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Effect of light spectra on *in vitro* multiplication, elongation and adventitious rooting stages of *Bambusa vulgaris* Schrad. ex J. C. Wendl.

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ABSTRACT

Bamboos occur throughout much of the temperate and tropical world, have rapid growth, and have various commercial and environmental applications. Clonal production of selected plants on a industrial scale is an important strategy for the bamboo sector. This study aimed to evaluate the effects of the light spectrum on *in vitro* multiplication, elongation, adventitious rooting, and anatomical features of the leaf surface of *Bambusa vulgaris*. In the multiplication and elongation stages, *in vitro*–established explants were transferred to a culture medium supplemented with 8.88 μmol of 6-benzylaminopurine (BAP) and 2.69 μmol of α-naphthalene acetic acid (NAA), and subjected to four light spectra (*i.e.*, white, blue, green, and red). At the adventitious rooting stage, the culture medium was supplemented with 9.84 μmol of indole-3-butyric acid (IBA), 5.37 μmol NAA, and 2.22 μmol BAP under identical light spectra. Explant survival was not influenced by light spectra in the multiplication and elongation stages. White (2.2 shoots) and blue (1.8 shoots) light spectra were the most suitable for the number of shoots per explant. The white spectrum was associated with the highest average length of shoots (7.4 cm) and number of leaves per explant (3.0 leaves). The white light spectrum resulted in the highest average chlorophyll *a* contents (12.60 μg mg^{-1}), total chlorophyll (16.60 μg mg^{-1}), and carotenoids (10.10 μg mg^{-1}). White and blue light spectra resulted in the best responses for vigor, and least senescence and tissue oxidation. White and blue light spectra favored the chlorophyll *b* content, resulting in 4.60 and 3.60 μg mg^{-1}, respectively. Survival (80.0 %), adventitious rooting (50.0 %), vigor, senescence, and tissue oxidation were favored in the white light spectrum in the adventitious rooting stage. Scanning electron microscopy of leaves exposed to the white light spectrum revealed microtrichomes and spines on the adaxial surface of the leaf blade, papillae and stomata; on the abaxial surface, there were many unicellular trichomes arranged in rows, denoting normal growth and development. These results may help the production of micropropagated plants of *Bambusa vulgaris* on an industrial scale.

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1. Introduction

Bamboos show rapid growth throughout their range, mainly in Asia, Africa, and South America, and are versatile plants used for food, handicrafts, tool manufacturing, environmental recovery, erosion control, and soil protection (INBAR. INTERNATIONAL NETWORK FOR BAMBOO AND RATTAN, 2015; Liese and Köhl, 2015; Ahmad et al., 2021). Bamboos are also an alternative to fossil fuels for the production of renewable energy and cellulose for the paper manufacturing (INBAR. INTERNATIONAL NETWORK FOR BAMBOO AND RATTAN, 2015).

The wide distribution of bamboos is due to their high adaptability to various environmental regimes in tropical, subtropical, and temperate soil conditions (Bhandawat et al., 2019; Konzen et al., 2021a, 2021b). Brazil has the greatest bamboo diversity in Latin America, with approximately 232 species, and it is estimated that the country has a planted area of roughly 8 million hectares (INBAR. INTERNATIONAL NETWORK FOR BAMBOO AND RATTAN, 2015; Silveira et al., 2020). *Bambusa vulgaris* is widely cultivated, and it's propagation has been of particular interest to those working in the field of biotechnology due to its rapid growth, adaptation, and numerous commercial and industrial applications (Darabant et al., 2014; Furlan et al., 2018; García-Ramírez et al., 2014; Konzen et al., 2017; Ribeiro et al., 2020).

However, given limitations regarding the production of bamboo plants at a commercial-scale, such as low seed production and ineffective vegetative methods, the potential of *Bambusa vulgaris* production has not been adequately addressed. Some of the propagation methods have yet to be tested, or little is known about *in vitro* culture methods, especially the morphophysiological effects of light spectra. One method, micropropagation, has been shown to be a fast and secure method for clonal propagation (Lin et al., 2019; Konzen et al., 2021b; Furlan et al., 2018; Zhao et al., 2017; Teixeira et al., 2021).

In vitro culture can be adequate to produce clonal plants of economic importance in a fast and optimized system (Ribeiro et al., 2016). However, some major challenges often interfere with their success. One of the main obstacles is related to environmental factors (Batista et al., 2018; Souza et al., 2022a), and light spectra can interfere with the *in vitro* culture of plants, as previously demonstrated (Souza et al., 2022a, 2019, 2021). Several studies focussing on the effect of light spectra on *in vitro* culture have been presented, including the effects on plant growth, development, and metabolism (Souza et al., 2018; Gnasekaran et al., 2021). The aim of the study was to evaluate the effects of light spectra on the *in vitro* multiplication, elongation, and adventitious rooting stages of *Bambusa vulgaris*.

2. Material and methods

2.1. General characterization and tissue source

Culm sections were collected from a 12-year-old plant of *Bambusa vulgaris* Schrad. ex J. C. Wendl. (Fig. 1A) growing in Lavras, Minas Gerais, Brazil (21°13′29.16″ S; 44°58′1.87″ W). The propagules were transported and arranged in a greenhouse environment to promote sprouting (Fig. 1B). After adventitious rooting (60 days), the culms were placed in 4-L pots with washed sand and subsoil soil (1:1, v:v) as substrate, constituting the stock plants (Fig. 1C).

Stock plants were fertigated weekly with a nutrient solution to promote the growth and release of new shoots (Souza et al., 2022b). Irrigation was performed once a day directly on the substrate, avoiding water contact with the new propagules. Weed control was conducted manually.

Shoots (Fig. 1D) were collected from the stock plants (*i.e.*, middle position of shoot, 0.5–0.7 cm diameter, and containing an axillary bud wrapped by the leaf sheath). Subsequently, leaf sheaths were removed (Fig. 1E), and their remnant tissues were scraped (Fig. 1F) with the aid of a stylus for bud exposition, facilitating asepsis (Teixeira et al., 2021). The asepsis process of explants (Figs. 1G and 1H), preparation/sterilization of the MS culture medium (Murashige and Skoog, 1962), and inoculation of the explants were carried out according to Teixeira et al. (2021).

