



Spectral quality modulates the morphophysiology of *Sinningia rupicola* (Mart.) Wiehler micropropagated seedlings: an endangered species of the Ferruginous Rupestrian Grassland

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Abstract

The Ferruginous Rupestrian Grassland is a highly biodiverse ecosystem that harbors endemic species vulnerable to anthropogenic activities and climate change, such as *Sinningia rupicola*, classified as endangered. In vitro cultivation enables large-scale seedling production with high physiological quality. Among the factors influencing the success of the technique, spectral quality stands out, acting on photosynthesis and morphophysiology with species-specific responses. This study evaluated the effects of different spectral qualities on the morphophysiology of *Sinningia rupicola* seedlings in the multiplication and elongation/rooting stages of in vitro culture. Tissue oxidation, vigor, number and length of shoots, leaf number and area, root formation, photosynthetic pigments, leaf anatomy, and survival in the acclimatization were assessed. LED-R promoted lower vigor in multiplication. LED-B increased leaf area but reduced the number of roots. LED-W increased the number of leaves but reduced photosynthetic pigment levels. In contrast, LED-P resulted in higher pigment contents and secondary roots in 90.9% of the explants, indicating balanced growth. In elongation/rooting, LED-W increased mesophyll thickness but reduced photosynthetic pigment levels. LED-B showed greater leaf area, high stomatal density, and reduced rooting. LED-R promoted shoot length but reduced survival. In contrast, LED-P produced vigorous plants with an efficient photosynthetic apparatus, lower stomatal density, and greater rooting, with 90% survival. Spectral quality modulates the morphophysiological traits of *Sinningia rupicola* grown in vitro, with LED-P being the most suitable for both multiplication and elongation/rooting, representing a strategy for species conservation through the production of high-quality seedlings.

Key message

Purple LEDs optimize in vitro protocols for *Sinningia rupicola*, ensuring high-quality seedling production and acclimatization survival, fundamental for biodiversity restoration programs in Ferruginous Rupestrian Grasslands.

Keywords Endemic species · In vitro culture · LEDs · Photomorphogenesis

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Introduction

The Ferruginous Rupestrian Grasslands constitute an ecosystem of high biodiversity and are considered of great ecological importance due to the high degree of endemism, as well as the presence of rare species restricted to ferruginous soils (Messias et al. 2012; Porembski et al. 2016; Leite et al. 2017). This habitat occurs at altitudes ranging from 900 to 2300 m and is distributed in small fragments of rocky outcrops across Brazil, with its central area located along the Serra do Espinhaço mountain range, between the states of Minas Gerais and Bahia (Silveira et al. 2016). Endemic species occurring in restricted areas, such as the Rupestrian Grassland of the Quadrilátero Ferrífero, which have been experiencing area reduction due to intense anthropogenic activities and climate change, are more susceptible to some degree of extinction risk (Messias et al. 2012; Santos et al. 2021).

Among the species occurring in the Rupestrian Grassland of the 'Quadrilátero Ferrífero' is *Sinningia rupicola* (Mart.) Wiehler, an herbaceous plant belonging to the Gesneriaceae family. Measuring 20 to 40 cm in height, with ornamental potential and classified as endangered (EN) (Araujo et al. 2005; Ministério do Meio Ambiente 2014). Flowering occurs between October and March, with pollination mediated by hummingbirds (Araujo et al. 2005). The species develops an annual aerial stem, persisting as a tuber for the remainder of the year. Furthermore, while it produces small, light seeds, they exhibit low germination capacity (Ranieri et al. 2012). Flora rescue in Rupestrian Grassland areas contributes to the conservation of some individuals; however, it does not prevent the loss of natural habitat. In this context, the restoration of these areas may favor ecosystem conservation (Mendonça et al. 2019).

Therefore, studies aimed at improving the propagation process of endemic and endangered species, such as *Sinningia rupicola*, may assist in seedling production and, consequently, contribute to the conservation of the species and the biodiversity of Rupestrian Grassland. In this context, although in vitro protocols have been successfully developed for other species from the Ferruginous Rupestrian Grasslands, such as *Paliavana sericiflora* (Leite et al. 2024) and *Arthrocereus glaziovii* (Leite et al. 2025a), research on *Sinningia rupicola* is still in its early stages. Recent studies have begun to develop cultivation protocols for this species, focusing on sterilization techniques and the influence of gas exchange on its in vitro growth (Mendes et al. 2026). However, the optimization of spectral quality the in vitro cultivation stages still needs to be elucidated.

In vitro culture is a tool that provides an adequate cost–benefit ratio, as it enables large-scale production within a short period, with phytosanitary, nutritional, and

environmental control, in addition to allowing long-term in vitro storage of the species, which is of great importance for the conservation of native species (Xavier et al. 2013; Keret et al. 2018; Leite et al. 2024). The performance of in vitro culture at different stages is directly related to the adjustment of factors that favor the production and quality of the plantlets obtained. Among these factors, light stands out for acting both as an energy source for photosynthesis and as an environmental signal involved in photomorphogenesis.

Plants continuously respond to incident light conditions through photoreceptors such as phytochromes, cryptochromes, and phototropins, adjusting their growth and development (Tripathi et al. 2019). However, different spectral qualities (light intensity and wavelength) elicit different morphophysiological responses depending on the genotype (Miranda et al. 2020; Frade et al. 2023), making species-specific adjustments necessary to ensure the production of vigorous plantlets. Thus, the choice of the light source used on in vitro culture can influence process efficiency and survival in the acclimatization stage. In addition, light sources with higher energy efficiency and lower consumption may increase the chances of success in micropropagation (Monteuuis 2015; Leite et al. 2025b). In this regard, light-emitting diodes (LEDs) offer advantages over fluorescent lamps, mainly due to energy savings and the possibility of combining specific wavelengths, which may result in higher productivity and better quality of micropropagated plantlets (Batista et al. 2018; Leite et al. 2025b).

Therefore, the objective of the present study was to evaluate the effect of different spectral qualities on the morphophysiology of *Sinningia rupicola* plantlets in the multiplication and elongation/rooting stages of in vitro culture, as well as their acclimatization.

Materials and methods

Study site and plant material

For the development of this study, shoots of *Sinningia rupicola* (Mart.) Wiehler previously established in vitro (Mendes et al. 2026) were used, with registration in the National System for the Management of Genetic Heritage (SisGen: ACF88E2). The genetic material was provided by the Research and Innovation Unit of Gerda (GERDAU Açominas S.A.) and was collected from natural occurrences in the Ferruginous Rupestrian Grasslands in the region of Ouro Branco, Minas Gerais, Brazil. This material constitutes a representative sample of the local genetic resources of *Sinningia rupicola* from this specific ferruginous area.

Effect of spectral quality on in vitro multiplication

To evaluate the influence of spectral quality in the multiplication stage, *Sinningia rupicola* shoots previously established in vitro were isolated and standardized to 1 cm in length with four leaves per explant. Under aseptic conditions, one explant was inoculated per test tube (25 × 150 mm) containing 10 mL of MS culture medium (Murashige and Skoog 1962), supplemented with 30 g L⁻¹ sucrose (Synth Ltda., Diadema, Brazil), 0.5 mg L⁻¹ 6-benzylaminopurine (BAP; Sigma®, Barueri, Brazil), 0.05 mg L⁻¹ α -naphthaleneacetic acid (NAA; Sigma®, Barueri, Brazil), and 6 g L⁻¹ agar (Merck S.A., Rio de Janeiro, Brazil). The pH of the culture medium was adjusted to 5.8 (± 0.05) prior to the addition of agar, and the medium was autoclaved in a Digitale 20 L autoclave (BS Equipamentos Ltda., Varginha, Brazil) at 121 °C and 1.0 kgf cm⁻² for 20 min.

The explants were cultured in a growth room at 24 °C (± 1 °C), under a 16-h photoperiod, with spectral quality according to the following treatments: (a) Blue (LED-B) [blue LED lamp / photosynthetic photon flux density (PPFD) of 12 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Tubular LED T8, 150 lm, 10 W, LuzSolar®, China)]; (b) Red (LED-R) [red LED lamp / PPFD of 8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Tubular LED 488 lm, 9 W, CTB®, China)]; (c) Purple (LED-P) [combination of red and blue LED lamps / PPFD of 6.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$, at a 1:1 ratio, respectively (Tubular LED 488 lm, 9 W, CTB®, China) and (Tubular LED T8,

150 lm, 10 W, LuzSolar®, China)]; and (d) White (LED-W) [white LED lamp / PPFD of 38 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (SuperLED 6500 K, 100 lm W⁻¹, 9 W, OuroLux®, São Paulo, Brazil)]. The White LED treatment (LED-W) was established as the control, as it provides a broad-spectrum light source that represents the standard lighting conditions for in vitro cultivation in modern plant tissue culture laboratories. Spectral quality and PPFD were determined using a spectroradiometer (Ocean Optics SpectraSuite, Ocean Optics, Dunedin, Florida) (Fig. 1).

