

Plant growth regulators and organic additives on the proliferation of protocorm-like bodies and plantlet regeneration of *Cattleya gaskelliana* (N.E.Br.) B.S.Williams

Reguladores de crescimento vegetal e aditivos orgânicos na proliferação de corpos semelhantes a protocormos e na regeneração de plântulas de *Cattleya gaskelliana* (N.E.Br.) B.S.Williams

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ABSTRACT

In this study, a tissue culture method was developed for the propagation and conservation of *Cattleya gaskelliana* (N.E.Br.) B.S. Williams. Initially, protocorms with leaf primordium, obtained via asymbiotic seed germination, were used as explants. Half-strength Murashige and Skoog (MS) medium supplemented with 1.5 mg L⁻¹ 6-benzyl aminopurine (BA) and 2.0 mg L⁻¹ indole butyric acid (IBA) was found to be optimal for the proliferation of protocorm-like bodies (PLB). Most explants (81%) proliferated, each producing 11.1 ± 1.87 units per explant. The optimum number of PLB differentiated to plantlets on a cultured medium with 0.5 mg L⁻¹ Thidiazuron (TDZ) + 100 mL L⁻¹ coconut water. Firmly rooted plantlets with vigorous growth were regenerated on ½ MS medium + 1.0 mg L⁻¹ Indole-3-acetic acid (IAA) and 50 g L⁻¹ banana puree. Plants regenerated via *in vitro* processes were wrapped with sphagnum moss and acclimatized in a substrate containing equal proportions of bark, perlite, and peat with a 100% *ex-vitro* survival rate after acclimation for 70 days. The protocol developed in this study can be used to obtain several thousand plants within one year, and thus, is an effective method.

Index terms: Orchids; PLB; auxin; cytokinin; organic extract.

RESUMO

Neste estudo foi desenvolvido um método de cultura de tecidos para propagação e conservação de *Cattleya gaskelliana* (N.E.Br.) B.S. Williams. Inicialmente, protocormos com primórdio foliar, obtidos via germinação assimbiótica de sementes, foram utilizados como explantes. Meio Murashige e Skoog (MS) de meia concentração suplementado com 1,5 mg L⁻¹ de 6-benzil aminopurina (BA) e 2,0 mg L⁻¹ de ácido indol butírico (AIB) foi considerado ideal para a proliferação de corpos semelhantes a protocormos (PLB). A maioria dos explantes (81%) proliferou, cada um produzindo 11,1 ± 1,87 unidades por explante. O número ótimo de PLB diferenciados para plântulas em meio de cultura com 0,5 mg L⁻¹ de Thidiazuron (TDZ) + 100 mL L⁻¹ de água de coco. Plântulas firmemente enraizadas e com crescimento vigoroso foram regeneradas em meio ½ MS + 1,0 mg L⁻¹ de ácido indol-3-acético (AIA) e 50 g L⁻¹ de pasta de banana. Plantas regeneradas via processos *in vitro* foram envoltas com musgo esfagno e aclimatadas em substrato contendo proporções iguais de casca, perlita e turfa com taxa de sobrevivência *ex-vitro* de 100% após aclimação por 70 dias. O protocolo desenvolvido neste estudo pode ser usado para obter milhares de plantas por um ano sendo, portanto, um método eficaz.

Termos para indexação: Orquídeas; PLB; auxinas; citocinina; extrato orgânico.

Introduction

Orchids are commonly used in landscape design and have different shapes, colors, and sizes. They constitute the second-largest family in the plant kingdom, accounting for about 800 genera and 30,000 species. About 30% of them are terrestrial, while the remaining 70% thrive in highly stressful epiphytic and lithophytic habitats (Xing et al., 2019; Misirova, 2023). Among the most popular epiphytic orchids, *Cattleyas* are widely known for their beautiful and large flowers. These plants are also used as floral decorations, including potted plants, bouquets, and flower arrangements. Additionally, they are used in breeding programs aimed at generating new hybrids for ornamental purposes (De, 2020).

Cattleya Lindl. belongs to the subfamily Epidendroideae Lindl., tribe Epidendreae Kunth, and subtribe Laeliinae Benth. This genus includes 114 species, most of which are epiphytic

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and have horticultural importance (Van den Berg, 2014; Ferreira et al., 2021). Members of *Cattleya* are distributed throughout Mexico and Central America, as well as, along the slopes of the Andes in South America, including Colombia and Venezuela. They are found in different habitats, from mountain ranges and arid forests to transitional zones leading to humid, misty hillsides, and canyons, mainly on trees and rocks (Pupulin, 2015; Menezes, Giordani, & Mendes, 2022). *Cattleya* species are often called “Queens of Flowers”. They have large, beautiful, brightly colored, and occasionally fragrant flowers, which are very popular in both the cut flower and indoor plant markets. These attributes contribute to the importance of their cultivation and propagation at a commercial scale (Pant et al., 2020). The flowers of *Cattleya gaskelliana* (N.E.Br.) B.S. Williams are fragrant and large, and their size is similar to that of the flowers of *C. warscewiczii*. The petals and sepals are purple-violet with a white tinge. The trumpet-shaped lip is similar in color to the petals, although the lower part may be paler. Plants of this species are short-day *Cattleya* and bloom under photoperiods of 9 h of light per day, specifically from July to September, and they develop rapidly immediately after the shoots start to grow (Lopez & Runkle, 2004). They grow vigorously, with flowers occasionally appearing in less than two months after emerging from dormancy (Chadwick & Chadwick, 2021). Plants of this species are found in Venezuela and Trinidad and Tobago; this species is considered to be critically endangered and is included in the Venezuelan Red Book. The wild populations of this species have a restricted habitat and are under the threat of overharvesting for their use as ornamental plants; thus, they need to be protected and conserved (Salazar & Arcia-Barreto, 2020; Plants of the World Online - POWO, 1885). Orchid seeds have an undifferentiated embryo, lack endosperm, and possess testa-covered air spaces—these features help seeds disperse across farther distances. Under natural conditions, the propagation of orchids by seeds is difficult due to their small size (0.1–6.0 mm) and their nutritional requirements for germination and growth. Also, the biggest challenge in seed germination is the absence of mycorrhizal symbiosis, considering that mycorrhizae provide most of the nutrients, minerals, vitamins, and water needed for germination and seedling development. Moreover, different mycorrhizae have different relationships with their orchid hosts (Zhao et al., 2021; Jolman et al., 2022). *Cattleyas*, like other sympodial plants, can be propagated asexually by dividing back pseudobulbs while transplanting. Sometimes this method of propagation may be ineffective for these plants as obtaining pseudobulbs to produce new roots is difficult. This often happens because the older roots are damaged or destroyed during the replanting process (De, 2015). Tissue culture can effectively reduce the pressure on the natural distribution of orchids and promote their sustainable utilization (Farinacio, Galdiano, & Lemos, 2018; Dawa et al., 2019). PLB production is a type of vegetative propagation *in vitro* and an important technique