2.2. In vitro multiplication and elongation stages

Established explants were transferred to glass flasks (72 × 72 × 100 mm) containing 50 mL of MS culture medium supplemented with 8.88 μmol of 6-benzylaminopurine (BAP), 2.69 μmol of α-naphthalene acetic acid (NAA), 30 g L^{-1} of sucrose, and 7 g L^{-1} of agar. The treatments consisted of four light spectra: T1: white; T2: blue; T3: green; and T4: red (Fig. 2A–D). Light spectra were provided by filtering the light output of fluorescent lamps with double sheets of cellophane paper (*i.e.*, internal irradiation ranged from 38 to 40 μmol m^{-2} s^{-1}), according to Souza et al. (2020a) and Frade et al. (2023). For all experiments, flasks were placed in a growth room at a temperature of 24 °C (± 1 °C), 40 μmol m^{-2} s^{-1} irradiation (*i.e.*, external fluorescent lamp), and a photoperiod of 16 h. Light spectra were measured with a SPECTRA PEN Z850 portable spectroradiometer (Qubit Syculms-Kingston, Ontario, USA).

The experiment was conducted in a complete randomized design with four light spectra (white, blue, green, and red) as treatments. Each treatment consisted of 15 replicates, with one explant per replicate. Survival percentage, number of shoots per explant, length of shoots (cm), number of leaves per explant, photosynthetic pigment contents (μg mg^{-1}) [chlorophyll *a*, chlorophyll *b*, total chlorophyll ($a + b$), and carotenoids], vigor, senescence, and tissue oxidation were evaluated at 30 days.

Photosynthetic pigment contents of the leaves were measured according to Souza et al. (2020b) adapted from Lichtenthaler (1987). A scoring scale from 1 to 4 was adapted from Oliveira et al. (2016) to assess vigor, senescence, and tissue oxidation evaluation (Fig. 3A–C).

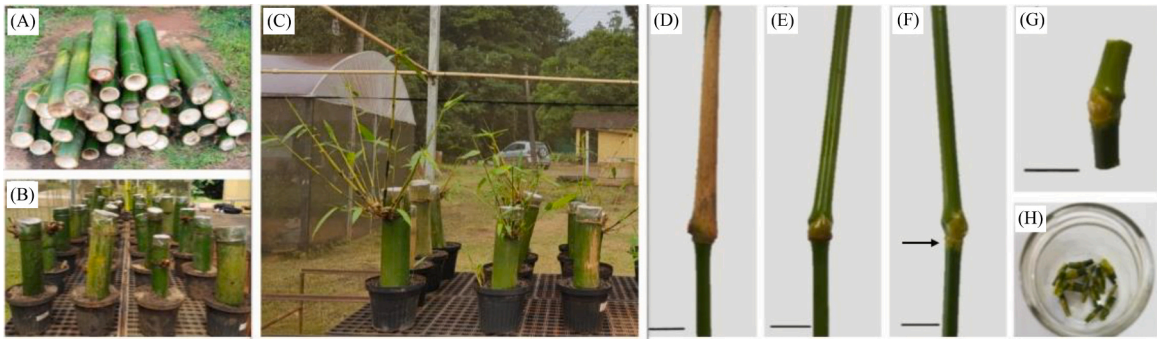

Fig. 1. Vegetative rescue and asepsis stages of *Bambusa vulgaris*. (A) Sectioned culm. (B) Culm arrangement in a greenhouse. (C) Culms rooted and emergence of new shoots. (D) Collected shoot. (E) Leaf-sheaths removed. (F) Remnant tissues scraped with the aid of a stylus. (G) Standardized explant (*i.e.*, 1.5–2.0 cm long nodal segment, containing an axillary bud). (H) Transfer of explant to clean work bench. Bars = 1 cm.

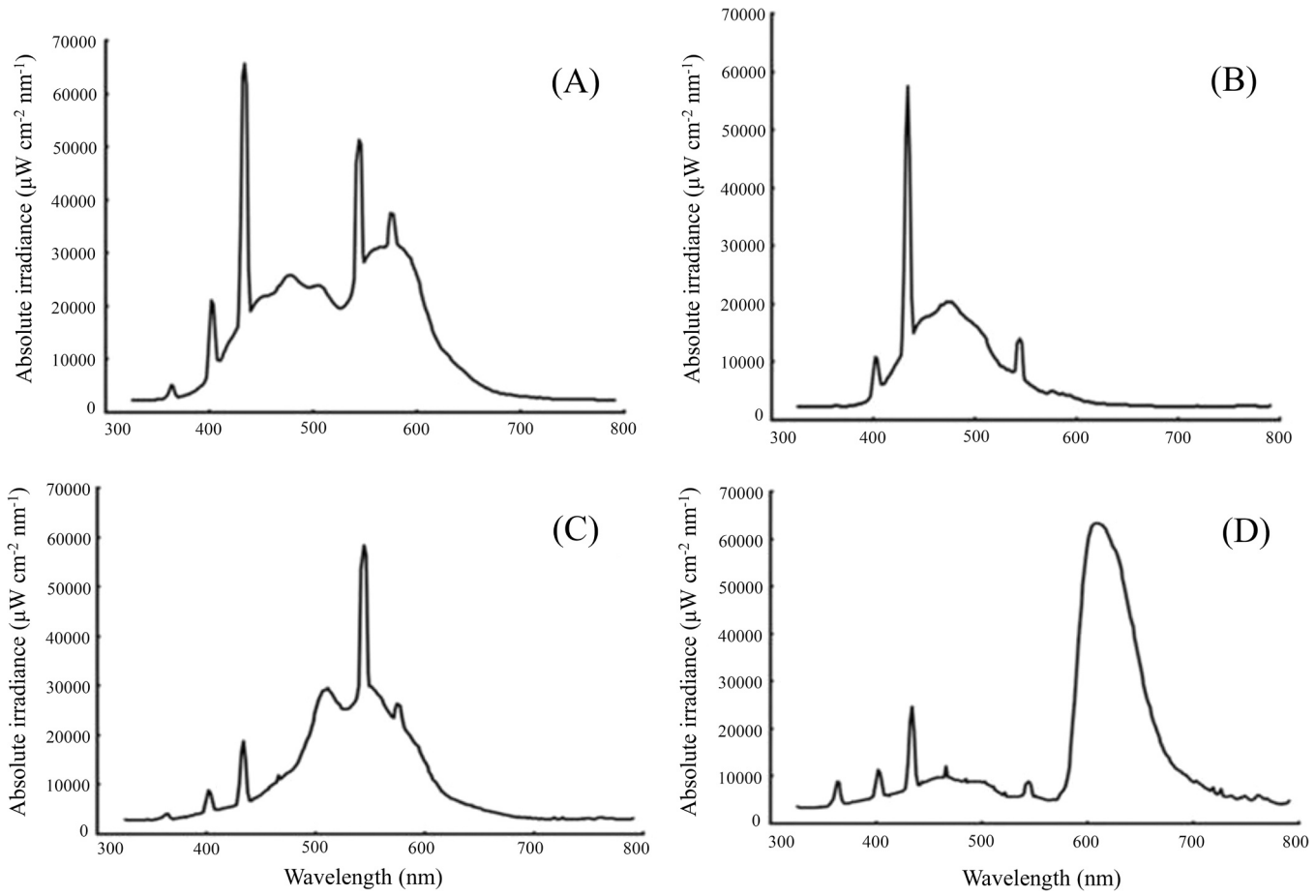

Fig. 2. Distributions of light spectra (wavelength, nm) used in the experiment emitted by the fluorescent lamps and filtered by the double cellophane sheets (absolute irradiance, μW cm^{-2} nm^{-1}). (A) White. (B) Blue. (C) Green. (D) Red.

