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Influence of different spectral qualities on the morphology and physiology of Cattleya crispa lindl. (Orchidaceae) during in vitro cultivation and plant acclimatization

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Abstract

This study evaluated the influence of different spectral light qualities during *in vitro* cultivation on the morphological and physiological development of *Cattleya crispa* L. and the persistence of these effects in *ex vitro* acclimatization. To this end, seeds were germinated asymbiotically in ½MS medium and, after 180 days, the seedlings were cultivated under white (400–700 nm), red (660 nm), blue (450 nm) and blue/red combination LEDs (40%/60%; 450 and 665 nm). At 270 days, the plants were transferred to commercial substrate and maintained for 120 days under the same treatments. During *in vitro* cultivation, white, red and blue/red lights favored greater fresh mass and root development, while isolated blue light showed a restrictive effect. During acclimatization, the blue/red combination promoted greater fresh mass, root length, and shoot size, while red light especially favored the number of roots. Leaves of plants grown under red light showed a lower SPAD index, and white and blue with red lights stimulated greater chlorophyll accumulation and photosynthetic efficiency (Fv/Fm and ETRmax). Carotenoid contents did not differ among treatments, whereas anthocyanins were not detected in the evaluated plants.. It is concluded that the blue/red combination is the most suitable for optimizing growth, vegetative development, and *ex vitro* acclimatization of *Cattleya crispa*, and is recommended for micropropagation and conservation protocols of the species.

Introduction

The family Orchidaceae is one of the largest and most diverse among angiosperms, exhibiting high taxonomic diversity and a high degree of endemism (Pessoa et al., 2025; Wang et al., 2024; Van Den Berg, 2020). However, restricted geographic distribution, combined with extractive pressure and habitat loss, has increased the number of threatened species, reinforcing the importance of ex situ conservation strategies (Pessoa et al., 2025; Vitt et al., 2023; CNFLORA, 2023; Van Den Berg, 2020).

Cattleya crispa L. is endemic to the Atlantic Forest, occurring at altitudes between 700 and 800 m in the states of Rio de Janeiro, Espírito Santo, and Minas Gerais. The species exhibits slow growth, with an average generation time of ten years (CNCFLORA, 2023). In the Brazilian Flora Red List, under the synonym *Hadrolaelia crispa* (Lindl.) Chiron & V.P. Castro, it is classified as Vulnerable, with a projected population reduction of at least 30% over the next 30 years (BRASIL, 2022).

In vitro cultivation has become an important tool for conserving genetic diversity and propagating orchids, particularly due to their seeds having low nutritional reserves and dependence on specific conditions for germination (Phillips et al., 2020; Pujasatria *et al.*, 2020; Yeung *et al.*, 2019). Among environmental factors, light spectral quality plays a central role in seedling growth and development, acting both as an energy source and as a regulatory signal for physiological and morphogenetic processes, including germination, photosynthesis, photomorphogenesis, biomass accumulation, chlorophyll biosynthesis, and photosynthetic efficiency (Zheng et al., 2019; Stefanowski *et al.*, 2022; Samkumar et al., 2021; Costa et al., 2021; Huang et al., 2022; Landi et al., 2020).

Manipulation of the light spectrum therefore constitutes a relevant strategy for controlling *in vitro* growth (Qiao et al., 2025; Tarakanov et al., 2022; Talib et al., 2020). Wavelengths in the red, blue, green, and yellow ranges have shown promising effects on plant development (Cavallaro et al., 2023; Stefanowski *et al.*, 2022). In orchids, different spectral qualities influence germination and the protocorm development index, altering growth rates according to the applied wavelength (Pereira et al., 2022; Nadal et al., 2023).

Plant responses to light radiation are spectrum-specific: red light is associated with chloroplast development, organ elongation, and formation of the photosynthetic apparatus, whereas blue light regulates growth, leaf expansion, stomatal opening, photosynthetic activity, and the accumulation of pigments and phenolic compounds (Li et al., 2020, 2021; Jin et al., 2023). The combination of blue and red light enhances growth, promoting increases in fresh and dry mass, root development, leaf area, and the biosynthesis of carbohydrates, pigments, and proteins (Wang et al., 2022a, 2022b; Liu et al., 2023). In this context, the use of LEDs in *in vitro* cultivation has reduced energy costs and enabled the application of specific wavelengths to optimize growth and metabolite production (Pang et al., 2023).

