Influence of spectral qualities on morphophysiological and biochemical parameters at the end of the in vitro period and during acclimatization of Gomesa flexuosa

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Abstract

Light spectral quality directly influences growth, photosynthetic physiology, and acclimatization processes in orchids cultivated in vitro. In this context, the present study aimed to evaluate the effects of different light spectra on the morphophysiological development of Gomesa flexuosa plantlets cultivated in vitro and during ex vitro acclimatization. Plantlets were exposed to different light qualities provided by light-emitting diodes (LEDs), including white, blue, red, and combined spectra. Morphometric traits, photosynthetic pigments, chlorophyll a fluorescence, and photosynthetic performance were evaluated throughout in vitro cultivation and after acclimatization. The results demonstrated that spectral quality significantly modulated plant physiological and structural responses. Red light promoted greater leaf emission and vegetative growth, whereas blue light increased chlorophyll and carotenoid contents and improved the photochemical efficiency of photosystem II. Plants grown under combined spectra showed intermediate responses and greater physiological balance during acclimatization. Chlorophyll fluorescence parameters indicated higher photosynthetic stability under blue and blue/red light treatments, evidencing lower photoinhibition occurrence. It can be concluded that manipulating light spectral quality is an efficient strategy to optimize in vitro cultivation and improve ex vitro adaptation of Gomesa flexuosa, contributing to more efficient protocols for micropropagation and conservation of ornamental orchids.

Introduction

The family Orchidaceae is among the most diverse groups of angiosperms, exhibiting high ecological, ornamental, and economic relevance (TIWARI et al., 2024). In Brazil, the genus *Gomesa* stands out due to its high endemism and broad distribution across the Atlantic Forest and Cerrado biomes (CASTRO; SINGER, 2018). Among its species, *Gomesa flexuosa* (Lodd.) M.W. Chase & N.H. Williams exhibits an epiphytic habit, high floral production, and crassulacean acid metabolism (CAM), characteristics associated with elevated physiological plasticity and adaptation to environments with variable light and water availability (CORDEIRO et al., 2018).

In vitro micropropagation represents an important tool for orchid production and conservation, enabling high multiplication rates and genetic uniformity of plantlets (NONGDAM et al., 2023). However, the artificial conditions of in vitro cultivation, such as low irradiance, high sucrose availability, and restricted gas exchange, frequently impair photosynthetic functionality and plant acclimatization during the ex vitro phase (XIAO; NIU; KOZAI, 2011).

In this context, light spectral quality plays a fundamental role in the regulation of photomorphogenesis and plant metabolism through photoreceptors such as phytochromes and cryptochromes (CHENG et al., 2021; FRAIKIN; BELENIKINA; RUBIN, 2023). The use of light-emitting diodes (LEDs) has expanded the possibilities for manipulation of the light environment due to their precise spectral control, high energy efficiency, and low heat emission (LIVADARIU et al., 2023). Recent studies have demonstrated that

different light spectra directly influence the photosynthetic apparatus, pigment synthesis, and growth of plants cultivated in vitro (CIOĆ et al., 2025).

The transition from the in vitro to the ex vitro environment represents a critical stage in micropropagation, requiring physiological and structural reorganization of plants to adapt to new environmental conditions (GRZELAK; PACHOLCZAK; NOWAKOWSKA, 2024). In this process, chlorophyll *a* fluorescence analysis enables rapid and non-destructive assessment of photosynthetic performance and the functional status of photosystem II (MOUSTAKA; MOUSTAKAS, 2023; SWOCZYNA et al., 2022).

Despite advances related to the use of light-emitting diodes (LEDs) in in vitro cultivation, studies integrating physiological, biochemical, and morphometric responses during the in vitro–ex vitro transition in native Brazilian species remain scarce. Therefore, the present study aimed to evaluate the effects of different LED spectral qualities on physiological, biochemical, and morphometric parameters of *Gomesa flexuosa*, seeking to understand the relationship between in vitro photosynthetic performance and ex vitro adaptive success.

Materials and Methods

Plant material and experimental design

The experiment was conducted in two distinct stages: the in vitro phase and the ex vitro phase (acclimatization). During the in vitro phase, seeds of *Gomesa flexuosa* were inoculated into 300 mL culture flasks containing 30 mL of MS culture medium according to the formulation of Murashige and Skoog (1962), supplemented with Morel and Wetmore (1951) vitamins, 30 g L^{-1} sucrose, 2 g L^{-1} activated charcoal, and 6 g L^{-1} agar. The medium pH was adjusted to 5.8 prior to sterilization in an autoclave at 121°C and 1.3 atm for 20 min.

The flasks were maintained in a growth chamber at 25 ± 2°C under a 16 h light/8 h dark photoperiod and average irradiance of approximately 50 µmol m^{-2} s^{-1} provided by LED lighting systems with different spectral qualities (Table 1). After seven months of cultivation, bimonthly subcultures were performed under a horizontal laminar airflow cabinet, transferring the plantlets to new flasks containing the same culture medium until they reached approximately 5 cm in height. Experimental evaluations were performed after the third subculture.

The experiment was arranged in a completely randomized design consisting of four treatments corresponding to different LED light spectra (Table 1). During the in vitro phase, 16 replicates were used per treatment, with each replicate consisting of one culture flask containing two plantlets.

Table 1
Treatments with different spectral light qualities and their respective wavelengths used in experiments with plants of the specie *Gomesa flexuosa* during *in vitro* cultivation.

Treatments	Spectral Quality	Wavelength
Blue	Blue LED	450 nm
Red	Red LED	660 nm
Blue/Red	Bue/Red LED	450 nm e 665 nm
White	White LED	400 a 700 nm

Lighting was provided by tubular LED lamps with a light intensity of 50 μmol m^{-2} s^{-1} per lamp, positioned 50 cm above the culture flasks.

