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BIOLOGY

Culture medium, sucrose and cytokinin: effects on the *in vitro* propagation of the orchid *Epidendrum carpophorum* Barb. Rodr

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ABSTRACT: *Epidendrum carpophorum* is an epiphytic orchid native to Brazil. This study investigated the influence of culture media and sucrose on protocorm development, and 6-benzylaminopurine concentrations on the micropropagation of this orchid. Closed, maturing capsules were used for seed germination, inoculated into flasks with MS and ½ MS culture media, sucrose concentrations (20 g L^{-1} and 30 g L^{-1}), and activated charcoal (2.5 g L^{-1}), resulting in eight treatments with five replicates. Protocorm development was assessed based on height, number of leaves, and number and length of roots, after 90 and 180 days. For micropropagation, shoot apices and nodal segments of *E. carpophorum* served as explants and were cultured in MS and ½ MS media with 6-benzylaminopurine concentrations of 0.0; 0.5; 1.0 mg L^{-1}. Parameters analyzed included height, number of shoots, and number and length of roots, after 90 days. Germination occurred after 60 days, with chlorophyllous protocorms. The ½ MS medium with 20 g L^{-1} sucrose was most effective for protocorm development after 180 days, with median values of 4 ± 0 and 0.6 ± 0.3 cm for root number and length, respectively, and 1.3 ± 0.0 cm and 3 ± 0 for seedling height and number of leaves, respectively. In micropropagation, neither the cytokinin concentrations nor culture media exhibited a significant effect. Results contributed to the development of optimized *in vitro* propagation protocols for this species.
Key words: protocorm, Orchidaceae, growth regulator, micropropagation.

Meio de cultura, sacarose e citocinina: efeitos na propagação *in vitro* da orquídea *Epidendrum carpophorum* Barb. Rodr

RESUMO: *Epidendrum carpophorum* é uma orquídea epífita nativa no Brasil. Este estudo investigou a influência de meios de cultura e sacarose no desenvolvimento dos protocormos e concentrações de 6-benzilaminopurina na micropropagação dessa orquídea. Utilizaram-se cápsulas fechadas em maturação para germinação das sementes, inoculadas em frascos com meios de cultura MS e ½ MS, concentrações de sacarose (20 g L^{-1} e 30 g L^{-1}) e carvão ativado (2,5 g L^{-1}), resultando em oito tratamentos com cinco repetições. O desenvolvimento dos protocormos foi avaliado em termos de altura, número de folhas, número e comprimento de raiz, após 90 e 180 dias. Para a micropropagação, ápices caulinares e segmentos nodais de *E. carpophorum* serviram como explantes e cultivados em meio MS e ½ MS com concentrações de 6-benzilaminopurina (0,0; 0,5; 1,0 mg L^{-1}), e os parâmetros analisados incluíram altura, número de brotações, número e comprimento de raiz, após 90 dias. A germinação ocorreu após 60 dias, com protocormos clorofilados. O meio ½ MS + 20 g L^{-1} de sacarose foi o mais eficaz para o desenvolvimento dos protocormos após 180 dias, destacando-se pelos valores medianos de 4 ± 0 e 0,6 ± 0,3 cm para o número e comprimento de raiz, respectivamente e, 1,3 ± 0,0 cm e 3 ± 0 para altura de plântulas e número de folhas, respectivamente. Na micropropagação, as concentrações da citocinina e os meios de cultivo testados não mostraram influência significativa. Os resultados contribuem para o desenvolvimento de protocolos otimizados para a propagação *in vitro* da espécie.
Palavras-chave: protocormo, Orchidaceae, regulador de crescimento, micropropagação.

INTRODUCTION

The Orchidaceae family has the highest number of species among angiosperms worldwide. Their significant economic importance and increasing medicinal use (CAKOVA et al., 2017) have attracted the interest of botanists and floriculture collectors both nationally and internationally. This widespread interest stems from the broad potential applications of orchids across different sectors, including the

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food industry, breeding programs, and cosmetics. Furthermore, the distinct physiological requirements of different orchid species contributed to their adaptability to diverse environments, reflecting their wide genetic variability (MENEZES-SÁ et al., 2019).

Among the genera within the Orchidaceae family, *Epidendrum* L. stands out for encompassing species with different growth habits, such as epiphytes, lithophytes and terrestrial plants (SANTOS & SILVA, 2020; GOMES et al., 2021). The species *Epidendrum carpophorum* Barb. Rodr. is epiphytic, found predominantly in tropical regions, and morphologically characterized by cream-colored to whitish flowers, lanceolate sepals and petals, a trilobed labellum, and a short peduncle (SILVA et al., 2022). In Brazil, *E. carpophorum* is primarily found in the Amazon, Caatinga, and Atlantic Forest biomes (RÊGO & AZEVEDO, 2017). In the state of Pará, in the Amazon region, it can be found in riparian and upland teras firmeforests (SILVA & SILVA, 2010).

The propagation of *E. carpophorum* in its native habitat is threatened by extractivism, predatory collection, and habitat destruction caused by deforestation or adverse climate change (ABREU et al., 2018).