After 45 d, contamination, tissue oxidation, vigor, number of shoots, shoot length (cm), number of leaves per shoot, leaf area (cm²), number of roots, secondary roots, and photosynthetic pigments [chlorophyll *a*, *b*, total (*a* + *b*), carotenoids, *a* / *b*, and carotenoids / total (*a* + *b*)] were evaluated. Vigor and oxidation were assessed using a rating scale, in which 1=excellent, 2=good, and 3=poor, according to the methodology adopted by Souza et al. (2019). For the variables contamination and secondary roots, absence or presence was recorded. The experiment was conducted in a completely randomized design with 20 replicates per treatment.

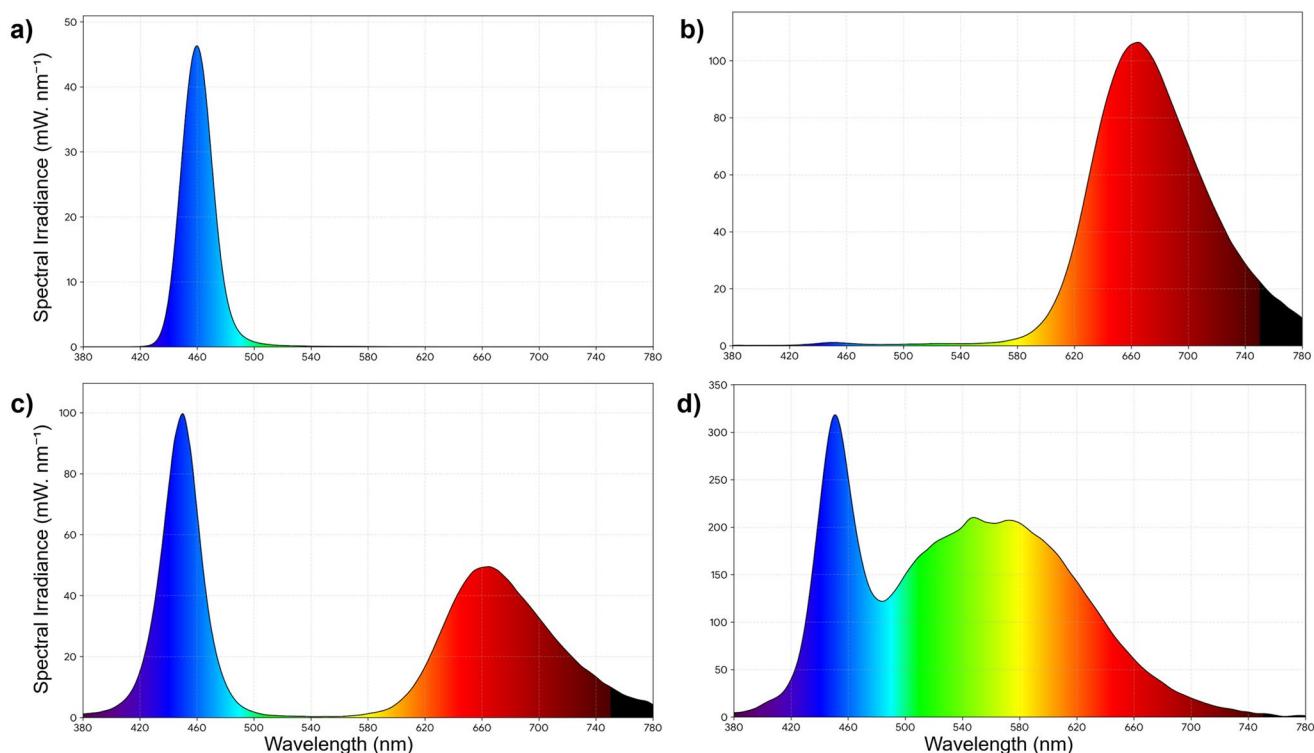


Fig. 1 Absolute spectral irradiance distribution as a function of wavelength of the light sources used on in vitro culture of *Sinningia rupicola*. (a) Blue LED (LED-B); (b) Red LED (LED-R); (c) Purple LED (combination of Red and Blue LED lamps, 1:1) (LED-P); (d) White LED (LED-W)

Effect of spectral quality on in vitro elongation and rooting

Shoots measuring 1.0 cm in length obtained from the multiplication stage were isolated and subcultured in test tubes (25×150 mm) containing 10 mL of MS culture medium, supplemented with 30 g L^{-1} sucrose (Synth Ltda., Diadema, Brazil), 0.05 mg L^{-1} BAP (Sigma[®], Barueri, Brazil), 0.1 mg L^{-1} α -naphthaleneacetic acid (NAA; Sigma[®], Barueri, Brazil), 0.1 mg L^{-1} indole-3-butyric acid (IBA; Sigma[®], Barueri, Brazil), and 6 g L^{-1} agar (Merck S.A., Rio de Janeiro, Brazil). The explants were maintained in a growth room under the different spectral qualities used in the previous experiment (i.e., LED-B, LED-R, LED-P, and LED-W).

After 45 d, contamination, tissue oxidation, vigor, number of shoots, shoot length (cm), number of leaves per shoot, leaf area (cm^2), number of roots, secondary roots, photosynthetic pigments [chlorophyll *a*, *b*, total (*a* + *b*), carotenoids, *a* / *b*, and carotenoids / total (*a* + *b*)], and leaf anatomy were evaluated through transverse leaf sections (measurement of mesophyll tissues) and leaf epidermis analysis [stomatal density (μm^2), stomatal size, stomatal index (%), and epidermal cell density (cells mm^{-2})]. The experiment was conducted in a completely randomized design with 20 replicates per treatment.

Acclimatization

Plantlets produced in vitro were transplanted into polypropylene containers using a mini-incubator system (Brondani et al. 2012) filled with a substrate composed of decomposed pine bark and vermiculite at a 2:1 (v/v) ratio. The plantlets were grown in a growth room at a temperature of 24°C ($\pm 1^\circ\text{C}$), under a 16-h photoperiod (white LED lamps, SuperLED, 6500 K, 9 W, OuroLux[®], São Paulo, Brazil) and an irradiance of $38 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (quantified using a radiometer, Ocean Optics SpectraSuite, Ocean Optics, Dunedin, Florida), with irrigation every 48 h, as described by Mendes et al. (2026). After 30 days, plant survival was assessed, concluding the in vitro culture phase (Fig. 2), with 20 plants per light treatment acclimatized, originating from the elongation and rooting stages.

Analysis of photosynthetic pigment content

At the end of the multiplication and elongation/rooting experiments, 25 mg of fresh leaf tissue per treatment were collected. The samples were immersed in 5 mL of dimethyl sulfoxide (DMSO) solution (Sigma[®], Barueri, Brazil) and kept in the dark for 48 h (Lichtenthaler 1987). Absorbance was determined in triplicate using a quartz cuvette with a 10 mm optical path length in a Genesys 10UV spectrophotometer (Thermo Scientific, São Paulo, Brazil). Sample readings were taken at wavelengths of 665, 649, and 480 nm, and the equations used to calculate the concentrations of

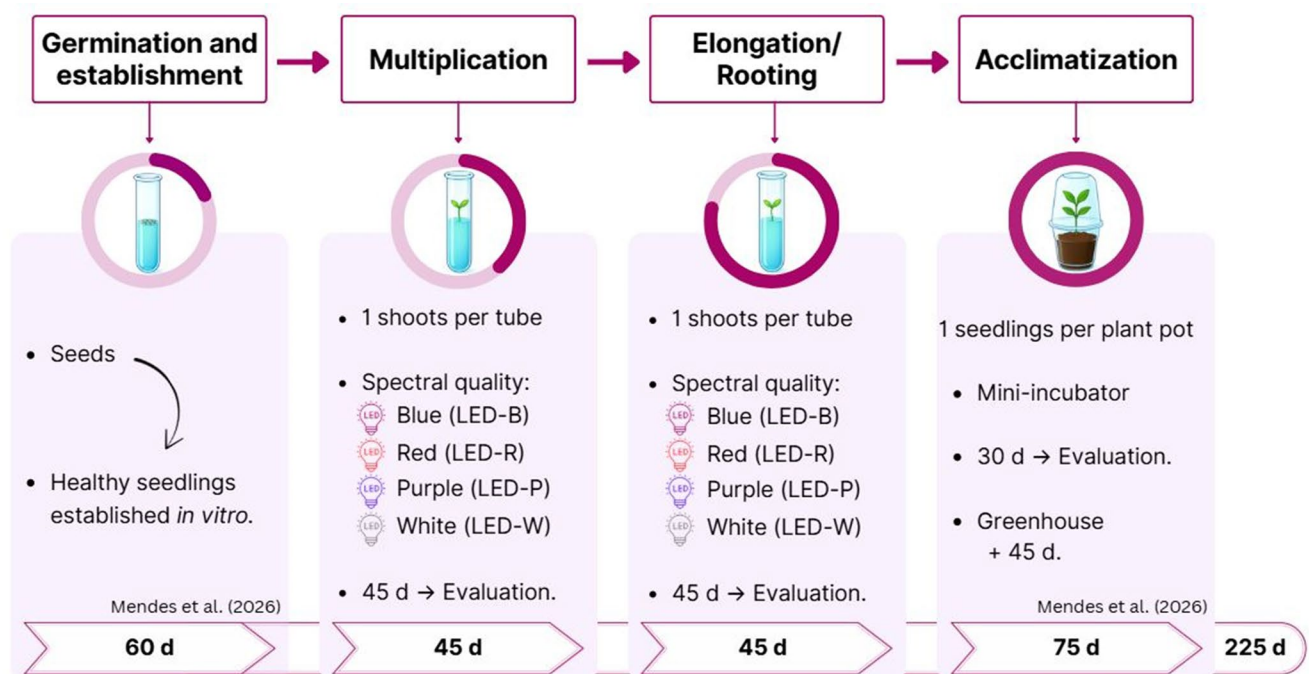


Fig. 2 Flowchart of the stages carried out in the in vitro culture of *Sinningia rupicola*

chlorophyll *a*, chlorophyll *b*, total chlorophyll (*a*+*b*), and carotenoids were based on the method described by Wellburn (1994).