At the end of the in vitro phase, the plantlets were carefully removed from the culture flasks and washed under running water to completely remove the culture medium adhered to the roots. Subsequently, they were transplanted into expanded polystyrene trays containing 72 cells filled with the commercial substrate Agrinobre TN MIX CE 0.4®, composed of sphagnum peat, expanded vermiculite, carbonized rice husk, dolomitic limestone, agricultural gypsum, and fertilizers.

The trays were maintained in a greenhouse covered with 50% shade cloth under average temperature conditions of 24 ± 5°C and daily sprinkler irrigation, supplying approximately 600 mL of water per day. The acclimatization period lasted 45 days.

Physiological analyses

At the end of the ex vitro phase, chlorophyll *a* fluorescence analyses were performed using a portable fluorometer MINI-PAM (Walz®, Germany). Leaves were dark-adapted for 2 h prior to measurements, and the initial fluorescence (Fo), maximum fluorescence (Fm), and maximum quantum yield of photosystem II (Fv/Fm) were determined. Evaluations were carried out at 13 time points throughout the acclimatization period.

Biochemical analyses

Biochemical analyses were conducted at the end of both the in vitro and ex vitro phases. Chlorophyll *a* and chlorophyll *b* contents were determined using 0.05 g of fresh leaf mass per replicate. Pigment extraction was performed using dimethyl sulfoxide (DMSO), and samples were incubated in the dark for 48 h at 25 ± 2°C. Subsequently, absorbance readings were obtained using a spectrophotometer, and photosynthetic pigment concentrations were calculated according to the equations proposed by Wellburn (1994).

Morphometric analyses

Morphometric evaluations were performed at the end of the in vitro and ex vitro phases using six replicates per treatment. Fresh mass, plantlet height, number of leaves, and shoot formation were evaluated. Plant height was measured from the base of the plantlet to the tip of the longest leaf.

Statistical analysis

The data were subjected to analysis of variance (ANOVA). When significant differences were detected, means were compared using Tukey's test at a 5% probability level. The assumptions of normality and homogeneity of variances were assessed prior to the analyses. For chlorophyll *a* fluorescence data collected throughout the acclimatization period, linear and quadratic regression models were fitted, and the model with the best fit was selected. Statistical analyses were performed using Statistica® 10.0 software.

Results

Morphometric parameters

Different light spectra did not significantly affect plantlet fresh mass during either the in vitro or ex vitro phases ($p > 0.05$). In the in vitro phase, mean values ranged from 1.3 to 1.9 g, whereas in the ex vitro phase values ranged from 1.2 to 1.9 g. The coefficients of variation were 32.83% and 26.91%, respectively (Table 2), indicating moderate experimental variability.

Shoot formation was also not significantly influenced by the light treatments in either cultivation phase ($p > 0.05$). Nevertheless, the red spectrum resulted in the highest mean values under both in vitro and ex vitro conditions. The coefficients of variation ranged from 22.30% to 22.53%, indicating moderate variability among replicates.

Leaf number was significantly influenced by the light spectra only during the in vitro phase ($p < 0.05$). Under this condition, the red treatment promoted greater leaf emission compared with the blue treatment, while the white and blue + red treatments showed intermediate responses. During the ex vitro phase, no significant differences were observed among treatments ($p > 0.05$), although the blue + red treatment presented the lowest numerical mean. The coefficients of variation were 12.30% and 15.41% for the in vitro and ex vitro phases, respectively, indicating good experimental precision.

Plantlet height did not differ significantly among treatments during the in vitro phase ($p > 0.05$). In contrast, during the ex vitro phase, light spectra exerted a highly significant effect on this variable ($p < 0.01$). The blue + red treatment resulted in lower mean plant height compared with the other treatments, which remained statistically similar to one another. The low coefficient of variation observed for this variable (5.54%) indicates high experimental precision.

Table 2
Morphometric data of *Gomesa flexuosa* plants after 13 months of in vitro cultivation and after 45 days of the ex vitro phase under different LED spectral qualities.

Treatments	Fresh Mass (g)		Shoots		Number of Leav		Height (cm)	
	In vitro	*Ex vitro*	*In vitro*	*Ex vitro*	*In vitro*	*Ex vitro*	*In vitro*	*Ex vitro*
Blue	1,3 ± 0,33a	1,6 ± 0,25a	1,5 ± 0,28a	2,0 ± 0,30a	9,2 ± 0,76b	9,2 ± 0,75a	9,5 ± 0,66a	10,2 ± 0,32a
Red	1,8 ± 0,33a	1,4 ± 0,25a	2,7 ± 0,28a	3,0 ± 0,30a	12,7 ± 0,76a	8,5 ± 0,75a	8,5 ± 0,66a	10,2 ± 0,32a
Blue/Red	1,9 ± 0,3a	1,2 ± 0,25a	2,0 ± 0,28a	1,8 ± 0,30a	9,8 ± 0,76ab	6,8 ± 0,75a	9,5 ± 0,66a	8,2 ± 0,32b
White	1,8 ± 0,33a	1,9 ± 0,25a	2,3 ± 0,28a	2,5 ± 0,30a	10,8 ± 0,76ab	9,0 ± 0,75a	10,7 ± 0,66a	11,3 ± 0,32a
CV (%)	32,83	26,91	22,53%	22,30%	12,3	15,41	11,96	5,54

Means followed by the same letter within the column do not differ significantly according to Tukey's test at the 5% probability level.

The morphometric parameters of *Gomesa flexuosa* integratively reflected the cumulative effects of the physiological and biochemical changes induced by the different light spectra, demonstrating that light quality plays a central role in modulating plant growth, particularly during in vitro cultivation (Fig. 1). Among the evaluated treatments, red light promoted greater leaf emission after 13 months of cultivation, suggesting that this spectrum directly affects organogenesis and cell expansion processes.