2.3. In vitro adventitious rooting stage

Shoots (*i.e.*, explants) 3 cm long (derived from multiplication and elongation stages) were inoculated into glass flasks (72 × 72 × 100 mm) containing 50 mL of MS culture medium supplemented with 9.84 μmol of indole-3-butyric acid (IBA), 5.37 μmol NAA, and 2.22 μmol BAP. The treatments consisted of the four light spectra (Fig. 2A–D), which were provided by filtering the light output of the fluorescent lamps with double sheets of cellophane paper, as described in the multiplication and elongation stages.

The experiment was conducted in a complete and randomized design with the same four light spectra evaluated above. Each treatment consisted of 15 replicates, with one explant for each replicate. Survival percentage, adventitious rooting percentage, vigor, senescence, tissue oxidation were evaluated at 30 days. The same scoring scale from 1 to 4, adapted from Oliveira et al. (2016) to assess vigor, senescence, and tissue oxidation was used here (Fig. 3A–C). Leaf samples were collected from the best treatment to perform an anatomical description through scanning electron microscopy (SEM).

2.4. Scanning electron microscopy

Leaf samples from *in vitro* rooted plants were collected and sectioned into 0.5 cm^2, and were then submerged in microtubes (1.5 mL) with Karnovsky (1965) fixative (2.5 % glutaraldehyde, 2.0 % paraformaldehyde, 0.05 M cacodylate buffer at pH 7.2 + 0.001 M $CaCl_2$) for 72 h in the refrigerator. The samples were washed three times in cacodylate buffer (0.05 M) for 10 min each. The dehydration began with immersing the sections in a graded acetone series (25 %, 50 %, 75 %, 90 %, and 100 %) for 10 min each. The step for 100 % concentration was performed three times. After dehydration, the samples were placed on a porous support containing acetone and sent to the critical-point drying stage, where the acetone was volatilized and replaced by carbon dioxide (CO_2). For mounting of the leaf samples on the stubs, supports were wrapped in aluminum foil and fixed in double-sided carbon tape, where the samples were deposited. Finally, the gold metallization step was performed. The observation was performed with a scanning electron microscope (LEO EVO-40), and the images were recorded.

2.5. Statistical analysis

Data analyses were performed with R Core Team (2018) (version 4.2.1) using the ExpDes package, version 1.1.2 (Ferreira et al., 2013). Data of survival percentage, number of shoots per explant, length of shoots, number of leaves per explant, photosynthetic pigment contents [chlorophyll *a*, chlorophyll *b*, total chlorophyll (*a* + *b*), and carotenoids], and adventitious rooting were analyzed for homoscedasticity and their fitting to normal distribution of residuals, respectively with the Hartley's test ($p > 0.05$) and Shapiro-Wilk's test ($p > 0.05$). Subsequently, the data were transformed using the Box-Cox test. The data were then processed by analysis of variance (ANOVA, $p < 0.05$), and the means were compared using the Tukey's test ($p < 0.05$). Data on vigor, senescence and tissue oxidation were analyzed for principal component analysis (PCA), performed with the R software (R Core Team, 2018) (version 4.2.1), using the "factoextra" R package (version 1.0.7) (Kassambara and Mundt, 2020).

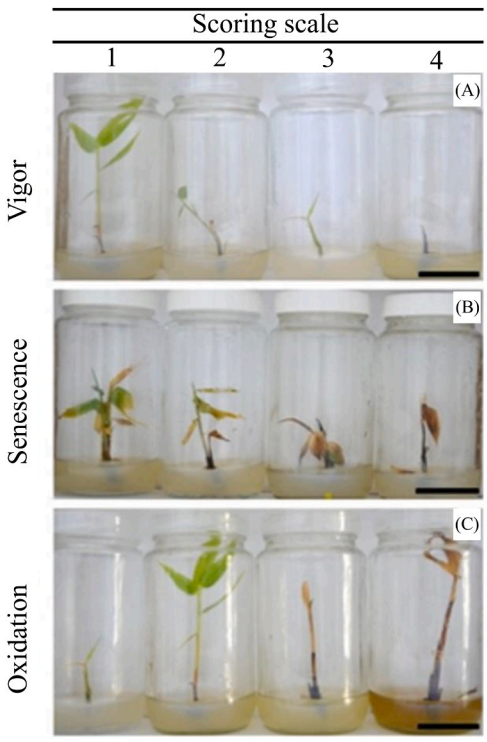

Fig. 3. Vigor, senescence and tissue oxidation evaluations of *Bambusa vulgaris* according to the scoring scale. (A) Vigor: Score_1 = Excellent: induction of bud and shoot with active growth, without apparent nutritional deficiency; Score_2 = Good: induction of bud and shoot but with reduced growth; Score_3 = Regular: small bud and shoot development and/or apparent nutritional deficiency; Score_4 = Poor: mortality of the explant; (B) Senescence: Score_1 = Null/low: absence of senescence or its presence, with symptoms of chlorosis/browning on leaf border; Score_2 = Intermediate: presence of senescence throughout the leaf blade; Score_3 = Intermediate/high: presence of senescence throughout the leaf blade and stem; Score_4 = High: mortality of explant; (C) Oxidation: Score_1 = Null: no oxidation; Score_2 = Low: reduced oxidation of the explant base; Score_3 = Intermediate: oxidation and color change in the culture medium; Score_4 = High: complete shoot oxidation and mortality (*i.e.*, darkened of the culture medium). Bars = 2.0 cm.
Adapted from Oliveira et al. (2016).

3. Results

3.1. In vitro multiplication and elongation stages

The light spectra used in the *in vitro* culture of *Bambusa vulgaris* did not significantly affect the explant survival at 30 days after inoculation, with 100.0 % survival for white, 90.0 % for blue, 80.0 % for green, and 80.0 % for red (Fig. 4A).