Orchids generally have a lower germination rate under natural conditions. This may be related to the minute size of their seeds (0.05-6.0 mm in length and 0.01-0.9 mm in diameter) (PARAMANIK et al., 2021). This trait, combined with the absence of nutrient-rich tissues such as the endosperm, and the fact that many species require association with mycorrhizal fungi, hinders seed dispersal and germination, since specific substrates and optimal conditions are needed for germination (VALENCIA-GLUSHCHENKO et al., 2024). These factors underscore the need for conservation strategies that ensure the survival of these plants.

In vitro cultivation has emerged as an important tool for orchid conservation, enabling studies ranging from physiological responses to *ex situ* conservation methods (SOARES et al., 2023; KALADHARAN et al., 2024). It can also be used in native species reintroduction programs through asymbiotic seed germination (LAKSHMI et al., 2023). Furthermore, techniques such as micropropagation significantly contribute to the rapid reproduction and multiplication of these plants, especially when the goal is to preserve individuals with specific traits of interest (YAM & ARDITTI, 2018).

The composition of the culture medium influences *in vitro* cultivation (REDDY, 2016), since the presence of macro- and micronutrients, sugars, vitamins, plant growth regulators (auxins, cytokinins, gibberellins, among others), and activated charcoal reduces oxidation and promotes *in vitro* rooting (SORGATO et al., 2020; MERCADO & JAIMES, 2022).

The most used culture medium for orchids is MS medium (MURASHIGE & SKOOG, 1962) and its variation (½ MS), with adjustments depending on the composition and purpose of the culture (SILVA et al., 2017). Therefore, this study assessed the influence of two culture media (MS and ½ MS), sucrose, and cytokinin on the early development of protocorms and the micropropagation of the orchid *Epidendrum carpophorum* under *in vitro* conditions.

MATERIALS AND METHODS

Plant material collection and disinfection

Experiments were conducted at the Biotechnology Laboratory of the Universidade Federal Rural da Amazônia, Campus of Belém, Pará, Brazil. Two closed capsules were collected from a single, four-year-old adult *Epidendrum carpophorum* Barb. Rodr. plant. The plant was taxonomically identified from herbarium specimens prepared by researchers at the Orquidário do Bosque Rodrigues Alves – Jardim Zoobotânico da Amazônia, Belém, Pará (coordinates: 1°25'44" S, 48°27'40" W). The mother plant was maintained in a greenhouse with 70% shading, no artificial light, and daily manual irrigation. The brown cross-pollinated capsules were collected at the maturing stage, six months after pollination and before dehiscence (Figure 1A). After collection, the fruits (capsules) were stored in paper envelopes in a refrigerator at 4 ºC for two weeks until the onset of the *in vitro* germination assay.

The following steps were performed for the asepsis and disinfection of the capsules: a) washing under running water with neutral detergent; b) soaking in 70% (v/v) ethyl alcohol solution for two minutes; c) treatment with a 2.5% (v/v) sodium hypochlorite solution for thirty minutes; and d) rinsing three times with distilled water sterilized in an autoclave at 120 ºC and 1 atm pressure for twenty minutes. The capsules were then opened, and small portions of seeds were removed and germinated in 150 mL glass flasks sealed with cling film, containing the culture media. All of these procedures were carried out under a laminar flow hood.

Asymbiotic germination

A completely randomized design was used, with eight treatments and five replicates, each replicate consisting of a single flask containing the

