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Research Article

Effects of LED light quality on leaf anatomy and photosynthetic efficiency of *in vitro*-grown *Genipa americana*[1]

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ABSTRACT

The viability loss of *Genipa americana* seeds highlights the need for alternative propagation methods. In this context, *in vitro* culture and light manipulation can stimulate the production of higher-quality and more efficient seedlings, achieving large-scale propagation with higher yield and year-round production. This study aimed to establish the *in vitro* cultivation of *G. americana* under LED light. The seedlings were maintained under four spectral ranges: white (400-700 nm), blue (400-490 nm), red (600-700 nm), and blue + red (2:3). Leaf anatomical, biometric, and photosynthetic characteristics were evaluated. The blue + red treatment promoted the greatest palisade parenchyma thickness and total leaf thickness, differing significantly from all the other treatments. The white light resulted in higher photosynthetic pigment contents and promoted greater stomatal conductance. The blue light alone showed the lowest root length and photosynthetic pigment levels, whereas the red light alone represented an intermediate condition. The principal component analysis explained 88.7 % of the total variance, with vegetative growth and leaf thickening loading in opposite directions. The blue + red light favors leaf thickening, whereas the white light favors moderate leaf expansion, gas exchange, and pigment accumulation without promoting excessive leaf thickening.

KEYWORDS: Genipap, Brazilian Savanna, plant tissue culture, photomorphogenesis.

RESUMO

Efeitos da qualidade da luz LED sobre a anatomia foliar e a eficiência fotossintética de *Genipa americana* cultivada *in vitro*

A perda da viabilidade de sementes de *Genipa americana* ressalta a necessidade de métodos alternativos de propagação. Nesse contexto, o cultivo *in vitro* e a manipulação da luz podem favorecer a produção de mudas de maior qualidade e eficiência, possibilitando a propagação em larga escala, com maior rendimento e produção ao longo de todo o ano. Objetivou-se estabelecer o cultivo *in vitro* de *G. americana* sob luz LED. As plântulas foram mantidas sob quatro faixas espectrais: branca (400-700 nm), azul (400-490 nm), vermelha (600-700 nm) e azul + vermelha (2:3). Foram avaliadas características anatômicas foliares, biométricas e fotossintéticas. O tratamento com luz azul + vermelha promoveu a maior espessura do parênquima paliçádico e a maior espessura total da folha, diferindo significativamente de todos os demais tratamentos. A luz branca resultou em maiores teores de pigmentos fotossintéticos e promoveu maior condutância estomática. A luz azul isolada apresentou os menores valores de comprimento radicular e de pigmentos fotossintéticos, enquanto a luz vermelha isolada caracterizou uma condição intermediária. A análise de componentes principais explicou 88,7 % da variância total, com o crescimento vegetativo e o espessamento foliar apresentando cargas em direções opostas. A luz azul + vermelha favorece o espessamento foliar, enquanto a luz branca favorece a expansão foliar moderada, as trocas gasosas e o acúmulo de pigmentos, sem promover espessamento foliar excessivo.

PALAVRAS-CHAVE: Jenipapo, Cerrado, cultura de tecidos vegetais, fotomorfogênese.

INTRODUCTION

A member of the Rubiaceae family, *Genipa americana*, commonly known as genipap, is a native fruit-bearing species widely distributed throughout the Brazilian territory, occurring in the Caatinga, Pantanal, Cerrado, Atlantic Forest, and Amazon biomes (Ruzza et al. 2020, Silva et al. 2020).

G. americana is recognized by farmers and traditional communities for its grayish stem and elongated dark green leaves, as well as its ecological importance in feeding wildlife and restoring degraded

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areas, and its cultural and economic significance for local and indigenous populations (Ruzza et al. 2020). Its fruits are sold in farmers' markets and public markets, generating income for family farmers and boosting the regional economy (Silva et al. 2020). The species has been identified as a priority for biodiversity conservation and silvicultural management, although knowledge gaps remain in ecology, genetics, and forest management.

G. americana seeds are classified as intermediate, in relation to storage, exhibiting moderate tolerance to desiccation, with a critical moisture content between 9 and 6 % (Salomão 2004). However, they are sensitive to low temperatures, as exposure to -20 ºC drastically reduces germination, which rules out their conservation using conventional seed bank methods (Salomão 2004). Consequently, seeds must be sown shortly after dispersal, as they lose viability within approximately 60 days, imposing restrictions on large-scale production throughout the year (Cardoso et al. 2022).

Silica gel desiccation followed by cryopreservation has shown promising results for maintaining seed viability, although the approach still presents significant limitations, with germination rates reaching only 35 % after 20 hours of desiccation and subsequent cryopreservation (Souza et al. 2023). Given this scenario, further research is needed to explore the species' potential contribution to biodiversity and its sustainable use in agroecosystems (Sousa-Santos et al. 2024). In this context, it is necessary to develop integrated conservation and large-scale propagation strategies for *G. americana* using biotechnological approaches such as *in vitro* cultivation, which overcome the difficulties of traditional propagation methods and allows large-scale plant multiplication and the use of conservation technologies (Souza et al. 2019).

In the case of *G. americana*, studies on its *in vitro* establishment and multiplication remain limited and face challenges related to the absence of efficient, standardized protocols, limited exploration of growth regulators and genotypes, lack of histological confirmation of regeneration processes, low rooting and *ex vitro* acclimatization efficiency, and a scarcity of studies integrating advanced physiological and biotechnological analyses (Rocha et al. 2008, Lédo et al. 2015, Sá et al. 2016, Souza et al. 2016, Oliveira et al. 2017, Nascimento et al. 2024). Therefore, the need to optimize specific culture protocols that favor its large-scale propagation is reinforced, contributing to its conservation and biotechnological use.

In this context, the use of light-emitting diodes (LEDs) in controlled environments has stood out as an efficient and economically viable alternative due to their specific spectral peaks favorable for plant growth, high luminous efficiency, minimal heat generation, long lifespan, absence of heavy metals, and low energy consumption. These features improve the cost-effectiveness of *in vitro* plant propagation (Scolaro et al. 2025). Different LED wavelengths and light intensities influence growth, photosynthetic pigment accumulation, phytochemical content, and antioxidant activity (Coelho et al. 2021, Djangalina et al. 2023, Nacheva et al. 2023, Pang et al. 2023).

These variations in the influence of light wavelength on plant physiology highlight the importance of investigating how different LED light spectra affect the *in vitro* growth and propagation of *G. americana*. Such investigations can contribute to the development of optimized *in vitro* protocols for both commercial and conservation purposes.

The present study aimed to evaluate how LED light qualities influence the growth and anatomical, biometric, and physiological development of *in vitro*-grown *G. americana*.

MATERIAL AND METHODS

The study was conducted with fruits collected in December 2025 from the ground at the Instituto Federal Goiano, in Rio Verde, Goiás state, Brazil (17º48'06.1"S; 50º54'25.7"W), with a voucher specimen (nº 2508) registered in the herbarium of the institution.