Bud multiplication and shoot elongation occurred at the same time, with the light spectra having a significant effect on the number of shoots per explant (Fig. 4B), length of shoots (Fig. 4C), and number of leaves (Fig. 4D). White (2.2 shoots) and blue (1.8 shoots) light spectra resulted in the greatest number of shoots per explant, but there were no statistical differences among them (Fig. 4B). The longest shoots (7.4 cm, Fig. 4C) and greatest number of leaves (3.0 leaves per explant, Fig. 4D) were observed in the white light spectrum, which had the highest averages and was statistically different when compared to the other treatments (*i.e.*, blue, green, and red light spectra).

The white light spectrum resulted in the highest average of chlorophyll *a* (12.60 μg mg^{-1}), total chlorophyll (16.60 μg mg^{-1}), and carotenoid content (10.10 μg mg^{-1}), and was statistically different when compared to the other treatments (Fig. 4E). White and blue light spectra were not statistically different for chlorophyll *b* content, with 4.60 μg mg^{-1} and 3.60 μg mg^{-1}, respectively (Fig. 4E). *In vitro* culture of *Bambusa vulgaris* explants in a green spectrum resulted in the lowest values for photosynthetic pigment contents (Fig. 4E).

Given the main results for the *in vitro* multiplication and elongation stages, the white and blue light spectra were the best treatments for most of the evaluated features, and green and red resulted in the worst *in vitro* responses.

According to the PCA, 70.0 % of the vigor frequencies of explants cultured under the white light spectrum were classified with a score of 1, resulting in a high correlation. The blue light spectrum was correlated with scores of 2 (50.0 % of frequencies), and red with scores of 3 (60.0 % of frequencies). The green light spectrum had low correlations, with no distinct responses (Fig. 5A).

The white light spectrum showed a high frequency for senescence, with 90.0% of the frequencies in scores 1 and 2. The red light spectrum had a high frequency in the score 2 (50.0 % of frequencies). Blue and green light spectra showed similar results, both highlighted in scores 1 and 3 (Fig. 5B).

When analyzing the scoring scale for tissue oxidation, the white (70.0 % of frequencies) and blue (80.0 % of frequencies) light spectra showed high frequency in score 1. The red light spectrum was more frequent in score 2 (50.0 % of frequencies). The green light spectrum showed low correlations with the scores, indicating the lack of a distinct response (Fig. 5C).

3.2. In vitro adventitious rooting stage

The highest survival was observed under the white light spectrum (80.0 %), which statistically differed from the blue (30.0 %), green (20.0 %), and red (30.0 %) spectra (Fig. 6A).

The light spectrum had an effect on the adventitious rooting of the explants. The clearest result was obtained when using the white light spectrum (50.0 %), which differed from the green (20.0 %), and no roots were observed under the blue or red light spectra (Fig. 6B). Thus, the efficiency of light spectrum in the production of clonal plants of *Bambusa vulgaris* may vary, and the use of a broad light spectrum is required, as in the white spectrum.

According to the PCA of the vigor, the highest frequency for score 1 (66.6 % of frequencies) was observed with the white light spectrum, resulting in a high correlation. The blue light spectrum showed a higher frequency for score 3 (44.4 % of frequencies); and green (50.0 % of frequencies) and red (57.1 % of frequencies) for score 4 (Fig. 7A).

The white light spectrum showed a higher frequency for senescence between scores 1 and 2, totaling 70.0 % of the observed frequencies. The blue light spectrum showed a higher frequency for score 3 (66.6 % of frequencies); and the green (50.0 % of frequencies) and red (57.1 % of frequencies) spectra for score 4 (Fig. 7B).

The white light spectrum showed a higher frequency of score 1 (50.0 % of the frequencies) for tissue oxidation. The blue light spectrum had the highest frequency for score 3 (77.7 % of frequencies), and the green (33.3–44.4 % of frequencies) and red (28.5–4 2.8 % of frequencies) spectra for scores 3 and 4 (Fig. 7C).

Leaf samples of *Bambusa vulgaris* explants cultured *in vitro* in the white light spectrum were collected to evaluate the anatomical characteristics of their surfaces, considering that this spectrum presented the strongest results in the adventitious rooting stage at 30 days (Fig. 6B). SEM revealed few microtrichomes and spines on the adaxial surface of the leaf blade (Fig. 8A), in addition to papillae and stomata (Fig. 8B). On the abaxial surface (Fig. 8C), there were many unicellular trichomes arranged in rows. Fig. 8D shows the papillae in greater detail. These were often associated with stomata, unicellular trichomes, hook-shaped trichomes, and microtrichomes. Overall, the SEM revealed the normal features of *in vitro* development in micropropagated plants under a white spectrum light.