This response may be associated with the activation of phytochromes, photoreceptors responsible for the perception of red and far-red light. Phytochromes occur in two forms: Pr, the inactive form that absorbs red light, and Pfr, the active form generated after absorption of this spectral range. Once activated, Pfr migrates to the cell nucleus and regulates transcription factors, particularly proteins of the PIF (Phytochrome Interacting Factors) family, modulating genes involved in cell division, tissue expansion, and leaf development (ROMANOWSKI et al., 2021). In addition, phytochromes regulate several plant developmental processes, including chloroplast differentiation, photomorphogenesis, shade responses, and elongation of vegetative organs (LI et al., 2025; ROMANOWSKI et al., 2021).

In this context, the higher number of leaves observed under red light in *G. flexuosa* indicates that this spectrum favored morphogenic responses associated with vegetative expansion. Similar results have been reported for other micropropagated orchids. In *Cattleya walkeriana*, for example, red LEDs promoted increased leaf emission without a proportional increase in biomass accumulation (NADAL et al., 2023). Likewise, in *Phalaenopsis*, changes in spectral quality significantly altered vegetative growth and plant architecture during in vitro cultivation (ANUCHAI; HSIEH, 2017).

The tendency toward greater shoot emission under red light observed in the present study reinforces this morphogenic pattern associated with phytochrome activation and stimulation of meristematic activity. Although the differences were not statistically significant, the higher mean values recorded under this spectrum suggest that red light may favor vegetative proliferation in *G. flexuosa*. In Orchidaceae, shoot responses to light are highly dependent on species, developmental stage, and the applied light regime (ZHANG et al., 2018; ONA, 2021). Studies with *Cymbidium* have demonstrated that red light stimulates shoot formation, whereas blue light primarily favors rooting and physiological differentiation of plantlets (CAVALLARO et al., 2022).

However, the increase in leaf and shoot number under red light was not accompanied by a significant increase in fresh mass, indicating a decoupling between organogenesis and biomass accumulation. This pattern suggests that although red light stimulates organ formation, it may limit photosynthetic efficiency when supplied alone, as demonstrated by studies reporting reduced photosystem II (PSII) efficiency and lower photosynthetic performance under this spectrum (ZHENG; CEUSTERS; VAN LABEKE, 2019). In orchids, this effect may result in thinner, elongated, and yellowish leaves, characteristics associated with lower chlorophyll density and reduced light absorption capacity (KHOTSKOVA et al., 2019; NADAL et al., 2023). This phenotype has also been described in other crop species, in which growth under red light promotes cell elongation without a proportional increase in leaf thickness or pigment content (SPANINKS, 2023; GAI, 2024; JEONG, 2024).

Conversely, blue light resulted in lower numbers of leaves and shoots during the in vitro phase, but with indications of greater leaf thickness and individual leaf area, suggesting more compact and structurally efficient growth. This pattern is consistent with the literature, which indicates that the blue spectrum promotes chloroplast organization, increased chlorophyll *b* content, and the development of a more efficient photosynthetic apparatus (ZHANG et al., 2018; FARROKHZAD et al., 2022). Moreover, blue light is associated with the formation of denser tissues and the control of excessive elongation, resulting in more robust plants despite lower leaf emission.

The blue/red combination, often described as ideal for maximizing growth and photosynthesis in several species, did not show superiority for *G. flexuosa*, particularly during acclimatization, when it resulted in lower plant height. Although previous studies indicate that combining these spectra may optimize the activation of different photoreceptors and increase photosynthetic rates (PARADISO; PROIETTI, 2021), the observed response reinforces that light effects are highly dependent on genotype and cultivation conditions. Similar results have been reported for orchids such as *Phalaenopsis amabilis*, in which responses to light spectra varied among cultivars (MASSARO et al., 2019), and for *Cattleya walkeriana*, demonstrating that the superiority of combined spectra is not universal (NADAL et al., 2023).

During acclimatization, a tendency toward homogenization of morphometric parameters was observed, including shoot emission, except for plant height, which was greater under white light (Fig. 2). This behavior indicates that after transfer to ex vitro conditions, more complex environmental factors, such as natural light, humidity, and gas exchange, exert greater influence on plant growth, reducing the effects

previously induced by artificial spectra. The superiority of white light at this stage may be associated with its broader spectrum, which more closely resembles sunlight and promotes more balanced growth (CABRAL et al., 2022). This convergence pattern has also been reported in other species, in which initial differences induced by LEDs tend to disappear during acclimatization (MASSARO et al., 2019).

The absence of significant differences in fresh mass after acclimatization, combined with a 100% survival rate, reinforces the high phenotypic plasticity of *G. flexuosa*. This behavior is characteristic of orchids, which exhibit a strong capacity for morphophysiological adjustment under environmental changes by reorganizing their photosynthetic and structural apparatus during the ex vitro transition (LANDO et al., 2016; MANI et al., 2020). Studies with *Epidendrum denticulatum* also demonstrated that different light spectra affect physiological parameters without compromising ex vitro establishment, highlighting the high adaptive capacity of these species (CABRAL et al., 2022).

Biochemical Parameters

Different light spectra significantly influenced chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in *Gomesa flexuosa* during both the *in vitro* and *ex vitro* phases, whereas the chlorophyll *a/b* ratio remained statistically unchanged among treatments ($p > 0.05$) (Table 3).

Table 3
Biochemical parameters of *Gomesa flexuosa* plants after 13 months of in vitro cultivation and after 45 days of the ex vitro phase under different LED light spectra.