In addition to *in vitro* cultivation, the *ex vitro* acclimatization phase is critical, as it involves the transition from controlled conditions to an uncontrolled environment, requiring metabolic shifts from heterotrophy to autotrophy (Rineksane et al., 2023). The evaluation of physiological parameters such as chlorophyll a and b contents, carotenoids, and anthocyanins, combined with chlorophyll fluorescence and Photosystem II (PSII) efficiency, is an important tool for analyzing photosynthetic performance under different spectral qualities.

Although several studies have investigated the effects of light spectrum on the *in vitro* cultivation of orchids, information regarding the persistence of these effects on plant morphology and physiology during acclimatization remains scarce. Therefore, this study aimed to evaluate the influence of different spectral qualities on the morphological development and physiological parameters of *Cattleya crispa* plantlets cultivated *in vitro*, as well as to verify the maintenance of these effects during the acclimatization phase.

Material and Methods

Study site and plant material

The experiment was conducted at the Center for Research in Biotechnology and Plant Development (NPBV) and at the Laboratory of Developmental Physiology and Plant Genetics (LFDGV), Department of Crop Science, Federal University of Santa Catarina (UFSC).

Seeds were obtained from mature capsules produced by hand pollination between adult plants maintained in the orchid collection of the São Paulo State Institute of Botany (São Paulo, Brazil). The parental plants were maintained under greenhouse conditions and belonged to ex situ conservation collections of the institution. Seeds were obtained from plants cultivated in a greenhouse at the São

Paulo State Institute os Botany (23°38'30.7"S; 46°37'14.2"W), manually pollinated and provided to the NPBV.

Inoculation and asymbiotic germination

In vitro germination was performed in a laminar airflow cabinet according to Suzuki et al. (2010). Samples of 10 mg of seeds were soaked in autoclaved distilled water for 10 min, surface-sterilized in sodium hypochlorite solution (0.5% active chlorine), and rinsed three times with autoclaved distilled water.

After disinfestation, the seeds were inoculated into glass flasks (240 mL) containing 30 mL of half-strength MS medium (Murashige & Skoog, 1962), supplemented with 2 mL L^{-1} Morel vitamins, 30 g L^{-1} sucrose, 2.0 g L^{-1} activated charcoal, and 6.0 g L^{-1} agar. The pH was adjusted to 5.8 ± 0.1, and the medium was autoclaved at 120°C for 20 min.

The flasks were maintained in a growth room under white fluorescent lamps (35 W; 60 ± 5 µmol m^{-2} s^{-1}), at 25 ± 2°C and a photoperiod of 16 h light/8 h dark.

In vitro phase (spectral treatments)

At 180 days after inoculation, the plantlets were subcultured (three plants per flask containing 30 mL of half-strength MS medium) and subjected to different spectral qualities (Table 1), under a temperature of 24 ± 2°C, relative humidity of 42.5 ± 5%, and a photoperiod of 16 h light/8 h dark.

Table 1
Treatments using different light spectral qualities and their respective wavelengths during the *in vitro* cultivation of orchids of the genus *Cattleya* Lindl.

Light	Spectral Quality	Wavelength
White (100%)	White LED	400 a 700 nm
Red (100%)	Red LED	660 nm
Blue/Red (40%/60%)	Blue/Red LED	450 nm e 665 nm
Blue (100%)	Blue LED	450 nm

After 90 days of growth under these conditions, the plants were superficially washed, evaluated, and transferred to the acclimatization phase.

Acclimatization phase

At 270 days after inoculation, plants derived from the *in vitro* phase were transferred to plastic cups (50 mL) containing Carolina Soil II® substrate (sphagnum peat, expanded vermiculite, dolomitic limestone, agricultural gypsum, and NPK fertilizer), with pH 5.5 ± 0.5, density of 145 kg m^{-3}, water retention capacity of 55%, and maximum moisture of 50%.

After transplanting, the plants were maintained in a growth room under the same spectral qualities used during the *in vitro* phase (Table 1), at 24 ± 5°C, relative humidity of 42.5 ± 5%, and a photoperiod of 16 h light/8 h dark, with weekly irrigation by manual spraying. The acclimatization period lasted 120 days. At the end of this period, the plants were superficially washed and evaluated.