To obtain seeds, mature fruits were depulped. Subsequently, the seeds were submerged in 70 % ethanol for 1 min, followed by 10 % sodium hypochlorite (NaOCl) for 20 min for surface disinfestation. The seeds were rinsed three consecutive times with autoclaved osmolyzed water in a laminar flow hood and inoculated into flasks containing 50 mL of Murashige & Skoog (MS) medium with 50 % salt concentration and 15 g L^{-1} of sucrose, with the pH adjusted to 5.7 ± 0.3 before the addition of 4.0 g L^{-1} of agar and subsequent autoclaving (Souza et al. 2016). The flasks were inoculated, capped, and sealed with PVC film in a laminar flow hood. After inoculation, they were kept in a growth room at

25 ± 2 ºC in the dark. After 15 days, they were kept under a 16/8-hour (light/dark) photoperiod.

After germination, *G. americana* plantlets were subcultured into flasks (25 × 150 mm) containing 50 mL of 50 % MS medium with 30 g L^{-1} of sucrose, with pH adjusted to 5.7 ± 0.3. The flasks were maintained under a 16-h light/8-h dark photoperiod with four spectral ranges: white (400-700 nm), blue (400-490 nm), red (600-700 nm), and blue/red (2:3), provided by 20 W LED tubes (Lanao series Tubes, China) installed on a metal structure (1.10 × 0.90 m), with a PPFD of 150 µmol m^{-2} s^{-1} at a distance of 20 cm between the lamp and the plantlets. The LED spectral curve was measured under the experimental conditions (Figure 1), with an average temperature of 26 ± 3 ºC. At 30 days, the plantlets were transferred and subcultured onto the same medium. The experimental unit was a flask containing a single plantlet. Flasks were randomly assigned to the light treatments and kept in the same position throughout the experiment.

At 60 days, evaluations were performed for leaf anatomy (tissue thicknesses), photosynthetic parameters (pigments and gas exchange), and biometric traits (shoot length, root length, total length, leaf area, number of leaves, and number of nodes) to identify the effects of lighting on the *in vitro* growth process, using the ImageJ® software. The experimental design was completely randomized, with four treatments of distinct spectral conditions, each with ten replicates. Each replicate consisted of one flask containing one plantlet.

Pigment extraction was performed according to Lichtenthaler & Wellburn (1983), with modifications. Leaf tissue samples (50 mg) were incubated overnight in the dark in tubes containing 4 mL of 80 % ethanol. After incubation, they were filtered, and the absorbance of the resulting supernatant was measured

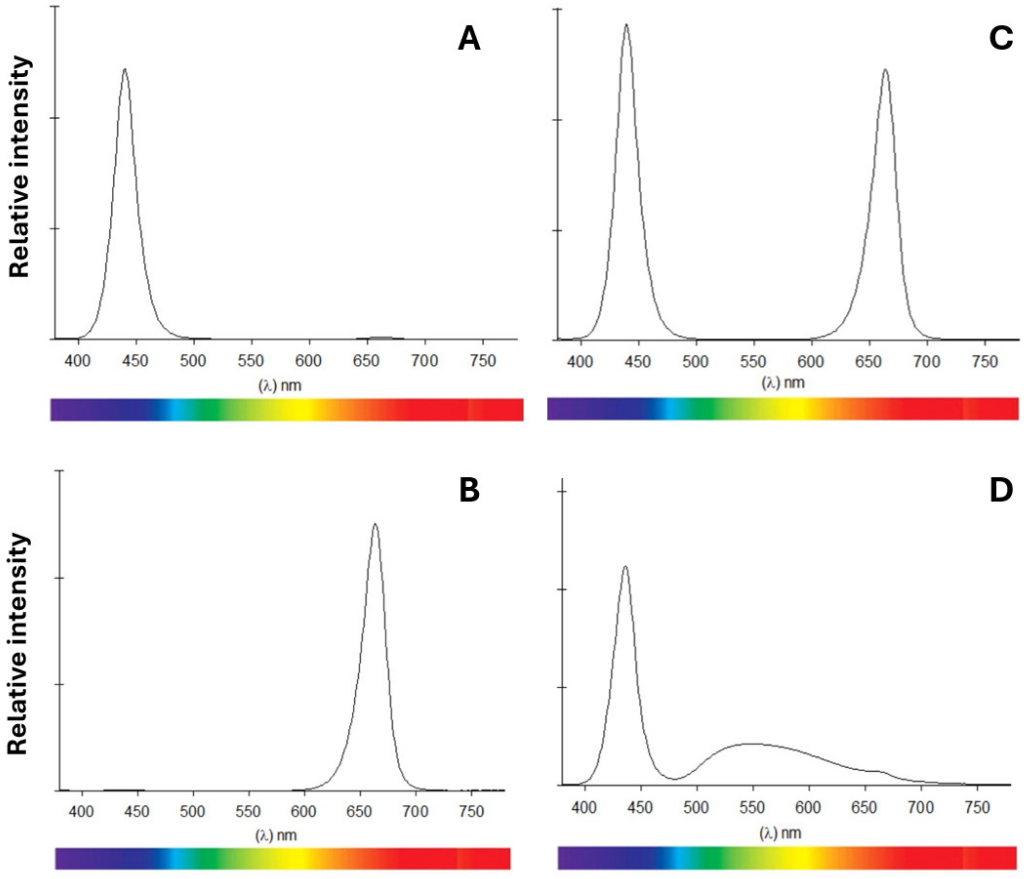


Figure 1. Relative spectral distributions of the LED light treatments used during the *in vitro* cultivation of *Genipa americana*: A) blue light, with an emission peak at 441 nm; B) red light, at 664 nm; C) combined blue and red light in a 2:3 ratio, with emission peaks at 440 and 664 nm, respectively; D) white light, characterized by a broad emission spectrum across the visible region. The curves represent relative light intensity as a function of wavelength (λ; nm).

using a spectrophotometer at wavelengths of 470, 647, and 663 nm. The concentrations of chlorophyll *a* (*C*a), chlorophyll *b* (Cb), total chlorophyll (Tc), and carotenoids were calculated using the following equations: *C*a = [(12.25 x A663) - (2.79 x A647)] x V; Cb = [(21.50 x A647) - (5.10 x A663)] x V; Tc = [20.29 A647 + 8.02 A663] x V; and carotenoids = [(1,000 A470 - 1.82 *C*a - 95.15 Cb) x V]/225; where *A* represents the absorbance of the extract at the specified wavelength, and *V* the final volume of the ethanolic extract (mL). Pigment concentrations were expressed as μg mL^{-1} of extract.

