report that *Dendrobium bigibbum* Lindl. plants

cultivated in a natural ventilation system also initiated their hardening process during in vitro cultivation, due to reduced in vitro humidity and increased aeration. On the other hand, the *C. nobilior* and *S. crispa* plants that developed the most during the acclimatization phase were those previously cultivated in MS + BP and MS + BF + CW media, respectively, which had a lower survival rate but continued their development.

Regarding the *C. walkeriana* species, previous cultivation in medium supplemented with coconut water (MS + CW) was the critical factor determining survival in the ex vitro phase. While plants from all other treatments failed to survive, possibly due

Table 4. Survival percentage (%SURV) and increments (%) of leaf number (LN), longest leaf length (LL), root number (RN), longest root length (LR), plant height (PH), pseudobulb diameter (PD), shoot number (SN) and fresh mass (FM) of *Cattleya nobilior* Rchb.f., as a function of previous cultivation in different media (MS; MS + banana pulp (BP); MS + coconut water (CW); MS + banana flour (BF); MS + BP + CW and MS + BF + CW) after 180 days of ex vitro cultivation

Media	Characteristics								
	%SURV	LN	LL	RN	LR	PH	PD	SN	FM
MS	13.04 e	0.00 b	34.63 a	0.00 c	0.00 c	0.00 b	58.76 a	0.00 b	14.17 c
MS + BP	47.22 b	12.38 a	7.96 c	5.00 a	0.00 c	0.00 b	60.54 a	0.00 b	29.68 b
MS + CW	9.52 f	0.00 b	21.44 b	0.00 c	7.72 b	1.08 a	31.05 b	0.00 b	34.71 a
MS + BF	35.71 c	0.00 b	2.22 d	0.00 c	5.67 b	0.00 b	0.00 d	0.00 b	0.00 d
MS + BP + CW	100.00 a	0.00 b	0.08 d	0.00 c	0.00 c	0.00 b	20.77 c	0.00 b	0.00 d
MS +BF + CW	23.80 d	0.00 b	11.94 c	4.00 b	57.55 a	0.00 b	28.01 b	40.00 a	13.98 c
CV%	7.08	9.91	17.56	4.84	10.70	0.82	8.86	7.57	9.31

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test ($p \leq 0.05$); CV - Coefficient of variation

Table 5. Survival percentage (%SURV) and increments (%) of leaf number (LN), longest leaf length (LL), root number (RN), longest root length (LR), plant height (PH), pseudobulb diameter (PD), shoot number (SN) and fresh mass (FM) of *Cattleya walkeriana* Gardner, as a function of previous cultivation in different media (MS; MS + banana pulp (BP); MS + coconut water (CW); MS + banana flour (BF); MS + BP + CW and MS + BF + CW) after 180 days of ex vitro cultivation

Media	Characteristics								
	%SURV	LN	LL	RN	LR	PH	PD	SN	FM
MS	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
MS + BP	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
MS + CW	54.54 a	2.50 a	47.29 a	3.33 a	0.10 a	0.48 a	0.96 a	6.66 a	6.81 a
MS + BF	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
MS + BP + CW	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
MS +BF + CW	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
CV%	1.19	8.65	19.31	16.62	2.73	9.38	13.66	18.44	13.52

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test ($p \leq 0.05$); CV - Coefficient of variation

Table 6. Survival percentage (%SURV) and increments (%) of leaf number (LN), longest leaf length (LL), root number (RN), longest root length (LR), plant height (PH), shoot number (SN) and fresh mass (FM) of *Schomburgkia crispa* Lindl., as a function of previous cultivation in different media (MS; MS + banana pulp (BP); MS + coconut water (CW); MS + banana flour (BF); MS + BP + CW and MS + BF + CW) after 180 days of ex vitro cultivation