Leaf area

At the end of the multiplication and elongation/rooting experiments, 20 leaves per treatment were measured. Leaves were collected from the second and third pairs of young leaves of the shoots, counted from the shoot apex toward the base. Leaf images were captured using an HP Deskjet Ink Advantage 1516 scanner (HP Inc., Palo Alto, California), and leaf area was measured using ImageJ software.

Leaf anatomy

At the end of the elongation/rooting stage, five fully expanded leaves from the median region were collected and fixed in FAA 70% solution (formaldehyde, acetic acid, and 70% ethanol at a ratio of 1:1:18) for 48 h and then transferred to 70% ethanol solution (Johansen 1940). These samples were used for transverse leaf sections and leaf epidermis analysis.

For transverse leaf sections, leaf samples were sectioned using a semi-automatic rotary microtome (Yidi; Jinhua Yidi Medical Appliance Co., Ltd., Jinhua, China) at 7 μ m thickness, then stained with 0.05% toluidine blue in acetate buffer for two min, followed by three washes with deionized water. Slides were mounted with colorless Acrilex varnish (Acrilex S.A., São Bernardo do Campo, Brazil) and covered with a coverslip. Microscopic analyses were performed using a 40 $\times$ objective on a light microscope (Eclipse E100-LED; Nikon, Tokyo, Japan) coupled to an Infinity-1 digital camera (Lumenera Corporation, Ottawa, Canada), with the aid of Infinity Analyze software (version 6.3). Mesophyll thickness (μ m), adaxial epidermis thickness (μ m), palisade parenchyma thickness (μ m), and spongy parenchyma thickness (μ m) was measured. Images were analyzed using ImageJ Fiji software.

For leaf epidermis analysis, leaf samples were cleared in a solution of 2% sodium hypochlorite and deionized water at a 1:1 ratio until complete epidermal transparency was achieved. Subsequently, samples were stained with 1% safranin solution for 2 min and then washed three times with deionized water.

Slides were mounted with 50% glycerinated water, covered with a coverslip, and observed under a 40 $\times$ objective using a light microscope. For each treatment, five microscopic fields from the median region of the abaxial leaf epidermis were photomicrographed. Each field corresponded to a leaf area of 0.1036 mm², within which the number of stomata and epidermal cells were counted to calculate

stomatal density (SD) and stomatal index (SI). Images were analyzed using ImageJ Fiji software with the Cell Counter plugin.

Stomatal density (SD) was obtained as the ratio between the number of stomata and the analyzed area (mm²), i.e., $SD = \text{number of stomata} / \text{analyzed area (mm}^2\text{)}$. The stomatal index (SI) was calculated as the proportion between the number of stomata and the total number of cells (stomata+epidermal cells), multiplied by 100, i.e., $SI = 100 \times [\text{number of stomata} / (\text{number of stomata} + \text{number of epidermal cells})]$. Mean stomatal size was also measured.

Data analysis

The collected data were analyzed for homoscedasticity and normal distribution using Hartley's test ($P > 0.05$) and the Shapiro–Wilk test ($P > 0.05$), respectively. The results were subjected to analysis of variance (ANOVA, $P < 0.05$), followed by Tukey's mean comparison test ($P < 0.05$). R software version 4.0.3 (2020) was used for data analysis, considering the easyanova package (Arnhold 2013).

For leaf anatomy data, normality was verified using the Shapiro–Wilk test ($\alpha = 0.05$) in Sisvar (v. 5.8). For normally distributed data, ANOVA followed by Tukey's test (5%) was applied. For non-normal data, the Kruskal–Walli's test was used in PAST (v. 4.03), followed by Dunn's test with Bonferroni correction for multiple comparisons.

Results

Effect of spectral quality on in vitro multiplication

No contamination or tissue oxidation was observed in any of the treatments. Analysis of shoot vigor showed that the treatment light spectrum LED-R exhibited good vigor (score 2.0), differing statistically from the Blue (LED-B), Purple (LED-P), and White (LED-W) treatments, which showed excellent vigor with scores of 1.0 (Fig. 3a).

Regarding the number of shoots, no statistical differences were observed among the different light spectra, with mean values of 2.0, 1.7, 2.4, and 2.2 for the LED-B, LED-R, LED-P, and LED-W treatments, respectively. For shoot length, LED-R showed the best result, with a mean value of 4.1 cm, differing statistically from the LED-P, LED-B, and LED-W treatments, which presented mean values of 2.4 cm, 1.9 cm, and 1.9 cm, respectively (Fig. 3b).

The number of leaves per explant under the LED-W treatment showed the best result, with a mean of 10.8 leaves per explant, differing statistically from the LED-P treatment, which showed 8.1 leaves. Shoots cultivated under LED-R and LED-B exhibited intermediate values compared

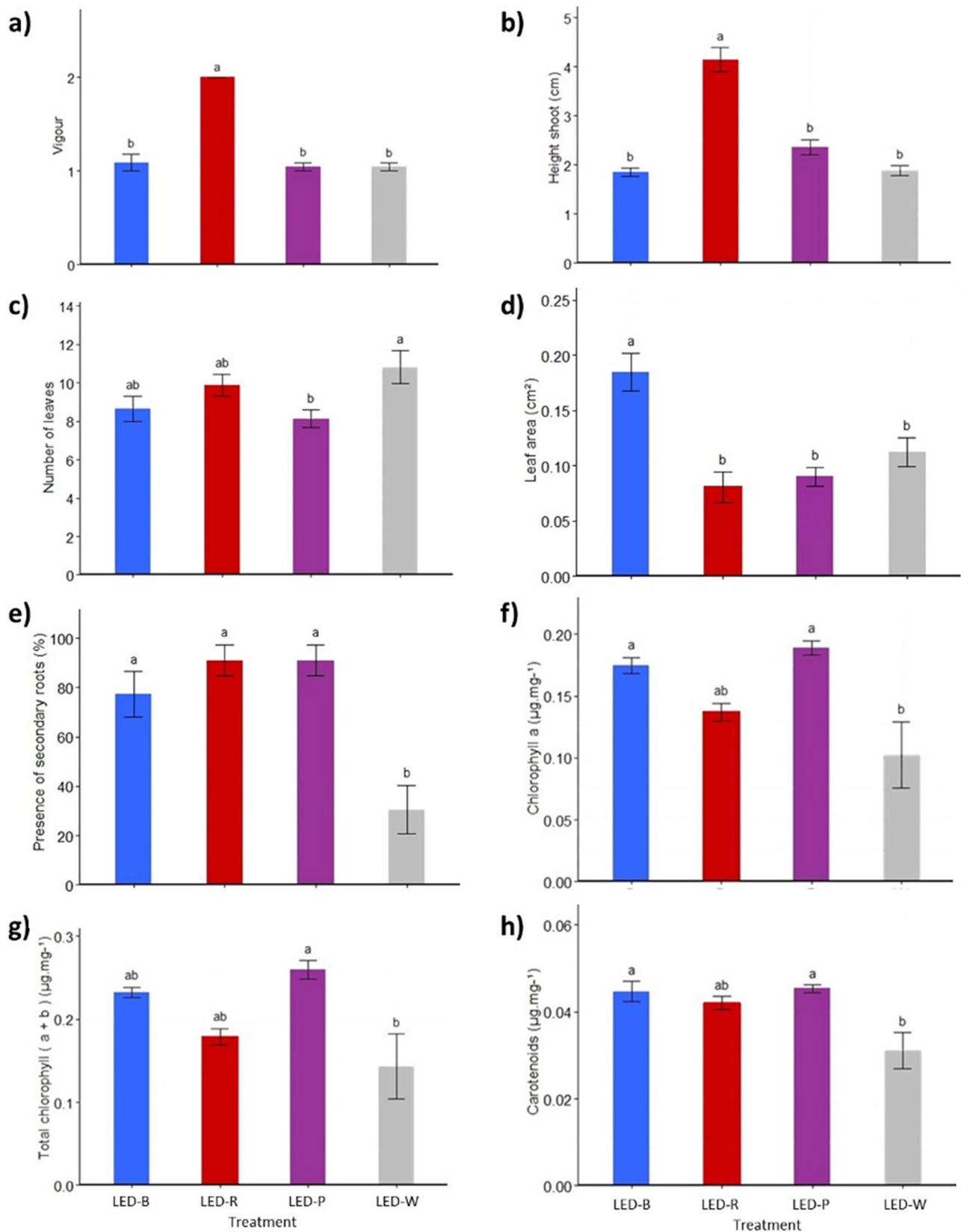


Fig. 3 Features evaluated after 45 d on in vitro multiplication of *Sinningia speciosa* under different spectral qualities (LED-B, LED-R, LED-P, and LED-W) that showed significant differences. **(a)** Vigor, according to a rating scale: 1=excellent, 2=good, and 3=poor, as described by Souza et al. (2018); **(b)** shoot length (cm); **(c)** number of leaves per explant; **(d)** leaf area (cm²); **(e)** presence of secondary roots (%); **(f)** chlorophyll *a*; **(g)** total chlorophyll (*a* + *b*); and **(h)** carotenoids. Means followed by the same letter do not differ significantly according to Tukey's test at the 5% significance level. Error bars represent the standard error of the mean

