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Scientific Article

Impact of monochromatic lights on the *in vitro* development of *Cattleya walkeriana* and effects on acclimatization

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Abstract

Light quality is an important factor for the adequacy of plant production through plant tissue culture, as it directly interferes with morphogenesis and photosynthetic capacity of explants. The objective of this study was to analyze the impact of monochromatic lights such as light emitting diode (LED) on the *in vitro* development of *Cattleya walkeriana* G. and their effects on acclimatization. The plants were developed *in vitro* under the colors of green, blue, yellow, red, 2 red:1 blue, and white LED lights. For *in vitro* cultivation, Knudson medium was used, supplemented with 20 mg L^{-1} sucrose, 5.5 mg L^{-1} agar, 2% activated charcoal, 100 mL coconut water, and pH 6.0. For *ex vitro* cultivation, the plants were acclimatized in styrofoam trays containing sphagnum as a substrate. In general, the supplied light lengths impacted*in vitro* growth and acclimatization analyses. There was influence on the cuticle thickness of plants *in vitro*. Chlorophyll and carotenoid contents were not significant. We can conclude that light lengths formed by 2 red:1 blue, red and yellow LEDs can be indicated for better performance in the production of *C. walkeriana*. The 2 red:1 blue and red LEDs provide superior *in vitro* development than the others, with gains for the species in acclimatization. The yellow LED provided a possible *in vitro* hardening, which ensured the greatest success of the seedlings during acclimatization.
Keywords: light emitting diode - LED, light length, micropropagation, Orchidaceae, ornamental plants.

Resumo

Impacto de luzes monocromáticas no desenvolvimento *in vitro* de *Cattleya walkeriana* e seus efeitos na fase de aclimatização

A qualidade luminosa é um fator importante para a adequação da produção de plantas através da cultura de tecidos vegetais, pois interfere diretamente nos processos de morfogênese e na capacidade fotossintéticas dos explantes. O objetivo deste estudo foi analisar o impacto de luzes monocromática do tipo diodo emissor de luz (*light-emittingdiode* - LED) no desenvolvimento *in vitro* de *Cattleya walkeriana* G. e os efeitos na fase aclimatização. As plantas foram desenvolvidas *in vitro* sob as cores de luzes do tipo LED verde, azul, amarelo, vermelho, 2 vermelhos: 1 azul, e branco. Para cultivo *in vitro* foi utilizado o meio Knudson acrescido de 20 mg L^{-1} de sacarose, 5,5 mg L^{-1} de ágar, 2% de carvão ativado, 100 mL de água de coco, e pH 6,0. Para o cultivo *ex vitro* as plantas foram aclimatizadas em bandejas de isopor contendo esfagno como substrato. De maneira geral, os comprimentos luminosos fornecidos apresentaram impactos nas análises de crescimento *in vitro* e na de aclimatização. Houve influência na espessura da cutícula das plantas *in vitro*. Os teores de clorofilas e carotenoides não foram significativos. Podemos concluir, que comprimentos luminosos formados pelos LEDs 2 vermelhos: 1 azul, vermelho e amarelo podem ser indicados para melhor desempenho na produção de *C. walkeriana*. Os LEDs 2 vermelhos: 1 azul, e o LED vermelho propiciam desenvolvimento *in vitro* superior ao demais com ganhos na fase de aclimatização para espécie. O LED amarelo propiciou um possível processo de rustificação *in vitro* o que garantiu o maior sucesso das mudas no período de aclimatização.
Palavras-chave: comprimento luminoso, diodo emissor de luz - LED, micropropagação, Orchidaceae, plantas ornamentais.

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Introduction

Brazil has one of the greatest orchid diversity in the world; such plants are found in all Brazilian biomes. Among many, *Cattleya walkeriana* G. is a small orchid, with a more expressive occurrence in the Cerrado biome, being a native and endemic species of Brazil (REFLORA, 2020). It has pink flowers in an attractive format for sale, in addition to being highly appreciated by collectors. This plant is among the species considered vulnerable, due to excessive collection for marketing and pressure on its natural habitat (Brasil, 2022).