Morphological analyses

Morphological evaluations were performed at the end of the *in vitro* phase and after *ex vitro* acclimatization. The following variables were measured: fresh mass (mg), number of shoots, number of leaves, shoot length (mm), number of roots, and root length (mm). Length measurements were obtained using a digital caliper, and fresh mass was determined using a Shimadzu AUY 220 analytical balance.

Physiological analyses

Physiological analyses were performed at 390 days after inoculation (120 days *ex vitro*), including chlorophyll a and b contents, a/b ratio, total chlorophyll, carotenoids, anthocyanins, SPAD index, and chlorophyll a fluorescence of Photosystem II (PSII) and associated parameters. For chlorophyll and carotenoid analyses, 0.03 g of fresh mass from fully expanded leaves was incubated in test tubes (10 mL) wrapped in aluminum foil containing 5 mL of dimethyl sulfoxide (DMSO) for 48 h in the dark at 25 ± 2°C, without maceration. Absorbance readings were performed using a PRÓ-ANÁLISE V-1600 spectrophotometer at wavelengths of 665, 649, and 470 nm. Chlorophyll a, chlorophyll b, carotenoid contents, total chlorophyll, and the a/b ratio were calculated according to Wellburn (1994):

Chlorophyll a = 12.19 × A665 − 3.45 × A649

Chlorophyll b = 21.99 × A649 − 5.3 × A665

Carotenoids = (1000 × A470 − 2.86 × Chlorophyll a − 129.2 × Chlorophyll b) / 221

Chlorophyll a/b ratio = Chlorophyll a / Chlorophyll b

Total chlorophyll = Chlorophyll a + Chlorophyll b

For anthocyanin determination, 0.05 g of fresh mass from fully expanded leaves was placed in Falcon tubes (15 mL) wrapped in aluminum foil containing 5 mL of extraction solution (3 M HCl:water:methanol, 1:3:16). Samples were kept in the dark for 48 h at 4°C and centrifuged at 3,000 × g for 20 min (4°C) using a Klindly KC16R centrifuge.

Absorbance was measured with a PRÓ-ANÁLISE V-1600 spectrophotometer at 530 and 653 nm. Anthocyanin content was estimated according to Murray & Hackett (1991) using: Anthocyanin = A530 − (0.24 × A653), with results expressed as mmol g^{-1} fresh mass (FM).

SPAD index

At 120 days of acclimatization, after 1 h of dark adaptation, measurements were taken at three points in the middle third of the leaves, obtaining the mean from five plants per treatment. Measurements were performed on the third fully expanded leaf because it represents a physiologically mature leaf and minimizes variability associated with leaf age (Uddling *et al.*,2007).

Chlorophyll a fluorescence (PSII)

Fluorescence was evaluated using a MINI-PAM fluorometer in the median region of the third fully expanded leaf (five measurements per treatment). After 1 h of dark adaptation, initial fluorescence (Fo) was determined using a far-red pulse (1–2 μmol m^{-2} s^{-1}), and maximum fluorescence (Fm) was measured using a saturating pulse (6,000 μmol m^{-2} s^{-1}).

The maximum quantum yield of PSII was calculated as:

$Fv/Fm = (Fm - Fo)/Fm$

The following parameters were also obtained: PSII quantum efficiency (α), maximum electron transport rate (ETRmax), and photoinhibition constant (Ik). Data were generated using Win-Control 3 software.

Experimental design and statistical analyses

The *in vitro* and *ex vitro* experiments were conducted in a completely randomized design with four treatments and ten replicates for morphological analyses (experimental unit: three plants). For physiological analyses, five replicates were used, except for pigment analyses (triplicate).

Data were subjected to Hartley's test for homogeneity of variances (Fmax) and analysis of variance (F test, 5%). Means were compared using the Scott & Knott test (5%). Statistical analyses were performed using GENES software v. 7.0 (Cruz, 2006).

Post-acclimatization management

After evaluations, plants were wrapped in sphagnum moss and transferred to clay pots containing Orquivale® substrate (70% pine bark, 20% charcoal, 15% coconut fiber, and 10% gravel), remaining under the same previously established environmental conditions.