Treatments	**Chlorophyll *a***		**Chlorophyll *b***		**Total Chlorophylls**		**Chlorophylls Ratio *a/b***	
	(mg. g^{-1} MF)		**(mg.g^{-1} MF)**		**(mg. g^{-1} MF)**			
	In vitro	*Ex vitro*	*In vitro*	*Ex vitro*	*In vitro*	*Ex vitro*	*In vitro*	*Ex vitro*
Blue	1,49 ± 0,16 a	1,05 ± 0,07 a	0,38 ± 0,03 a	0,33 ± 0,02 a	1,87 ± 0,18 a	1,39 ± 0,10 a	3,93 ± 0,22 a	3,14 ± 0,05 a
Red	1,33 ± 0,36 ab	0,84 ± 0,08 ab	0,36 ± 0,03 a	0,29 ± 0,02 ab	1,69 ± 0,39 ab	1,17 ± 0,10 ab	3,50 ± 0,68 a	3,03 ± 0,05 a
Blue/Red	0,92 ± 0,11 ab	0,90 ± 0,04 ab	0,27 ± 0,03 ab	0,30 ± 0,01 ab	1,19 ± 0,13 ab	1,20 ± 0,05 ab	3,39 ± 0,20 a	2,99 ± 0,04 a
White	0,64 ± 0,08 b	0,73 ± 0,05 b	0,21 ± 0,02 b	0,240 ± 0,01 b	0,85 ± 0,10 b	0,97 ± 0,07 b	2,97 ± 0,12 a	3,04 ± 0,05 a
CV (%)	23,8	16,47	15	15,12	21,8	16,11	12,3	3,42

During the in vitro phase, the blue spectrum promoted the highest contents of chlorophyll *a* (1.49 ± 0.16 mg g^{-1} FW), chlorophyll *b* (0.38 ± 0.03 mg g^{-1} FW), and total chlorophyll (1.87 ± 0.18 mg g^{-1} FW), differing statistically from the white treatment, which showed the lowest values for these variables. Red and blue/red treatments exhibited intermediate responses. The chlorophyll *a/b* ratio did not differ

significantly among treatments, indicating proportional stability between photosynthetic pigments regardless of spectral light quality. Shapiro–Wilk tests confirmed normality of residuals for all evaluated variables, although heterogeneity of variances was detected for chlorophyll *a*, total chlorophyll, and chlorophyll *a/b* ratio according to Bartlett's test, indicating the need for caution when interpreting these analyses.

At the end of the ex vitro acclimatization period, a pattern similar to that observed during in vitro cultivation was detected. Plants grown under blue LED maintained the highest levels of chlorophyll *a* (1.05 ± 0.07 mg g^{-1} FW), chlorophyll *b* (0.33 ± 0.02 mg g^{-1} FW), and total chlorophyll (1.39 ± 0.10 mg g^{-1} FW), differing statistically from the white treatment, which again showed the lowest pigment contents. Red and blue/red treatments remained statistically intermediate. The chlorophyll *a/b* ratio ranged from 2.99 to 3.14, with no significant differences among the evaluated spectra, demonstrating stability in pigment proportion regardless of light quality. Unlike the in vitro phase, all assumptions of normality and homogeneity of variances were fully met during the ex vitro phase.

These results suggest that blue light favors chlorophyll synthesis and accumulation, particularly chlorophyll *a*, which agrees with studies reporting that wavelengths in the blue region stimulate the photosynthetic apparatus and promote greater pigment accumulation under in vitro conditions (MORAŃSKA et al., 2023; ZHANG et al., 2023; SOHANI; SHARIATI; SHARIATI, 2022), although frequently associated with lower proportions of chlorophyll *b* and reduced development of the light-harvesting complex (BANAŚ et al., 2024; KOCOT et al., 2026).

The low chlorophyll *b* concentration observed across all treatments in both in vitro and ex vitro phases resulted in a high chlorophyll *a/b* ratio, a characteristic commonly associated with plants cultivated under low irradiance and high relative humidity, as occurs in conventional in vitro systems. Under these conditions, the photosynthetic apparatus tends to exhibit reduced development of light-harvesting antenna complexes (LHCII), reflecting adaptation to environments with lower demands for photon capture efficiency (TARAKANOV et al., 2021; FILIMON; ROTARU; FILIMON, 2016). This behavior reinforces that, although chlorophyll *a* accumulation may be enhanced under specific spectra, the structural organization of the photosynthetic apparatus remains limited during in vitro cultivation.

At the end of the ex vitro phase (45 days of acclimatization), differences among light treatments were reduced, although chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents still differed significantly among treatments. Plants cultivated under blue light maintained the highest chlorophyll *a* (1.05 ± 0.07 mg g^{-1} FW), chlorophyll *b* (0.33 ± 0.02 mg g^{-1} FW), and total chlorophyll (1.39 ± 0.10 mg g^{-1} FW) contents, whereas the lowest values were observed under red light. The higher chlorophyll *b* content under blue light suggests greater development of the light-harvesting complex, since this pigment is directly associated with photosystem II antenna complexes. These results corroborate previous studies reporting increased chlorophyll and carotenoid contents, as well as greater photochemical efficiency under blue light, reflecting improved performance of the photosynthetic apparatus (MOOSAVI-NEZHAD et al., 2021; YIN et al., 2025; BANAŚ et al., 2024).

The reduction in differences among treatments during acclimatization indicates a convergent adjustment of the photosynthetic apparatus, probably in response to more homogeneous ex vitro environmental conditions, such as higher irradiance, thermal variation, and lower relative humidity. Similar responses have been reported in several micropropagated species, including orchids, in which initial differences induced by light spectra tend to diminish after transfer to natural conditions, resulting in greater uniformity of pigment contents (CABRAL et al., 2022; DE PAULA ALVES et al., 2022; TARAKANOV et al., 2021). This process reflects the high physiological plasticity of plants, allowing reorganization of the photosynthetic apparatus to maximize efficiency under the new environmental conditions.

Furthermore, the tendency for reduction in the chlorophyll *a/b* ratio during acclimatization suggests the transition from a typical “shade-leaf” photosynthetic apparatus to a more balanced “sun-leaf” condition, with greater development of light-harvesting antenna complexes and improved energy distribution between photosystems. This adjustment is essential for ex vitro adaptation, as it increases photosynthetic capacity and improves plant performance under higher irradiance conditions (TARAKANOV et al., 2021).