Gas exchange was evaluated to record the net photosynthetic rate (*A*; μmol CO_2 m^{-2} s^{-1}), transpiration rate (*E*; mmol H_2O m^{-2} s^{-1}), stomatal conductance (*gs*; mol H_2O m^{-2} s^{-1}), internal CO_2 concentration (*Ci*), and ambient CO_2 concentration (Ca) in fully expanded leaves. From these data, instantaneous carboxylation efficiency (*A*/*Ci)* and water-use efficiency (*A*/*E*) were calculated. These evaluations were performed using an infrared gas analyzer (LI-6800, Licor®, Lincoln, Nebraska, USA) under constant photosynthetically active radiation (PAR) (1,200 μmol photons m^{-2} s^{-1}), ambient CO_2 concentration (Ca) (~400 μmol mol^{-1}), temperature (~25 ºC), and humidity (~50 %). The LI-6800 IRGA chamber has a fixed area of 2 x 3 cm. Because the evaluated leaves were small and originated from *in vitro* plants, they did not always fill this entire area. To correct for this, each leaf was photographed at the time of measurement, and its actual area was determined using the ImageJ software. This value (in cm^2) was then used to correct the raw data exported from the IRGA spreadsheet, replacing the standard chamber area (2 x 3 cm) automatically assumed by the equipment, ensuring that the final gas exchange values reflected the leaf area effectively exposed to radiation and airflow within the chamber. Readings were taken one hour after the photoperiod began, allowing up to 120 seconds for stabilization, with one reading per replicate. The experimental design was completely randomized, with four treatments of distinct spectral conditions, each with five replicates. Each replicate consisted of one flask containing one plantlet.

Leaf samples were collected from plantlets grown under different light treatments and fixed in 70 % formaldehyde, acetic acid, and ethanol solution for 24 hours, a fundamental procedure to preserve cell integrity and prevent tissue degradation during processing. Subsequently, the samples were subjected to an increasing ethanol series (70, 80, 90, and 100 %) for tissue dehydration (Johansen 1940). After this process, they were transferred to an infiltration solution (Leica®, Germany) for 24 hours, to ensure the complete penetration of the embedding medium and removal of water from the tissues. The samples were then embedded in resin blocks for subsequent sectioning using a microtome. Sections were stained with 0.05 % toluidine blue in 0.1 M phosphate buffer, pH 6.8 (O'Brien et al. 1964), and the obtained sections were analyzed using photomicrographs, with observations focused on internal plant tissue anatomy, evaluating the leaf blade under different light treatments. The experimental design was completely randomized, with four treatments of distinct spectral conditions, each consisting of three replicates. Each replicate consisted of two leaf fragments from one flask containing one plantlet.

Data were checked for compliance with the assumptions of analysis of variance (Anova). Subsequently, they were subjected to Anova, and, when significant differences were detected, means were compared using the Tukey test ($\alpha = 0.05$), with the aid of the R software version 4.0.4. Tables containing means, standard errors, p-values, and number of replicates for all variables are presented in Table 1.

RESULTS AND DISCUSSION

Shoot length was significantly affected by light spectral quality ($p = 0.027$). The highest values were observed under red (4.64 cm) and white (4.62 cm) light, both of which were greater than under blue + red light (3.06 cm). The blue light (4.50 cm) did not differ from the other treatments (Figure 2A).

Root length was also affected by spectral quality ($p < 0.001$). The white (0.90 cm) and blue + red (0.86 cm) light promoted the greatest root development, significantly surpassing the red (0.32 cm) and blue (0.14 cm) light, which did not differ from each other (Figure 2B). Total length followed a similar trend ($p = 0.046$), with the white light (5.52 cm) resulting in greater total length than the blue + red light (3.92 cm). The red (4.96 cm) and blue (4.64 cm) light showed intermediate values that did not differ from either extreme (Figure 2C).

Leaf area also responded to spectral quality ($p = 0.009$). The white (1.78 cm^2) and blue (1.55 cm^2) light

Table 1. Means, standard error, p-values, and number of replicates for all variables evaluated in *Genipa americana* seedlings under LED light spectral qualities (blue, red, blue + red, and white).

Variable (unit)	Blue	Red	Blue + red	White	p-value	n
Growth (biometric) parameters						
Shoot length (cm)	4.500 ± 0.385 ab	4.640 ± 0.385 a	3.060 ± 0.385 b	4.620 ± 0.385 a	0.0275*	5
Root length (cm)	0.1400 ± 0.0846 b	0.3200 ± 0.0846 b	0.8600 ± 0.0846 a	0.9000 ± 0.0846 a	< 0.0001*	5
Total length (cm)	4.640 ± 0.365 ab	4.960 ± 0.365 ab	3.920 ± 0.365 b	5.520 ± 0.365 a	0.0456*	5
Leaf area (cm²)	1.546 ± 0.189 a	1.222 ± 0.189 ab	0.7675 ± 0.1890 b	1.780 ± 0.189 a	0.0094*	5
Number of leaves (count)	6.800 ± 0.745 a	5.800 ± 0.745 a	7.000 ± 0.745 a	5.800 ± 0.745 a	0.5442	5
Number of nodes (count)	8.200 ± 0.648 a	7.600 ± 0.648 ab	5.200 ± 0.648 b	5.800 ± 0.648 ab	0.0137*	5
Photosynthetic pigments						
Chlorophyll *a* (μg mL^{-1})	31.37 ± 7.05 c	72.48 ± 7.05 b	73.18 ± 7.05 b	118.06 ± 7.05 a	0.0002*	3
Chlorophyll *b* (μg mL^{-1})	12.92 ± 3.87 b	25.64 ± 3.87 b	26.54 ± 3.87 b	47.71 ± 3.87 a	0.0016*	3
Total chlorophyll (μg mL^{-1})	48.79 ± 11.68 c	108.14 ± 11.68 b	109.89 ± 11.68 b	182.63 ± 11.68 a	0.0003*	3
Carotenoids (μg mL^{-1})	35.31 ± 9.35 b	75.22 ± 9.35 b	73.15 ± 9.35 b	128.51 ± 9.35 a	0.0008*	3
Gas exchange						
Stomatal conductance (*gs*; mol H_2O m^{-2} s^{-1})	0.4899 ± 0.0810 c	0.8932 ± 0.0810 b	0.4110 ± 0.0810 c	1.256 ± 0.081 a	< 0.0001*	5
Transpiration rate (*E*; mmol H_2O m^{-2} s^{-1})	7.199 ± 0.980 b	13.30 ± 0.98 a	6.708 ± 0.980 b	17.19 ± 0.98 a	< 0.0001*	5
Net CO_2 assimilation rate (*A*; μmol CO_2 m^{-2} s^{-1})	8.479 ± 0.895 a	10.44 ± 0.90 a	4.516 ± 0.895 b	10.05 ± 0.90 a	0.0009*	5
Intercellular CO_2 concentration (*Ci*; μmol mol^{-1})	380.10 ± 2.93 a	383.47 ± 2.93 a	388.45 ± 2.93 a	390.58 ± 2.93 a	0.0857	5
Leaf anatomy						
Adaxial epidermis thickness (mm)	0.0120 ± 8.78e-4 b	0.0245 ± 8.78e-4 a	0.0230 ± 8.78e-4 a	0.0130 ± 8.78e-4 b	< 0.0001*	3
Abaxial epidermis thickness (mm)	0.00900 ± 9.93e-4 b	0.0135 ± 9.93e-4 a	0.0147 ± 9.93e-4 a	0.0115 ± 9.93e-4 ab	0.0171*	3
Palisade parenchyma thickness (mm)	0.0270 ± 0.0027 b	0.0225 ± 0.0027 b	0.0443 ± 0.0027 a	0.0220 ± 0.0027 b	0.0013*	3
Spongy parenchyma thickness (mm)	0.0553 ± 0.0043 a	0.0490 ± 0.0043 a	0.0440 ± 0.0043 a	0.0540 ± 0.0043 a	0.2981	3
Total leaf thickness (mm)	0.1030 ± 0.0031 b	0.1095 ± 0.0031 b	0.1260 ± 0.0031 a	0.1005 ± 0.0031 b	0.0016*	3

Values are means ± standard error (SE) of the mean, calculated as SE = √(MSE/n), where MSE is the residual mean square from the one-way Anova and n the number of replicates per treatment. Means followed by the same lowercase letter within a row do not differ significantly by the Tukey test ($p < 0.05$). p-values correspond to the one-way Anova F-test for the effect of light treatment (treatment df = 3), and * indicates a significant effect ($p < 0.05$).

provided the largest areas, whereas the blue + red light resulted in the smallest value (0.77 cm²) (Figure 2D). The number of leaves did not respond to light quality ($p = 0.544$), with means ranging from 5.8 to 7.0 and no significant differences among the treatments (Figure 2E). The number of nodes was significantly higher under the blue light (8.2), when compared to the blue + red light (5.2) (Figure 2F).