Results

The results of the morphological and physiological variables of *Cattleya crispa* Lindl., obtained through asymbiotic germination and cultivated under different spectral qualities during *in vitro* culture and acclimatization, are presented in Tables 2, 3 and 4 and Figs. 1 and 2.

In vitro cultivation

Analysis of variance indicated a significant effect of spectral qualities ($p \leq 0.05$) on fresh mass, number of roots, and main root length. No significant differences were observed for number of shoots, number of leaves, or shoot length (Table 2 and Fig. 1).

Plants grown under white (400–700 nm), red (660 nm), and blue/red (450/665 nm) light showed similar fresh masses (0.2918, 0.2305, and 0.2276 g, respectively), whereas those grown under blue light (450 nm) showed a significantly lower fresh mass (0.1606 g).

Regarding root number, the highest values were obtained under white (5.06), red (4.80), and blue/red (4.63) light, differing significantly from blue light (3.45). A similar trend was observed for main root length, with values of 31.48 mm under white light, 27.72 mm under red light, 24.71 mm under blue/red light, and 20.46 mm under blue light.

No significant differences were observed for number of leaves, number of shoots, or shoot length. On average, plants exhibited a shoot length of 21.45 mm, approximately five leaves, and low emission of lateral shoots after 270 days of in vitro cultivation.

Table 2
Fresh mass (g), number of shoots, number of leaves, shoot length (mm), number of roots, and primary root length (mm) of *Cattleya crispa* Lindl. plants subjected to different spectral qualities during *in vitro* cultivation.

Spectral Quality	***In vitro***					
	Fresh mass (g) **	**Number of shoots^{ns}**	**Number of leaves^{ns}**	**Shoot length (mm)^{n}**	**Number of roots****	**Root length (mm)***
White LED	0.291 ± 0.10 a	0.818 ± 0.07	5.278 ± 0.85	24.515 ± 4.30	5.060 ± 1.94 a	31.484 ± 4.21a
Red LED	0.230 ± 0.08 a	0.865 ± 0.07	5.065 ± 0.89	21.393 ± 4.35	4.803 ± 0.58 a	27.727 ± 4.11 a
Blue/Red LED	0.227 ± 0.07 a	0.622 ± 0.06	5.008 ± 0.81	20.775 ± 4.82	4.636 ± 1.14 a	24.719 ± 4.27 a
Blue LED	0.160 ± 0.04 b	0.577 ± 0.05	4.722 ± 0.41	19.152 ± 4.41	3.454 ± 1.03 b	20.469 ± 3.68 b
CV (%)	34.73	27.00	14.84	25.73	22.02	34.91

[1] Coefficient of variation; ^{n}: not significant by the F test; * significant at 5% probability by the F test; ** significant at 1% probability by the F test; means followed by the same letter in the column do not differ

according to the Scott-Knott test at 5% probability.

Acclimatization phase

During acclimatization, spectral quality significantly affected fresh mass, number of roots, main root length, and shoot length ($p \leq 0.05$), whereas no significant differences were observed for number of shoots or number of leaves (Table 3)

Monochromatic blue light resulted in the lowest values for fresh mass (1.0048 g), number of roots (4.425), root length (39.20 mm), and shoot length (29.11 mm). Red and white light produced intermediate values. Fresh mass reached 1.3632 g under red light and 1.3152 g under white light. Root number was 6.841 under red light and 6.338 under white light.

The blue + red treatment produced the highest fresh mass (1.6538 g), root length (57.07 mm), and shoot length (44.88 mm), exceeding the values obtained under the monochromatic treatments.

Table 3
Fresh mass (g), number of shoots, number of leaves, shoot length (mm), number of roots, and primary root length (mm) of *Cattleya crispa* Lindl. plants subjected to different spectral qualities during *in vitro* acclimatization.