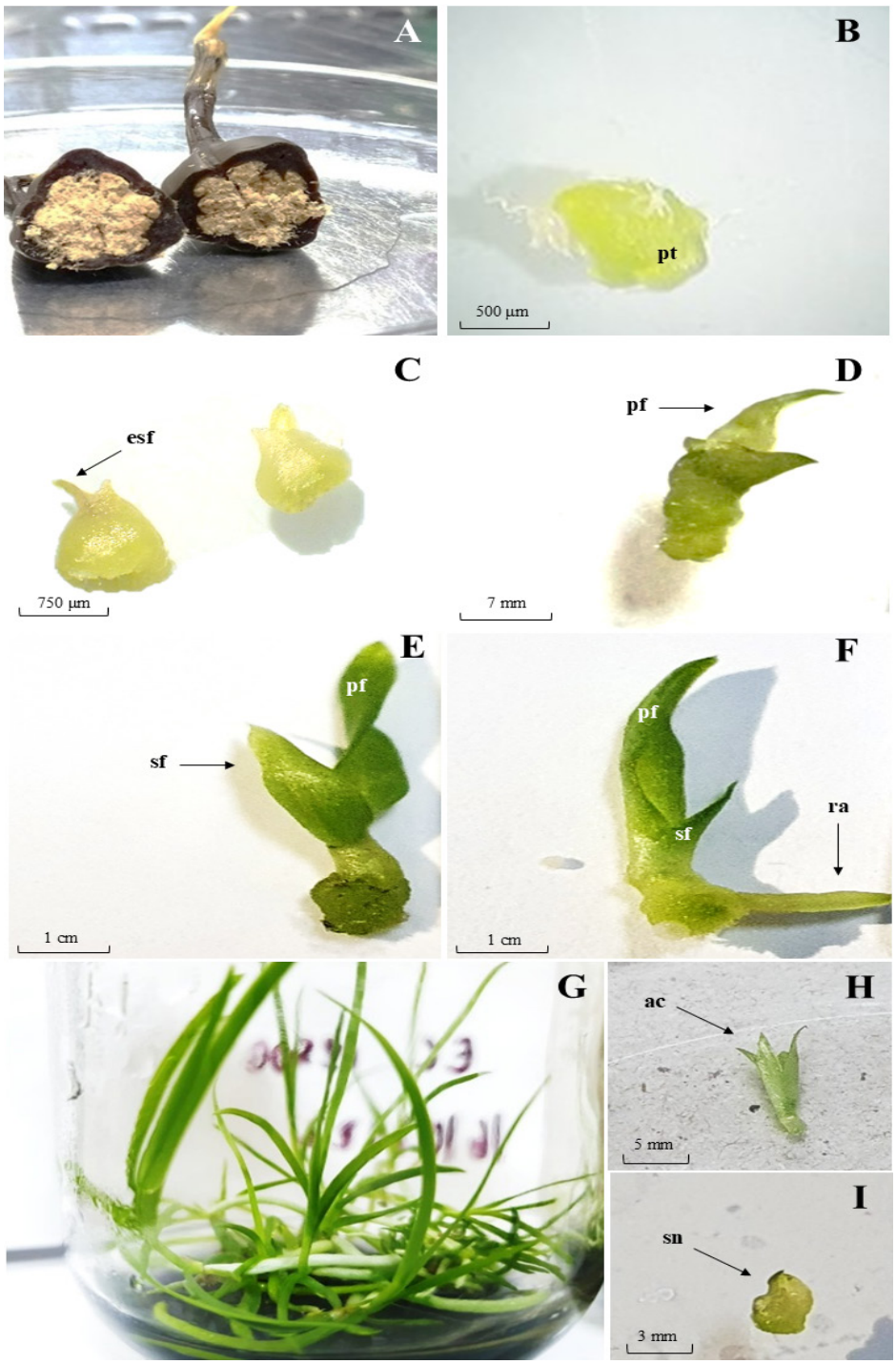

Figure 1 - *In vitro* propagation and early developmental stages of *Epidendrum carpophorum* Barb. Rodr. (A) Capsules containing *Epidendrum carpophorum* seeds; (B) Chlorophyllous embryo at the onset of germination, 30 days after sowing (DAS); (C) Swollen protocorm beginning the development of leaf primordia at 60 DAS (stage 1); (D) Formation of the first leaf at 60 DAS (stage 2);(E) Plantlet with two leaves at 90 DAS (stage 3);(F) Root formation at 180 DAS (stage 4);(G) *Epidendrum carpophorum* plantlets at 280 DAS grown in ½ MS medium with 30 g L^{-1} sucrose, used as donor plants for (H) Shoot apex explants and(I) Nodal segment for *in vitro* micropropagation.pt - protocorm; esf - leaflet-like structure; pf - first leaf; sf - second leaf; ra - root; ac - shoot apex; sn - nodal segment.

seeds. The culture media tested were MS, MS with activated charcoal (2.5 g L^{-1}), MS with half-strength macronutrients (½ MS), and ½ MS with activated charcoal (2.5 g L^{-1}). Additionally, two sucrose concentrations (20 g L^{-1} and 30 g L^{-1}) were tested (Table 1). All media were supplemented with 2.0 g L^{-1} of Phytagel.The pH was adjusted to 5.8 before adding the Phytagel. Next, the medium was homogenized using a magnetic stirrer with heating to dissolve the gelling agent, and 30 mL of each medium was poured into 150 mL glass flasks, which were then sealed with plastic film. Sterilization was carried out in an autoclave at 121 ºC and 1 atm for 20 minutes. The culture media were maintained in a growth room under a 16-hour photoperiod using LED lights with a photon flux density of 25 µmol m^{-2} s^{-1} at a temperature of 27 ± 2 ºC.

In vitro protocorm development

After 60 days of *in vitro* germination, five protocorms were removed from each sample and isolated into new flasks containing the same culture media used in the initial seed germination treatments (Table 1). They were then maintained under the same environmental conditions as the previous experiment. A completely randomized design with eight treatments and five replicates was used. Each replicate consisted of one flask containing five protocorms. At 90 and 180 days, the following morphological variables were assessed: plantlet height (cm), number of leaves, number of roots, and length of the longest root (cm). In treatments where most plantlets died or failed to develop the morphological traits evaluated during the experiment, the statistical analysis returned a value of 0.

The different stages of early protocorm development up to plantlet formation were observed, following the methodology proposed by SUZUKI et al. (2009): stage 1: swollen and chlorophyllous protocorm initiating leaf primordia; stage 2: protocorm with the first developed leaf; stage 3: plantlet with two or more leaves; stage 4: plantlet with leaves and roots.

In vitro micropropagation

For this experiment, shoot apices (0.2 cm in diameter and 0.5 cm in length) and nodal segments (5 mm in length) were used as explants, obtained from *in vitro*-grownplantlets cultivated in ½ MS medium supplemented with activated charcoal and 30 g L^{-1} sucrose (Figure 1G, Figure 1H and Figure 1I).