Means followed by the same letter in the column do not differ from each other according to Tukey’s test at a 5% probability level, and are considered significant at 5% by the t-test.

Physiological Parameters

The behavior of initial fluorescence (Fo), characterized by elevated values at the beginning of acclimatization (except for the blue light treatment) followed by a subsequent reduction over time, suggests that the plants initially exhibited disturbances in PSII (Fig. 3). The literature demonstrates that increases in Fo are frequently associated with the inactivation or closure of PSII reaction centers (RCs), as well as reduced efficiency of energy transfer from the antenna complex to these centers (NAWROCKI et al., 2021; HONG; XU, 1999).

In this context, the elevated Fo values observed on day 0 of acclimatization indicate that the plants, upon transfer from the *in vitro* environmentchara, cterized by high humidity, low irradiance, and restricted gas Exchange, were under physiological stress, resulting in the accumulation of inactive or functionally impaired PSII reaction centers. This pattern may also reflect alterations in the connectivity between PSII units and in antenna–reaction center (RC) coupling, as described by Kalaji et al. and Ren et al..

On the other hand, the progressive reduction in Fo throughout the 45 days suggests a functional reactivation of PSII, with improved energy transfer efficiency and possible structural reorganization of antenna complexes. This behavior is consistent with acclimatization processes, in which the photosynthetic apparatus adjusts to new environmental conditions. Furthermore, it is important to consider that spectral variations in light, such as those imposed by different LED treatments, can directly modulate PSII efficiency by affecting electron transport, energy dissipation, and the functional antenna

size (JANEESHMA et al., 2022). This may explain the differences observed among treatments, particularly the distinct response under blue light.

Maximum fluorescence (Fm) and recovery of photochemical capacity

The Fm results showed an opposite trend compared to Fo, with lower values at the beginning of acclimatization and a progressive increase throughout the experimental period (Fig. 4). This pattern indicates a gradual recovery of the maximum fluorescence capacity, reflecting an improvement in the functional state of PSII.

The literature establishes that reductions in Fm are associated with severe disturbances in PSII, including reaction center inactivation, uncoupling of the light-harvesting complex (LHCII), and alterations in electron transport (YAMANE et al., 1997; KALAJI et al., 2016). Thus, the low initial Fm values reinforce the interpretation that the plants were under photosynthetic stress immediately after transfer to the *ex vitro* environment.

The progressive increase in Fm throughout acclimatization suggests a restoration of the plastoquinone A (QA) reduction capacity and electron transport efficiency, indicating reactivation of reaction centers and improvement in the functional connectivity of the system (KRAUSE et al., 1990). This process may involve both the repair of PSII damage and the reorganization of pigment–protein complexes.

However, the lower performance observed under the red-light treatment throughout the experimental period indicates that this spectrum may have imposed greater limitations on the photosynthetic apparatus. Studies have shown that certain spectral combinations can increase non-regulated energy losses (ΦNO) and induce greater stress in PSII (STEFANOV et al., 2024), which is consistent with the lower Fm values observed under this treatment.

Fv/Fm as an integrated indicator of PSII efficiency

The maximum quantum yield (Fv/Fm) showed a consistent increase throughout acclimatization, rising from reduced values under *in vitro* conditions (≈ 0.55) to values between 0.75 and 0.80 at the end of the experimental period (Fig. 5). This behavior is highly informative, since Fv/Fm is widely used as an indicator of the potential efficiency of PSII (MAXWELL; JOHNSON, 2000).

Low Fv/Fm values at the beginning of the experiment indicate photoinhibition or downregulation of photosynthesis, possibly resulting from the artificial conditions of in vitro culture and the sudden exposure to new light levels and environmental variability. Indeed, the literature shows that several abiotic stresses, including excessive light, temperature fluctuations, and water deficit, lead to reductions in Fv/Fm by impairing the functional integrity of PSII (KALAJI et al., 2025; HU et al., 2023).

However, the minimum values observed in this study do not indicate severe damage, since more drastic reductions (e.g., < 0.4) are generally associated with intense or irreversible stress. In many systems,

values between 0.4 and 0.6 indicate moderate and potentially reversible stress, as observed under drought or salinity conditions (GUO et al., 2018; WEI et al., 2024).

The gradual increase in Fv/Fm to values close to 0.80 by the end of acclimatization indicates recovery of photochemical efficiency and reestablishment of PSII function, approaching the values considered optimal for non-stressed plants (≈ 0.83) (BJÖRKMAN & DEMMIG, 1987). This result suggests that *G. flexuosa* exhibits high physiological plasticity, being able to adjust its photosynthetic apparatus to new environmental conditions. Additionally, the convergence of Fv/Fm values among treatments at the end of the period indicates that, regardless of the initial light spectrum, the plants were able to reach a similar functional state, reinforcing the idea of successful acclimatization.

The combined analysis of Fo, Fm, and Fv/Fm allows for a more robust interpretation of the results. The initial pattern of high Fo + low Fm + low Fv/Fm characterizes a typical PSII stress state, involving inactivation of reaction centers, alterations in the antenna complex, and limitations in electron transport.

As acclimatization progressed, the trend of decreasing Fo, increasing Fm, and rising Fv/Fm indicates progressive recovery of the photosynthetic system, with reactivation of reaction centers, improved energy connectivity, and restoration of photochemical efficiency. This behavior is consistent with current models of photoinhibition, which consider that changes in Fo and Fm result from a combination of factors, including the fraction of active/inactive reaction centers, energy dissipation processes, and structural reorganization of PSII (KONO et al., 2021; NIES et al., 2023).