for conserving different orchid species (Mondal & Banerjee, 2017). Several studies have shown the effectiveness of using PLB as an explant in the propagation of epiphytic orchids, such as *Phalaenopsis*, *Oncidium*, and *Dendrobium*. PLB has a unique embryonic structure and consists of meristematic centers similar to somatic embryos, which can divide and multiply rapidly; these structures can be later induced to form plantlets *in vitro* (Malhotra et al., 2023). After in-depth investigations, researchers have identified the optimal medium for *in vitro* regeneration, multiplication, and growth of orchid seedlings, using a combination of organic additives and growth regulators. Organic additives contain carbohydrates, vitamins, and amino acids, which enhance the effectiveness of the propagation of orchid plants and decrease laboratory production costs (Moraes et al., 2020; Poniewozik, Szot, & Parzymies, 2021; Samiei et al., 2021). However, few studies have investigated the clonal micropropagation of *C. gaskelliana* (Mercado & Jaimes, 2022). Therefore, we developed an effective protocol for PLB proliferation, plantlet regeneration, and rooting of this species.

Material and Methods

The study was conducted in the Laboratory of Plant Biotechnology of the Main Botanical Garden, named after N.V. Tsitsin of the Russian Academy of Sciences. All experiments were conducted using conventional biotechnological techniques and methods established in the Plant Biotechnology Laboratory (Molkanova et al., 2018).

The plant materials used in this study were initiated from protocorms with leaf primordium (0.1–0.2 cm in length) obtained via asymbiotic seed germination on Knudson C medium supplemented with 0.5 g L⁻¹ peptone and 0.5 g L⁻¹ activated charcoal. The aseptic culture was obtained from the Laboratory of Plant Cellular Biotechnology of the Central Botanical Garden of the National Academy of Sciences of Belarus.

Experimental design

All experiments were conducted using a completely randomized design (CRD) and repeated four times. Each replicate contained approximately seven explants. The experimental data were analyzed using Microsoft Office Excel 2016 and PAST 3.2 (Paleontological Statistical) using descriptive statistics. Next, one-factor and two-factor analysis of variance was conducted using Duncan's test with the level of significance at $\alpha = 0.05$ to determine the significant differences between experimental variants.

Culture medium and cultivation conditions

The basal culture medium used for all experiments was Murashige and Skoog (1962) (MS) containing 50% macronutrient

and 100% micronutrient, and vitamin concentrations with 20.0 g L⁻¹ saccharose, 6.8 g L⁻¹ agar, 0.1 g L⁻¹ Myo-inositol, and 0.1 g L⁻¹ activated charcoal. Laminar boxes (Claire, Australia) were used to cultivate the plants on the nutrient medium, following the rules for working with sterile material. All cultures were incubated in a phytotron at 25 ±2 °C, 70 ±5% relative humidity, and a photoperiod of 16/8 h (light/dark). The light intensity was maintained at approximately 45–60 μmol·m⁻²·s⁻¹.

Proliferation of protocorm-like bodies (PLB)

To proliferate PLB, protocorms with the leaf primordium were subcultivated on the ½ MS basal medium containing different combinations of 6–benzylaminopurine (BA) and Indole-3-butyric acid (IBA) (Sigma, USA), as shown in Table 1.

Table 1: Combination of BA and IBA for the proliferation of PLB.

BA (mg L ⁻¹)	IBA (mg L ⁻¹)		
	1.0	1.5	2.0
1.0	+	+	
1.5	+	+	+
2.0		+	+

+: studied combination.

The pH of the medium was adjusted by KOH to 5.6 and autoclaved in a WAC–60 autoclave (Daihan Scientific, South Korea) at 121 °C and 15 psi for 20 min. The percentage of PLB formed and the number of PLB were assessed after 70–90 days.

Effect of organic additives and cytokinins on the regeneration of PLB and formation of plantlets

The PLB proliferated in the previous stage were transferred to ½ MS nutrient medium supplemented with different types of cytokinins (BA, kinetin (Kin), thidiazuron (TDZ), and various fresh organic additives (Table 2).

Table 2: Combination of cytokinin and organic additives for PLB regeneration and plantlet formation.

Cytokinin 0.5 mg L ⁻¹	Organic additives	Treatment
BA	100 mL L ⁻¹ Coconut water	A
	50 g L ⁻¹ Banana puree	B
TZD	100 mL L ⁻¹ Coconut water	C
	50 g L ⁻¹ Banana puree	D
Kin	100 mL L ⁻¹ Coconut water	E
	50 g L ⁻¹ Banana puree	F

After the pH was adjusted to 5.6 by KOH, the medium was sterilized using an autoclave (WAC–60; Daihan Scientific, South Korea) for 20 min at 121° C. The following parameters were recorded after 70–90 days: survival rate, number of adventitious shoots per explant, plantlet height, and number of leaves.

Rooting of plantlets

Small plantlets (> 1.0 cm long), formed at the previous stage, were placed on the same nutrient medium, but different auxins and various fresh organic additives were added, as shown in Table 3.

Table 3: Culture media for rooting C. gaskelliana plantlets.

Organic additives	Auxin type	Auxin (mg L ⁻¹)	Treatment
Banana puree 50 g L ⁻¹	IAA	1.0	A
		2.0	B
	IBA	1.0	C
		2.0	D
Potato puree 20 g L ⁻¹	IAA	1.0	E
		2.0	F
	IBA	1.0	G
		2.0	H
Coconut water 100 mL L ⁻¹	IAA	1.0	I
		2.0	J
	IBA	1.0	K
		2.0	L

The rooting percentage, number of leaves, seedling length, number of roots, and root length were recorded after 70–90 days. The formation of adventitious shoots during this stage was also recorded.