A previous study has shown that the seed germination of *G. americana* is indifferent to light quality, although continuous darkness yields the best results (Souza et al. 2016). Our results demonstrate that light spectral quality exerts a stronger effect on post-germination development (e.g., shoot and root elongation) than on germination itself, indicating that light quality plays a role in the early seedling morphology of *G. americana*. Furthermore, it has been demonstrated that *G. americana* plantlets exhibit high phenotypic plasticity, as biomass accumulation increases proportionally with light availability (Lima et al. 2010). The differences observed in leaf area and length under different light spectra, and not just light intensity, provide evidence for this plasticity at a more detailed physiological level, demonstrating that specific wavelengths finely tune *G. americana* development at the morphological level.

Light spectral quality significantly influenced the chlorophyll *a* content ($p < 0.001$). The white light resulted in the highest content (118.1 μg mL^{-1}), followed by the blue + red (73.2 μg mL^{-1}) and red (72.5 μg mL^{-1}) light, which did not differ from each other but were significantly higher than the blue light (31.4 μg mL^{-1}) (Figure 3A).

The chlorophyll *b* content showed a similar response ($p = 0.002$). The white light (47.7 μg mL^{-1}) was significantly higher than all the other treatments. The blue + red (26.5 μg ml^{-1}), red (25.6 μg mL^{-1}), and blue (12.9 μg mL^{-1}) light did not differ from each other (Figure 3B).

Total chlorophyll followed the same pattern ($p < 0.001$). The white light resulted in the highest content (182.6 μg mL^{-1}), the blue + red and red light showed intermediate and similar values (109.9 and 108.1 μg mL^{-1}, respectively), and the blue

light produced the lowest content (48.8 µg mL^{-1}) (Figure 3C).

The carotenoid content was also affected by light quality ($p = 0.001$). The white light (128.5 µg mL^{-1}) was superior to all the other treatments, whereas the red (75.2 µg mL^{-1}), blue + red (73.2 µg mL^{-1}), and blue (35.3 µg mL^{-1}) light did not differ from each other (Figure 3D).

The superiority of the white light may be attributed to its balanced spectral composition, which encompasses effective wavelengths in both the blue and red ranges, thereby maximizing photosynthesis and also accessory pigment synthesis. This pattern is consistent with observations in other Rubiaceae species. In *Anthocephalus chinensis*, the light intensity was also found to influence photosynthetic and anatomical traits (Feng et al. 2004, Nacheva et al. 2023).

Furthermore, the high carotenoid accumulation observed under white light aligns with the species' inherent biochemical capacity for producing these compounds. Indeed, *G. americana* fruits are known to contain high levels of carotenoids (Ribeiro et al. 2023), supporting the idea that this species possesses a robust metabolic potential for carotenoid biosynthesis.

The elevated carotenoid content under white light suggests a more pronounced photoprotective

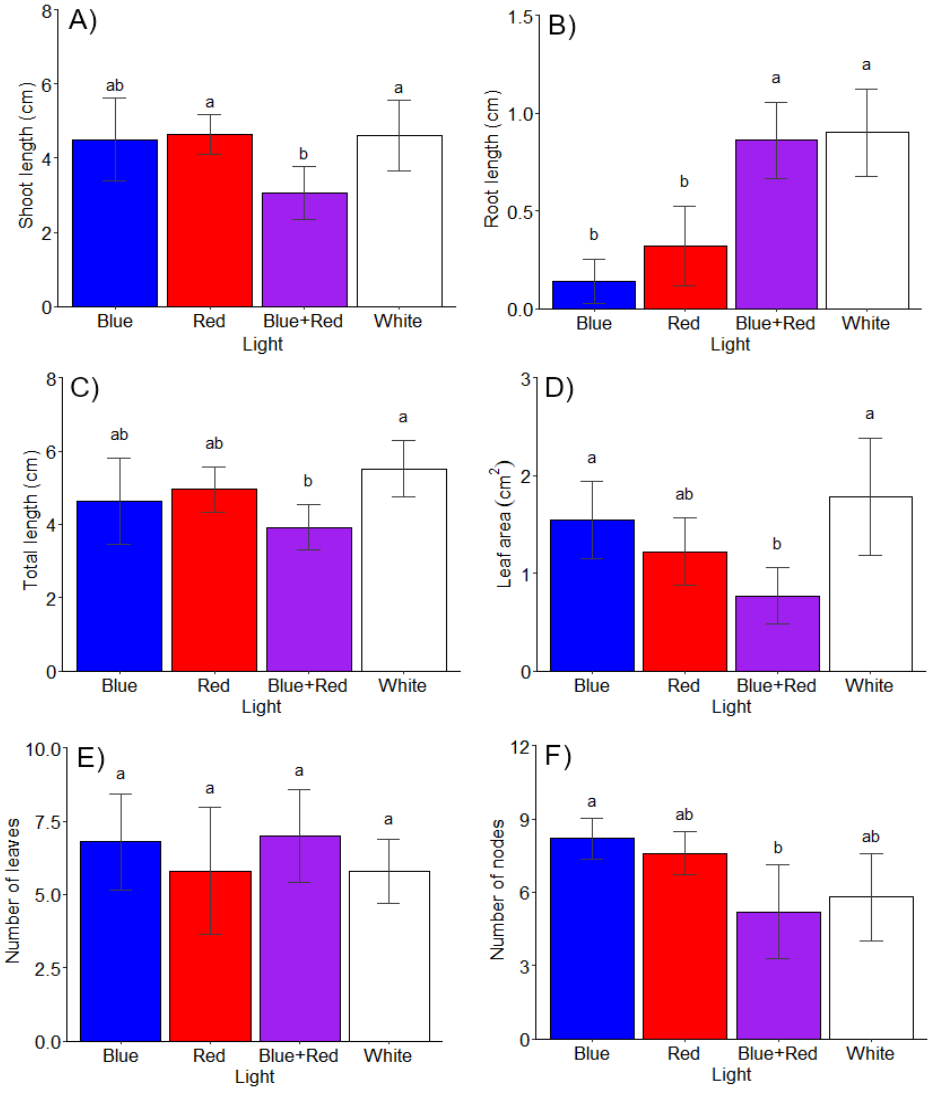


Figure 2. Growth parameters of *Genipa americana* seedlings under light spectra (white, red, blue, and blue + red): shoot length (A), root length (B), total length (C), leaf area (D), number of leaves (E), and number of nodes (F). Bars represent the means ± SD. Different letters indicate significant differences by the Tukey test ($p < 0.05$).