Conclusion

Light spectral quality significantly modulated the morphophysiological development of *Gomesa flexuosa* during in vitro culture, demonstrating that different light spectra promote distinct responses in the photosynthetic apparatus, pigment accumulation, and plant morphogenesis. Red light favored leaf emission and chlorophyll accumulation, whereas blue light was associated with the development of more compact structural characteristics and greater functional organization of the photosynthetic apparatus. In contrast, the blue/red combination did not show consistent superiority for the studied species, reinforcing the species-dependent nature of photomorphogenic responses in orchids.

Chlorophyll a fluorescence analyses demonstrated that the transition from in vitro to ex vitro conditions involves intense physiological reorganization, marked by the progressive recovery of photochemical efficiency of photosystem II and by the adjustment of photosynthetic metabolism to new environmental conditions. Despite the initial differences induced by light spectra, a trend of physiological and morphological convergence was observed during acclimatization, highlighting the high phenotypic plasticity of the species. The high ex vitro survival rate demonstrates that *Gomesa flexuosa* exhibits broad adaptive capacity, even after physiological changes induced during in vitro culture.

These results broaden the understanding of the mechanisms involved in photoautotrophic acclimatization of micropropagated orchids and demonstrate the potential of LED use as a tool to

optimize propagation and conservation protocols for native ornamental species.

Declarations

Ethics declaration

Not applicable

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Not applicable

Author Contribution

Nadhine Nostrani Cabral: Conceptualization, data analysis, statistical analysis, writing – review and editing.Fernanda Espíndola Assumpção Bastos: Writing – original draft (English translation), writing – review and editing.Elinton Soares Pontes: Data curation, data tabulation, formal analysis, interpretation of results, writing – original draft.Thiago Sanches Ornellas: Data tabulation, formal analysis, interpretation of results, writing – original draft.Rosete Pescador: Supervision, conceptualization, writing – review and editing.All authors have read and approved the final version of the manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

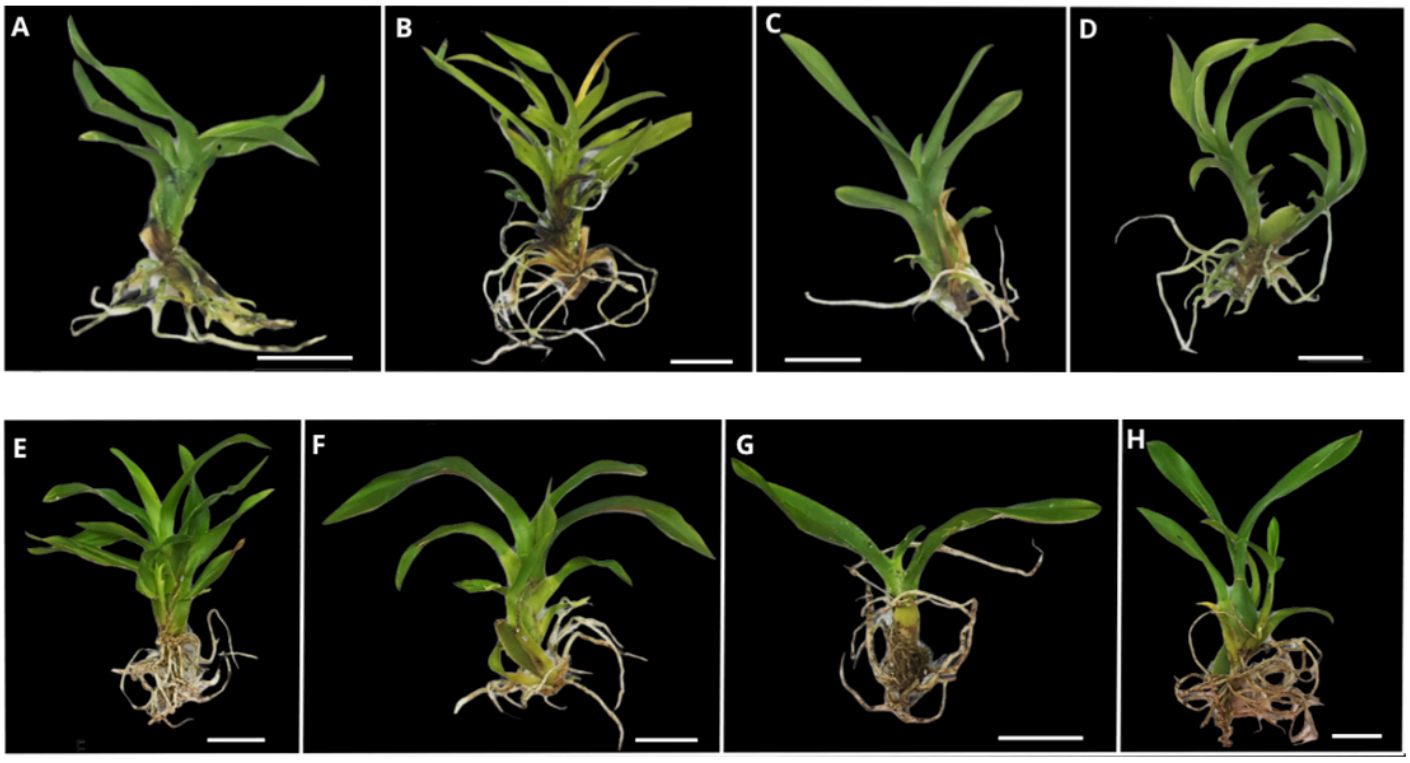

Figure 1

Plants of *Gomesa flexuosa* after 13 months of in vitro cultivation (A – Blue LED treatment, B – Red LED treatment, C – Blue/Red LED treatment, D – White LED treatment) and after 45 days of the ex vitro phase (E – Blue LED treatment, F – Red LED treatment, G – Blue/Red LED treatment, H – White LED treatment). Bars = 2 cm.

Figure 2

Gomesa flexuosa plants at the 45th day of acclimatization, showing a high number and good root length (A – Blue LED treatment, B – Red LED treatment, C – Blue/Red LED treatment, D – White LED treatment). Scale bars: 2 cm.