The explants were tested in six treatments, as follows: MS medium (T1), MS + 0.5 mg L^{-1} of the cytokinin 6-benzylaminopurine (BAP) (T2), MS + 1.0 mg L^{-1} of BAP (T3), medium with half-strength MS salts (½ MS) (T4), ½ MS + 0.5 mg L^{-1} of BAP (T5), and ½ MS + 1.0 mg L^{-1} of BAP (T6). All treatments were supplemented with 20 g L^{-1} of sucrose, 2.5 g L^{-1} of activated charcoal, and solidified with 2.0 g L^{-1} of Phytagel. The pH was adjusted to 5.8, and the media were sterilized in an autoclave at 121 ºC and 1 atm for 20 minutes.

During the experiment, the plantlets were sub-cultured twice at intervals of three to four weeks using the same culture media and maintained under the same environmental conditions as the previous experiments. A completely randomized experimental design was used, with six treatments, five replicates, and two explants per replicate. After 90 days, the following variables were evaluated: plantlet height (cm), number of shoots, number of roots and root length (cm).

Statistical analyses

The data were tested for normality and homoscedasticity of variance using the Shapiro-Wilk

Table 1 - Treatments, culture media, and sucrose concentrations used for the asymbiotic germination and early development of *Epidendrum carpophorum* protocorms *in vitro*.

Treatment	----------Culture medium----------	----------Sucrose----------
T1 (n = 5)	MS	20 g L^{-1}
T2 (n = 5)	MS + 2.5 g L^{-1}activated charcoal	
T3 (n = 5)	½ MS	
T4 (n = 5)	½ MS + 2.5 g L^{-1}activated charcoal	
T5 (n = 5)	MS	30 g L^{-1}
T6 (n = 5)	MS + 2.5 g L^{-1}activated charcoal	
T7 (n = 5)	½ MS	
T8 (n = 5)	½ MS + 2.5 g L^{-1}activated charcoal	

n - number of replicates

and Levene's tests, respectively. Both tests were conducted at a 5% probability level. Following these preliminary tests, a non-parametric analysis was performed to compare medians between treatments. The Kruskal-Wallis test was used to determine if significant intergroup differences existed. When a significant difference was found ($P < 0.05$), the medians were compared using Dunn's test ($P < 0.05$). Additionally, medians for sucrose concentrations within the same culture medium were compared using the Wilcoxon test ($P < 0.05$). All statistical analyses were carried out using R software version 4.3.0 (R CORE TEAM, 2023), and the FSA (OGLE et al., 2023) and companion (MANGIAFICO, 2024) packages.

RESULTS AND DISCUSSION

Initial in vitro protocorm development

After 60 days, all treatments exhibited germinated seeds and the formation of chlorophyllous protocorms (Figure 1B), with the emergence of the first leaf, corresponding to stages 1 and 2, respectively (Figure 1C and Figure 1D). In *Aciantheraprolifera*, chlorophyllous protocorm (stage 1) and shoot apex formation was observed two weeks after sowing (KOENE et al., 2019). Protocorm formation is considered a distinctive trait of post-seminal orchid development, and the uniform development of these structures is crucial for shoot apex formation and the onset of leaf primordia (YEUNG, 2017; LAL & SINGH, 2020).

At 90 days into the initial development experiment, protocorms in stages 3 and 4 were observed (Figure 1E and Figure 1F; Figure 2B, Figure 2C, Figure 2E and Figure 2F). In the same timeframe, the species *C. paludicolum* also reached stages 3 and 4 (FERREIRA et al., 2022), unlike the epiphytic orchid *P.scolopendrifolia* in ½ MS medium (KIM et al., 2021). Conversely, *C. nobilior* showed stage 3 development at 45 days in both MS and ½ MS media (SOARES et al., 2020).

After 90 days, treatments T2, T3, T5, and T6 differed significantly from the others for plantlet height, with lengths ranging from 0.3 ± 0.0 cm to 0.6 ± 0.0 cm (median ± IQR), with T3 showing the most favorable values for this variable (Table 2). For leaf number, T2 and T3 (both with 20 g L^{-1} of sucrose) and T5 (with 30 g L^{-1} sucrose) differed from the other treatments (Table 2). During the same evaluation period, T1 and T4 showed significantly slower development of the morphological variables