Spectral qualities	**Aclimatization**					
	Fresh mass (g) **	**Number of shoots^{ns}**	**Number of leaves^{ns}**	**Shoot length (mm)^{n}**	**Number of roots****	**Root length (mm)***
White LED	1.315 ± 0.20 a	1.422 ± 0.09	6.926 ± 0.09	47.663 ± 0.65 a	6.338 ± 0.60 a	49.356 ± 5,00 b
Red LED	1.363 ± 0.26 a	1.026 ± 0.10	6.851 ± 0.17	45.548 ± 0.70 a	6.841 ± 0.41 a	51.265 ± 5,60 b
Blue/Red LED	1.653 ± 0.50 a	0.744 ± 0.07	6.605 ± 0.13	44.884 ± 0.60 a	5.454 ± 0.64 a	57.065 ± 6,20 a
Blue LED	1.004 ± 0.15 b	0.708 ± 0.08	6.005 ± 0.07	29.105 ± 0.61 b	4.425 ± 0.70 b	39.200 ± 3,60 c
CV(%)[1]	25.80	19.00	21.51	16.26	19.37	11.82

[1] Coefficient of variation; ^{n}: not significant by the F test; * significant at 5% probability by the F test; ** significant at 1% probability by the F test; means followed by the same letter in the column do not differ according to the Scott-Knott test at 5% probability.

Physiological responses

Analysis of variance revealed significant differences ($p \leq 0.05$) for chlorophyll a, chlorophyll b, total chlorophyll, and SPAD index. No significant differences were observed for chlorophyll a/b ratio or carotenoid content (Table 4).

Chlorophyll a content was highest under white (0.1067 mg g^{-1}) and blue light (0.1000 mg g^{-1}), while lower values were observed under red (0.0400 mg g^{-1}) and blue + red light (0.0533 mg g^{-1}).

A similar pattern was observed for chlorophyll b. The highest contents occurred under blue (0.0234 mg g^{-1}) and white light (0.0233 mg g^{-1}), whereas red (0.0133 mg g^{-1}) and blue + red light (0.0132 mg g^{-1}) resulted in lower values.

Total chlorophyll followed the same trend, with higher concentrations under white (0.1300 mg g^{-1}) and blue light (0.1233 mg g^{-1}), and lower concentrations under red (0.0500 mg g^{-1}) and blue + red light (0.0667 mg g^{-1}).

The SPAD index was higher under white (46.6), blue (45.8), and blue + red (44.6) light than under red light (39.8).

Table 4
Chlorophyll a and b contents (mg g^{-1} fresh mass), total chlorophyll (mg g^{-1} fresh mass), a/b ratio chlorophyll, carotenoids (mg g^{-1} fresh mass), and anthocyanins (mg g^{-1} fresh mass) of *Cattleya crispa* Lindl. plants subjected to different spectral qualities during acclimatization.

Spectral qualities	**Chlorophyll a (mg g^{-1})***	**Chlorophyll b (mg g^{-1})***	**Total chlorophyll (mg g^{-1})***	**Chlorophyll a/b ratio^{ns}**	**Carotenoids (mg g^{-1})^{ns}**	**SPAD index****
White LED	0.1067 ± 0.01 a	0.0233 ± 0.001 a	0.1300 ± 0.02 a	5.1633 ± 0.52	0.0200 ± 0.001	46.6 ± 1.02 a
Red LED	0.0400 ± 0.03 b	0.0133 ± 0.001 b	0.0500 ± 0.04 b	4.3167 ± 0.64	0.0100 ± 0.000	39.8 ± 0.89 b
Blue/Red LED	0.0533 ± 0.02 b	0.0132 ± 0.001 b	0.0667 ± 0.03 b	4.8867 ± 0.72	0.0133 ± 0.000	44.6 ± 2.20 a
Blue LED	0.1000 ± 0.01 a	0.0234 ± 0.002 a	0.1233 ± 0.03 a	4.0533 ± 0.84	0.0132 ± 0.001	45.8 ± 2.90 a
CV(%)[1]	33.10	27.02	31.51	21.09	28.81	16.75

[1] Coefficient of variation; ^{ns}: not significant by the F test; * significant at 5% probability by the F test; ** significant at 1% probability by the F test; means followed by the same letter in the column do not differ according to the Scott-Knott test at 5% probability.

PSII quantum efficiency (α) was highest under the blue + red treatment (0.1048), whereas white (0.0734), red (0.0730), and blue (0.0630) light did not differ significantly (Fig. 1).