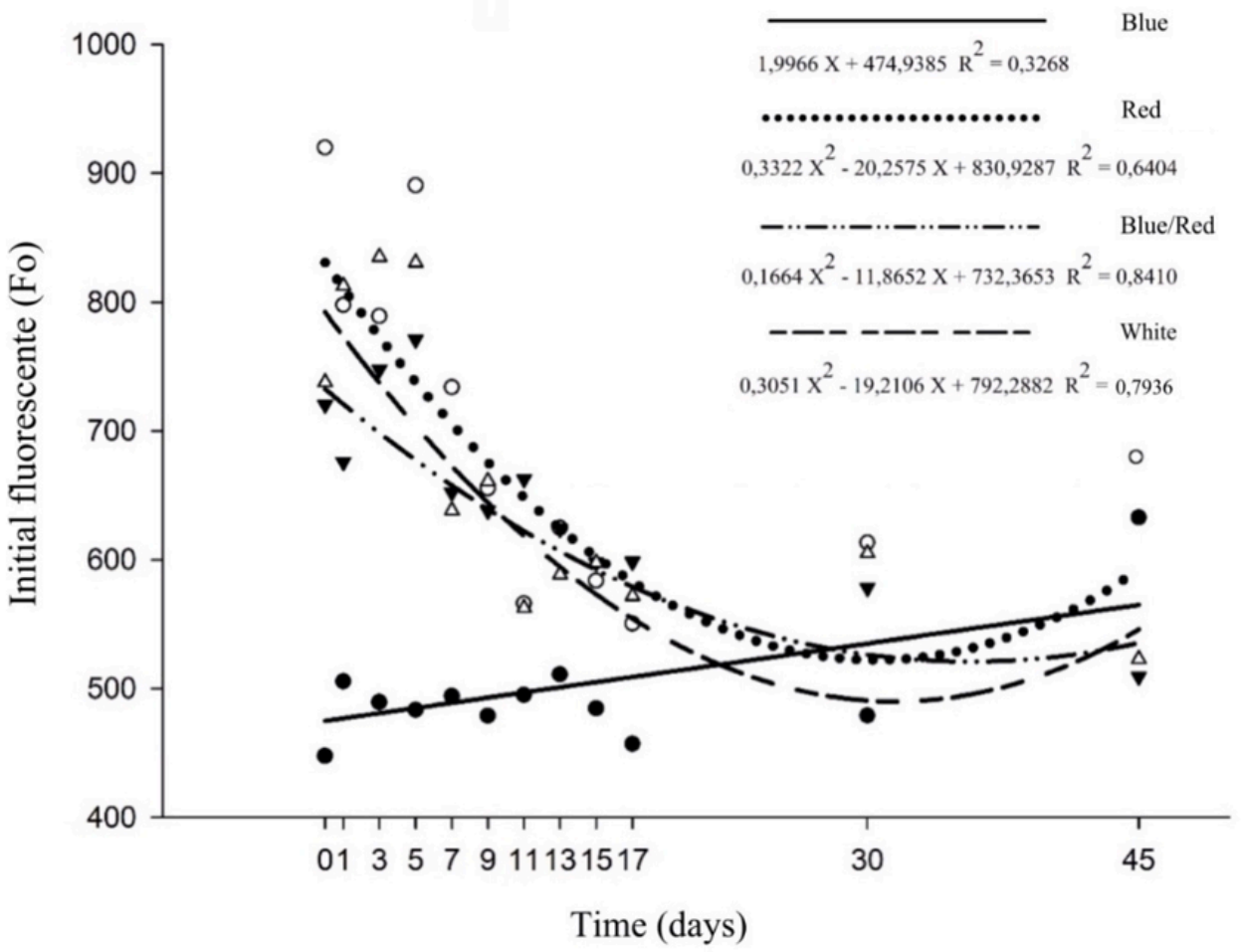

Figure 3

Dynamics of basal fluorescence (Fo) – arbitrary units – of *Gomesa flexuosa* plants during the *ex vitro* acclimatization period of micropropagated plants grown under different spectral qualities of LED lamps.