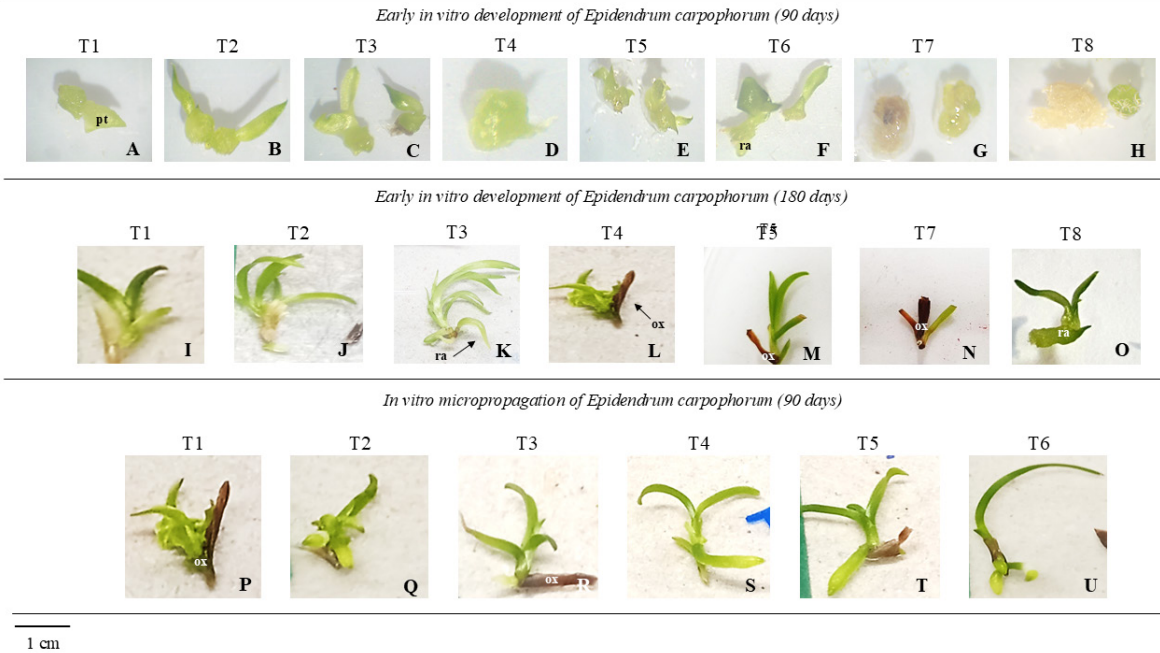

Figure 2 - Morphological aspects of early *in vitro* development at 90 days (A) (B) (C) (D) (E) (F) (G) (H) and at 180 days (I) (J) (K) (L) (M) (N) (O), and of *in vitro* micropropagation of *Epidendrum carpophorum* at 90 days (P) (Q) (R) (S) (T) (U). pt. protocorm, ra - root, ox - oxidation.

Table 2 - Median ± interquartile range for morphological variables of *Epidendrum carpophorum* plantlets cultured *in vitro*, subjected to two culture media (MS and ½ MS), with or without the addition of activated charcoal (2.5 g L^{-1}), and two sucrose concentrations (20 g L^{-1} and 30 g L^{-1}) at 90 days.

Treatment	------Sucrose------	------HT (cm)------	----------NL---------	----------NR---------	------RL (cm)------
T1	20 g L^{-1}	0 ± 0 Bb	0 ± 0 Bb	0 ± 0 b	0 ± 0 b
T2		0.3 ± 0 abc	1 ± 0 Aab	0 ± 0 Bb	0 ± 0 Bb
T3		0.6 ± 0 Aa	3 ± 0 Aa	0 ± 0 b	0 ± 0 b
T4		0 ± 0 bc	0 ± 0 b	0 ± 0 b	0 ± 0 b
T5	30 g L^{-1}	0.5 ± 0 Abc	0.3 ± 0 Aab	0 ± 0 b	0 ± 0 b
T6		0.3 ± 0 abc	2 ± 0 Bab	1 ± 0 Aa	0.7 ± 0.5 Aa
T7		0 ± 0 Bbc	0 ± 0 Bb	0 ± 0 b	0 ± 0 b
T8		0 ± 0 bc	0 ± 0 b	0 ± 0 b	0 ± 0 b

Lowercase letters indicate median comparisons between treatments, according to Dunn's test.
($P < 0.05$). Uppercase letters represent median comparisons between sucrose concentrations, according to the Wilcoxon test ($P < 0.05$). HT(cm) - plantlet height; NL - number of leaves; NR - number of roots; RL (cm) - root length. For treatments in which most plantlets died or did not develop the morphological variables evaluated during the experimental trial, the statistical analysis returneda value of 0.

analyzed, remaining in protocorm stage 1 (Figure 2A and Figure 2D). Consequently, all variables in these treatments maintained values of 0.0 ± 0.0 (Table 2). Additionally, some swollen seeds in T7 and T8 turned brown (Figure 2G and Figure 2H), potentially indicating the inhibition of chlorophyll formation due to an increased respiration rate (GEORGE et al., 2008). With respect to root development, satisfactory results were observed only in treatment T6, which included 30 g L^{-1} of sucrose, with values of 1 ± 0 for root number and 0.7 ± 0.5 cm for root length (Table 2).

After 180 days, T3 and T8 contained plantlets at stage 4 (Figure 1F, Figure 2K and Figure 2O), a stage also observed for *C. nobilior,* with promising results in MS, ½ MS, and KC media during the same assessment period (OLIVEIRA et al., 2021). Treatments T1, T2, T3, T4, and T5 obtained the best results for plantlet height (Figure 2I, Figure 2J, Figure 2K, Figure 2L and Figure 2M), with T3 showing a considerable height of 1.3 ± 0.0 cm (Table 3). For the number of leaves, all these treatments, along with T7 and T8, differed significantly from T6. For this same variable, the culture media supplemented with 20 g L^{-1} of sucrose (T1 and T3) benefited from this concentration (Table 3). However, despite favorable results for the aforementioned variables, treatments T4, T5, and T7 exhibited symptoms of toxicity and necrotic structures in the plants (Figure 2L, Figre 2M and Figure 2N). These outcomes may

Table 3 - Median ± interquartile range for the morphological variables of *Epidendrum carpophorum* plantlets cultured *in vitro* subjected to two culture media (MS and ½ MS), with or without the addition of activated charcoal (2.5 g L^{-1}), and two sucrose concentrations (20 g L^{-1} and 30 g L^{-1}), assessed at 180 days.