Acclimatization

The rooted plantlets were removed from the *in vitro* culture medium and washed thoroughly in tap water to remove any adhering medium. After washing, their bases were wrapped with sphagnum moss, and then, they were planted in containers filled with 1:1:1 (v: v: v) peat: perlite: bark. The seedlings were watered twice weekly and placed in greenhouse conditions for further acclimatization. The survival rate was determined after 70 days.

Results and Discussion

Proliferation of PLB

In our study, the ½ MS nutrient medium supplemented with a combination of BA and IBA was found to effectively increase the percentage of PLB formation and the number of PLB formed.

A high percentage of PLB was recorded (68.0% and 82.8%) for the following combinations 2.0 mg L⁻¹ BA: 1.5 mg L⁻¹ IBA and 1.5 mg L⁻¹ BA: 2.0 mg L⁻¹ IBA, respectively, whereas, the lowest percentage (27.4%) was recorded for the combination 1.0 mg L⁻¹ BA: 1.5 mg L⁻¹ IBA (Figure 1).

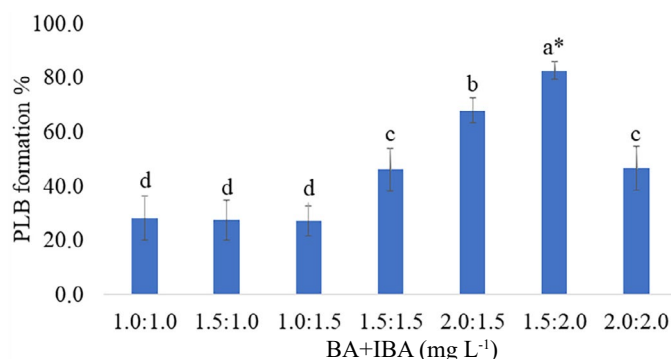


Figure 1: Effect of different combinations of BA and IBA (in mg L⁻¹) on PLB formation after 90 days of cultivation. *Values indicate the mean $\pm$ SD, the letters "a", "b", and "c" denote groups, determined by Duncan's multiple range test at the 5% level.

Many studies have reported that auxins and/or cytokinins must be used for the formation of PLB in many orchid species. Treatment with 2.5 mg L⁻¹ auxins or cytokinins yielded the highest percentage of dendrobium PLB (88.3% and 87.3%, respectively) compared to other treatments (Hossen et al., 2021). In another study, culturing the PLB of *Aerides crispum* Lindl. on ½ MS medium supplemented with BA (1.0 mg L⁻¹) + NAA (0.5 mg L⁻¹) stimulated the highest percentage of PLB formation (84.3%) (Pyati, 2022). These findings confirmed that orchid species vary in the percentage of PLB formed, as well as, the plant growth regulators (PGRs) needed, and the

combination and ratio between them for obtaining the highest percentage of PLB.

Also, the highest number of PLB (11.1 $\pm$ 1.87 units per explant) was recorded on ½ MS medium containing 1.5 mg L⁻¹ BA and 2.0 mg L⁻¹ IBA compared to the number of PLB recorded with other treatment variants (Figure 2). Combined treatment with cytokinins and auxins can promote the proliferation of the PLB of *Vanilla planifolia* Jacks. The largest number of PLB per explant was recorded on the MS medium containing 1.5 mg L⁻¹ BA and 2.0 mg L⁻¹ NAA (Malhotra et al., 2023). In another study, the number of *Oncidium* PLB was lower on ½ MS culture medium supplemented with auxin without cytokinin, but treatment with 0.5 mg L⁻¹ BA and 0.5, 1.0, or 1.5 mg L⁻¹ NAA yielded the highest number of PLB (Zakaria et al., 2021). This was consistent with the results of our study, and it can be explained by the combined effect of auxins and cytokinins on cell division and elongation. In this case, auxins dominated over cytokinins, which led to an increase in the uptake of dissolved salts, such as potassium in the cell, causing cell elongation (Majda & Robert, 2018).

Plantlet regeneration

For optimal growth and development under *in vitro* conditions, an appropriate nutrient medium must be used that helps in leaf formation, production of shoots, and root formation, because they grow from the protocorm, which is a mass of undifferentiated cells (Reddy et al., 2020). Optimal plantlet regeneration can be achieved by using organic additives along with growth regulators.

In our study, all treatments containing organic additives (coconut water and banana puree) in combination with different cytokinins showed high survival rates (80.0–96.0%), except for media containing kinetin, which showed low survival rates compared to the other treatments (46.0–50.0%) (Figure 3). These

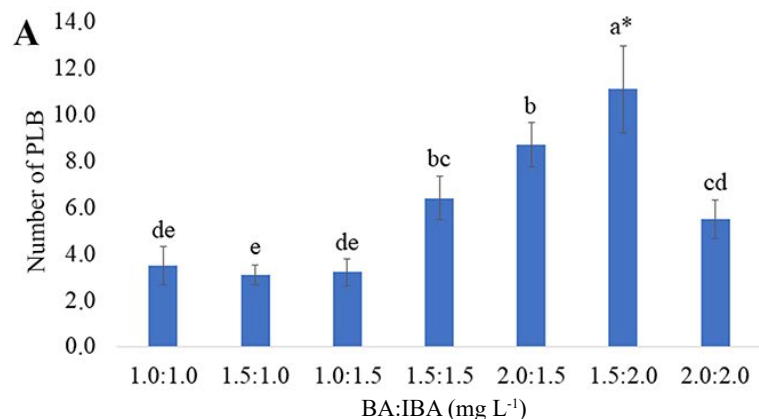


Figure 2: (A) Effect of the combination of BA and IBA (in mg L⁻¹) on PLB formation. *Values indicate the mean $\pm$ SD, the letters "a", "b", and "c" denote groups based on Duncan's multiple range test at the 5% level; (B) ½ MS medium supplemented with 1.5 mg L⁻¹ BA and 2.0 mg L⁻¹ IBA after 90 days of cultivation (bar = 1 cm).

results were consistent with the results of other studies, where the protocorms of orchid species such as *Cypripedium subtropicum* S.C.Chen & K.Y.Lang, and *Paphiopedilum* Alma Gavaert showed low survival rates, which was attributed to vitrification, followed by the browning of tissues, and subsequently, explant death (Hong, Chen, & Chang, 2008; Perner et al., 2022).