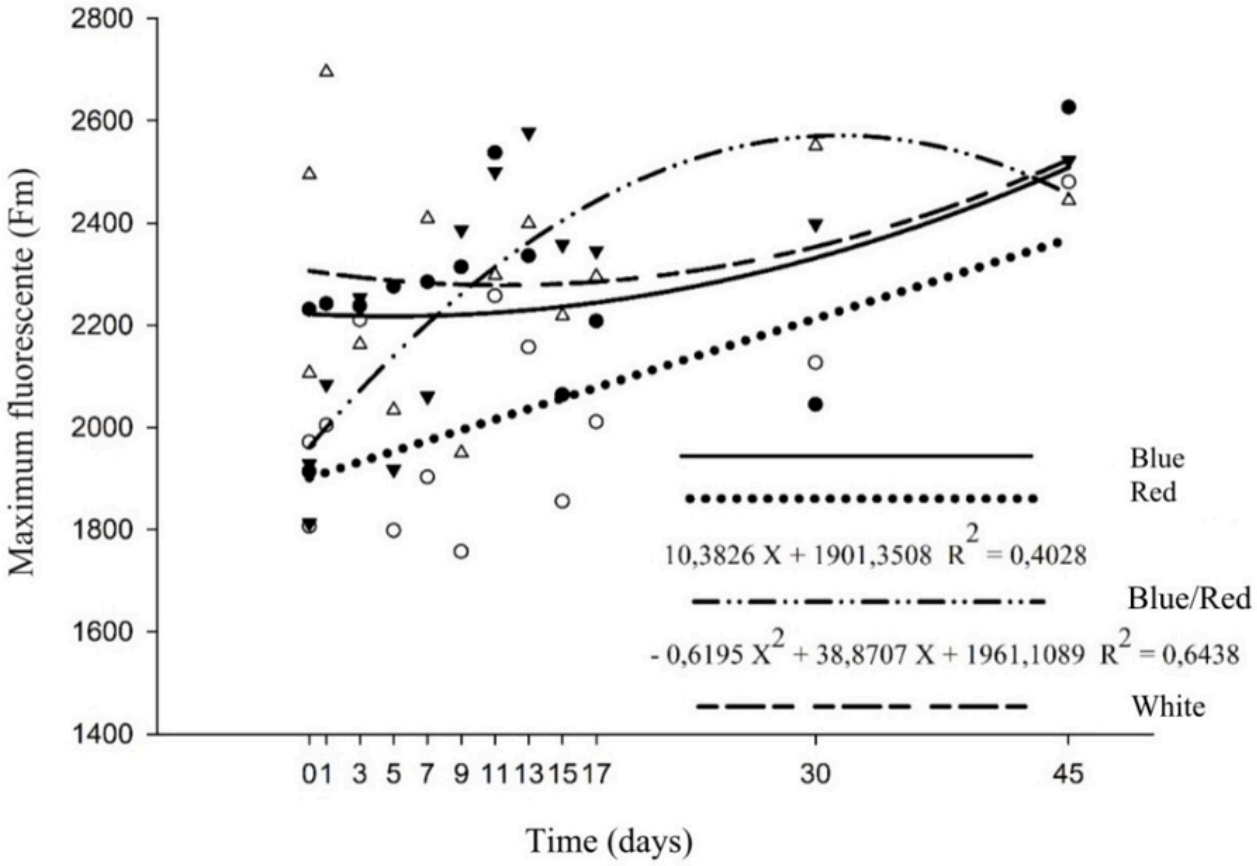

Figure 4

Dynamics of maximum fluorescence (Fm) – arbitrary units – of *Gomesa flexuosa*plants during the *ex vitro* acclimatization period of micropropagated plants grown under different spectral qualities of LED lamps.

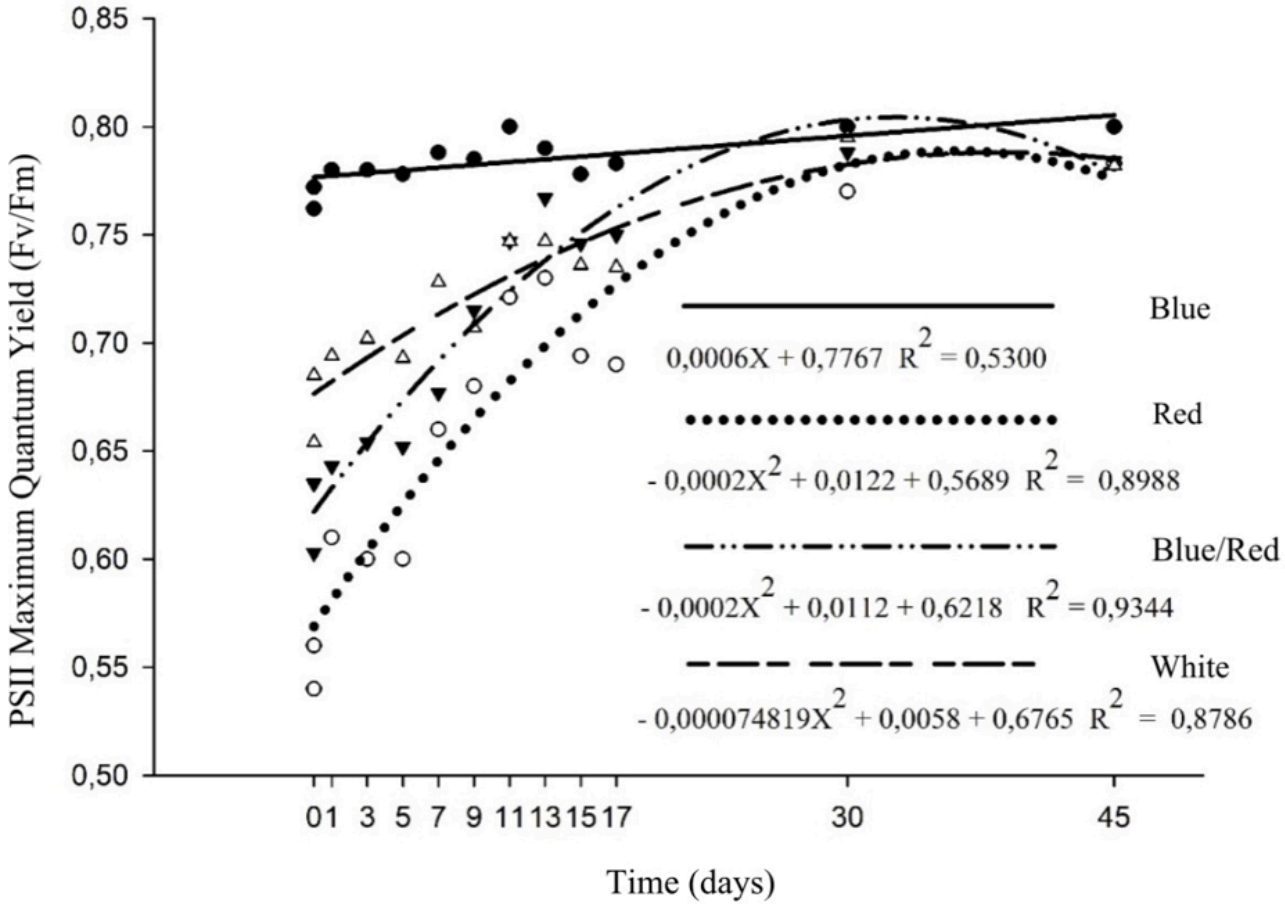

Figure 5

Dynamics of the maximum quantum yield (Fv/Fm) of *Gomesa flexuosa*plants during the *ex vitro* acclimatization period of micropropagated plants grown under different spectral qualities of LED lamps.