Treatment	-------Sucrose-------	------HT (cm)------	----------NL---------	----------NR---------	-------RL (cm)------
T1	20 g L^{-1}	0.5 ± 0 ab	3 ± 0 Aa	0 ± 0 b	0 ± 0 b
T2		1.0 ± 0 ab	2 ± 0 a	0 ± 0 b	0 ± 0 b
T3		1.3 ± 0 Aa	3 ± 0 Aa	4 ± 0 Aa	0.6 ± 0.3 Aa
T4		0.5 ± 0 ab	3 ± 0 a	0 ± 0 Bb	0 ± 0 Bb
T5	30 g L^{-1}	0.6 ± 0 ab	2 ± 0 Ba	0 ± 0 b	0 ± 0 b
T6		0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
T7		0.2 ± 0 Bb	2 ± 0 Ba	0 ± 0 Bb	0 ± 0 Bb
T9		0.2 ± 0 b	3 ± 0 a	2 ± 0 Aa	0.2 ± 0.3 Aa

Lowercase letters indicate median comparisons between treatments, according to Dunn's test.
($P < 0.05$). Uppercase letters indicate median comparisons between sucrose concentrations, according to the Wilcoxon test ($P< 0.05$). HT (cm) - plantlet height; Nl - number of leaves; NR - number of roots; RL (cm) - root length. For treatments in which most plantlets died or did not develop the morphological variables evaluated during the experimental trial, the statistical analysis returned a value of 0.

be due to a hormonal imbalance in the culture media. High nutrient concentrations can become toxic to early-stage plantlets, while low macronutrient concentrations at this phase can lead to nutritional deficiencies that compromise plant metabolic processes (SASAMORI et al., 2021). Furthermore, all plants in treatment T6 were lost to fungal contamination in the culture medium, precluding evaluation at 180 days for this treatment.

Both root number and root length were positively affected in T3 and T8. Additionally, the ½ MS medium supplemented with 20 g L^{-1} of sucrose (T3) favored all the variables analyzed over the 180-day period (Table 3). Similar results to those found for treatment T3 were reported for the *in vitro* growth and development of *Sedirea japonica* (AN et al., 2021) and in a study with *Miltoniaflavescens*, in which both MS and ½ MS media were effective for the initial development of the species (LEMES et al., 2020).

From the observations made on the initial development of *Epidendrum carpophorum* protocorms at 90 days, the medium with half-strength MS salts (½ MS) and 20 g L^{-1} of sucrose without activated charcoal (T3) promoted favorable shoot growth and leaf emission. However, root development was observed only in the MS medium with activated charcoal and increased sucrose concentration (30 g L^{-1}) (T6). These results are possibly associated with the interaction between nutrients and the osmotic effects of the culture medium, directly influencing root formation (GEORGE et al., 2008).

Subsequent observations at 180 days indicated that treatment T3 was effective for the development of *E. carpophorum* plantlets. Given that MS medium is considered one of the richest in nutrients (MORAES et al., 2023), it may have significantly contributed to the *in vitro* development of *E. carpophorum*, even in the treatment with reduced salt concentrations. Furthermore, it is important to consider that *E. carpophorum* is an epiphytic orchid, which allows the species to have low nutritional requirements and adapt even to nutrient-poor environments (RAMÍREZ-MOSQUEDA et al., 2019). This was also observed for the epiphytic orchid *Cattleya cernua*, in which the reduction of MS salts did not compromise *in vitro* growth and development (SASAMORI et al., 2021), as well as in epiphytic species of the genus *Dendrobium* (DIANTINA et al., 2020) and for the orchid *Epidendrum secundum* Jacq. (CAVALCANTE et al., 2018), where cultivation in ½ MS medium favored plant development.

At the highest sucrose concentration (30 g L^{-1}), values for morphological variables were lower compared to media with 20 g L^{-1} sucrose over a 180-day period. Lower sugar concentrations (10 g L^{-1} and 20 g L^{-1}) led to greater shoot growth in *Alatiglossum fuscopetalum* plantlets (FERREIRA et al., 2017) and other orchid species (ZAHARA et al., 2017; BOZDEMIR et al., 2018; ZAKARIA et al., 2021). Although, exogenous sucrose is important for providing charcoal and energy in culture media, the optimal supply of this carbohydrate is species-dependent. Both increased and reduced sugar concentrations can compromise plant growth (GUPTA, 2016).

The addition of activated charcoal to the culture media did not significantly influence the development phase of *E. carpophorum* protocorms, given that plantlets developed successfully in both the presence and absence of this component. This finding contrasts with observations for other species (WASIATI et al., 2021; BERNAL-BALTAZAR et al., 2022), suggesting that *E. carpophorum* might be less sensitive to compounds adsorbed by activated charcoal, or that metabolites released into the culture medium did not exert significant inhibitory effects on the growth of these plants. Therefore, the response to activated charcoal appears to be species-specific and dependent on the explant type, culture objective, and *in vitro* cultivation phase, since it can have either beneficial or antagonistic effects on plant growth and development (SOUZA et al., 2021).