with the other treatments, with means of 9.9 and 8.6 leaves, respectively (Fig. 3c). For leaf area, the LED-B treatment showed the best result, with a mean area of 0.19 cm², differing statistically from the LED-R, LED-P, and LED-W treatments, which presented mean values of 0.08 cm², 0.09 cm², and 0.11 cm², respectively (Fig. 3d).

The number of roots did not differ statistically among treatments, with mean values of 3.1, 3.6, 4.1, and 3.9 for the LED-B, LED-R, LED-P, and LED-W treatments, respectively. However, when secondary roots were considered, the LED-R, LED-P, and LED-B treatments differed statistically from the LED-W treatment, which showed secondary roots in only 30.4% of the explants. LED-R and LED-P showed the best results, both with 90.9%, followed by LED-B with a mean of 77.3% (Fig. 3e).

In the analysis of photosynthetic pigments, the LED-P treatment exhibited the highest concentrations of chlorophyll *a* (0.189 µg mg⁻¹) and total chlorophyll (*a* + *b*) (0.260 µg mg⁻¹), differing statistically from the LED-W treatment, which presented the lowest concentrations of photosynthetic pigments, chlorophyll *a* (0.102 µg mg⁻¹) and total chlorophyll (*a* + *b*) (0.143 µg mg⁻¹). The LED-B and LED-R treatments showed intermediate values compared with the other treatments for chlorophyll *a* (0.175 and 0.137 µg mg⁻¹) and total chlorophyll (*a* + *b*) (0.232 and 0.179 µg mg⁻¹), respectively (Fig. 3f–g).

For carotenoids, the LED-P and LED-B treatments showed the highest mean values, both at 0.045 µg mg⁻¹, differing statistically from the LED-W treatment (0.031 µg mg⁻¹). The LED-R treatment presented an intermediate value compared with the other treatments, with a mean of 0.042 µg mg⁻¹ (Fig. 3h). Chlorophyll *b* did not differ statistically among treatments, with mean values of 0.057, 0.042, 0.071, and 0.041 µg mg⁻¹ for the LED-B, LED-R, LED-P, and LED-W treatments, respectively. During the multiplication phase, neither the chlorophyll *a*/*b* ratio nor the carotenoid-to-total chlorophyll ratio showed statistically significant differences among treatments.

Effect of spectral quality on in vitro elongation and rooting

The treatment under the red-light spectrum (LED-R) exhibited good vigor (score 2.0), differing significantly from the LED-B, LED-P, and LED-W treatments, which showed excellent vigor with scores of 1.0 (Fig. 4a). No contamination or tissue oxidation was observed in any of the tested treatments.

The number of shoots did not differ statistically among treatments, with mean values of 1.4, 1.4, 1.8, and 1.9 for the LED-B, LED-R, LED-P, and LED-W treatments, respectively. For shoot length, the LED-R treatment showed the highest mean value (3.9 cm), differing statistically from LED-P, which presented a mean shoot length of 2.4 cm. This was followed by the LED-B and LED-W treatments, both with mean values of 1.5 cm, which also differed statistically from the other treatments (Fig. 4b).

The number of leaves per explant did not show significant differences among treatments, with mean values of 8.9, 9.0, 9.2, and 8.3 for LED-B, LED-R, LED-P, and LED-W, respectively. For leaf area, the LED-B treatment showed the best result, with a mean area of 0.19 cm², differing statistically from the other treatments. This was followed by the LED-P, LED-W, and LED-R treatments, with mean values of 0.11 cm², 0.09 cm², and 0.06 cm², respectively (Fig. 4c).

Regarding the number of roots, the LED-P and LED-W treatments showed the best results, with mean values of 4.8 and 4.7 roots, respectively, differing statistically from the LED-B treatment, which presented the lowest number of roots, with a mean of 3.2. The LED-R treatment showed an intermediate value compared with the other treatments, with a mean of 4.0 roots (Fig. 4d). For secondary roots, no significant differences were observed among the LED-B, LED-R, LED-P, and LED-W treatments, with the presence of secondary roots in 84.0%, 58.3%, 87.5%, and 70.8% of the samples, respectively; however, all treatments produced plants suitable for the subsequent seedling production.

In the analysis of photosynthetic pigments, the LED-P treatment exhibited the highest concentrations of chlorophyll *a* (0.189 µg mg⁻¹), chlorophyll *b* (0.074 µg mg⁻¹), and total chlorophyll (*a* + *b*) (0.262 µg mg⁻¹), whereas the LED-W treatment showed the lowest concentrations of photosynthetic pigments, chlorophyll *a* (0.078 µg mg⁻¹), chlorophyll *b* (0.021 µg mg⁻¹), and total chlorophyll (*a* + *b*) (0.099 µg mg⁻¹). The LED-B and LED-R treatments presented intermediate values for chlorophyll *a* (0.138 and 0.099 µg mg⁻¹), chlorophyll *b* (0.048 and 0.041 µg mg⁻¹), and total chlorophyll (*a* + *b*) (0.186 and 0.140 µg mg⁻¹), respectively (Fig. 4e–g). Carotenoids did not show significant differences among treatments. Likewise, the chlorophyll *a*/*b* ratio