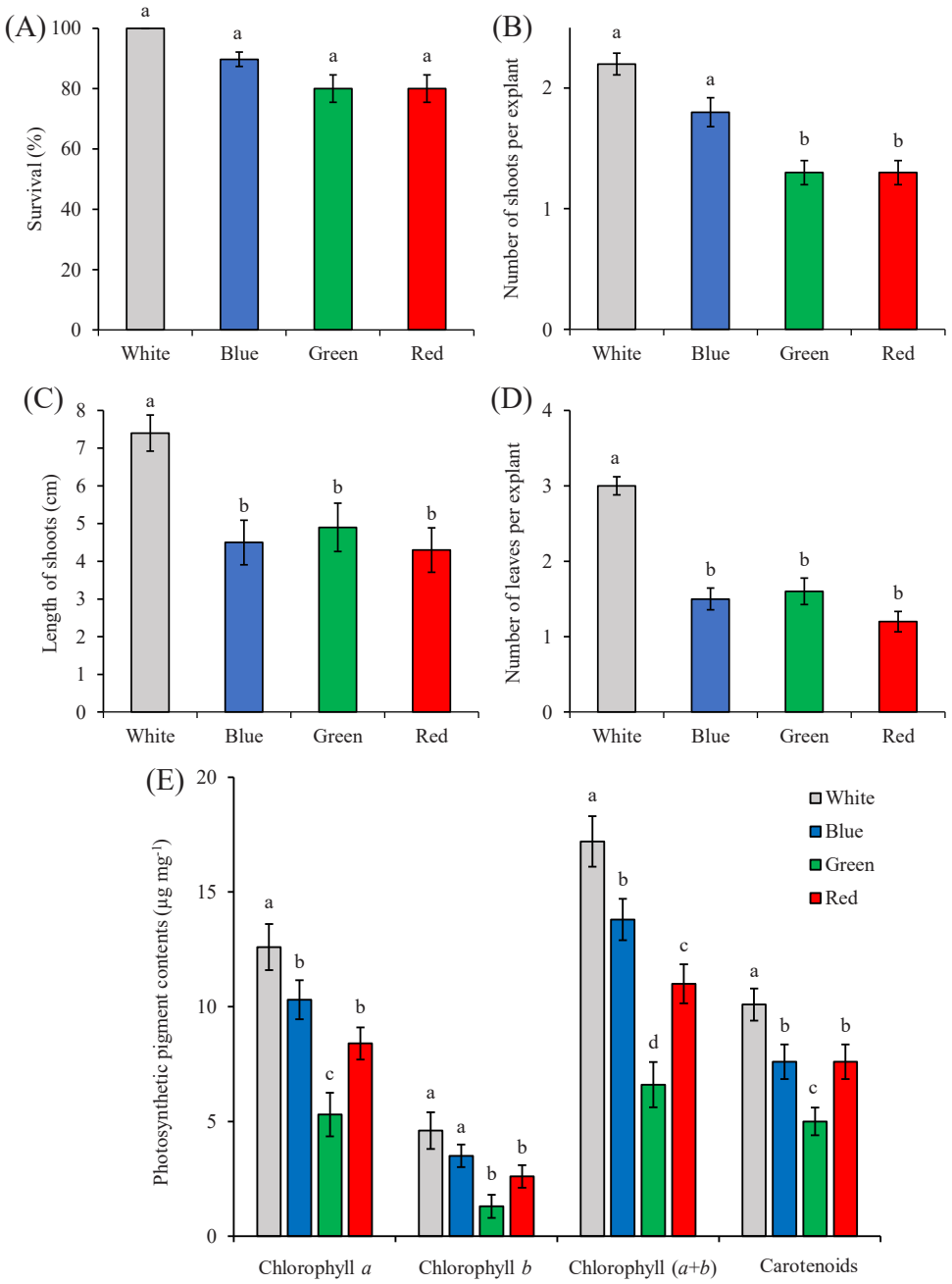

Fig. 4. Features of *Bambusa vulgaris* explants on *in vitro* multiplication and elongation stages at 30 days, based on the light spectra [white (Fig. 2A), blue (Fig. 2B), green (Fig. 2C), and red (Fig. 2D)]. (A) Survival. (B) Number of shoots per explant. (C) Length of shoots. (D) Number of leaves per explant. (E) Photosynthetic pigment contents. According to Tukey's test, means with the same letters were not significantly different ($p < 0.05$). Data presented as mean ± standard error.