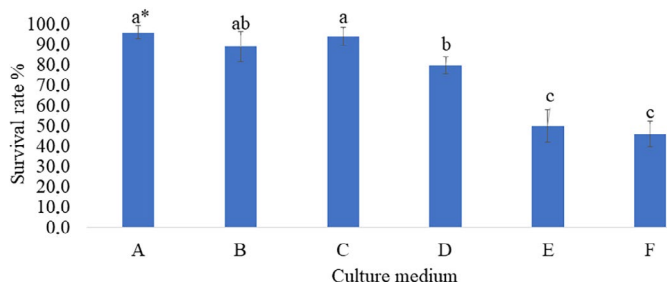


Figure 3: Influence of organic additives and cytokinins: (A) 0.5 mg L⁻¹ BA + coconut water, (B) 0.5 mg L⁻¹ BA + banana puree, (C) 0.5 mg L⁻¹ TDZ + coconut water, (D) 0.5 mg L⁻¹ TDZ + banana puree, (E) 0.5 mg L⁻¹ Kin + coconut water, and (F) 0.5 mg L⁻¹ Kin + banana puree on the survival rate of explant after 90 days of cultivation. *Values indicate the mean $\pm$ SD, the letters "a", "b", and "c" denote groups by Duncan's multiple range test at the 5% level.

The maximum number of adventitious shoots formed was recorded on the $\frac{1}{2}$ MS medium supplemented with TDZ and coconut water (6.50 ± 1.18 units per explant) and the $\frac{1}{2}$ MS medium supplemented with BA and banana puree (5.20 ± 1.45 units per explant); although no significant difference was found between them, a significant difference was found between each of them and other treatments (Figure 4 and Table 4).

A higher number of shoots was observed on the $\frac{1}{2}$ MS medium supplemented with TDZ compared to that in other cytokinins (Corbellini et al., 2020). In another study, the highest rate of shoot proliferation was found in the MS medium containing banana puree and BA for *Dendrobiumgaton sunray* (Herawati, Ganefianti, & Romeida, 2021). These findings were similar to those of our study, which confirmed that modification of the nutrient medium using growth regulators and organic additives together stimulates shoot formation and growth induction. The combined treatment was more effective than the single treatment in enhancing the division and differentiation of tissues because a balance was maintained between the elements and hormones contained in these compounds (Hartati et al., 2017; Akhiriana, Samanhudi, & Yunus, 2019).

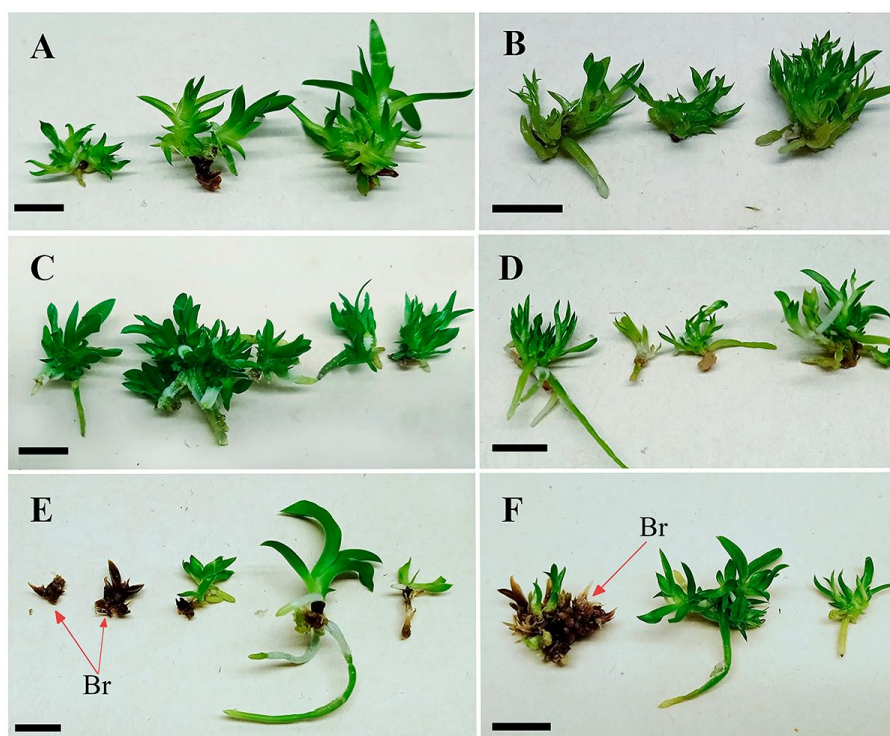


Figure 4: *Cattleya gaskelliana* plantlet regeneration from protocorm cultured in $\frac{1}{2}$ MS medium supplemented with different combinations of organic additives and cytokinins: (A) 0.5 mg L⁻¹ BA + coconut water, (B) 0.5 mg L⁻¹ BA + banana puree, (C) 0.5 mg L⁻¹ TDZ + coconut water, (D) 0.5 mg L⁻¹ TDZ + banana puree, (E) 0.5 mg L⁻¹ Kin + coconut water, and (F) 0.5 mg L⁻¹ Kin + banana puree; Br: browning after 90 days of cultivation (bar = 1 cm).

The highest values of plantlet height (1.65 ± 0.13 cm) and number of leaves (3.60 ± 0.37 -unit per plantlet) were observed on $\frac{1}{2}$ MS medium supplemented with 100 mL of coconut and 0.5 mg. L^{-1} TDZ compared to that recorded in other treatments (Table 4). This finding was similar to those reported in several studies, which confirmed that using TDZ along with coconut powder or water is more effective for shoot and leaf formation from PLB in different epiphytic orchid species (Naing et al., 2011; Khatun, Nath, & Rahman, 2020). The cultivation of PLB on a nutrient medium containing these cytokinins for more than

16 weeks can lead to somaclonal variations and morphological changes; future studies need to further investigate these findings from a mechanistic perspective (Cardoso, Zanello, & Chen, 2020).