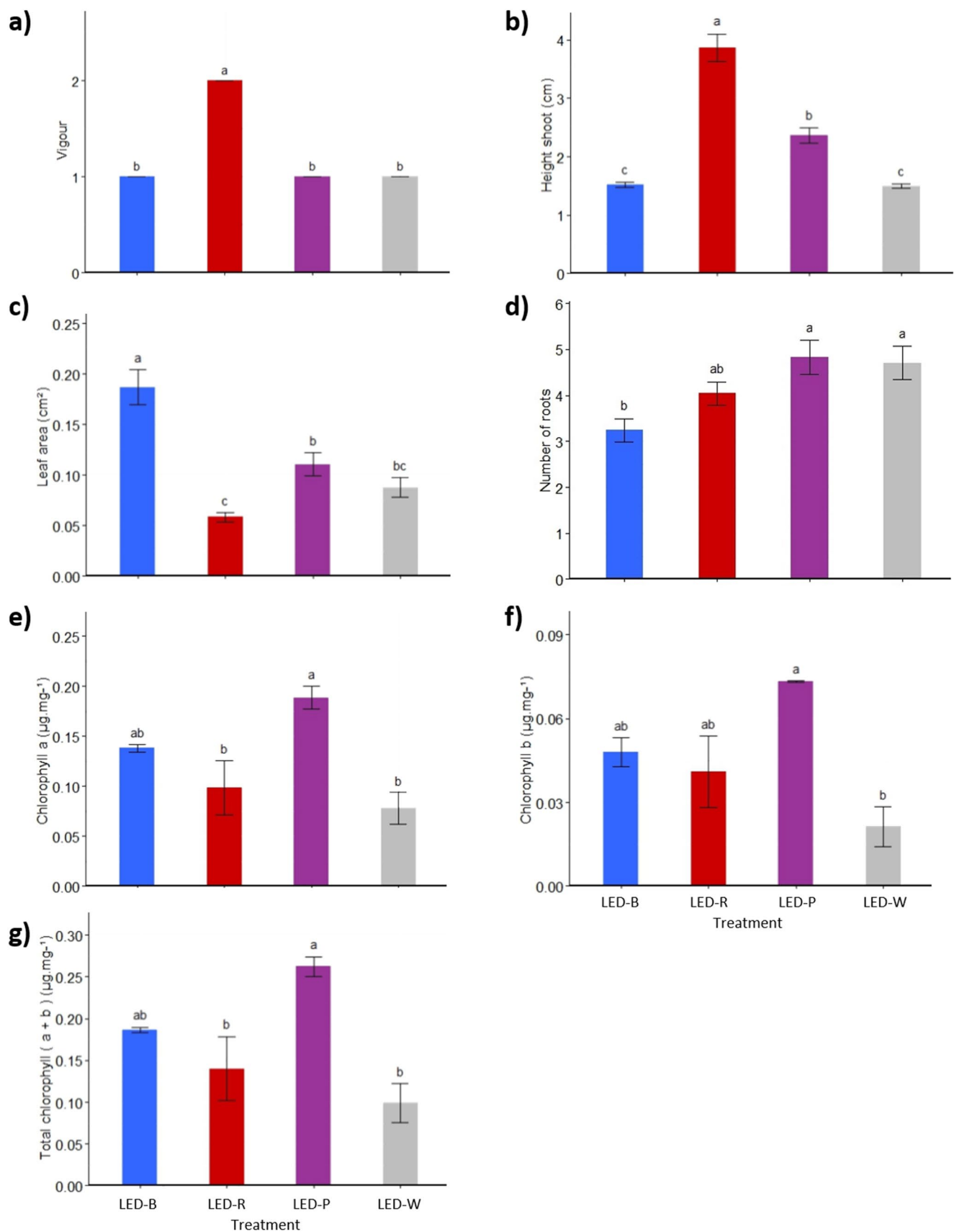


Fig. 4 Features evaluated after 45 d on in vitro elongation/rooting of *Sinningia rupicola* under different spectral qualities (LED-B, LED-R, LED-P, and LED-W), which showed significant differences. **(a)** Vigor, according to the rating scale: 1=excellent; 2=good; and 3=poor, from Souza et al. (2018). **(b)** Shoot length (cm); **(c)** Leaf area (cm²); **(d)** Number of roots; **(e)** Chlorophyll *a*; **(f)** Chlorophyll *b*; **(g)** Total chlorophyll (*a*+*b*). Means followed by the same letter do not differ significantly according to Tukey's test at the 5% significance level. Bars represent the standard error of the mean

and the carotenoid-to-total chlorophyll ratio did not differ significantly among treatments.

For leaf anatomy, transverse leaf sections show that *Sinningia rupicola* exhibits hypostomatic and dorsiventral leaves. In the mesophyll region, palisade parenchyma (pp) arranged in elongated rows beneath the adaxial epidermis and spongy parenchyma (ps) with intercellular spaces are observed. In the midrib region, a closed collateral vascular bundle (vb) is observed, surrounded by parenchymatic cells, and immediately below the epidermis, the differentiation of angular collenchyma (co) is noted, still at an early stage of thickening (Fig. 5a-d).

Spectral quality significantly affected the thickness of the leaf mesophyll in *Sinningia rupicola*. The LED-R and LED-W treatments promoted a thicker mesophyll, with values of $89.91 \pm 11.2 \mu\text{m}$ and $84.46 \pm 9.11 \mu\text{m}$, respectively, differing statistically from LED-B ($69.01 \pm 12.94 \mu\text{m}$) and LED-P ($45.66 \pm 6.21 \mu\text{m}$) (Fig. 6a). Additionally, leaves exposed to LED-R ($26.05 \pm 5.24 \mu\text{m}$) exhibited a thicker adaxial epidermis, differing significantly from the other treatments: LED-W ($20.60 \pm 3.88 \mu\text{m}$), LED-P ($20.24 \pm 3.53 \mu\text{m}$), and LED-B ($18.81 \pm 3.77 \mu\text{m}$) (Fig. 6b).

All treatments presented a single layer of palisade parenchyma, which was thicker under LED-W ($36.80 \pm 3.46 \mu\text{m}$) but did not differ statistically from LED-R ($34.77 \pm 2.74 \mu\text{m}$). The LED-P treatment had the lowest thickness ($22.28 \pm 3.02 \mu\text{m}$), differing statistically from the other treatments, as did LED-B ($28.78 \pm 5.54 \mu\text{m}$) (Fig. 6c). Regarding spongy parenchyma thickness (Fig. 6d), leaves under LED-R had the greatest thickness ($55.41 \pm 11.37 \mu\text{m}$), although this did not differ statistically from leaves under LED-W ($48.98 \pm 9.19 \mu\text{m}$). The lowest thickness values were again observed under LED-P ($24.58 \pm 5.65 \mu\text{m}$) and LED-B ($39.30 \pm 10.24 \mu\text{m}$).

The leaf epidermis of *Sinningia rupicola* exhibits anomocytic stomata, restricted to the abaxial surface (Fig. 7). The surface is covered with a dense layer of trichomes, including both glandular and tector trichomes, distributed on both leaf surfaces.

Spectral quality significantly influenced the leaf stomatal parameters of *Sinningia rupicola* (Fig. 8). Stomatal density was highest under LED-R (351.40 stomata mm⁻²) and LED-B (347.27 stomata mm⁻²), with no statistical difference between these treatments. LED-W showed an intermediate

value (276.98 stomata mm⁻²), while LED-P resulted in the lowest stomatal density (175.01 stomata mm⁻²).

Regarding stomatal size, the largest values were observed under LED-B (30.1 μm) and LED-R (29.9 μm), with a significant reduction in LED-W (25.5 μm) and, most notably, in LED-P (21.5 μm). This pattern was also reflected in the stomatal index, which was highest in LED-B (21.57%), followed by LED-R (19.41%) and LED-W (18.63%). The lowest stomatal index was observed in LED-P (8.32%), consistent with the low stomatal density and size recorded in this treatment. Conversely, epidermal cell density was significantly higher under LED-P (1852.09 cells mm⁻²), exceeding the values observed in LED-R (1468.16 cells mm⁻²), LED-B (1284.89 cells mm⁻²), and LED-W (1155.63 cells mm⁻²).

Based on the evaluated characteristics, variations in the developmental patterns of *Sinningia rupicola* explants grown under different spectral qualities can be observed in the multiplication and elongation/rooting stages, after 45 d of cultivation (Fig. 9).

Acclimatization

All treatments produced complete plants that were transferred to the acclimatization stage. Seedlings grown under LED-B, LED-P, and LED-W exhibited 90% survival after 30 d, demonstrating the feasibility of cultivating the species under these spectral qualities. However, seedlings grown under LED-R showed only 5% survival after 30 d of acclimatization.

Discussion

In vitro cultivation has advanced as an efficient strategy for producing seedlings of threatened native species, with spectral quality being one of the main environmental factors affecting plant growth and development due to photoreceptors capable of perceiving and interpreting incident light signals (Paik and Huq 2019). Spectral quality activates different photoreceptors, such as phytochromes, cryptochromes, and phototropins, triggering morphophysiological responses, including shoot emission, stem elongation, leaf expansion, and pigmentation changes, as well as influencing chlorophyll and carotenoid composition in the photosynthetic process (McMahon et al. 1991; Souza et al. 2011, 2024; Wei et al. 2023). Thus, controlling spectral quality via LEDs in the in vitro culture represents a promising approach to optimize seedling production of threatened species, such as *Sinningia rupicola*, contributing to their propagation and conservation.

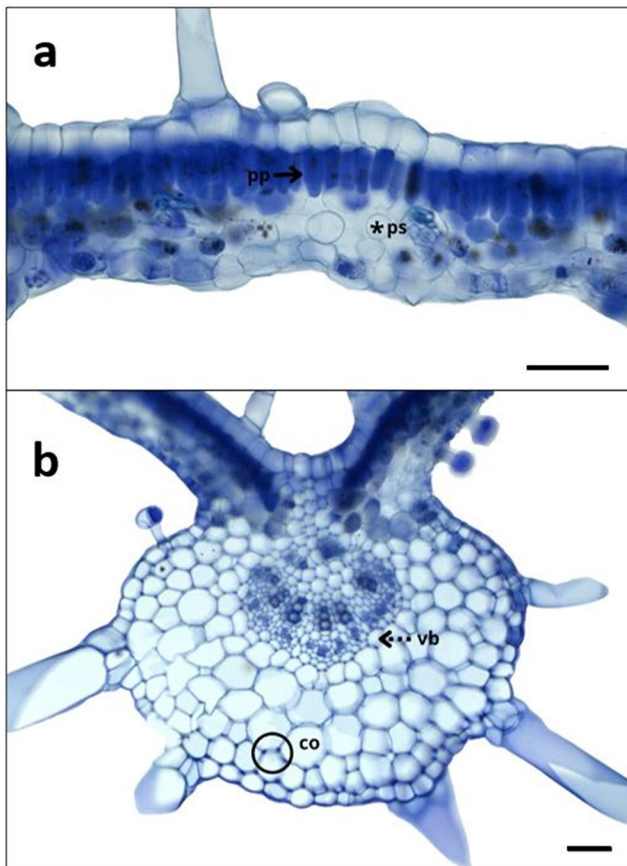


Fig. 5 Transverse leaf sections of *Sinningia rupicola* *in vitro* cultivated under different spectral qualities, after 45 d in the elongation/rooting stage. **(a)** LED-B; **(b)** LED-R; **(c)** LED-P; **(d)** LED-W. In the mesophyll region, the palisade parenchyma (pp), arranged in elongated rows beneath the adaxial epidermis (ade), and the spongy parenchyma (sp), stand out. Stomata (st) are observed in the abaxial epidermis (abe). In the midrib region, the collateral vascular bundle (vb) and the collenchyma tissue (co) located in the periphery are observed. Scale bars = 100 µm

The absence of tissue oxidation across treatments indicates that spectral quality did not impose oxidative stress under the tested conditions. According to Dutta Gupta and Sahoo (2015), spectral quality can influence tissue oxidation by regulating the biosynthesis of secondary metabolites responsible for oxidative processes. Therefore, the results of this study suggest that tissue oxidation in *Sinningia rupicola* may be more related to other factors, such as the species' capacity to respond to oxidative stress, rather than spectral quality.