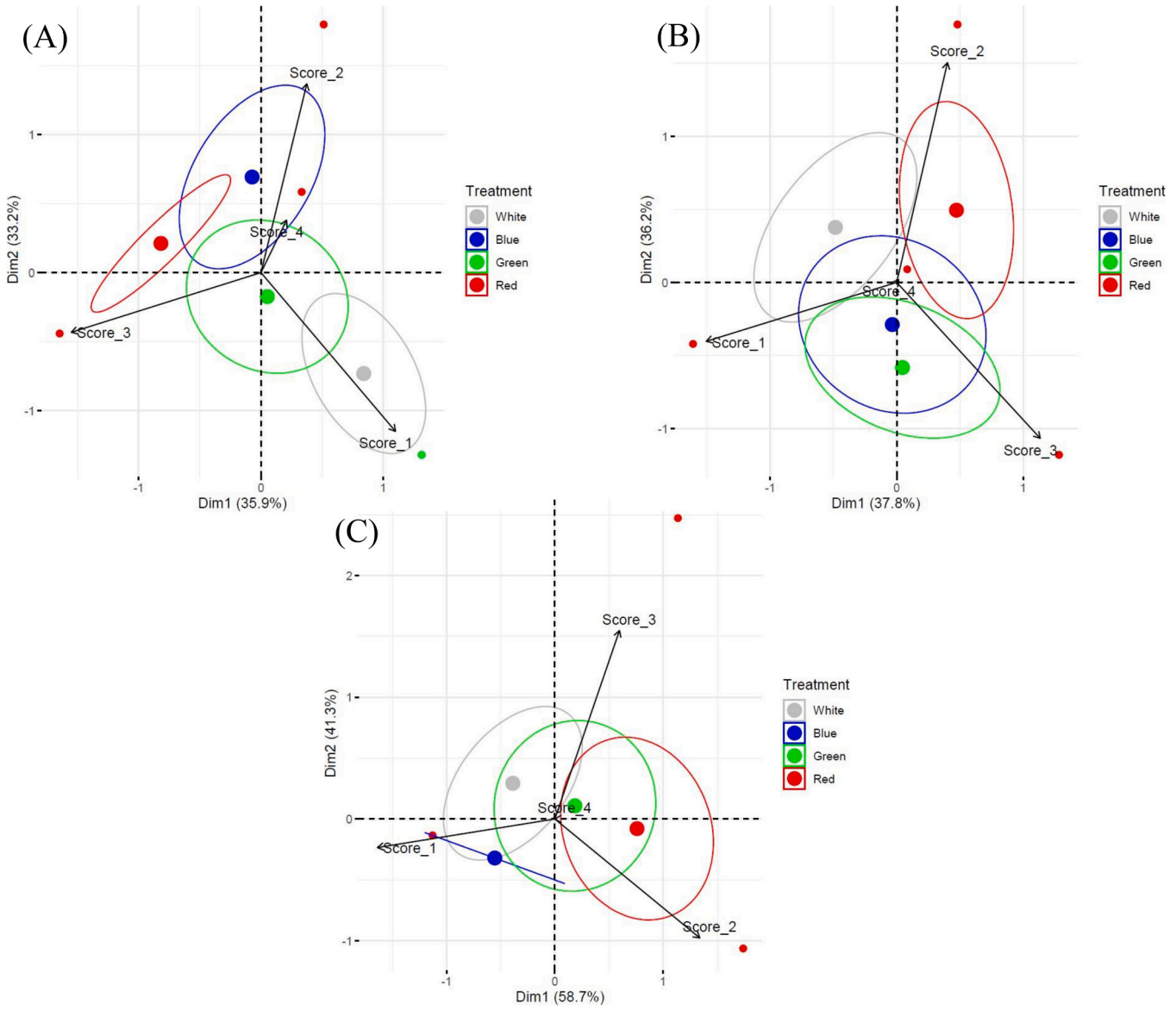

Fig. 5. Principal component analysis (PCA) of frequency of the vigor (Fig. 3A), senescence (Fig. 3B) and oxidation (Fig. 3C) of *Bambusa vulgaris* explants on *in vitro* multiplication and elongation stages at 30 days, based on the scoring scale under light spectra [white (Fig. 2A), blue (Fig. 2B), green (Fig. 2C), and red (Fig. 2D)]. (A) Vigor: 1 = Excellent: induction of bud and shoot with active growth, without apparent nutritional deficiency; 2 = Good: induction of bud and shoot but with reduced growth; 3 = Regular: small bud and shoot development and/or apparent nutritional deficiency; 4 = Poor: mortality of the explant; (B) Senescence: 1 = Null/low: absence of senescence or its presence on leaf border; 2 = Intermediate: presence of senescence throughout the leaf blade; 3 = Intermediate/high: presence of senescence throughout the leaf blade and stem; 4 = High: mortality of explant; (C) Oxidation: 1 = Null: no oxidation; 2 = Low: reduced oxidation of the explant base; 3 = Intermediate: oxidation and color change in the culture medium; 4 = High: complete shoot oxidation and mortality (*i.e.*, darkened of the culture medium). Dim1 = Principal component 1 (PC1), Dim2 = Principal component 2 (PC2).

4. Discussion

4.1. *In vitro* multiplication and elongation stages

The light spectra used for *in vitro* multiplication and elongation did not significantly affect the survival of *Bambusa vulgaris* explants (Fig. 4A). Trueman et al. (2018) reported that only a few explants needed to release shoots free of contamination for successful micropropagation. When micropropagated material is required, more explants with buds are necessary to rapidly increase the number of buds. It is possible to continue the elongation and rooting stages with more buds, which shows the importance of high survival percentages.

White and blue light spectra provided the highest means for the number of shoots per explant (Fig. 4B); and the white spectrum for the length of shoots (Fig. 4C) and number of leaves per explant (Fig. 4D). There are similar reports for *Bambusa oldhamii* Munro, which showed the highest number of shoots under 30.0 % of blue + 70.0 % of red light spectra, while the white light spectrum promoted a significant increase in the length of shoots (Silveira et al., 2020).

Plants have different morphophysiological responses to light spectra, with genetic and metabolic changes that result in plant growth and development (Oliveira et al., 2021; Zhou et al., 2021), and the light spectrum can influence the photosynthetic processes even with the supplementation of carbohydrates in the culture medium (George et al., 2008). This requirement varies according to the plant species (Jung et al., 2021). Regarding the biosynthesis of chlorophyll *a*, chlorophyll *b*,

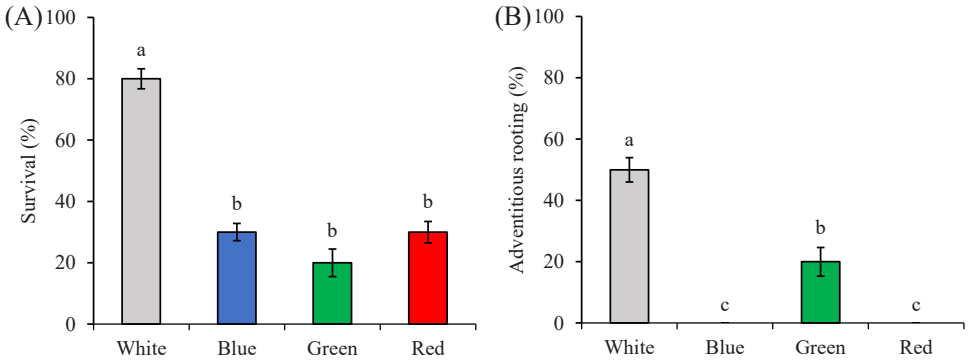

Fig. 6. Features of *Bambusa vulgaris* explants on *in vitro* adventitious rooting stage at 30 days, based on the light spectra [white (Fig. 2A), blue (Fig. 2B), green (Fig. 2C), and red (Fig. 2D)]. (A) Survival. (B) Adventitious rooting. According to Tukey's test, means with the same letters were not significantly different ($p < 0.05$). Data presented as mean ± standard error.

total chlorophyll (*a*+*b*) and carotenoids, the mixture of light spectra and the intensity of the light influenced the absorption of the light energy and the efficiency of the photosynthetic process (Jung et al., 2021; Gupta and Karmakar, 2017). In the cultivation of *Bambusa oldhamii*, the use of 100.0 % red LEDs resulted in relatively low chlorophyll and carotenoid contents. These results corroborate those found in the present study when comparing the fluorescent white light group with the other groups, as this light spectrum was associated with the highest photosynthetic pigment contents (Silveira et al., 2020).

Chlorophyll *b* content showed similar results between the white and blue light groups (Fig. 4E). This result may have occurred because blue light plays an essential role in synthesizing photosynthetic pigments, besides being responsible for the allocation of plastids to the leaves (Brenner et al., 2005). When evaluating the amounts of chlorophyll and carotenoids in the bamboo species *Guadua chacoensis* (Rojas Acosta) Londoño & P. M. Peterson, Polesi et al. (2019) observed that individuals with senescence (*i.e.*, yellowing or whitening of leaves and shoots) had less chlorophyll and carotenoids than individuals that did not show signs of senescence (*i.e.*, green shoots and leaves). This association was observed in the present study, where the explants subjected to the white light spectrum had the highest proportion of individuals with low senescence (Fig. 5B) and had higher photosynthetic pigment contents (Fig. 4E). The absorption of a broad light spectrum promotes a higher-energy state (Oliveira et al., 2021); thus, the efficiency of monochromatic light at producing plants varies between species, often requiring a combination of light spectra (Souza et al., 2021).

Senescence in bamboo species can be a natural process throughout the evolution of plastids or induced by some stress conditions (Polesi et al., 2019), such as poor light quality and intensity. In addition, these results may be influenced by internal environmental factors (*i.e.*, which affect the tissue vigor, senescence, and oxidation), such as smaller vials, temperature, and humidity (Souza et al., 2019; Ashrafzadeh and Leung, 2021).