Maximum electron transport rate (ETRmax) was higher under white (6.4610) and blue + red (8.0452) light and lower under red (4.9836) and blue (5.0898) light.

Maximum fluorescence (Fm) and maximum quantum yield of PSII (Fv/Fm) were higher under white (Fm = 1.923; Fv/Fm = 0.657) and blue + red light (Fm = 1.918; Fv/Fm = 0.693) than under red (Fm = 1.459;

Fv/Fm = 0.580) and blue light (Fm = 1.327; Fv/Fm = 0.549).

Discussion

Light spectral quality markedly influenced the morphophysiological performance of *Cattleya crispa* throughout both the in vitro cultivation and acclimatization phases. Although all light treatments supported plant development, the responses demonstrated that spectral composition differentially regulates biomass accumulation, root system architecture, pigment biosynthesis, and photosynthetic efficiency. These responses indicate that the effects of light extend beyond its function as na energy source, acting as na environmental signal that coordinates developmental and physiological processes through multiple photoreceptors.

Morphological responses and photomorphogenic regulation

During the in vitro phase, monochromatic blue light consistently reduced fresh mass, root number, and root length compared with white, red, and blue/red spectra. Similar reductions in biomass under blue LEDs have been reported for orchids and other micropropagated species (Nakonechnaya et al., 2021), suggesting that this wavelength alone is insufficient to sustain vigorous vegetative growth. Blue light predominantly activates cryptochromes and phototropins, which regulate photomorphogenic responses associated with compact growth, stomatal opening, and chloroplast development rather than extensive cell expansion (Kim & Lee, 2023; Massaro et al., 2019). Consequently, plants exposed exclusively to blue wavelengths frequently allocate fewer resources to biomass accumulation and root elongation.

In contrast, red light promoted greater root formation, while the combined blue and red spectrum generated the most balanced vegetative development, particularly during acclimatization. These responses are consistent with the complementary action of phytochromes and cryptochromes. Activation of phytochromes by red wavelengths stimulates cell elongation, biomass partitioning, and hormonal pathways involved in rhizogenesis (Murillo-Talavera et al., 2016; Pawłowska et al., 2018; Abushattal et al., 2025), whereas blue light enhances chlorophyll biosynthesis, stomatal regulation, and photosynthetic competence. When combined, these signaling pathways optimize carbon assimilation and resource allocation, resulting in more vigorous plants, as demonstrated in several horticultural species cultivated under red/blue LED combinations (Gam et al., 2020; Si et al., 2024; Saeedi et al., 2023).

Interestingly, shoot number and leaf production were not affected by spectral quality, indicating that vegetative organ initiation in *C. crispa* is less sensitive to wavelength composition than biomass accumulation and root development. Similar species-dependent responses have been described for orchids, where the influence of LED lighting depends on genotype, developmental stage, irradiance, and culture conditions (Ma et al., 2021; Tarakanov et al., 2022; Chashmi et al., 2022; Flores et al., 2022). This

reinforces that light quality alone does not universally determine morphogenesis but interacts with intrinsic developmental programs.

Photosynthetic apparatus and pigment accumulation

Spectral quality also altered the composition of the photosynthetic pigments. White and blue light promoted greater accumulation of chlorophyll a, chlorophyll b, and total chlorophyll, whereas red light resulted in the lowest pigment concentrations. These findings agree with previous reports demonstrating that blue wavelengths play a central role in chlorophyll biosynthesis and chloroplast differentiation (Landi et al., 2020). Accordingly, wheat seedlings grown exclusively under red light require subsequent exposure to blue wavelengths to complete chlorophyll synthesis (Tripathy & Brown, 1995), and similar reductions in chlorophyll content under monochromatic red LEDs have been reported in *Cattleya loddigesii* (Galdiano Jr. et al., 2012a) and *Triticum monococcum* (Bartucca et al., 2020).

Despite the differences in chlorophyll content, carotenoid concentrations remained unchanged among treatments. Since carotenoids participate primarily in photoprotection through the dissipation of excess excitation energy (Taiz & Zeiger, 2013), their stability suggests that none of the spectral treatments imposed sufficient oxidative stress to induce adjustments in carotenoid metabolism. Likewise, anthocyanins remained below the detection limit in all samples, indicating that the light environments did not trigger stress responses associated with anthocyanin biosynthesis. These observations collectively suggest that the differences among treatments primarily reflected modifications in photosynthetic organization rather than activation of photoprotective pathways.