Effects of different MS medium concentrations and cytokinin on in vitro micropropagation

After 90 days of *in vitro* cultivation, plantlet heights ranged from 0 ± 0 cm to 0.9 ± 0.2 cm, corresponding to treatments T2 and T1 (MS medium). In MS medium with 1.0 mg L^{-1} BAP (T3), plantlets reached 0.5 ± 0.6 cm in height, while those in ½ MS medium + 0.0 mg L^{-1} BAP (T4) exhibited a height of 0.6 ± 0.8 cm (Figure 3).

For the number of shoots, only T1 (MS without BAP) and T4 (½ MS without BAP) showed median values greater than 0. For root number and root length, all treatments exhibited a median of 0. However, statistical analysis revealed no significant treatment effects (Figure 3) or BAP concentrations (Figure 4) on any of the variables assessed ($P > 0.05$).

These results corroborated those found for *Epidendrum lilas*, where no significant responses to the same BAP concentrations were observed, likely due to the balance between exogenous and endogenous cytokinins (LONDE et al., 2021). The absence of BAP effects has also been reported for the orchids *Stanhopeatigrina* (CASTILLO-PÉREZ et al., 2021) and *Oncidium baueri* Lindl. (CAMARGO et al., 2015); although, the latter showed satisfactory *in vitro* multiplication even without this cytokinin.

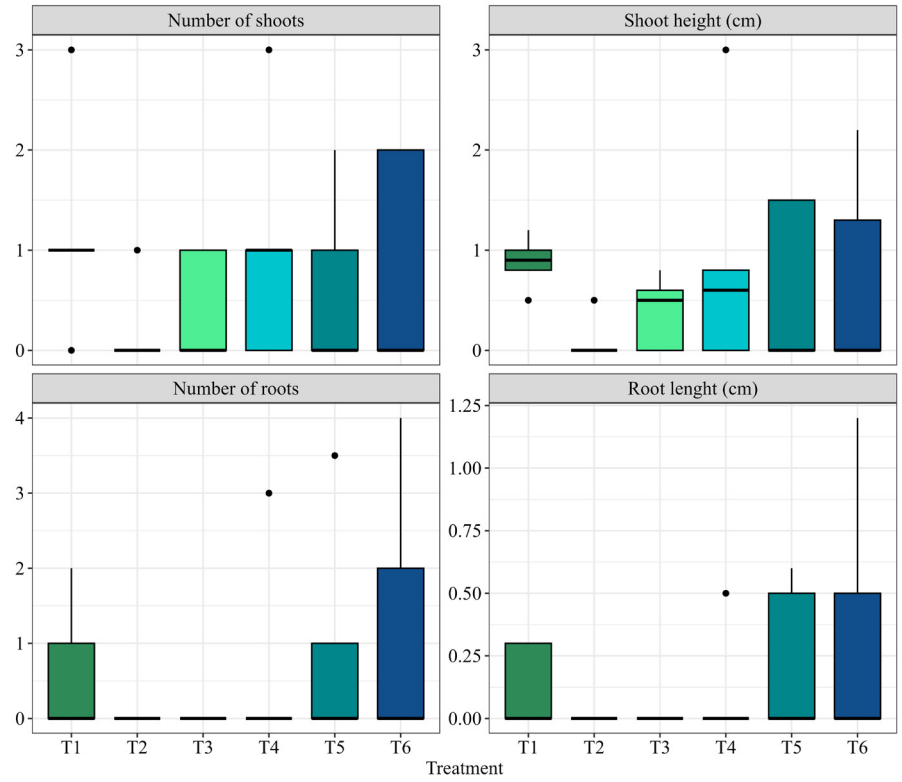

Figure 3 - Medians ± Interquartile Range of morphological variables (shoot height, number of shoots, number of roots, and root length) of *Epidendrum carpophorum* plantlets subjected to different treatments. (T1) MS + 0.0 mg L^{-1} BAP; (T2) MS + 0.5 mg L^{-1} BAP; (T3) MS + 1.0 mg L^{-1} BAP; (T4) ½ MS + 0.0 mg L^{-1} BAP; (T5) ½ MS + 0.5 mg L^{-1} BAP; (T6) ½ MS + 1.0 mg L^{-1} BAP. The absence of comparison letters indicates no statistical difference among treatments according to the Kruskal-Wallis test ($P > 0.05$).Black dots represent outliers for each variable. For treatments in which most plantlets died or failed to develop the morphological variables analyzed during the experimental trial, the statistical analysis returned a value of 0.

A high number of plantlets with abnormal growth were found in MS control medium without BAP (T1), MS medium with 0.5 mg L^{-1} BAP (T2), and MS medium with 1.0 mg L^{-1} BAP (T3). In these treatments, multiple shoots at the base of the explants, tissue oxidation, and impaired root development were observed (Figure 2P, Figure 2Q and Figure 2R). According to SASAMORI et al. (2021), plants can experience stress conditions during micropropagation, leading to morphological and physiological alterations.