According to Fig. 5C, higher scores of tissue oxidation (score 4) in *Bambusa vulgaris* tissues were little observed. These data align with the literature, which shows that *Bambusa vulgaris* explants do not offer high tissue oxidation when cultured *in vitro* (Furlan et al., 2018; Teixeira et al., 2021). In work with different light spectra for *Eucalyptus cloeziana* F. Muell. (Oliveira et al., 2015), *Corymbia citriodora* × *C. torelliana* (Souza et al., 2018) and *E. urophylla* × *E. grandis* (Souza et al., 2022a), low phenolic oxidation and adequate tissue vigor in the *in vitro* multiplication systems were observed. In general, tissue oxidation has been a common problem in micropropagation (Teixeira et al., 2021; Souza et al., 2019; Oliveira et al., 2016), and its reduction may improve the *in vitro* culture conditions of plants (Hartmann et al., 2011). Thus, according to our results, white and blue light spectra were the most appropriate for the *in vitro* multiplication and elongation stages of *Bambusa vulgaris*.

4.2. *In vitro adventitious rooting stage*

Variations in the response to light spectra have also been observed in the adventitious rooting stage in *Eucalyptus grandis* × *E. urophylla* explants (Souza et al., 2020b), which indicates that the white fluorescent light source has a positive effect on the production of clonal plants. Thus, knowledge of the relationship between light spectra, growth patterns, and plant rooting will better define the protocols for obtaining *Bambusa vulgaris* propagated plants, considering the positive results observed in the white light spectrum (Fig. 6A–B).

Vigor (Fig. 7A), senescence (Fig. 7B) and tissue oxidation (Fig. 7C) of the explants differed according to the light spectrum, and according to our results, the white light spectrum can be used to obtain vigorous plants, and with reduced senescence/tissue oxidation in the *in vitro* adventitious rooting of *Bambusa vulgaris*, considering that the highest frequency values for score 1 (Fig. 7A–C) were observed. One cause of this variation is related to the regulation of physiological processes, such as photomorphogenesis, which results in higher quality, production, and development of micropropagated plants (Oliveira et al., 2021; Gupta and Karmakar, 2017). In contrast, different culture environments can alter the metabolism, leading to denaturation of enzymes and low nutrient absorption, which affect vigor, senescence, and consequently the rhizogenic capacity at the base of the propagules (Batista et al., 2018; Miranda et al., 2020).

Microtrichomes and spines (Fig. 8A), and many stomata in leaves of *Bambusa vulgaris in vitro* cultured under white light spectrum were observed (Figs. 8B and 8D), denoting normal development. Stomata are fundamental structures in assisting plants to adjust physiological processes due to environmental variation. Stomata are responsible for optimizing the homeostasis of plants by modulating gas exchange between cells and the external environment (Hartmann et al., 2011; Molinari et al., 2020). This modulation may occur during *in vitro* culture when plants are subjected to different light spectra (Batista et al., 2018; Oliveira et al., 2021). Many papillae were seen on the abaxial and adaxial surfaces in combination with the stomata, which according to Luis et al. (2017), occur frequently and are essential for the taxonomy of some bamboo species.

Anatomical and structural studies of bamboo species *in vitro* grown are frequent, such as those of *Bambusa vulgaris* (Furlan et al., 2018; Teixeira et al., 2021; García-Ramírez et al., 2019), *Dendrocalamus asper* Backer ex K. Heyne (Montiel and Sánchez, 2006), and *Dendrocalamus giganteus* Munro (Yasodha et al., 2010), which highlights the importance of studies such as this one for establishing micropropagation protocols for these species.