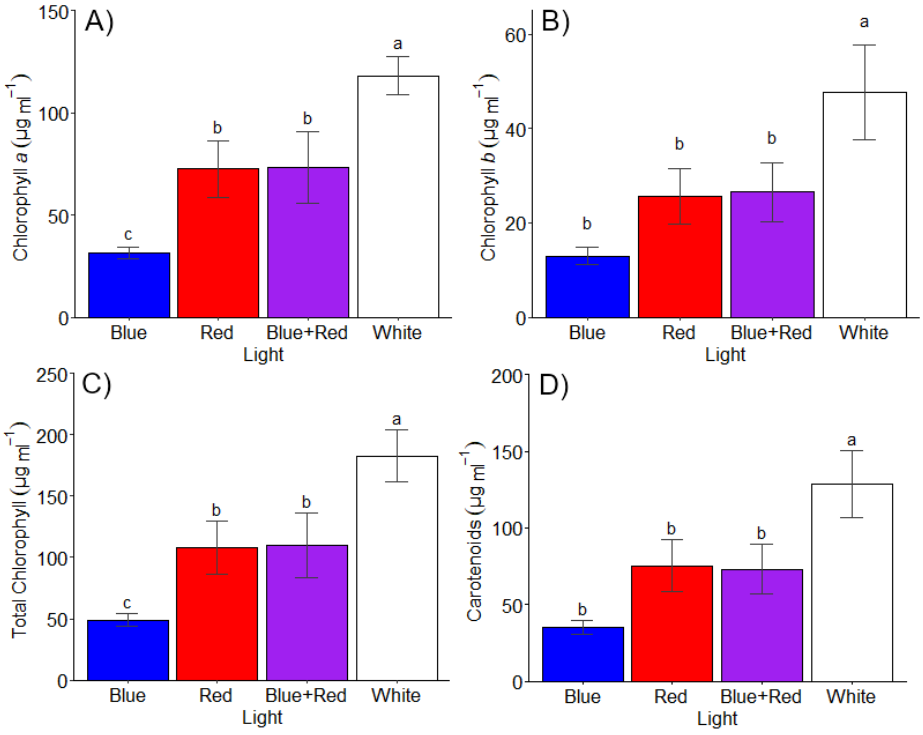


Figure 3. Photosynthetic pigment content in *Genipa americana* seedlings under light spectra (white, red, blue, and blue + red): chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C), and carotenoids (D). Bars represent the means ± SD. Different letters indicate significant differences by the Tukey test ($p < 0.05$).

response, potentially contributing to the photosynthetic integrity of the seedlings. Carotenoids are known to dissipate excess excitation energy as heat through non-photochemical quenching, and to quench reactive-oxygen species that would otherwise damage the photosynthetic apparatus (Demmig-Adams et al. 2025).

This interpretation is corroborated by findings during *G. americana* acclimatization, where increased antioxidant enzyme activity was associated with successful *ex vitro* establishment and high survival rates (Souza et al. 2021). Therefore, the positive effect of the white light on both chlorophyll and carotenoid synthesis likely enhanced the overall photosynthetic performance and stress tolerance in *G. americana* seedlings, being consistent with the high phenotypic plasticity reported for this species.

Stomatal conductance (*gs*) was higher under the white light (1.256 mol m^{-2} s^{-1}), followed by the red (0.893 mol m^{-2} s^{-1}) light. The blue (0.490 mol m^{-2} s^{-1}) and blue + red (0.411 mol m^{-2} s^{-1}) light showed the lowest values, with no significant difference between them ($p < 0.001$) (Figure 4A). Transpiration rate (*E*) followed the same pattern ($p < 0.001$), with the white (17.19 mmol m^{-2} s^{-1}) and red (13.30 mmol m^{-2} s^{-1}) light presenting superior values, when compared to the blue (7.20 mmol m^{-2} s^{-1}) and blue + red (6.71 mmol m^{-2} s^{-1}) light (Figure 4B).

Net CO_2 assimilation (*A*) was also affected by spectral quality ($p = 0.001$). The blue + red light (4.52 µmol m^{-2} s^{-1}) was significantly lower than the red (10.44 µmol m^{-2} s^{-1}), white (10.05 µmol m^{-2} s^{-1}), and blue (8.48 µmol m^{-2} s^{-1}) light, which did not differ from each other (Figure 4C). Intercellular CO_2 concentration (*Ci*) did not respond significantly to spectral quality ($p = 0.086$), ranging from 380.1 to 390.6 µmol mol^{-1} (Figure 4D).

Unlike in *Brassica napus* (Saleem et al. 2020), where the blue light induced oxidative stress and reduced gas exchange, in *G. americana* this spectrum, alone or in combination with red light, promoted a more efficient adjustment of the stomatal apparatus. In *B. napus*, stomatal closure under blue light limited internal CO_2 concentration (*Ci*). In contrast, in *G. americana* seedlings, the *Ci* values were maintained at levels similar to those observed under white light, but with lower water loss (*E*).

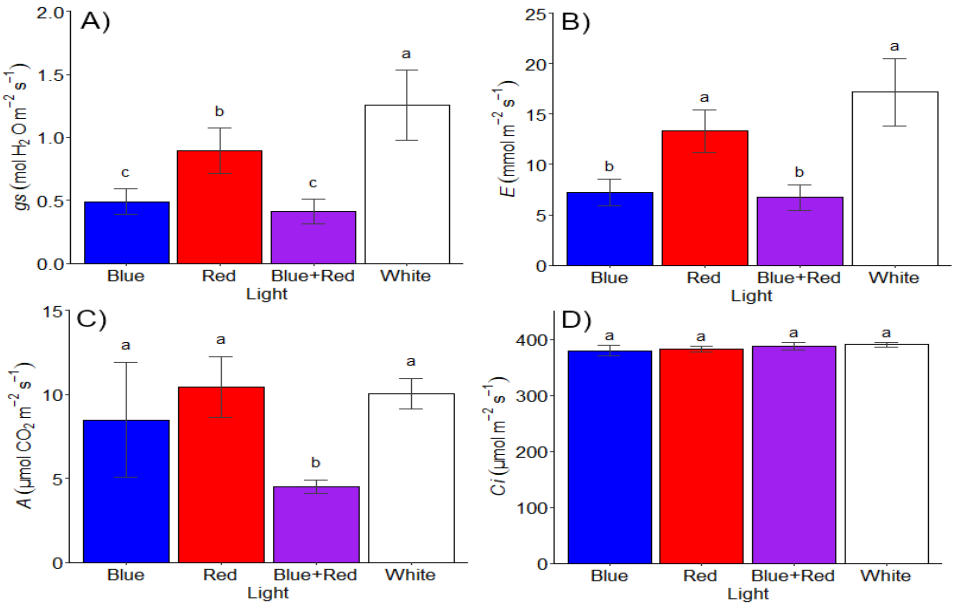


Figure 4. Gas exchange parameters of *Genipa americana* seedlings under light spectra (white, red, blue, and blue + red): stomatal conductance (*gs*; A), transpiration rate (*E*; B), net CO_2 assimilation rate (*A*; C), and intercellular CO_2 concentration (*Ci*; D). Bars represent the means ± SD. Different letters indicate significant differences by the Tukey test ($p < 0.05$).