Shoot vigor was classified as good under red LED and optimal under the other spectral qualities in both the multiplication and elongation/rooting stages. Similar results were reported for *Cattleya crispata*, where plants grown under red light exhibited lower vigor than those cultivated under white LED light (Souza et al. 2021), highlighting that responses are species-specific. In contrast, for *Alstroemeria*

plantaginea, multiplication under LED-R produced more vigorous plants compared to LED-W and LED-B (Leite et al. 2025c).

However, the number of shoots did not differ statistically between treatments, although LED-P and LED-W showed the highest means, yet lower than those reported by Mendes et al. (2026) when cultivating the species under white light using 250 mL flasks with 40 mL of MS medium in the multiplication and elongation. These findings suggest that shoot induction in *Sinningia rupicola* may be influenced more by factors other than spectral quality. Considering that multiplication success depends on the formation of new shoots with adequate vigor and free from contamination (Abiri et al. 2020), using larger flasks combined with LED-W or LED-P spectral quality could be a more effective alternative for *in vitro* multiplication of the species.

Shoot length was significantly greater under LED-R in the multiplication (4.1 cm) and elongation/rooting (3.9 cm). These results align with Chen et al. (2021), who observed shoot elongation and reduced leaf area in *Solanum tuberosum* under red light. This may be related to phytochromes activated by red light, triggering physiological responses similar to shade conditions, inducing auxin and gibberellin biosynthesis, which promote cell elongation and division, resulting in stem and petiole elongation, reduced leaf mass, and lower chlorophyll content (Casal 2013; Wei et al. 2023). Moreover, these results indicate the potential of integrating multiplication and elongation/rooting stages, as shoot elongation occurred in the multiplication stage.

Plants grown under low irradiance also exhibited smaller leaves and elongated petioles towards the light source (Eckstein et al. 2011), characterizing etiolation. In this study, LED-R and LED-P were the treatments with the lowest irradiance; however, LED-P seedlings did not exhibit etiolation, unlike LED-R. This may be explained by the presence of blue light in LED-P, which is known to prevent morphological disorders (Batista et al. 2018). Therefore, shoot vigor should be considered in addition to shoot length.

Regarding leaf number, in the multiplication, LED-W produced the highest number of leaves. Increased leaf production under white light has also been reported for *Alpinia purpurata* (Pinheiro et al. 2019) and *Solanum tuberosum* (Xu et al. 2018), indicating that white light, by providing multiple wavelengths, stimulates metabolic pathways essential for both photosynthesis and morphogenic development. For leaf area, LED-B produced the largest averages in both multiplication and elongation/rooting stages. This suggests that blue spectral quality positively influenced leaf expansion in *Sinningia rupicola*. As discussed by Batista et al. (2018), blue light is important for proper leaf development, preventing morphological disorders and maintaining stomatal functionality. Similar structural effects on leaves

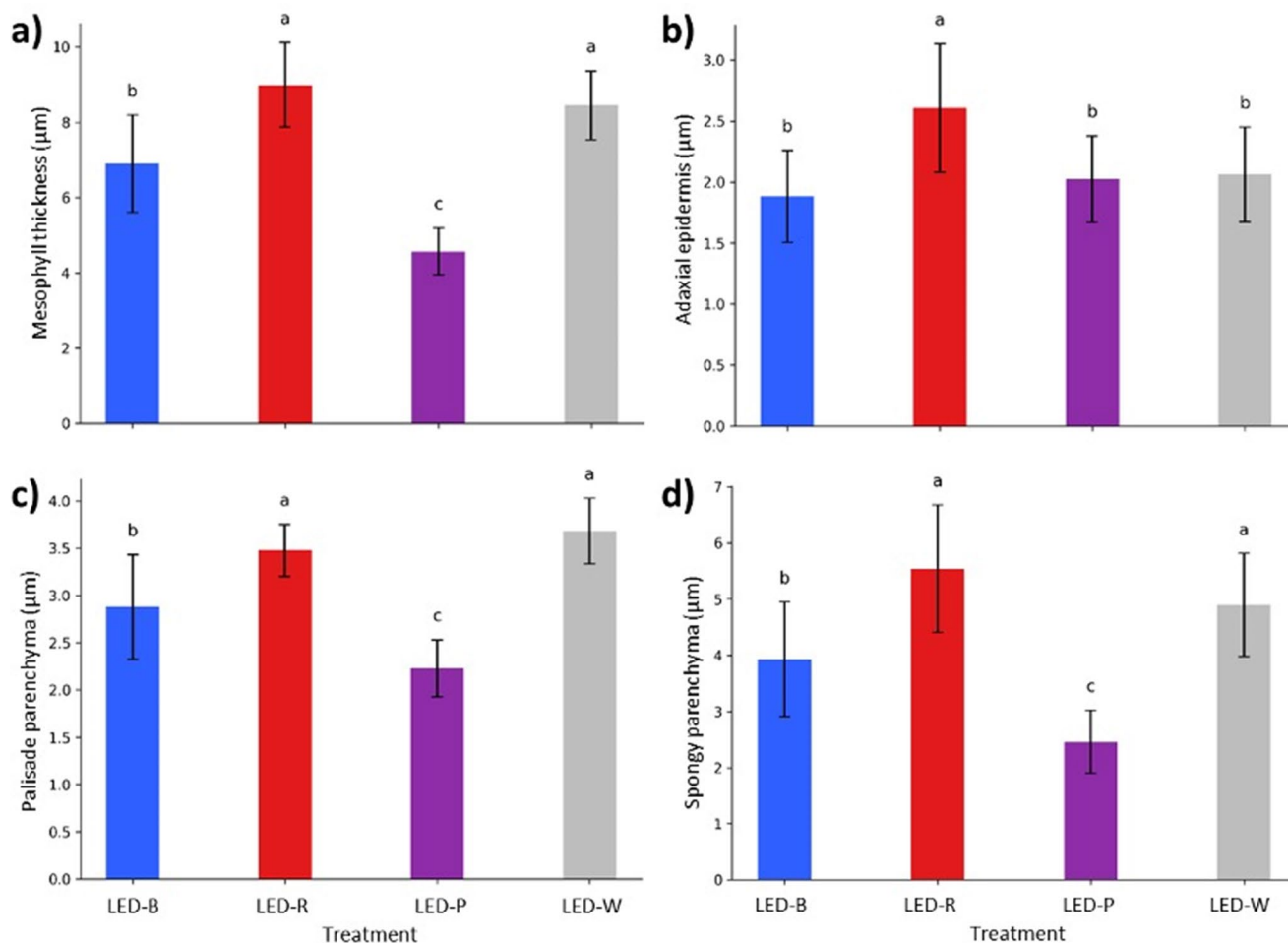


Fig. 6 Anatomical characteristics of the leaf mesophyll of *Sinningia rupicola* *in vitro* grown under different spectral qualities [LED-B, LED-R, LED-P, and LED-W], after 45 d of elongation/rooting. (a) Mesophyll thickness (μm); (b) Adaxial epidermis (μm); (c) Palisade

parenchyma (μm); (d) Spongy parenchyma (μm). Means followed by the same letter do not differ significantly according to Tukey's test at a 5% significance level. Error bars represent the standard error of the mean

have been observed in *Sinningia speciosa*, *Ficus benjamina*, and *Cordyline australis* (Zheng and Van Labeke 2017), reinforcing the role of blue light in leaf area formation across species.

In vitro cultivation stages of multiplication, elongation, and rooting have distinct objectives, often due to the specific use of plant growth regulators, resulting in differentiated physiological responses. However, some species allow simultaneous occurrence of these stages, as reported by Mendes et al. (2026) for *Sinningia rupicola*, where intense shoot multiplication occurred in the elongation. In this study, in the elongation/rooting, LED-P and LED-W produced the highest number of roots, but root development occurred under all treatments, similar to multiplication. These results are consistent with Leite et al. (2025b), who observed roots in both multiplication and elongation/rooting of *Paliavana sericiflora*, a species in the same family. Additionally, secondary root formation in the multiplication reached 90.9%

under LED-P and LED-R. In the elongation/rooting, there was no significant difference among treatments for this variable, yet all treatments produced seedlings suitable for acclimatization. These results indicate the possibility of merging stages in the *in vitro* cultivation, potentially reducing time and costs for seedling production.