Orchids have a complex life cycle. For seeds to germinate under natural conditions, symbiosis with fungi is necessary (Andrade et al., 2023; Makwela et al., 2022; Ogórek et al., 2020).Furthermore, these plants exhibit slow growth and go through an extensive vegetative period until they reach reproductive maturity (Zhang et al., 2018). In this context, *in vitro* cultivation is extremely useful for species propagation and maintenance, in addition to accelerating seedling development. Acclimatization is a very important phase requiring studies that help improve productivity to guarantee market demand (Mercado and Delgado, 2020).

One of the factors that affect *in vitro* cultivation is luminosity (Ribeiro et al., 2022). Light is a source of energy for chlorophyllated plants, essential for plant life, interfering in morphological, biochemical and anatomical processes. Light quality or wavelength aspects are related to luminosity (Al Murad et al., 2021).

Light energy is used to boost electron transfer and generate proton driving force across membranes, crucial for the formation of adenosine triphosphate (ATP). Plants absorb the entire length of the light spectrum. For carbon fixation, they use photosynthetically active radiation (PAR), which corresponds to the blue (400 to 500 nm) and red (greater than 600 nm) zones of the visible spectrum (Taiz et al., 2017). The other bands, such as green and yellow, are absorbed in smaller amounts and are involved in processes such as increasing the amount of secondary metabolites, flowering and improvements in plant nutrition (Al Murad et al., 2021).

To provide different light lengths in *in vitro* cultivation, LEDs (light emitting diodes) are used. The different LED colors can be used alone or combined with each other, and the intensity of the supplied light length can also be controlled. According to a recent review by Al Murad et al. (2021), it is necessary to test how plant species respond to the amount of light, thus identifying light regimes that improve growth, development, nutrition, flowering, defense mechanisms, and even fruit flavor. There is a lack of studies on the impact of monochromatic light combinations on plant growth and development aspects.

For ornamental plants, information on how the monochromatic light lengths provided by LEDs affect micropropagation are concentrated in the *in vitro* multiplication and rooting. Different light lengths, many in combination with other factors relevant to tissue culture, have been studied on different ornamental plants (Miler et al., 2019), such as Anthurium (*Anthurium andreanum)* (Martínez-Estrada et al., 2016), *Oncidium tigrinum* and *Laelia autumnalis* (Murillo-Talavera et al., 2016), *Microlaelia lundii* (Favetta et al., 2017), red ginger (*Alpinia purpurata*) (Pinheiro et al., 2019), *Phalaenopsis amabilisalba* Blume (Massaro et al., 2019), *Moluccella laevis* (Zielińska et al., 2020), Gerbera (*Gerbera jamesonii*) (Cioć et al., 2021), *Pteris aspericaulis* var. tricolor (Yu et al., 2021), and *Brassavola nodosa* (Vendrame et al., 2022).

In general, there is little information on the impact of monochromatic lights on acclimatization. In orchids, it was studied by Sorgato et al. (2015) with *Dendrobium phalaenopsis*, and the use of colored shading screens was discussed by Massaro et al. (2019) in *Phalaenopsis amabilis alba* Blume. This phase is a very critical step in micropropagation, with seedling losses occurring, as plants move from a heterotrophic condition, with a fully controlled environment, to an autotrophic condition. In this phase, predominant factors observed are related to humidity, temperature, shading and substrate (Faria et al., 2018; Nadal et al., 2022).

Given the above, the objective of this study was to analyze the impact of monochromatic LED lights on the *in vitro* development of *Cattleya walkeriana* G. and their effects on acclimatization.

Materials and Methods

Plant Material

Plants derived from *Cattleya walkeriana* seeds cultivated in an orchidarium at Universidade Federal de Lavras were used. Seeds were germinated in Knudson medium (Knudson, 1946), supplemented with 20 mg L^{-1} sucrose, 5.5 mg L^{-1} agar, 2% activated charcoal; pH was adjusted to 6.0. Subsequently, 40 mL of nutrient solution were added in glass flasks, and they remained in a growth room at 24 ºC with a 16-h photoperiod (40 to 56 µmol m^{-2} s^{-1}), for 60 days. After seed germination, the seedlings were transferred to the same medium with 100 mL of coconut water, remaining in a growth room for another 60 days, until the experiment was set.