Rooting of plantlets

To survive *ex vitro*, seedlings must have a suitable number of leaves and a strong root system. These parameters strongly influence the acclimation of *Cattleya* (Dewir et al., 2015).

In this study, the use of organic additives and auxin strongly affected the growth and rooting of *C. gaskelliana* seedlings (Figure 5).

Table 4: Effect of different organic additives and cytokinins on the morphometric parameters of *C. gaskelliana* plantlets.

Treatment	Plantlet height (cm)	Number of leaves (unit)	Number of adventitious shoots (unit)
A	1.24 ± 0.28 b	3.30 ± 0.87 ab	4.70 ± 0.44 ab
B	0.99 ± 0.15 bc	2.60 ± 0.38 bc	5.20 ± 1.01 b
C	1.65 ± 0.13 a	3.60 ± 0.37 a	6.50 ± 0.47 a
D	0.60 ± 0.11 de	2.10 ± 0.22 c	4.00 ± 0.87 bc
E	0.82 ± 0.29 cd	2.30 ± 0.25 c	2.40 ± 0.80
F	0.50 ± 0.17 e	2.10 ± 0.22 c	2.30 ± 0.90 c

Values are mean $\pm$ SD, the letters "a", "b" and "c" denote groups by Duncan's multiple range test at 5% level *: represent significant or n. s.: non-significant at $p \leq 0.05$.

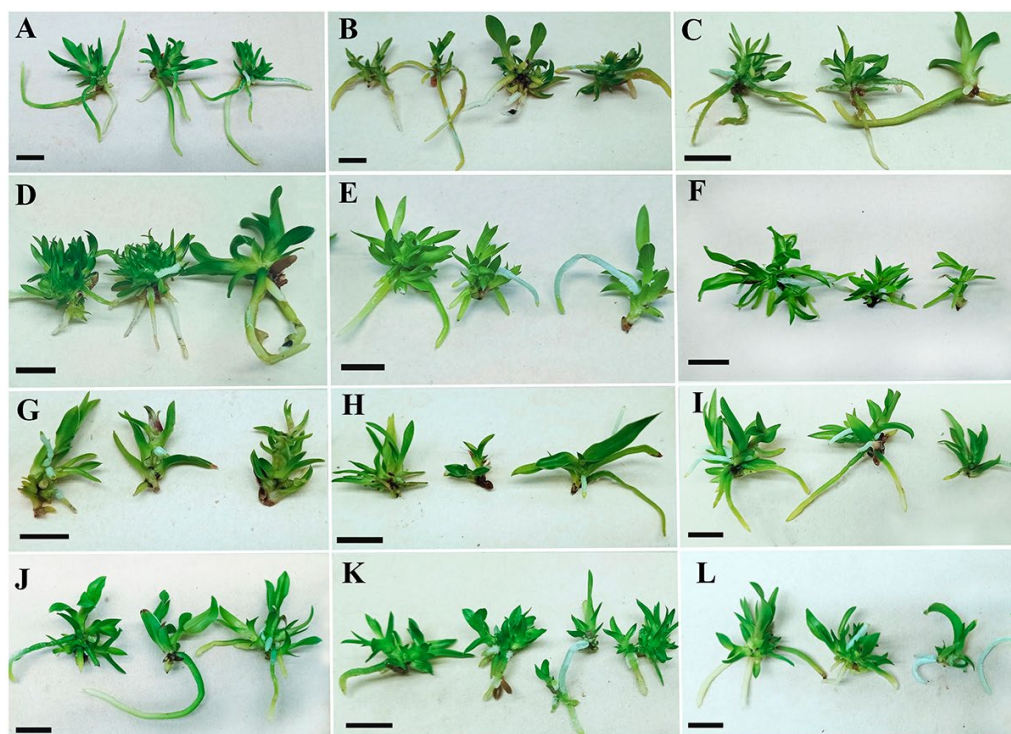


Figure 5: Individual plants grown in different media at the rooting stage are shown; (A) 1.0 mg L^{-1} IAA + banana puree, (B) 2.0 mg L^{-1} AA + banana puree, (C) 1.0 mg L^{-1} IBA + banana puree, (D) 2.0 mg L^{-1} IBA + banana puree, (E) 1.0 mg L^{-1} IAA + potato puree, (F) 2.0 mg L^{-1} AA + potato puree, (G) 1.0 mg L^{-1} IBA + potato puree, (H) 2.0 mg L^{-1} IBA + potato puree, (I) 1.0 mg L^{-1} IAA + coconut water, (J) 2.0 mg L^{-1} IAA + coconut water, (K) 1.0 mg L^{-1} IBA + coconut water, and (L) 2.0 mg L^{-1} IBA + coconut water (bar = 1 cm).

The differences in the percentage of rooting between different treatments were significant. Seedlings grown on $\frac{1}{2}$ MS media supplemented with banana puree had higher rooting percentages (88.3–95.0%) compared to those fortified with coconut water and potato puree, irrespective of the type and concentration of the hormones used (Table 5).

In many studies on different species of *Cattleya*, adding banana to the nutrient medium, either as a powder or a puree, was found to improve the growth and rooting of plantlets under *in vitro* conditions. However, species-specific differences in their responses were recorded. Vilcherrez–Atoche, Rojas-Idrogo and Delgado-Paredes (2020) studied *Cattleya maxima* Lindl. and found that the MS nutrient medium supplemented with 30 or 40 g L⁻¹ banana powder was optimal for the growth and rooting of plantlets at the rooting stage, although all treatments, including those fortified with coconut water or only growth regulators (0.5 mg L⁻¹ NAA and 0.1 mg L⁻¹ BA), showed 100% rooting. This finding was different from that recorded in our study, where only the nutrient media containing banana puree promoted 100% rooting. The lowest percentage of rooting (50 ± 7.32 – $70 \pm 8.61\%$) was observed in treatments fortified with potato puree, which occurred probably because potato puree contains large quantities of starch that interferes and prevents the absorption of nutrients, as it acts as a gelling agent in the nutrient medium (Lee et al., 2022). The results showed that the interaction between organic additives and auxin type affected plant height, number of leaves, number of adventitious shoots, number of roots, and their length. The average number of roots per plantlets in a medium supplemented with 1.0 and 2.0 mg L⁻¹ IBA, 1.0 mg L⁻¹ NAA,

and 50 g L⁻¹ banana puree was 4.71 ± 1.70 , 4.85 ± 1.86 , and 4.78 ± 1.28 units per explant, respectively. The number of roots in the banana puree-supplemented medium was significantly higher than that recorded in other treatments. Regarding the growth of the roots and leaves of *C. gaskelliana*, adding 50 g L⁻¹ banana puree with 1.0 mg L⁻¹ IAA to the medium promoted the highest root length (4.10 ± 0.97 cm), leaf number (6.14 ± 0.89 units per explant), and plant height (2.78 ± 0.30 cm) (Table 5).