In some cases, cytokinin concentrations in the culture medium can become detrimental to the explant, resulting in undesirable morphological traits (GARCIA et al., 2021), as observed in a study on *Dryadellazebrina*, where high concentrations caused tissue oxidation and reduced morphological structure development (ANJOS et al., 2021). PICOLOTTO et al. (2017) reported that certain growth regulators can have synergistic or antagonistic effects in the culture medium, and that optimal phytoregulator concentrations and requirements are specific to the plant species and explant type used. On the other hand, studies with other orchid species have demonstrated the importance and efficiency of growth regulators in *in vitro* propagation, showing that these compounds promoted explant regeneration and adventitious shoot formation, particularly when auxins and cytokininsare combined in the culture medium (BHOWMIK & RAHMAN, 2020; HOSSEN et al., 2021; PATAVARDHAN et al., 2022).

As observed in our study, the BAP concentrations tested did not result in statistically significant differences in the morphological variables analyzed, indicating that *Epidendrum carpophorum* development occurs both in the absence of this cytokinin and across its various concentrations. These

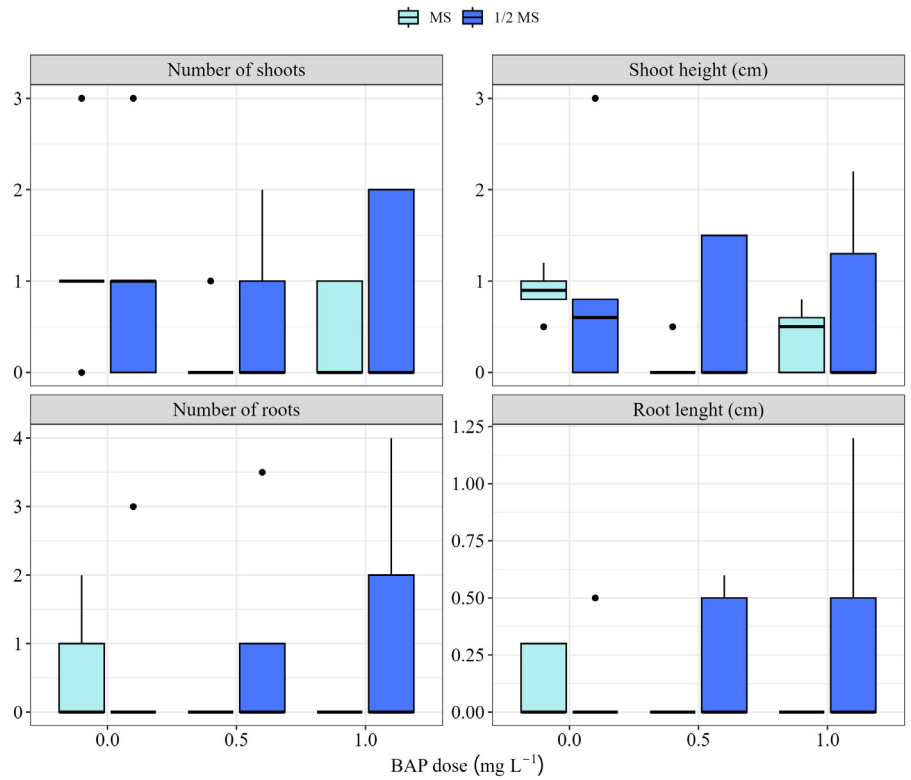

Figure 4 - Medians ± interquartile range of morphological variables (shoot height, number of shoots, number of roots, and root length) of *Epidendrum carpophorum* plantlets subjected to different concentrations of BAP (6-benzylaminopurine) in both MS and ½ MS culture media. The absence of comparison letters indicates no statistically significant difference among treatments according to the Kruskal-Wallis test ($P > 0.05$). The black dots represent outliers for each variable. For treatments where most plantlets died or failed to develop the evaluated morphological variables during the experimental trial, the statistical analysis returned a value of 0.

results suggested that exogenous supplementation with this plant growth regulator was not decisive for micropropagation of the species. However, when MS salts were reduced by half (½ MS), uniform growth and vigorous plants were observed (Figure 2S, Figure 2T and Figure 2U). It is important to emphasize that, as argued by GEORGE (2008), the effectiveness of exogenous growth regulators depends on specific factors such as the cultivation stage, type of explant, culture medium, and species-specific traits. In this context, QUIROZ et al. (2017) reinforced that germination, uniform development, multiple shoot formation, and the induction of embryogenic calli may occur at appropriate concentrations or even in the absence of any growth regulator.

CONCLUSION

This study showed that *Epidendrum carpophorum* exhibits uniform germination after 60 days of cultivation. The ½ MS medium supplemented with 20 g L^{-1} of sucrose was the most effective for initial protocorm developmentand benefitted all the morphological variables analyzed after 180 days. Furthermore, the cytokinin BAP had no significant influence on the micropropagation of *E. carpophorum*. The results presented in this study demonstrated that *in vitro* propagation of *E. carpophorum* is a viable strategy for *ex situ* conservation and can support further research that aim to acclimatize and reintroduce the species into its natural habitat.

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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 revised the manuscript and approved of the final version.

DATA AVAILABILITY STATEMENT

Not applicable.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

The manuscript was written without the use of artificial intelligence.

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