In vitro multiplication and rooting under monochromatic light regimes

For multiplication and rooting, Knudson medium (Knudson, 1946) was used, with 20 mg L^{-1} sucrose, 5.5 mg L^{-1} agar, 2% activated charcoal, supplemented with 100 mL of coconut water, pH adjusted to 6.0; 40 mL of nutrient solution were used in each flask.

The plants were kept in a growth room with a 16-hour photoperiod at 25 °C±2° C, under different monochromatic LED lights, comprising the treatments: red (mean irradiance: 31.1 µmol m^2 s^{-1}), blue (mean irradiance: 67.7 µmol m^2 s^{-1}), 2 red :1 blue (mean irradiance: 42.1 µmol m^2 s^{-1}), green (mean irradiance: 47.6 µmol m^2 s^{-1}), yellow (mean irradiance: 23.8 µmol m^2 s^{-1}), and white (mean irradiance: 76.9 µmol m^2 s^{-1}).

Eight experimental units were used, each experimental unitconsistedof a flask with three plants. The plants had, on average, 1.5 cm in height and 1.0 cm in root. They remained in the treatments for 120 days.

Acclimatization

After 120 days of *in vitro* development (multiplication and rooting), the plants were transferred to a greenhouse. The seedlings were individualized and acclimatized in styrofoam trays containing sphagnum as a substrate. Irrigation was carried out manually, according to the plant needs. The material remained in a greenhouse for 90 days. Six replications with six plants each were used.

Plant Analysis

Survival, number of sprouts, number of leaves, number of roots, shoot length (cm), longest root length (cm) were evaluated for the *in vitro* and acclimatization phases. Shoot (mg) and root (mg) dry matter were evaluated only in acclimatization.

A digital caliper was used to analyze the measured variables. Dry matter was evaluated after drying in a forced air oven at 65 ºC for 24 hours. For dry matter qualification, a precision scale was used.

Pigment Analysis

To estimate the content of chlorophylls *a*, *b*, total and carotenoids, 10 mg of fresh leaves were added in 80% acetone and the extract formed was filtered using a paper filter. The absorbances of the solution were read at 663 nm chlorophyll *a*, 645 nm chlorophyll *b* and 470 nm carotenoids. Pigment content was calculated according to Lichtenthaler's methodology (Lichtenthaler, 1987).

Anatomical analysis of cuticle thickness

In vitro plant leaves were collected and fixed in 70% alcohol (v v^{-1}) (Johansen, 1940) and, after 72 hours, they were placed in a new 70% alcohol solution (v v^{-1}) to preserve the material at room temperature until analysis.

The plant material was dehydrated in an increasing ethylic series (80%, 90% and 100% - v v^{-1}) and, after dehydration, it underwent infiltration and polymerization based on methacrylate historesin (Leica Microsystems, Wetzlar, Germany).

Subsequently, it was sectioned with about 8 μm thick, and leaf cross-sections were obtained, with the aid of a semi-automatic rotary microtome (MRP 2015, LupetecTecnologiaAplicada – Lupe IndústriaTecnológica de Equipamentos para Laboratório, Brazil). Sections were stained with 1% (m v^{-1}) (O'Brien et al., 1964) and slides were prepared with vitreous varnish (Acrilex Tintas Especiais S.A.) as a mounting medium.

The slides were observed and photographed in an optical microscope (Red 200, Kasvi/Motic), coupled to a digital camera (Moticam 5MP, Motic). For each treatment, triplicates of cuticle photographs were used. The images were then analyzed using the UTHSCSA-Image Tool software, version 3.0.

Experimental design and statistical analysis

For both experiments, a completely randomized experimental design was used. The data were submitted to the normality test, and subsequent analysis of variance. Means were compared using the 5% Scott-Knott test. The R© software was used.