Photosynthetic efficiency and acclimatization potential

Although plants cultivated under white light accumulated higher chlorophyll concentrations, the combined blue/red spectrum produced superior photochemical performance, as demonstrated by higher values of PSII quantum efficiency (α), maximum electron transport rate (ETRmax), and maximum quantum yield of PSII (Fv/Fm). These results indicate that photosynthetic performance depends not only on pigment concentration but also on the efficiency with which absorbed light energy is converted into photochemical reactions.

Fv/Fm is widely recognized as an indicator of the maximum efficiency of Photosystem II under non-stressed conditions, whereas ETRmax reflects the capacity of the photosynthetic electron transport chain to sustain carbon assimilation (Maxwell & Johnson, 2000; Baker, 2008). Therefore, the higher values obtained under white and blue/red light suggest a better balance between energy absorption, electron transport, and carbon fixation, resulting in greater metabolic stability during plant development. Similar improvements in Fv/Fm and ETRmax under combined red and blue LEDs have been reported for *Brasenia schreberi* (Li et al., 2021) and *Dendrobium candidum* (Wang et al., 2017), supporting the hypothesis that complementary spectral composition optimizes the functionality of the photosynthetic apparatus.

Conversely, the higher photoinhibition constant (Ik) observed under monochromatic red light indicates that greater irradiance is required before photosynthesis becomes light-saturated, suggesting lower efficiency in light utilization under this spectrum. This physiological limitation may explain why red light alone did not translate into the highest biomass accumulation despite promoting root development.

Implications for acclimatization and ex situ conservation

The superior morphophysiological performance obtained under the combined blue/red spectrum has important implications for orchid conservation. Acclimatization remains one of the most critical stages of micropropagation because plantlets produced in vitro often possess poorly developed cuticles, inefficient stomatal control, and limited autotrophic capacity, making them highly susceptible to environmental stress after transplanting (Sharma et al., 2023). Consequently, producing plantlets with a more efficient photosynthetic apparatus before transfer to ex vitro conditions is essential for increasing establishment success.

Micropropagation has become a cornerstone of orchid conservation, supporting germplasm collections, seed banking, cryopreservation, and restoration programs for threatened species (Merritt et al., 2013; Phillips et al., 2020; Sarmah, 2025). Successful examples include high post-acclimatization survival in *Vanda brunnea*, *Aerides crispa*, and cryopreserved *Caladenia huegelii* (Bustam et al., 2016; Nowakowska et al., 2022), demonstrating that optimized propagation protocols can substantially strengthen ex situ conservation strategies.

In this context, the present results indicate that optimizing light spectral quality represents a simple and low-cost approach to improve not only biomass accumulation but also the physiological quality of micropropagated plantlets. Particularly, the combined blue/red spectrum promoted a favorable integration between growth and photosynthetic performance, characteristics that are expected to enhance acclimatization success and increase the efficiency of conservation programs for endangered Orchidaceae.

Conclusions

The blue/red LED combination was the most effective spectral treatment for promoting vigorous growth and improving acclimatization performance of Cattleya crispa. Beyond its effects on plant development, this light regime represents a promising strategy for ex situ conservation programs, sustainable propagation of genetic resources, and the production of high-quality plants intended for restoration and conservation actions involving threatened orchid species therefore contributing to the establishment of germplasm banks and restoration initiatives involving threatened orchid species *in vitro*.

Declarations

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Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the author(s) used Consensus for research articles. The author(s) reviewed and edited the output as needed and take full responsibility for the content of the published article.