Photosynthetic pigments are essential for assessing plant morphogenesis, as they reflect the functionality of the photosynthetic apparatus (Esteban et al. 2014). LED-P produced the highest concentrations of chlorophyll *a*, total chlorophyll (*a* + *b*), and carotenoids in the multiplication. In the elongation/rooting, LED-P also yielded the highest concentrations of chlorophyll *a*, *b*, and total (*a* + *b*), while LED-W produced the lowest. Similar outcomes were observed in *Solanum tuberosum*, where a blue–red light combination resulted in the highest chlorophyll and carotenoid levels and promoted vegetative growth (Chen et al. 2021). In *Sinningia speciosa*, white light led to the lowest chlorophyll

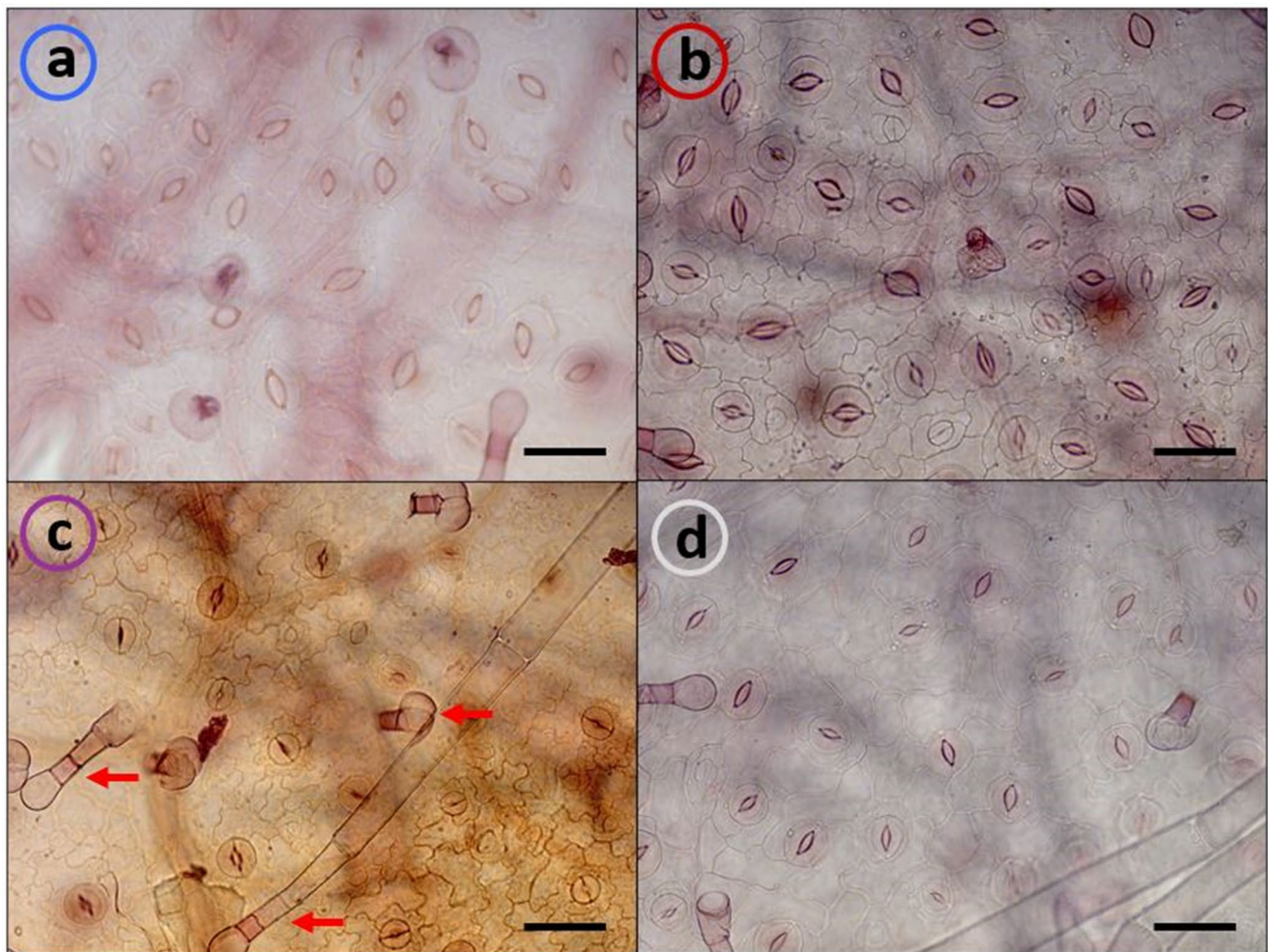


Fig. 7 Photomicrographs of the abaxial epidermis of *Sinningia rupicola* leaves in vitro grown under different spectral qualities, after 45 d in the elongation/rooting stage. (a) LED-B; (b) LED-R; (c) LED-P, showing trichome details; (d) LED-W. Scale bars = 50 μ m

concentrations (Zheng and Van Labeke 2017). Chlorophyll absorption is maximized in the blue (400–500 nm) and red (600–700 nm) spectra, which also activate essential metabolic pathways for synthesis and accumulation (Chen et al. 2021).

In vitro environmental conditions can alter stomatal characteristics, which are critical for successful acclimatization (Asayesh et al. 2017). In this study, spectral quality influenced stomatal distribution and epidermal cell density. LED-B and LED-R promoted higher stomatal densities, while LED-P produced lower stomatal density and higher epidermal cell density, possibly reflecting an adaptive anatomical strategy for water conservation (Shimazaki et al. 2007). Similar anatomical responses have been reported in related species. In *Sinningia speciosa*, although total leaf thickness did not change with spectral quality, specific adjustments occurred in epidermal and palisade parenchyma thickness, particularly under blue and combined red–blue light. These structural adjustments optimize

light interception and redistribution within the leaf, enhancing chloroplast absorption and photosynthetic efficiency without increasing total leaf thickness (Zheng and Van Labeke 2017). This suggests coordinated anatomical rearrangements between epidermis, stomata, and mesophyll in *Sinningia* species, reflecting morphofunctional strategies adapted to light environments.

Regarding leaf anatomy, under LED-B, *Sinningia rupicola* exhibited larger leaf area and intermediate chlorophyll content, consistent with blue-light photoreceptor activation (cryptochromes and phototropins), which modulate photomorphogenesis, stimulate stomatal opening, and induce pigment biosynthesis (Batista et al. 2018; Cavallaro et al. 2022). While LED-B typically thickens mesophyll (Shafiq et al. 2021; Zheng and Van Labeke 2017), this species showed thicker leaves under LED-R and LED-W, suggesting functional limitations, as photosynthetic efficiency depends on the interaction between pigment content and leaf architecture (Favarin et al. 2002). Although mesophyll