Results and Discussion

In the commercial multiplication of orchids through *in vitro* seeding, it is essential that the plants survive successfully, both in *in vitro* development and in acclimatization (Shah et al., 2019). In acclimatization, different measures are applied so that the plants adapt to the *ex vitro* environment and develop properly. In general, we observed distinct positive effects on the development of the species at all light lengths studied *in vitro*. Plants that were grown *in vitro* under yellow, 2 red:1 blue and red light showed better development in the acclimatization period. This treatment stood out from the others in most of the variables analyzed in acclimatization.

As for *in vitro* survival rates, plants grown *in vitro* under yellow, blue, red, and 2 red:1 blue light showed 100% survival. Under white light, 60% of the plants survived and, under green light, 80% of the plants. In acclimatization, the only plant loss occurred with seedlings from cultivation under blue light, where survival was 93%. In the other treatments, the plants had 100% survival (Table 1).

Table 1. Survival, sprouts, number of leaves and roots of *Cattleya walkeriana* grown under monochromatic LED lights during *in vitro* development and effects on acclimatization.

***In vitro* development**							
	Survival (%)	**Sprouts**		**No. of leaves**		**No. of roots**	
White	60.00	4.55	a*	3.30	NS*	5.33	b
Green	80.00	3.71	b	3.40		4.15	c
Blue	100.00	5.96	a	3.28		3.98	c
Yellow	100.00	4.74	a	3.33		3.06	d
2 red:1 blue	100.00	2.46	b	3.24		6.27	a
Red	100.00	2.96	b	2.94		5.78	b
Mean	-	12.09		3.26		4.46	
CV (%)	-	43.15		42.65		53.98	
Acclimatization							
	Survival (%)	**Sprouts**		**No. of leaves**		**No. of roots**	
White	100.00	-		2.25	NS	3.41	NS
Green	100.00	-		2.20		3.51	
Blue	93.00	-		2.38		3.41	
Yellow	100.00	-		2.19		3.59	
2 red:1 blue	100.00	-		2.02		4.00	
Red	100.00	-		2.28		4.01	
Mean	-	-		2.22		3.69	
CV (%)	-	-		47.95		35.79	

Scott-Knott test at 5%. *Same letters in the row do not differ statistically. NSNot significant.

For *Dendrobium phalaenopsis*, the use of the combination white + red demonstrated superior results during intermediate acclimatization, in addition to a higher survival rate (Sorgato et al., 2015).

Different LED treatments provide a more noticeable effect on plant growth and morphological changes, as light plays a crucial role in controlling plant development, especially through photosynthesis (Al Murad et al., 2021). In the initial phase of *in vitro* development, cell division is intense, especially due to the controlled conditions that generate a greater stimulus to the tissues. It is possible to state that the viability of using the studied colors in the 16-hour light exposure regime, with the exception of the green color, is greater in comparison with the white LED for this orchid. Other aspects related to the light regime may be involved in the lower performance of the white light in species survival, since this is the sum of all light lengths, acting in a complete way in plant growth and development.

In some studies, it was observed that the green light has effects similar to those of the blue light, affecting the formation of chlorophyll, in the development of chloroplasts, but this light can also reverse the effects of blue and red lights, through the so-called inductive biological antagonistic systems (Al Murad et al., 2021).

Plants grown under yellow, blue and white light had the highest number of sprouts, differing from plants grown under green, 2 red:1 blue and red light. Plants that went through the acclimatization period did not develop sprouts during the period (Table 1). Seedlings of the Brazilian orchid *Cattleya lundii* (formerly *Microlaelia lundii*) showed lower numbers of sprouts when exposed to red and blue LED (Favetta et al., 2017). However, in *in vitro* propagation of Alpinia cultivars, exposure to white LED for Red Ginger cultivar provided a higher number of sprouts (Pinheiro et al., 2019). This variation in the formation of sprouts between species and cultivars was also reported in the *in vitro* banana crop, where Rocha et al. (2017) evaluated the use of fluorescent lights and LEDs and demonstrated that the use of LED helps sprout development with variations between cultivars.