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Figures

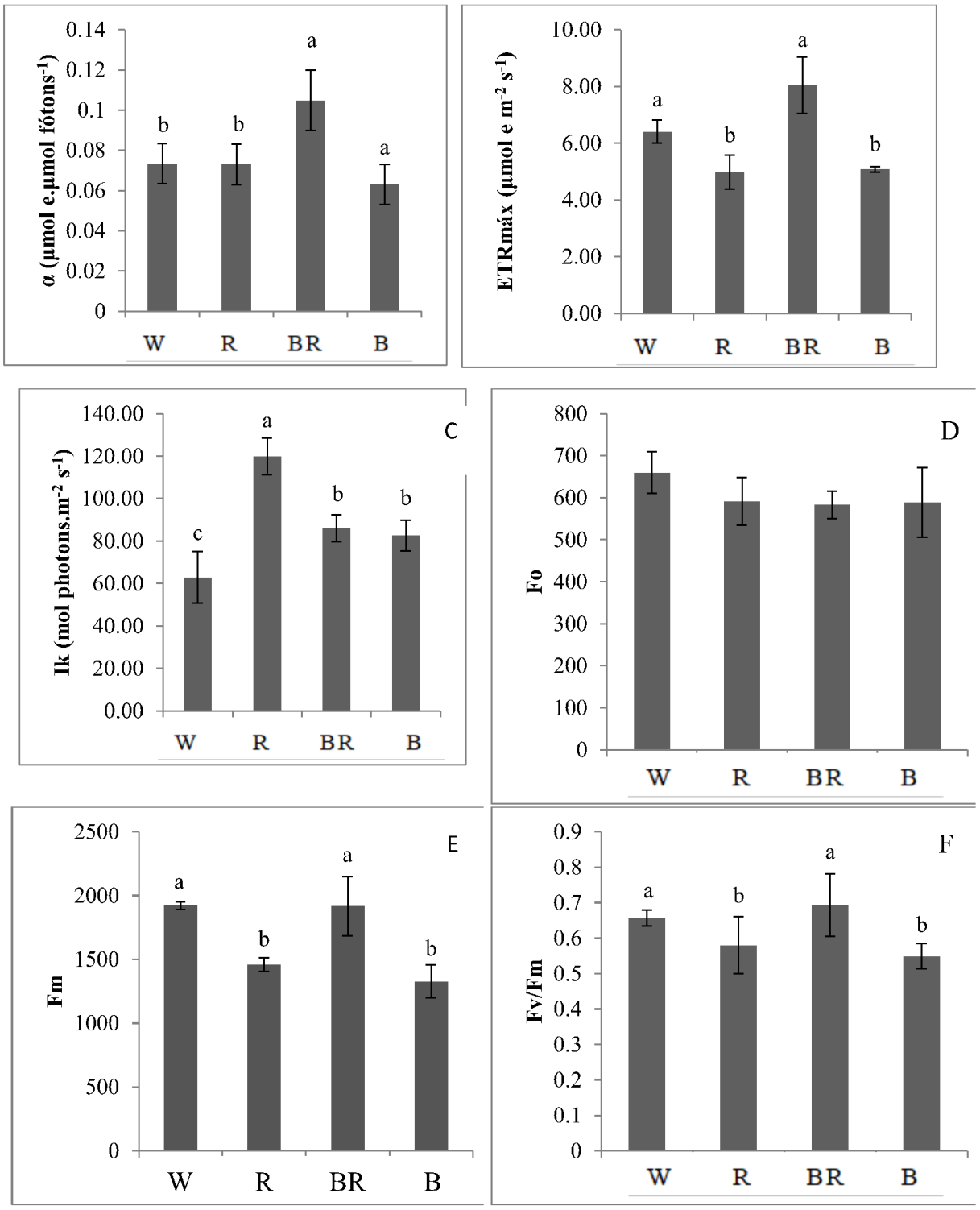


Figure 1

(A) Quantum efficiency of photosystem II (PSII) (α), (B) maximum electron transport rate (ETRmax), (C) photoinhibition constant (Ik), (D) basal chlorophyll *a* fluorescence (Fo), (E) maximum chlorophyll *a* fluorescence (Fm), and (F) maximum quantum yield of PSII (Fv/Fm) in *Cattleya crispa* Lindl. plants at 390 days after inoculation, subjected to different light spectra during *in vitro* cultivation and acclimatization.

W (White light — 400 to 700 nm); R (Red light — 660 nm); BR (Blue/Red light — 450 nm and 665 nm); B (Blue light — 450 nm). Bars indicate the standard deviation of the mean.



Figure 2

Cattleya crispa Lindl. plants at 120 days, subjected to different spectral qualities during *in vitro* cultivation. A) White light — 400 to 700 nm; B) Red light — 66.