Many researchers have shown that banana puree or banana powder can promote the growth and formation of roots for many species of orchids. This occurs because of two reasons: the presence of high levels of carbohydrates, ions, and other minerals, such as potassium, manganese, zinc, vitamins (thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, ascorbic acid, folic acid), and natural growth regulators, such as cytokinins, IAA, and GA₃ (Utami & Hariyanto, 2020), and the ability of banana extract to adjust cellular pH, which plays a crucial role in many functions, such as nutrient absorption, rooting, and cellular growth in plants (Acemi, 2020). At the rooting stage, adventitious shoots were formed in all variants. The lowest number of adventitious shoots was recorded on the medium with potato puree and different levels of auxin compared to that in other treatments. The highest number of adventitious shoots (4.1 ± 0.49 shoots per explant) was recorded in the medium supplemented with 50 g L⁻¹ banana puree and 2.0 mg L⁻¹ IBA (Table 5). While studying the effect of auxins and cytokinins on the formation of shoots and roots in *Cattleya maxima* seedlings, we found that the application of IBA stimulates the formation of a large number of shoots and leaves at the rooting stage (Saravia-Castillo et al., 2022).

Table 5: Effect of organic additives, auxin type, and their concentration on *in vitro* rooting and growth of *C. gaskelliana* plantlets after 180 days of cultivation.

Treatment	Plantlet height (cm)	Number of roots (unit)	Number of leaves (unit)	Root length(cm)	Rooting (%)	Number of adventitious shoots (unit)
A	2.78 ± 0.30 a	4.78 ± 1.28 ab	6.14 ± 0.89 a	4.10 ± 0.97 a	95.0 ± 8.36 a	1.64 ± 0.28 cdef
B	1.90 ± 0.26 c	3.57 ± 1.51 bc	5.42 ± 1.27 a	3.12 ± 0.75 bc	91.7 ± 6.83 ab	1.48 ± 0.32 ef
C	2.00 ± 0.31 c	4.71 ± 1.70 ab	5.28 ± 0.75 ab	3.22 ± 0.37 ab	90.0 ± 7.07 ab	1.83 ± 0.46 bcdef
D	2.22 ± 0.34 bc	4.85 ± 1.86 a	4.85 ± 0.69 abc	2.92 ± 0.26 bcd	88.33 ± 6.83 ab	4.10 ± 0.50 a
E	1.84 ± 0.57 c	2.28 ± 0.95 cde	3.42 ± 0.53 cd	2.28 ± 0.26 cdef	70.8 ± 8.61 c	2.40 ± 0.56 bc
F	2.08 ± 0.31 c	2.28 ± 0.48 cde	3.57 ± 0.78 cd	2.04 ± 0.39 def	68.3 ± 6.05 cd	2.45 ± 0.73 b
G	2.05 ± 0.39 c	1.28 ± 0.47 e	3.85 ± 0.69 bcd	1.57 ± 0.21 f	51.7 ± 7.5 e	1.31 ± 0.24 f
H	2.18 ± 0.24 bc	1.85 ± 0.81 de	3.42 ± 1.24 cd	1.96 ± 0.36 ef	60.0 ± 7.07 de	1.50 ± 0.32 ef
I	2.64 ± 0.65 ab	3.14 ± 1.06 cd	3.85 ± 0.89 bcd	3.24 ± 0.64 ab	84.0 ± 4.7 b	2.27 ± 0.55 bcde
J	1.88 ± 0.27 c	2.17 ± 0.37 de	3.57 ± 0.53 cd	2.79 ± 0.66 bcde	83.3 ± 6.6 b	1.62 ± 0.36 def
K	2.21 ± 0.51 bc	2.42 ± 0.53 de	3.28 ± 0.48 d	2.22 ± 0.31 cdef	85.0 ± 8.9 b	1.57 ± 0.32 ef
L	2.37 ± 0.54 abc	2.85 ± 0.69 cd	3.42 ± 0.53 cd	3.08 ± 0.32 bc	83.3 ± 8.7 b	2.36 ± 0.28 bcd

Values are mean $\pm$ SD, the letters "a", "b" and "c" denote groups by Duncan's multiple range test at 5% level *: represent significant or n. s.: non-significant at $p \leq 0.05$.

Indole butyric acid (IBA) also positively affects the multiplication rate compared to NAA (Xu, Beleski, & Vendrame, 2022), which matches the results of our study. However, an increase in the concentration of IBA to 2.0 mg L⁻¹ may negatively affect the development of plantlets and the survival rate in other orchid species, such as *Brassavola nodosa* Lindl. (Xu, Beleski, & Vendrame, 2022).

In vitro, plantlets cannot develop resistance to many biotic and abiotic stresses caused by their cultivation in the laboratory, which is characterized by a sterile environment with controlled temperature, high relative humidity, and high nutrient availability. Therefore, acclimatization strongly influences the success or failure of the mass propagation process of various orchid species *in vitro* (Da Silva et al., 2017). Various media are used to promote the growth of plantlets most effectively under *ex vitro* conditions, as they are characterized by weak roots and a few root hairs under *in vitro* conditions (Irsyadi, 2021). The plantlets of *Cattleya tigrina* A. Rich. showed a high survival rate and optimal growth in coconut coir and crushed pine bark: coconut coir (2:1) substrates, 120 days after transplantation (Menezes-Sá et al., 2021). The best acclimatization medium for *Cattleya warneri* T. Moore. was found to be coconut fiber and vermiculite (1:1, v/v) with a survival rate of 84.0% (Navarro et al., 2023). In our study, the treatment of the base of *C. gaskelliana* plantlets with sphagnum moss and the use of bark, perlite, and turf in a ratio of 1:1:1 resulted in a 100% survival rate after 70 days of acclimatization (Figure 6). This finding was similar to the results reported by (Dewir et al., 2015)

who showed that *Cattleya* hybrid plantlets cultivated in a growing substrate containing bark: perlite: peat in a 1:1:1 ratio had a 98.3% survival rate. This high survival rate was attributed to a suitable texture of the substance for epiphytic plants, which facilitated gas exchange between the roots and the environment (Menezes-Sá et al., 2021). Also, the treatment of the base of plantlets with sphagnum moss helped retain moisture and increased the ability to buffer pH (Khlebova et al., 2019).