For the number of leaves *in vitro*, there were no significant differences between treatments. However, for the number of roots *in vitro*, the light length provided by the use of 2 red:1 blue stands out from the others. On average,

plants grown under this light incidence had 6.27 roots. The lowest number of roots found in plants *in vitro* was with the use of yellow LED (3.06 roots). In acclimatization, the number of leaves and roots did not differ statistically (Table 1).

For the *in vitro* cultivation of *Oncidium tigrinum*, the use of red and blue LEDs at a 1:1 ratio inhibited the development of the root system. For this species, it was observed that the increase in the proportion of blue showed a relationship with the inhibition of root development (Murillo-Talavera et al., 2016). Undoubtedly, the combination of colors impacts plant response to light, given the opposite behavior observed in *C. walkeriana* with the use of 2 reds:1 blue.

For shoot length in *in vitro* cultivation, 2 red:1 blue and red LED lights provided better results. In these treatments, the plants have a shoot length of 3.42 and 3.72 cm. Regarding the length of the longest root in the *in vitro* cultivation, 2 red:1 blue, red and white LEDs stood out, providing an average of 7.96, 7.72 and 7.42 cm, respectively. However, in acclimatization, for shoot length, plants from yellow and red light stood out, with 38.02 and 39.71 cm in length. In relation to root length, the plants that were in the yellow (47.81 cm), 2 red :1 blue (50.50 cm) and red (47.48 cm) light are highlighted. Plants from white, green and blue light had the smallest root length (Table 2).

Table 2. Shoot length (SL) and longest root length (LRL) of *Cattleya walkeriana* grown under monochromatic LED lights during *in vitro* development and effects on acclimatization.

***In vitro* development**				
	SL (cm)		**LRL (cm)**	
White	2.73	b*	7.42	a
Green	2.68	b	5.90	b
Blue	2.61	b	5.46	b
Yellow	2.24	b	5.56	b
2 red:1 blue	3.42	a	7.91	a
Red	3.72	a	7.72	a
Mean	2.79		6.36	
CV (%)	49.85		60.70	
Acclimatization				
	SL (cm)		**LRL (cm)**	
White	26.80	c	32.07	c
Green	30.29	c	38.22	b
Blue	28.97	c	41.40	b
Yellow	38.02	a	47.81	a
2 red:1 blue	34.73	b	50.50	a
Red	39.71	a	47.48	a
Mean	33.22		43.20	
CV (%)	28.11		32.79	

Scott-Knott test at 5%. *Same letters in the row do not differ statistically.

The different bands of the light spectrum generate changes in the concentration of auxins, affecting apical elongation (Hanus-Fajerska and Wojciechowska, 2017). The *in vitro* cultivation of *Cattleya loddigesii* under red light through cellophane caused the plants to elongate (Araújo et al., 2009). Regarding root length, the literature shows that the red light yielded a shorter root length, since root elongation is inhibited by phytochrome in response to the red light. It was observed during the acclimatization of *Cattleya loddigesii* (Galdiano Júnior et al., 2012), and *Oncidium tigrinum* (Murillo-Talavera et al., 2016). However, it was not found in *C. walkeriana*. In addition, yellow light proved to be efficient when plants are taken for *ex vitro* development. In the literature, yellow light is involved in flowering processes (Al Murad et al., 2021).

In addition, acclimatized plants fromyellow, 2 red:1 blue and red light showedhighervaluesof shoot fresh and dry matter and root dry matter. For rootfresh matter, the plants from the yellow and 2 red:1 blue lights can be highlighted (Table 3). Figure 1 shows the plants after the acclimatization period.

Table 3. Shoot fresh matter (SFM), shoot dry matter (SDM), root fresh matter (RFM), root dry matter (RDM) of acclimatized *Cattleya walkeriana* grown under monochromatic LED lights during *in vitro* development.