This pattern indicates that, in this species, the blue light acts as a modulator of physiological efficiency, allowing the maintenance of carbon supply with lower water cost. Consistently, in *Prunus domestica* subsp. *insititia*, cultivation under blue light also resulted in lower transpiration rates (*E*) and stomatal conductance (*gs*), without compromising net CO_2 assimilation (*A*), which remained similar to that observed under white, red or mixed LEDs (Nacheva et al. 2023).

These results reinforce that the response to spectral quality is species-specific, allowing certain plants to use short wavelengths to optimize water-use efficiency without compromising carbon assimilation.

The leaf anatomy analyses revealed the typical characteristics of *G. americana* leaves, including juxtaposed epidermal cells (adaxial and abaxial) and the presence of palisade and spongy parenchyma.

The adaxial epidermis thickness was significantly affected by light spectral quality ($p < 0.001$), being thicker under the red (0.0245 mm) and blue + red (0.0230 mm) light, whereas the white (0.0130 mm) and blue (0.0120 mm) light showed the lowest values, with no significant difference between them (Figure 5A). The abaxial epidermis showed an opposite pattern ($p = 0.017$), with the blue + red (0.0147 mm) and red (0.0135 mm) light exhibiting higher values, whereas the blue light resulted in the lowest thickness (0.0090 mm). The white light showed an intermediate value (0.0115 mm) that did not differ significantly from either extreme (Figure 5B).

The palisade parenchyma thickness was greatest under the blue + red treatment ($p = 0.001$). This treatment resulted in a considerably thicker palisade parenchyma (0.0443 mm), when compared to the blue (0.0270 mm), red (0.0225 mm), and white (0.0220 mm) light, which did not differ from each other (Figure 5C). The spongy parenchyma thickness was not influenced by the light spectral quality ($p = 0.298$), with values ranging from 0.044 to 0.055 mm across all the treatments (Figure 5D).

The total leaf thickness followed the same pattern as the palisade parenchyma ($p = 0.002$). The blue + red light produced the thickest leaves (0.126 mm), differing from all the other treatments, whereas the red (0.110 mm), blue (0.103 mm), and white (0.101 mm) light did not differ from each other (Figure 5E).

The leaf anatomical responses observed in this study demonstrate that light spectral quality significantly influences the tissue development in *in vitro* cultivated *G. americana* seedlings. The blue + red light treatment promoted a greater thickening of both adaxial and abaxial epidermises and, most notably, of palisade parenchyma, when compared

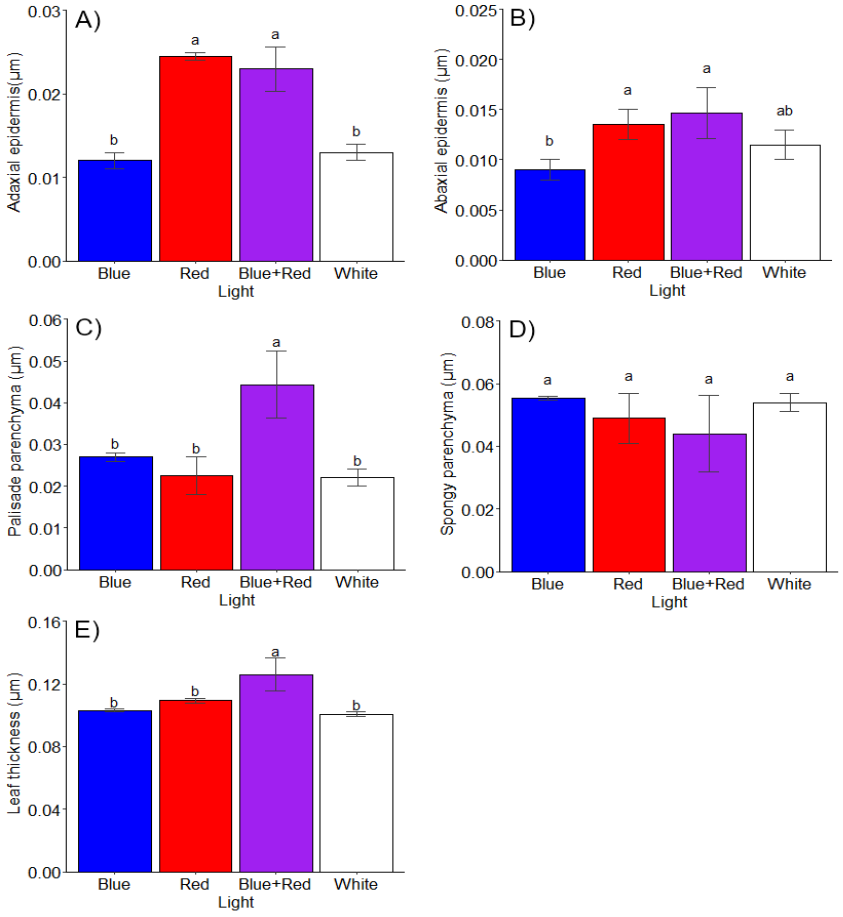


Figure 5. Leaf anatomical parameters of *Genipa americana* seedlings under light spectra (white, red, blue, and blue + red): adaxial epidermis thickness (A), abaxial epidermis thickness (B), palisade parenchyma thickness (C), spongy parenchyma thickness (D), and total leaf thickness (E). Bars represent the means ± SD. Different letters indicate significant differences by the Tukey test ($p < 0.05$).

to all the other treatments (Figures 5A-E; 6 and 7). The red light alone promoted epidermal thickening, but did not enhance the palisade parenchyma development beyond levels observed under the blue or white light (Figures 5A, 5B, and 5C).

These findings indicate that the combination of red and blue wavelengths is required to promote palisade parenchyma differentiation, whereas red light alone is sufficient only for epidermal thickening.

In contrast, the lack of significant differences in spongy parenchyma thickness across the treatments (Figure 5D) suggests that this tissue is less responsive to light spectral variation, when compared to the adaxial epidermis and palisade parenchyma. This may reflect the primary roles of spongy parenchyma in gas exchange and light scattering rather than in direct photoperception, making it structurally more stable under different light conditions. Consistent with this finding, in *Corylus avellana*, spongy parenchyma thickness did not differ significantly among plants grown under contrasting light conditions, whereas the palisade parenchyma thickness and palisade-to-spongy parenchyma ratio were significantly reduced under shade (Catoni et al. 2015).

The increased total leaf thickness (Figure 5E) was primarily driven by enhanced palisade parenchyma development rather than by changes in spongy parenchyma or epidermal layers alone, consistent with findings in sweet pepper seedlings, which also exhibited the greatest leaf thickness under mixed red and blue light, mainly attributed to a significant increase in palisade parenchyma thickness (Li et al. 2020).