5. Conclusions

Light spectra affect *in vitro* morphogenetic events in *Bambusa*

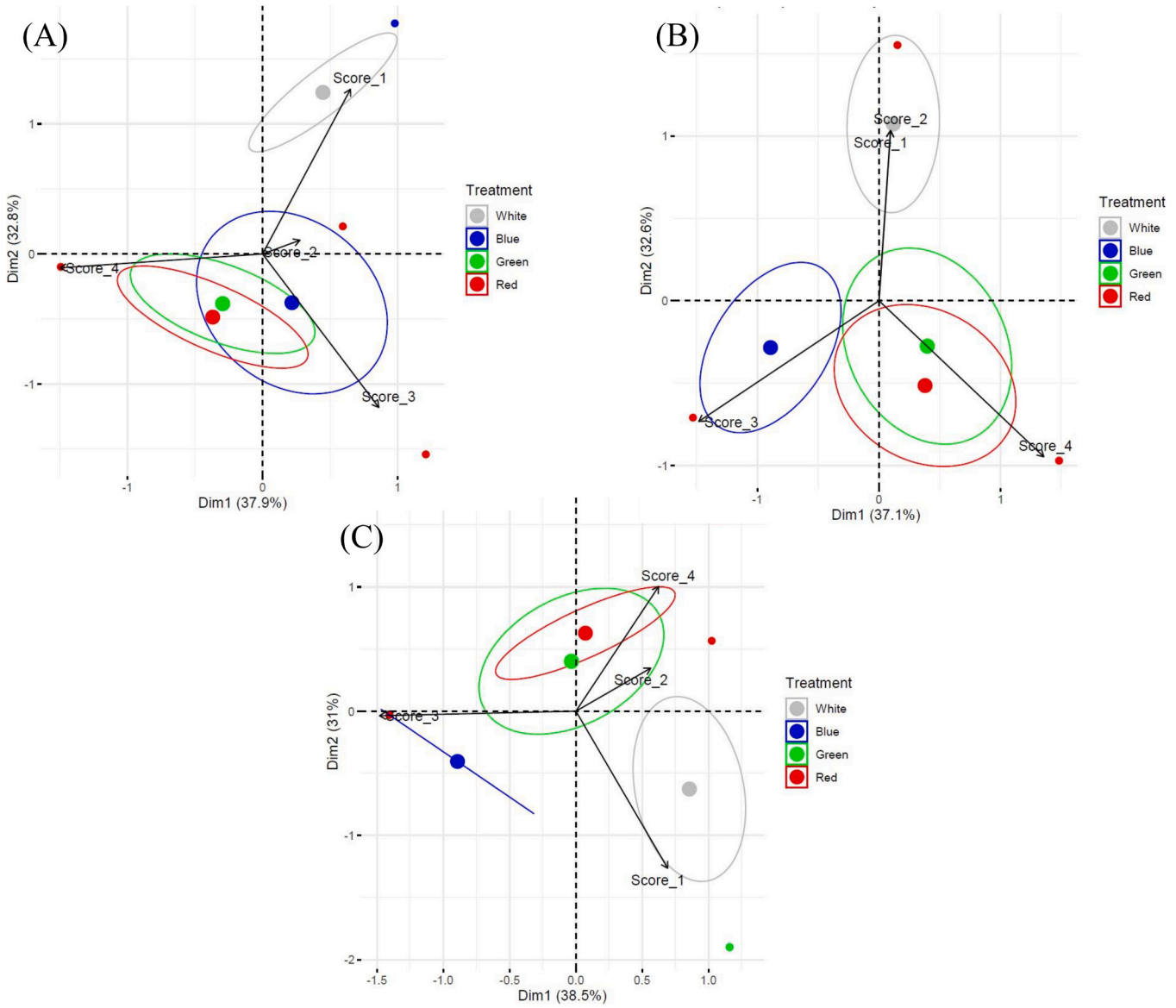

Fig. 7. Principal component analysis (PCA) of frequency of the vigor (Fig. 3A), senescence (Fig. 3B) and oxidation (Fig. 3C) of *Bambusa vulgaris* explants on *in vitro* adventitious rooting stage at 30 days, based on the scoring scale of the light spectra [white (Fig. 2A), blue (Fig. 2B), green (Fig. 2C), and red (Fig. 2D)]. (A) Vigor: 1 = Excellent: induction of bud and shoot with active growth, without apparent nutritional deficiency; 2 = Good: induction of bud and shoot but with reduced growth; 3 = Regular: small bud and shoot development and/or apparent nutritional deficiency; 4 = Poor: mortality of the explant; (B) Senescence: 1 = Null/low: absence of senescence or its presence on leaf border; 2 = Intermediate: presence of senescence throughout the leaf blade; 3 = Intermediate/high: presence of senescence throughout the leaf blade and stem; 4 = High: mortality of explant; (C) Oxidation: 1 = Null: no oxidation; 2 = Low: reduced oxidation of the explant base; 3 = Intermediate: oxidation and color change in the culture medium; 4 = High: complete shoot oxidation and mortality (*i.e.*, darkened of the culture medium). Dim1 = Principal component 1 (PC1), Dim2 = Principal component 2 (PC2).

vulgaris. White and blue light spectra were the most suitable for the *in vitro* multiplication and elongation stages of *Bambusa vulgaris*. The adventitious rooting was higher in explants cultured *in vitro* in the presence of a white light spectrum. Green and red light spectra were not favorable for the *in vitro* culture of the species. Explants grown *in vitro* in the white light spectrum showed normal leaf surface anatomy. These results are important for the production of clonal plants of the species using the micropropagation technique, in addition to favoring the optimization of *in vitro* culture.

CRediT authorship contribution statement

DSG conducting the experiment, statistical analysis, writing, and discussion. DMSCS statistical analysis, writing and discussion. SBF, LVM, AFD and ERK conducting the experiment and writing. GLT review and interpretation of results. GEB supervisor, was responsible for the obtention of funds and the administration of project, review and discussion. All authors revised the manuscript and approved the final version.

Declaration of Competing Interest

The authors declare that they have no conflict of interest and this manuscript was not submitted in another journal.

Data Availability

No data was used for the research described in the article.

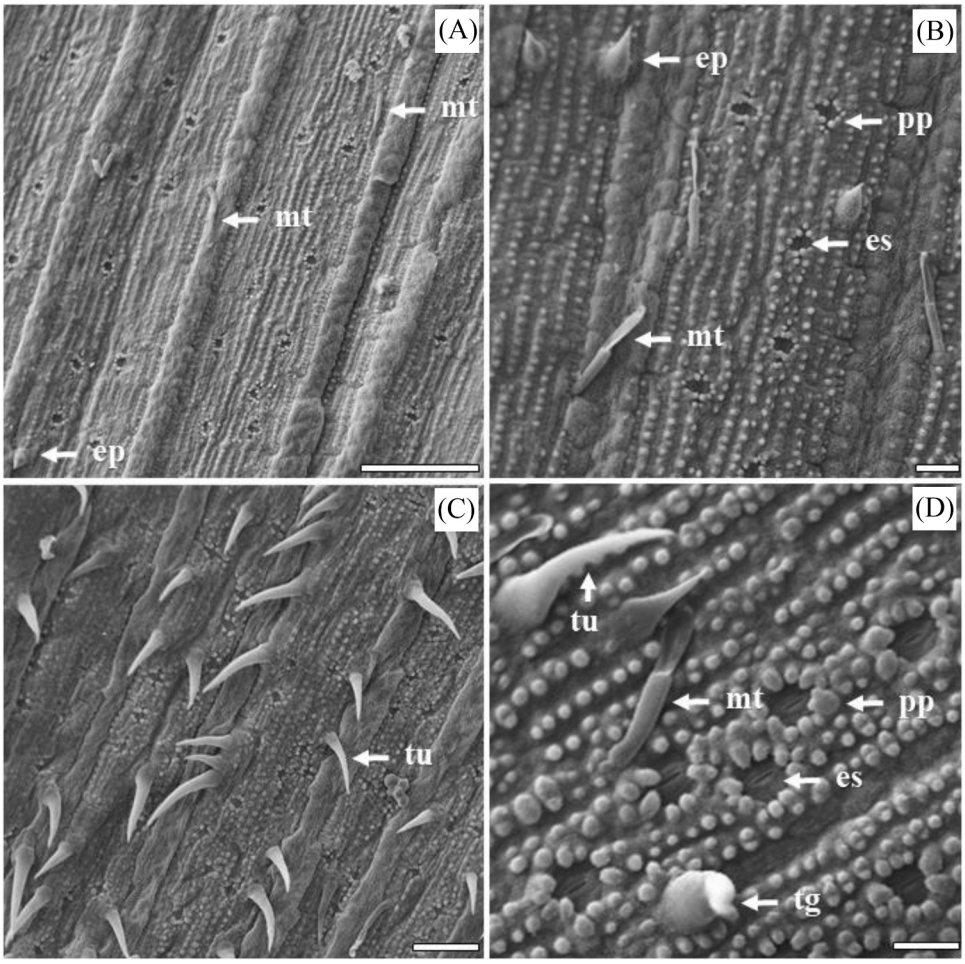

Fig. 8. Anatomical features of the leaf surface of *Bambusa vulgaris in vitro* grown under a white light spectrum (Fig. 2A) at 30 days. (A) Adaxial face with the presence of unicellular microtrichomes and spines. (B) Adaxial face. Details of the stomata. Presence of microtrichomes, spines, and papillae. (C) Abaxial face. Unicellular trichomes can be observed. (D) Abaxial face. Details of stomata with papillae, a hook-shaped trichome, stomata, a unicellular trichome, and a microtrichome. Abbreviations: (sp) spine, (st) stomata, (mt) microtrichome, (pp) papilla, (ht) hook-shaped trichome, (ut) unicellular trichome. Bars = (A) 100 μm; (B–C) 30 μm; and (C–D) 20 μm.

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