Acclimatization								
	SFM (mg)		**SDM (mg)**		**RFM (mg)**		**RDM (mg)**	
White	2.35	b*	0.47	b	2.90	b	0.15	b
Green	2.93	b	0.45	b	2.70	b	0.18	b
Blue	2.78	b	0.37	b	3.01	b	0.20	b
Yellow	4.84	a	1.27	a	4.00	a	0.27	a
2 red:1 blue	4.97	a	1.04	a	4.99	a	0.36	a
Red	4.39	a	1.19	a	3.54	b	0.29	a
Mean	3.71		0.80		3.52		0.24	
CV (%)	39.31		72.93		38.44		41.99	

Scott-Knott test at 5%. *Sameletters in the row do not differ statistically.

Figure 1. *Cattleya walkeriana* after acclimatization period. The treatments are represented by: A - White; B - Green; C - Blue; D - Yellow; E - 2 red:1 blue; F- Red.

The better performance under the yellow light possibly occurred due to plant hardening or stress still *in vitro* which, when transplanted, regulated more quickly in relation to the others. Plants from treatments 2 reds:1 blue and the red alone, presented a superior *in vitro* development, and might have also regulated themselves more quickly to the external environment. Plants cultivated under different light regimes commonly have a higher concentration of

enzymes involved in antioxidant metabolism. Some of these enzymes also act as signals for plant growth, such as hydrogen peroxide (Al Murad et al., 2021).

There are few studies on the impact of using specific light lengths in acclimatization. *Paphiopedilum* seedlings showed greater accumulation *in vitro* of root fresh and dry matter under the red light (Lee et al., 2011) , as well as for shoot and root fresh and dry matter in *Dendrobium phalaenopsis* (Sorgato et al., 2015).

Photosynthetic pigments play an important physiological role for plant growth and development, as they are light receptors, a vital energy source for photosynthesis and other metabolic processes. In addition, the biosynthesis of plant pigments is dependent on light (Taiz et al., 2017). The analyzed contents of chlorophylls and carotenoids were not significant for plants *in vitro* or in acclimatization (Table 4).

Table 4. Chlorophyll a content (ChlA), chlorophyll b content (ChlB),total chlorophyll content (ChlT) and carotenoids (Carot) of *Cattleya walkeriana* grown under monochromatic LED lights during *in vitro* development and effects on acclimatization.

In vitro development								
	ChlA		ChlB		ChlT		Carot	
White	0.3162	NS*	0.1755	NS	0.5128	NS	0.1457	NS
Green	0.6895		0.4278		1.0427		0.2667	
Blue	0.4099		0.4837		1.1670		0.2369	
Yellow	0.3874		0.3373		0.7447		0.1727	
2 red:1 blue	0.4430		0.4670		0.9254		0.2243	
Red	0.2802		0.2425		0.6465		0.1759	
Mean	0.4210		0.3557		0.8404		0.2037	
CV (%)	58.60		72.56		67.01		54.08	
Acclimatization								
	ChlA		ChlB		ChlT		Carot	
White	0.0352	NS	0.0301	NS	0.0622	NS	0.0166	NS
Green	0.0391		0.0242		0.0406		0.0142	
Blue	0.0257		0.0254		0.0438		0.0112	
Yellow	0.0263		0.0126		0.0399		0.0245	
2 red:1 blue	0.0529		0.0427		0.0871		0.0203	
Red	0.0537		0.0289		0.0888		0.0202	
Mean	0.0393		0.0272		0.0604		0.0178	
CV (%)	63.31		88.27		68.34		80.19	

[NS]Not significant.

According to Bridgen et al. (2018), plants cultivated *in vitro* grow in an artificial environment with high humidity and this factcauses them to have a reduced layer of epicuticular wax, leaving them prone to desiccation when placed in environments with lower humidity. During acclimatization, plants undergo a period of stomatal regulation and epicuticular wax development. The thicknesses of the adaxial cuticle (Figure 2) of the plants that were cultivated *in vitro* varied between 1.96 and 1.20 µm, and the plants cultivated under blue (1.96 µm), red (1.94 µm) and white (1.66 µm) light exhibited greater adaxial cuticle thickness, differing from plants grown under other light lengths. As forthe thickness of the abaxial cuticle (Figure3), there were no statistical differences between treatments (Table 5).