This anatomical adaptation is functionally important for restoring plant autotrophy, since thicker

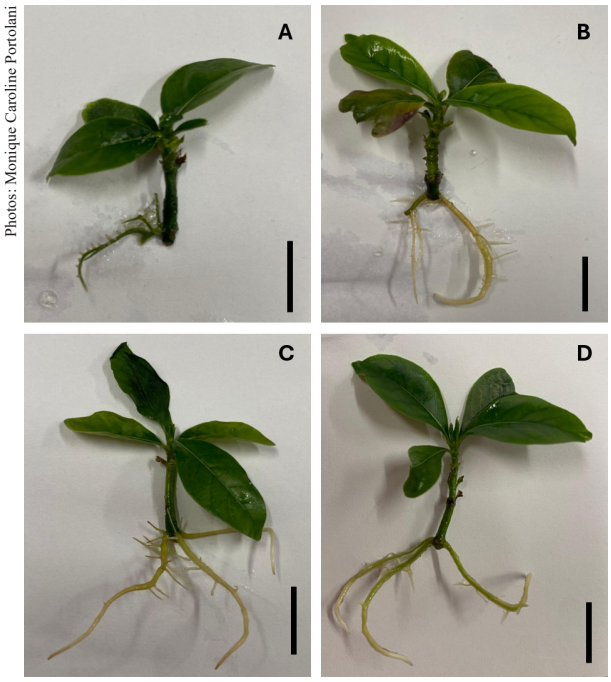


Figure 6. Morphological appearance of *Genipa americana* plants grown *in vitro* under LED light qualities: blue (A), red (B), combined blue and red at a 2:3 ratio (C), and white (D). Scale bars = 1 cm.

leaves with well-developed palisade parenchyma typically exhibit greater photosynthetic capacity, as the vertically elongated palisade cells minimize light scattering and allow deeper light penetration into chloroplasts (Li et al. 2020). Furthermore, the improved leaf structure observed under blue + red light has been associated with better water-use efficiency, as thicker leaves with more developed mesophyll tend to reduce mesophyll conductance to water vapor (Li et al. 2020). Finally, the coordinated thickening of both epidermal layers and palisade parenchyma likely contributed to better mechanical resistance, facilitating the successful transition from *in vitro* to *ex vitro* environments (Souza et al. 2021).

Therefore, the anatomical modifications observed under blue + red light, particularly the coordinated thickening of both epidermal layers and palisade parenchyma, likely contributed to autotrophism by optimizing leaf architecture for light capture and CO_2 assimilation (Tholen et al. 2012).

The principal component analysis (PCA) provided a comprehensive overview of the relationships among the 19 variables measured

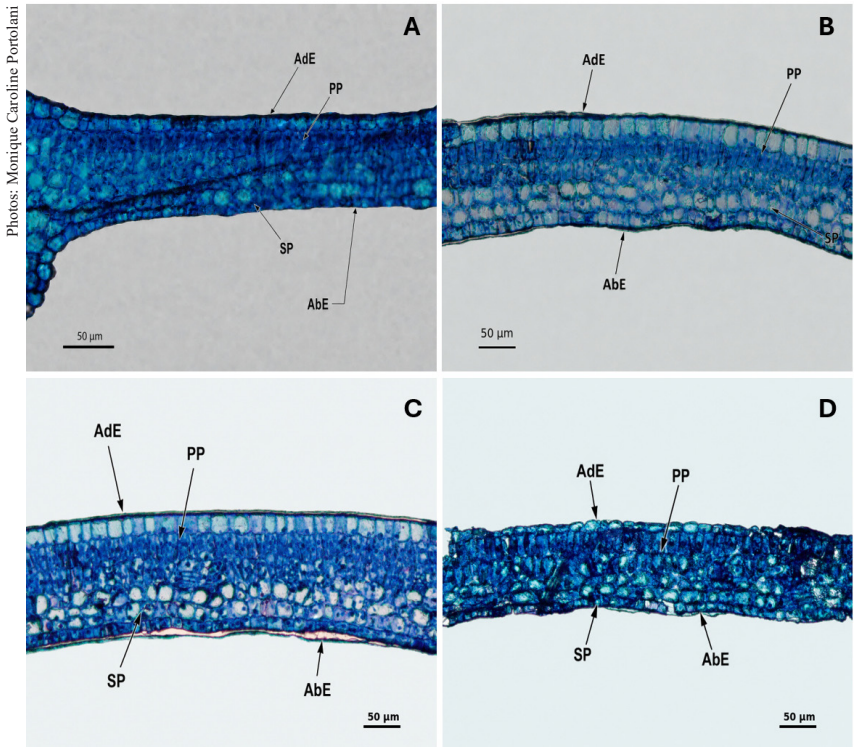


Figure 7. Photomicrographs of transverse leaf blade sections of *Genipa americana* grown *in vitro* under LED light qualities: blue (A), red (B), combined blue and red at a 2:3 ratio (C), and white (D). AdE: adaxial epidermis; PP: palisade parenchyma; SP: spongy parenchyma; AbE: abaxial epidermis. Scale bars = 50 µm.

across the four light quality treatments. The first two principal components (PC) explained 88.7 % of the total data variance (PC 1 = 49.6 %; PC2 = 39.1 %), suggesting that the evaluated treatments induced distinct physiological and anatomical response patterns (Figure 8).

In PC1, the variables that contributed the most were total length (10.3 %), leaf thickness (9.3 %), palisade parenchyma (9.5 %), leaf area (9.2 %), CO_2 assimilation (9.0 %), and shoot length (8.9 %). Indeed, variables associated with plant vigor (growth and gas exchange) loaded positively on PC1, whereas anatomical variables, particularly palisade parenchyma thickness (r = -0.946) and leaf thickness (r = -0.937), loaded negatively on this axis, suggesting a possible reciprocal relationship between growth-related variables and anatomical thickening.

The blue + red treatment was positioned at the negative pole of PC1 (contribution of 61.1 %; $cos^2 = 0.868$), suggesting that this spectral combination was associated with leaf thickening, whereas shoot elongation and gas exchange were less pronounced under this treatment. This pattern is consistent with findings in sweet pepper (*Capsicum annuum*), where mixed red and blue light significantly increased palisade parenchyma thickness and total leaf thickness, while promoting greater photosynthetic capacity and photoinhibition resistance (Li et al. 2020). Consistent with this pattern, different light conditions (shading levels) directly influence the relationship between stem diameter and height growth in *G. americana* (Lima et al. 2010). The negative correlation between growth and thickening on PC1 may reflect resource allocation patterns in *G. americana* seedlings, which appear to prioritize either vertical growth or leaf structural reinforcement depending on the spectral environment, a pattern that has also been observed in other species (Song & Jin 2023). However, given the exploratory nature of PCA and the limited number of treatments, these interpretations should be viewed with caution.