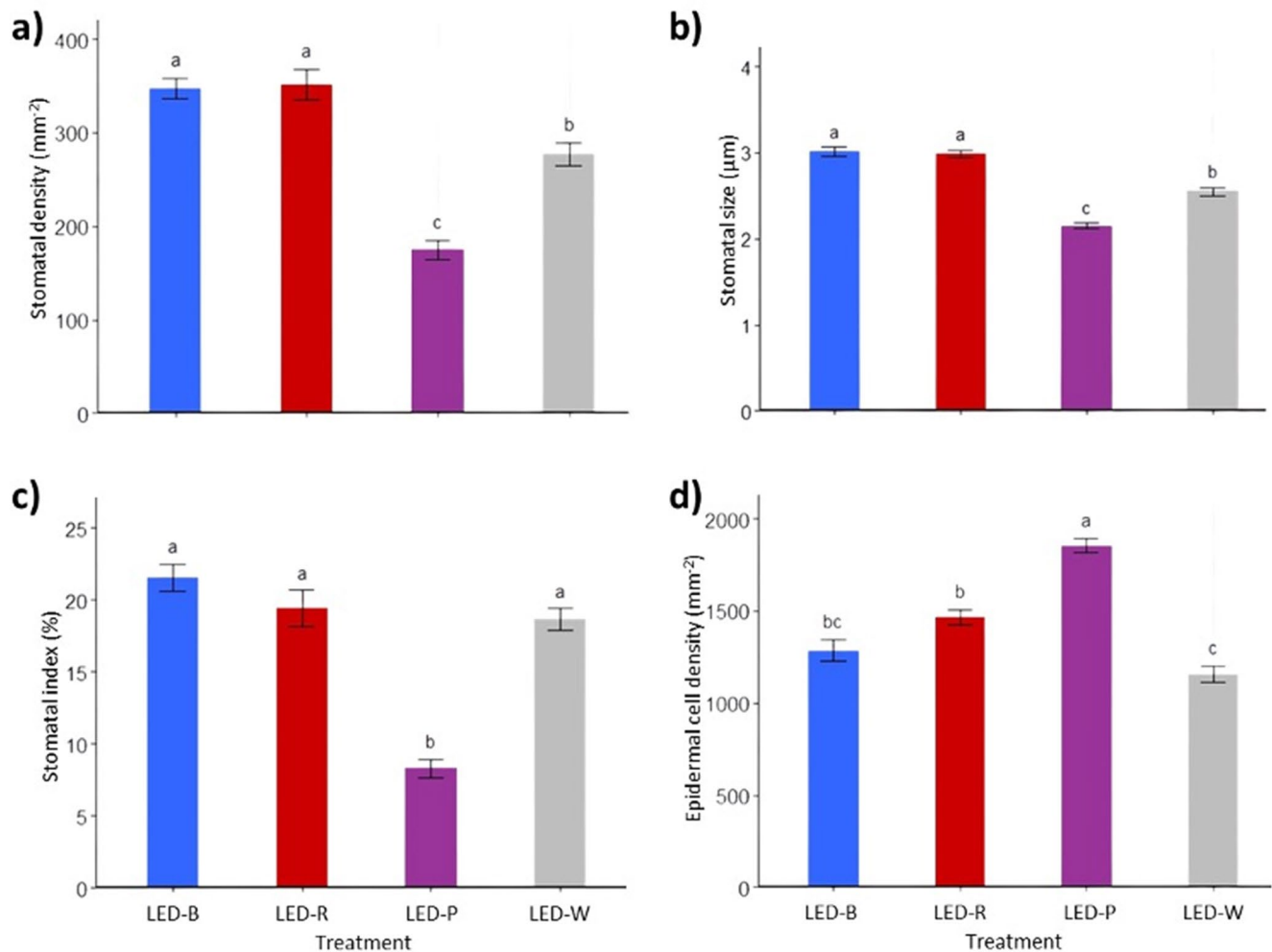


Fig. 8 Epidermal characteristics of *Sinningia rupicola* leaves in vitro grown under different spectral qualities (LED-B, LED-R, LED-P, and LED-W), after 45 d of elongation/rooting. **(a)** Stomatal density (stomata mm⁻²); **(b)** Stomatal size (μm); **(c)** Stomatal index (%); **(d)** Epi-

dermal cell density (cells mm⁻²). Means followed by the same letter do not differ significantly according to Tukey's test at a 5% significance level. Error bars represent the standard error of the mean

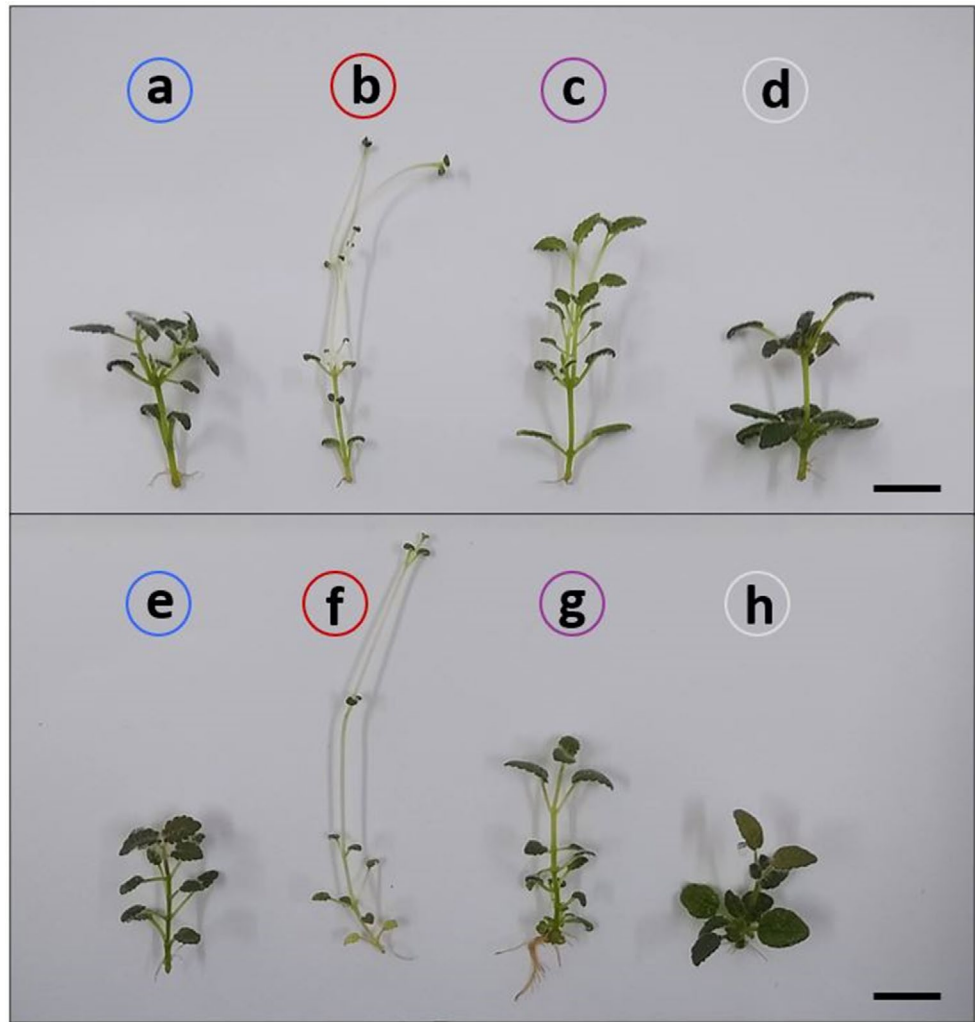
thickness was greatest under LED-R, leaf area was reduced. This increased thickness, particularly in palisade parenchyma, may compensate for smaller leaf area by increasing chloroplast density per unit area, maintaining average chlorophyll content (Zheng and Van Labeke 2017). This reflects a morphophysiological adjustment where the plant prioritizes tissue thickening over leaf expansion to optimize carbon assimilation (Favarin et al. 2002; Viana and Lombardi 2007).

Under LED-W, despite greater mesophyll thickness, chlorophyll content was lowest, likely due to broad spectral composition with peak blue emission combined with high green fraction, which is less efficiently absorbed at low light intensities (Liu and Van Iersel 2021). The combination of blue and red light generally produces more balanced responses (Paradiso and Proietti 2021). Under LED-P, although leaves were thinner, stomatal parameters were

lower, which can enhance water-use efficiency, particularly relevant in the species' natural habitat (Dubey et al. 2023; Petřík et al. 2024).

In vitro environmental conditions can induce morphophysiological changes that affect acclimatization (Asayesh et al. 2017). LED-B, LED-P, and LED-W produced 90% survival, whereas LED-R produced only 5%. Although LED-R promoted greater shoot elongation, this result should not be interpreted as indicative of improved plantlet quality. Plants developed under this condition exhibited reduced vigor, symptoms of etiolation, smaller leaf area, and higher stomatal density traits associated with poorer performance during acclimatization and increased senescence. This senescence may, at least in part, reflect an intrinsic characteristic of the species, as similarly reported for *Gymnopogon doellii* from the same environment (Lopes et al. 2024). Thus, the increased shoot length under LED-R

Fig. 9 *Sinningia rupicola* explants in vitro grown under different spectral qualities after 45 d. (a–b) Multiplication stage; (e–h) Elongation/Rooting stages. (a) and (e)=LED-B; (b) and (f)=LED-R; (c) and (g)=LED-P; (d) and (h)=LED-W. Scale bars=1.0 cm



reflects a morphological response that is not necessarily favorable, indicating that this light condition may not be suitable for the species.

Overall, in the multiplication, LED-P produced vigorous shoots with efficient photosynthesis and roots with secondary roots in 90.9% of samples, achieving a balance between shoot and root growth. LED-B produced larger leaf area with less root development, while LED-W induced many leaves but lower photosynthetic pigment content, indicating lower photosynthetic efficiency.

In the elongation/rooting, LED-W resulted in vigorous plants with robust roots and thicker mesophyll and palisade parenchyma but reduced photosynthetic efficiency. LED-B produced larger leaf area but higher stomatal density and fewer roots, potentially impairing water retention and adaptation in natural habitats. Under LED-P, thinner leaves were observed, yet chlorophyll content was high, stomatal density and size were lower, and the root system was robust, reflecting balanced growth and improved water regulation critical for species of the Rupestrian Grassland.

Thus, spectral quality can be optimized to improve in vitro multiplication and elongation/rooting stages for *Sinningia rupicola*, enabling high-quality seedling production in 225 d, supporting conservation and habitat restoration projects.

Conclusions

Different spectral qualities modulate the morphophysiology of *Sinningia rupicola* seedlings grown in vitro. LED-P spectral quality yielded superior results in the both multiplication and elongation/rooting stages by promoting balanced shoot growth, integrating aerial vigor with a robust root system. Seedlings cultivated under LED-P, LED-B, and LED-W showed 90% survival in acclimatization, in contrast to the poor performance under LED-R. The use of LED-P is recommended for optimizing the micropropagation of *Sinningia rupicola*, enabling the production of high-quality plantlets that can be applied in large-scale propagation and may contribute to the conservation of the species.

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Author contributions FMM: Investigation and formal analysis, writing - original draft. DML: Investigation and formal analysis, review. KIRS: Investigation, writing, review and editing. CMLA: Investigation, formal analysis, writing, review and editing. DPM: Formal analysis. GEB Supervisor, was responsible for the obtention of funds and the administration of project, review, discussion and editing. All authors revised the manuscript and approved the final version.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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