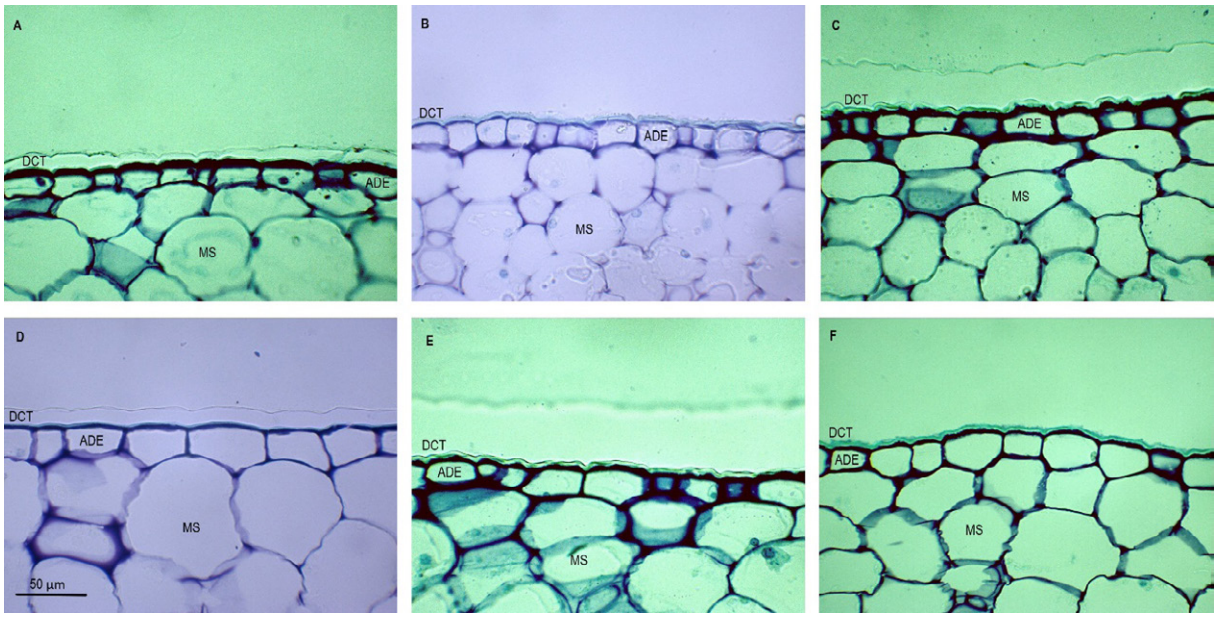

Figure 2. Adaxial cuticle anatomical images of cultivated *Cattleya walkeriana* grown under monochromatic LED lights during *in vitro* development. A - White; B - Green; C - Blue; D - Yellow; E - 2red:1blue; F- Red.ADE - adaxial epidermis; DCT - adaxial epidermis cuticle; MS - mesophyll cells. Images are at 40x magnification under light microscopy.