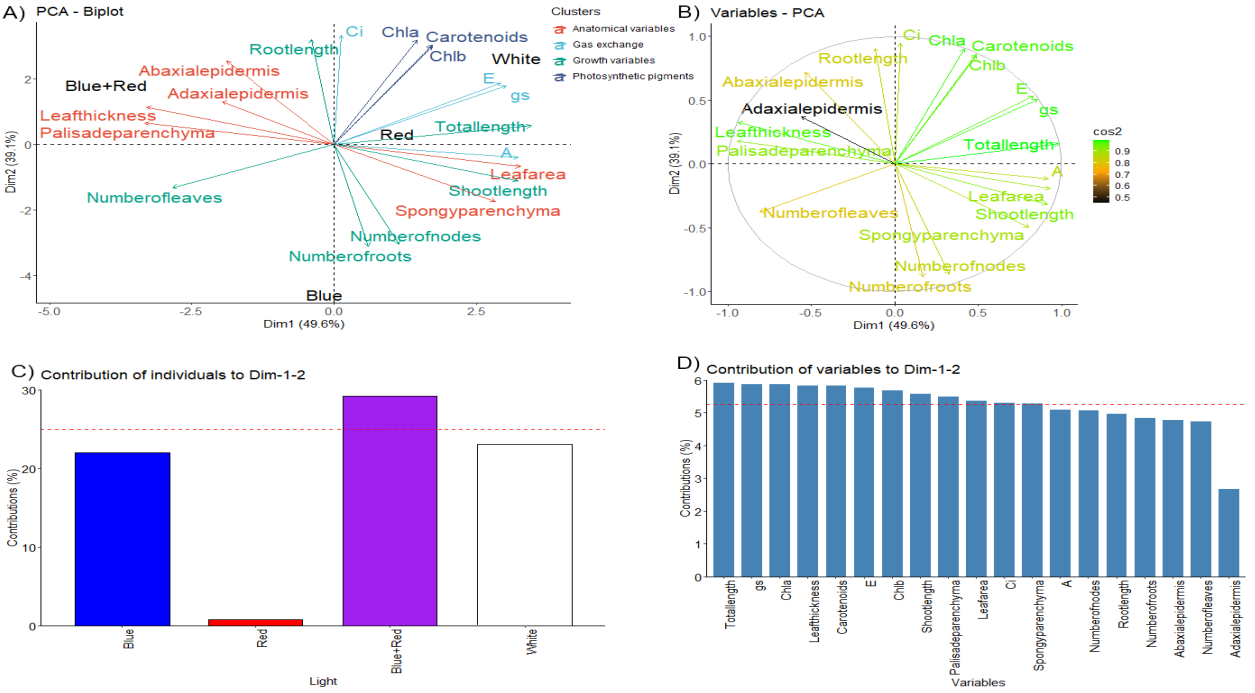


Figure 8. Principal component analysis (PCA) of growth, anatomical, photosynthetic pigment, and gas exchange variables of plants subjected to light qualities. A) PCA biplot showing the distribution of treatments (individuals) and the direction of the 19 variable vectors in the first two principal components, which together explained 88.7 % of the total variance (PC1 = 49.6 %; PC2 = 39.1 %); B) quality of representation of variables (cos^2) in the first two principal components, indicating the contribution degree of each variable to the factorial plane; C) contribution of treatments (individuals) to dimensions 1 and 2, highlighting the treatments that most influenced the structure of the principal components; D) contribution of variables to dimensions 1 and 2, highlighting those with the greatest discriminatory power among the evaluated treatments. The red dashed line indicates the expected contribution value under a uniform distribution (1/n × 100).

In contrast, the white treatment showed positive associations with growth variables, gas exchange, and photosynthetic pigments, distributing its variance across both PC1 (36.9 %) and PC2 (22.7 %), which reflects a more balanced physiological profile, when compared to the other treatments. Consistent with this pattern, *Cyclocarya paliurus* exposed to monochromatic light sources showed that the white light promoted greater biomass accumulation, photosynthetic capacity, leaf thickness, and pigment content than monochromatic sources, indicating that this wavelength provides a more balanced physiological profile (Liu et al. 2018).

In PC2, the greatest contributions came from *Ci* (12.0 %), root length (11.1 %), chlorophyll *a* (11.0 %), number of roots (10.6 %), and number of nodes (10.1 %). Among these, *Ci* (r = 0.946), root length (r = 0.908), and chlorophyll *a* (r = 0.904) correlated positively with this axis, whereas the number of roots (r = -0.886) and number of nodes (r = -0.865) showed negative correlations. The blue treatment predominantly determined the variance in PC2 (contribution of 66.3 %; $\cos^2 = 0.961$), characterized by a higher number of nodes, but lower root length and lower values of photosynthetic pigments and *Ci*.

It is interesting to note that the positive association of chlorophyll *a* and *Ci* with PC2 may suggest some role for the blue light in maintaining photosynthetic competence. However, absolute pigment levels under the blue light were among the lowest of all treatments (Figures 8A-D). This discrepancy highlights that the PCA associations reflect multivariate correlations rather than absolute performance, and should therefore be interpreted with caution.

The red-light treatment positioned itself near the origin of both axes, with reduced contributions to both PC1 (1.8 %) and PC2 (< 0.1 %). This intermediate profile indicates that the red light alone induced a moderate response across all measured variables without strongly polarizing toward either growth or anatomical thickening, aligning, for example, with the observation that the red light alone promoted epidermal thickening, but did not enhance palisade parenchyma development beyond the levels observed under blue or white light (Figure 8C).

Overall, these exploratory analyses captured the multivariate responses of *G. americana* seedlings to treatments with different light spectra, revealing distinct physiological and anatomical features associated with each wavelength.

Future studies should validate whether these structural advantages translate into improved survival and growth under *ex vitro* conditions, and evaluate two-stage lighting regimes as a strategy to combine the metabolic benefits of white light with the structural enhancement of the blue + red spectrum.

CONCLUSIONS

1. The blue + red treatment is the most effective in promoting leaf structural traits that are expected to favor acclimatization. Specifically, this combination significantly increased the palisade parenchyma thickness (0.0443 mm; $p = 0.001$) and total leaf thickness (0.126 mm; $p = 0.002$), when compared to all the other light treatments;
2. Monochromatic blue and red light both resulted in reduced root length (0.14 cm and 0.32 cm, respectively; $p = 0.001$), with the blue light also showing overall reduced photosynthetic pigment levels (e.g., total chlorophyll of 48.8 $\mu g\ mL^{-1}$), despite being associated with a higher number of nodes (8.2) in the multivariate analysis;
3. The white light provided the highest overall pigment accumulation (total chlorophyll of 182.6 $\mu g\ mL^{-1}$; $p < 0.001$) and stomatal conductance (1.256 mol $m^{-2}\ s^{-1}$; $p < 0.001$), while maintaining net CO_2 assimilation (10.05 $\mu mol\ m^{-2}\ s^{-1}$) and leaf area (1.78 cm^2) comparable to monochromatic red and blue light;
4. The red light represented an intermediate overall condition, promoting adaxial epidermal thickening (0.0245 mm; $p < 0.001$) but failing to enhance palisade parenchyma development (0.0225 mm) beyond the levels observed under other spectra;
5. These findings support the optimization of light spectra in establishment protocols for this species, with the blue + red combination emerging as the most promising for producing structurally robust plantlets.

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