Conclusions

PLB of *C. gaskelliana* can be induced using 1.5 mg L⁻¹ BA and 2.0 mg L⁻¹ IBA. The ½ MS medium supplemented with 0.5 mg L⁻¹ TDZ and 100 mL L⁻¹ coconut water was optimal for PLB regeneration and plantlet formation. At the rooting stage, the seedlings showed vigorous growth and root development on the ½ MS nutrient medium with the addition of 1.0 mg L⁻¹ IAA and 50 g L⁻¹ banana puree.

Author Contribution

Conceptual idea: Hussien, M.; Molkanova, O.I.; Methodology design: Hussien, M.; Molkanova, O.I.; Makarov, S.S.; Data collection: Hussien, M.; Makarov, S.S.; Data analysis and interpretation: Hussien, M.; Molkanova, O.I.; Makarov, S.S.; Writing and editing: Hussien, M.; Raeva-Bogoslovskaya, E.N.

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References

- Acemi, A. (2020). Growth enhancing effects of banana homogenate on a glucomannan-rich orchid species: *Serapias vomeracea* (Burm. f.) Briq. *Journal of Cultivated Plants*, 72(6):243-249.
- Akhiriana, E., Samanhudi, S., & Yunus, A. (2019). Coconut water and IAA effect on the *in vitro* growth of *Tribulus terrestris* L. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 67(1):9-18.
- Cardoso, J. C., Zanello, C. A., & Chen, J. T. (2020). An overview of orchid protocorm-like bodies: Mass propagation, biotechnology, molecular aspects, and breeding. *International Journal of Molecular Sciences*, 21(3):985.
- Chadwick, A. A., & Chadwick, A. E. (2021). *The classic Cattleyas*. Florida USA: University Press of Florida, 260p.



Figure 6: Acclimatized *C. gaskelliana* plantlets after 70 days of adaption *ex vitro*.

- Corbellini, J. R. et al. (2020). Effect of microalgae *Messastrum gracile* and *Chlorella vulgaris* on the *in vitro* propagation of orchid *Cattleya labiata*. *Journal of Applied Phycology*, 32:4013-4027.
- Da Silva, J. A. T. et al. (2017). Acclimatization of *in vitro*-derived *Dendrobium*. *Horticultural Plant Journal*, 3(3):110-124.
- Dawa, S. et al. (2019). Production technologies of orchid flowering species. *International Journal of Current Microbiology and Applied Sciences*, 8(1):147-161.
- De, L. C. (2015). Techniques for production of quality planting materials in orchids. *International Journal of Development Research*, 5(6):4587-4591.
- De, L. (2020). Good agricultural practices of *Cattleya* orchids. *Vigyan Varta*, 1(5):53-64.
- Dewir, Y. H. et al. (2015). Micropropagation of *Cattleya*: Improved *in vitro* rooting and acclimatization. *Horticulture, Environment, and Biotechnology*, 56:89-93.
- Farinacio, R., Galdiano, R. F., & Lemos, E. G. M. (2018). *Cattleya* orchids seedlings *in vitro* performance under artificial and natural light. *Acta Horticulturae*, 1224:45-50.
- Ferreira, N. P. et al. (2021). Chemical, chemophenetic, and anticancer studies of *Cattleya tigrina*. *Biochemical Systematics and Ecology*, 97:104303.
- Hartati, S. et al. (2017). Effects of organic additives and naphthalene acetic acid (NAA) application on the *in vitro* growth of black orchid hybrid (*Coelogyne pandurata* Lindley). *Bulgarian Journal of Agricultural Science*, 23(6):951-957.
- Herawati, R., Ganefianti, D. W., & Romeida, A. (2021). Addition of coconut water and banana extract on ms media to stimulate PLB (Protocorm Like Bodies) Regeneration of *Dendrobium gaton* sunray. *International Seminar on Promoting Local Resources for Sustainable Agriculture and Development*, 13:251-258.
- Hong, P. I., Chen, J. T., & Chang, W. C. (2008). Plant regeneration via protocorm-like body formation and shoot multiplication from seed-derived callus of a maudiae type slipper orchid. *Acta Physiologiae Plantarum*, 30:755-759.
- Hossen, M. M. et al. (2021). Effects of plant growth regulators on *in vitro* growth and development of orchid *Dendrobium* sp. from protocorm like bodies (PLBs). *Journal of Bangladesh Agricultural University*, 19(3):294-301.
- Irsyadi, M. B. (2021). Factors that effect of the optimal plantlet growth from tissue culture on the acclimatization stage. *Proceeding International Conference on Science and Engineering*, 4:100-104.
- Jolman, D. et al. (2022). The challenges of growing orchids from seeds for conservation: An assessment of asymbiotic techniques. *Applications in Plant Sciences*, 10(5):e11496.
- Khatun, K., Nath, U. K., & Rahman, M. S. (2020). Tissue culture of *Phalaenopsis*: Present status and future prospects. *Journal of Advanced Biotechnology and Experimental Therapeutics*, 3:273-285.
- Khlebova, L. P. et al. (2019). Adaptation to *ex vitro* conditions of *Stevia rebaudiana* (Bertoni) Hemsl. regenerants. *Ukrainian Journal of Ecology*, 9(3):376-380.
- Lee, Y. J. et al. (2022). The effects of banana, potato, and coconut water in the regeneration of *Ficus carica* cv. Japanese BTM 6. *Malaysian Applied Biology*, 51(1):163-170.
- Lopez, R., & Runkle, E. (2004). *The flowering of orchids: A reality check*. Orchids: The Bulletin of the American Orchid Society. Available in: <https://www.canr.msu.edu/uploads/resources/pdfs/19_-_flowering_of_orchids.pdf>.
- Majda, M., & Robert, S. (2018). The role of auxin in cell wall expansion. *International Journal of Molecular Sciences*, 19(4):951.
- Malhotra, E. V. et al. (2023). Regeneration of plantlets through protocorm-like bodies: An alternative method for vanilla micro propagation. *Indian Journal of Biotechnology*, 20:388-395.
- Menezes, E. L., Giordani, S. C. O., & Mendes, J. C. R. (2022). *Cattleya mireileiana*, a new species of Orchidaceae (Laeliinae) from the Southern Espinhaço Complex, Minas Gerais State, Brazil. *Phytotaxa*, 541(3):270-276.
- Menezes-Sá, T. S. A. et al. (2021). *In vitro* propagation and conservation of *Cattleya tigrina* A. Rich. *Ciência Rural*, 52(5):e20200517.
- Mercado, S. A. S., Jaimes, Y. M. O. (2022). Inclusion of organic components in culture medium to improve the *in vitro* propagation of *Cattleya warscewiczii* and *Cattleya gaskelliana*. *South African Journal of Botany*, 148:352-359.
- Misirova, S. (2023). Technology of growing orchid flowers from seeds. *E3S Web of Conferences EDP Sciences*, 390:07030.
- Molkanova, O. I. et al. (2018). Improving the technology of clonal micropropagation of valuable fruit and berry crops for production conditions. *Achievements of Science and Technology of the Agro-industrial Aomplex*, 32(9):66-69.
- Mondal, T., & Banerjee, N. (2017). Micropropagation and *in vitro* conservation of threatened orchids: A brief review. *CIBTech Journal of Biotechnology*, 6(3):1-12.
- Moraes, M. C. et al. (2020). Commercial fertilizers and organic additives in orchid micropropagation. *Plant Cell Culture & Micropropagation*, 16:16-e162.
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum*, 15:437-497.
- Naing, A. H. et al. (2011). Efficient plant regeneration of the endangered medicinal orchid, *Coelogyne cristata* using protocorm-like bodies. *Acta Physiologiae Plantarum*, 33:659-666.