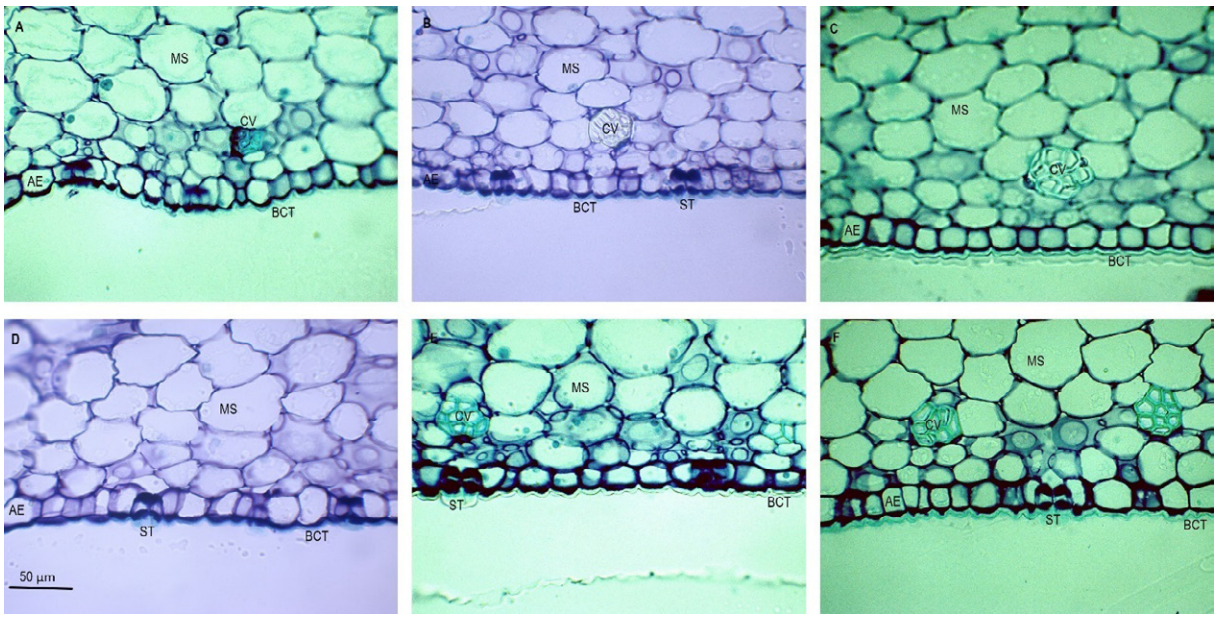

Figure 3. Abaxial cuticle anatomical images of cultivated *Cattleya walkeriana* grown under monochromatic LED lights during *in vitro* development. A - White; B - Green; C - Blue; D - Yellow; E - 2 red:1 blue; F - Red. AE - abaxial epidermis; BCT - abaxial epidermis cuticle; CV - conducting vessels; MS - mesophyll cells; ST- stomata. Images are at 40x magnification under light microscopy.

Table 5. Adaxial (DCT) and abaxial (BCT) cuticle thicknesses of *Cattleya walkeriana* grown under monochromatic LED lights during *in vitro* development.

***In vitro* development**				
	DCT (µm)		**BCT (µm)**	
White	1.66	a*	1.47	a
Green	1.40	b	1.25	a
Blue	1.96	a	1.71	a
Yellow	1.28	b	1.40	a
2 red:1 blue	1.20	b	1.51	a
Red	1.84	a	1.42	a
Mean	1.57		1.45	
CV (%)	25.42		20.18	

Scott-Knott test at 5%. *Same letters in the row do not differ statistically.

Plants from *in vitro* cultivation normally have a thinner cuticle. However, depending on light quality, there maybe an improvement in tissue development. Thicker cuticle thicknesses would help in a greater success in plant acclimatization. Nonetheless, these results, when observed together with phytotechnical data, possibly show other factors that have a more significant impact on acclimatization.

Finally, the results of this study offer subsidy to improve *Cattleya walkeriana* micropropagation. The idea used here can be adapted to other species of orchids that have high loss rates in acclimatization, as it was observed that the light provided during *in vitro* development impacts the process.

Conclusions

The light lengths formed by the 2 red:1 blue, red and yellow LEDs can be indicated for better performance in the production of *C. walkeriana*. The 2 red:1 blue and red LEDs provide superior *in vitro* development than the others, with gains in acclimatization for the species. The yellow LED provided a possible *in vitro* hardening, which ensured the greatest success of the seedlings during acclimatization.

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Author contribution

MCN: Conceptualization, Methodology, Investigation, Project administration. **MCN, NBM, CSS, JHNF**: Data curation. **MCN, JHNF**: Formal analysis, Validation,Visualization, Writing – review & editing. **MP, JD**: Funding acquisition. **MP**: Resources. **JHNF**: Software. **MCN, NBM**: Writing – original draft,

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