Media	Characteristics							
	%SURV	LN	LL	RN	LR	PH	SN	FM
MS	6.66 d	0.00 b	17.57 c	0.00 b	18.75 b	5.87 b	0.00 b	0.00 b
MS + BP	57.89 a	0.00 b	5.74 d	0.00 b	0.00 d	0.00 d	0.00 b	0.00 b
MS + CW	23.06 c	0.00 b	70.82 b	0.00 b	17.54 b	4.83 b	0.00 b	0.00 b
MS + BF	0.00 e	0.00 b	0.00 d	0.00 b	0.00 d	0.00 d	0.00 b	0.00 b
MS + BP + CW	50.00 b	0.00 b	70.72 b	0.00 b	5.97 c	2.40 c	0.00 b	0.00 b
MS +BF + CW	9.37 d	10.00 a	141.05 a	10.00 b	39.51 a	17.42 a	40.00 a	8.17 a
CV%	8.34	10.13	9.88	6.38	12.06	16.12	8.45	6.32

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test ($p \leq 0.05$); CV - Coefficient of variation

to a high sensitivity of this genotype to acclimatization stress or inadequate in vitro hardening, those from the MS + CW medium achieved a 54.54% survival rate. This protective effect might be associated with the characterized nutritive complex of the coconut water. In addition to natural cytokinins and antioxidants, it provides a highly available supply of essential minerals, notably potassium (152.00 mg/100 mL), calcium (31.95 mg/100 mL), and magnesium (13.93 mg/100 mL). This specific mineral matrix can potentially aid in maintaining cellular turgor, structural integrity, and photosynthetic efficiency, thereby helping to pre-condition the plantlets to withstand the severe abiotic stress of the ex vitro transition (Tuyekar et al., 2021).

In contrast, the mortality observed in other treatments suggests a genotype-dependent sensitivity, potentially linked to physiological disorders induced by the culture medium composition, such as toxicity arising from excessive nutrient accumulation or tissue hyperhydricity caused by the banana pulp, which may have compromised their ex vitro survival. Hyperhydricity, in particular, is a physiological disorder well-documented in the in vitro culture of plants, including orchids, known to severely compromise acclimatization (Hazarika, 2006). Furthermore, the successful establishment of plants from the MS + CW treatment underscores the role of root

functionality. Although these plants exhibited a lower root number in vitro compared to other treatments, their survival suggests that these roots were functionally better prepared for the transition, indicating that root quality is more decisive than absolute quantity. Therefore, further studies on the specific nutritional requirements of this species are suggested.

According to the results obtained in this study, the influence of culture media with organic compounds on plant development during the acclimatization phase was highly dependent on the species. For *C. nobilior* and *S. crispa*, media supplemented with banana pulp generally promoted superior plant development during acclimatization; for *C. walkeriana*, however, only coconut water supplementation ensured ex vitro survival, while banana-based media resulted in complete plant loss, underscoring the species-specific nature of these responses. These outcomes can be visually verified in Figure 2.

Overall, these findings underscore the highly species-specific responses to organic supplements. While these optimized protocols represent an important baseline for understanding the nutritional and physiological requirements of these orchids, further studies addressing scalability and production costs are necessary for large-scale commercial production or conservation and reintroduction programs.



Figure 2. Plants of *Cattleya nobilior* Rchb.f., *Cattleya walkeriana* Gardner, and *Schomburgkia crispa* Lindl., as a function of previous in vitro cultivation in different culture media (MS; MS + banana pulp (BP); MS + coconut water (CW); MS + banana flour (BF); MS + BP + CW and MS + BF + CW) after 180 days of acclimatization

CONCLUSIONS

1. For *C. nobilior*, Murashige & Skoog (MS) + banana pulp (BP) + fresh coconut water (CW) medium is recommended for in vitro growth, producing the highest values for leaf number, root number, shoot number, and fresh mass. For ex vitro establishment, the same medium ensured 100% survival; however, plants with the highest post-acclimatization growth increments were obtained from MS + CW (fresh mass and height) and MS + banana flour (BF) + CW (root length and shoot number), suggesting that medium selection should be guided by the specific goal of the propagation program.

2. For *C. walkeriana*, MS + BF + CW medium is recommended for maximizing in vitro vegetative growth. However, for successful ex vitro establishment and survival, previous cultivation in MS + CW medium is strictly required, highlighting a critical distinction between in vitro proliferation and physiological readiness for acclimatization.

3. For *S. crispa*, in vitro cultivation in MS + BP medium favored both in vitro growth and plant acclimatization.

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