- Navarro, Q. R. et al. (2023). Effect of microalga *Desmodesmus subspicatus* and plant growth regulators on the *in vitro* propagation of *Cattleya warneri*. *Plant Cell, Tissue and Organ Culture*, 153(1):77-89.
- Pant, M. et al. (2020). Cattleya orchids: A mini review. *Journal of Critical Reviews*, 7:4592-4598.
- Perner, H. et al. (2022). *Cypripedium subtropicum* embryo development and cytokinin requirements for asymbiotic germination. *Botanical Studies*, 63:28.
- Plants of the World Online - POWO. (1885). *Cattleya gaskelliana* (N.E.Br.) B.S.Williams. Available in: <<https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:50209-2>>.
- Poniewozik, M., Szot, P., & Parzymies, M. (2021). Tissue culture multiplication of *Paphiopedilum insigne* depending on the medium type, growth regulators and natural supplements. *Acta Scientiarum Polonorum Hortorum Cultus*, 20(4):125-134.
- Pupulin, F. (2015). A new form of *Cattleya dowiana* and the taxonomy of its color variations. *Orchids*, 84(1):46-54.
- Pyati, A. N. (2022). Multiple protocorm like body (MPLB) formation and plant regeneration from the PLB culture of a rare orchid *Aerides crispum* Lindl. *Indian Journal of Applied & Pure Biology*, 37(2):555-563.
- Reddy, J. et al. (2020). Plant growth regulators used for *in vitro* micropropagation of orchids: A research review. *International Journal of Biological Research*, 8(1):37-42.
- Salazar, S. K., & Arcia-Barreto, M. M. (2020). Ríos en la cuenca Caribe oriental y drenajes a los golfos de Cariaco y Paria. *Douglas Rodríguez Olarte*, 13.
- Samiei L. et al. (2021). Organic and inorganic elicitors enhance *in vitro* regeneration of *Rosa canina*. *Journal of Genetic Engineering and Biotechnology*, 19(1):60.
- Saravia-Castillo, G. et al. (2022). Auxins and Cytokinins elicit a differentiated response in the formation of shoots and roots in *Cattleya maxima* Lindl and *Phalaenopsis amabilis* (L) Blume. *Scientia Agropecuaria*, 13(1):63-69.
- Utami, E. S. W., & Hariyanto, S. (2020). Organic compounds: contents and their role in improving seed germination and protocorm development in orchids. *International Journal of Agronomy*, 2:1-12.
- Van Den Berg, C. (2014). Reaching a compromise between conflicting nuclear and plastid phylogenetic trees: a new classification for the genus *Cattleya* (Epidendreae; Epidendroideae; Orchidaceae). *Phytotaxa*, 186(2):75-86.
- Vilcherrez-Atoche, J. A., Rojas-Idrogo, C., & Delgado-Paredes, G. E. (2020). Micropropagation of *Cattleya maxima* J. Lindley in culture medium with banana flour and coconut water. *International Journal of Plant, Animal and Environmental Sciences*, 10(4):179-193.
- Xing, X. et al. (2019). The impact of life form on the architecture of orchid mycorrhizal networks in tropical forest. *Oikos*, 128(9):1254-1264.
- Xu, J., Beleski, D. G., & Vendrame, W. A. (2022). Effects of culture methods and plant growth regulators on *in vitro* propagation of *Brassavola nodosa* (L.) Lindl. hybrid. *In Vitro Cellular & Developmental Biology - Plant Tissue Culture*, 58:931-941.
- Zakaria, S. et al. (2021). Development of *in vitro* culture system for proliferation of protocorm-like bodies of *Oncidium* golden anniversary orchid. *Songklanakarin Journal of Science & Technology*, 43(6):1542-1549.
- Zhao, D. K. et al. (2021). Orchid reintroduction based on seed germination-promoting mycorrhizal fungi derived from protocorms or seedlings. *Frontiers in Plant Science*, 12:701152.