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Potential impact of different LED light spectra on callus induction, regeneration and plantlet growth of two cultivars of *Caladium bicolor*

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ABSTRACT

Purpose: *Caladium bicolor* is highly valued as both a landscape and indoor plant, primarily for its decorative appeal stemming from its diverse leaf shapes and vibrant, multicolored foliage. LED (light-emitting diode) lighting serves as a cost-efficient and potent means of promoting plant growth and development. The impact of different LED lights was investigated on callus induction, regeneration, and plantlet growth of two cultivars of *Caladium bicolor* ('White' and 'Red'). **Research Method**: Leaf explants were cultured on Murashige and Skoog (MS) medium supplemented with 1.5 mg L^{-1} IBA and 1 mg L^{-1} BA and moved to racks equipped with various LED lighting (100% red lights (R), 100% blue lights (B), 50% blue + 50% red lights (B+R), and 100% white fluorescent lamps (W)). **Findings**: Results showed W light was the best for maximum callus induction, leaf number, and plantlet height in both cultivars. Red + blue LED light spectrum motivated proliferation percentage of callus in both cultivars as compared to other light spectra. Conservation of 'White' caladium plantlets in R and B light spectra resulted in no hyperhydric micro shoot formation incidences. When examining various growth characteristics, it was evident that the B+R light spectrum of 'Red' caladium showed the best performance, while the B light spectrum in both cultivars had the least favorable outcomes compared to all other light spectra. **Research limitations**: There was no limitation. **Originality/Value**: Our findings offer a deeper understanding of how the quality of LED light impacts the *in vitro* propagation of caladium, potentially enhancing the cultivation of these plantlets through specific spectral exposure.

INTRODUCTION

Caladiums, specifically *Caladium bicolor*, are versatile plants that can thrive both indoors and outdoors. They are commonly used indoors as potted florists' plants due to their colorful foliage (Zhang et al., 2019). Caladiums are prized for their vibrant leaves and are known for their ability to add a pop of color to indoor spaces with relatively low light levels (Stamps & Savage, 2011). Their adaptability to different light conditions and the availability of various cultivars with unique leaf colors make caladiums a popular choice for indoor plant enthusiasts looking to brighten up their living or working spaces (Deng & Harbaugh, 2006). *In vitro* studies have shown successful micropropagation of caladium using specific hormone concentrations, leading to efficient shoot and root regeneration for propagation purposes (Kokalis-Burelle et al., 2017). Overall, caladium is not only a visually appealing house plant but also a subject of genetic and cytogenetic interest for breeding and cultivation advancements.

Different light spectrums have a significant impact on callus formation and germination in plant tissue cultures. Studies on wheat and *Prunella vulgaris* callus cultures have shown that specific light wavelengths influence biomass accumulation, secondary metabolite production, and antioxidant activity (Blidar et al., 2021; William & Carpenter, 1990). It has been reported that different light wavelengths have the ability to regulate various plant processes, including photosynthesis, germination, flowering, biomass accumulation, and phytochemical synthesis (Jafari et al., 2023). Additionally, research on lettuce, cucumber, and sweet pepper seedlings revealed that red and blue LEDs promote better growth responses compared to cool-white fluorescent light, affecting parameters like shoot length, root collar diameter, and dry matter content (Fazal et al., 2016). Furthermore, investigations on *Capsicum annuum* tissue culture demonstrated that red, white, blue, and green light can induce callus formation, with red light being optimal for bud differentiation while blue and green light inhibits it (da Silva et al., 2016). Therefore, the choice of light spectrum is crucial for optimizing callus formation and germination in different plant species.

Different light spectra have diverse effects on callus formation. Blue and purple light induce increased red coloration in callus, while yellow light promotes the greatest callus proliferation. White light enhances biomass production, total phenolic contents, and flavonoid contents in *Moringa oleifera* callus cultures (Bajwa et al., 2023). Red LED light significantly contributes the biomass accumulation in *Passiflora* calluses and induces the highest production of bioactive substances (Santos-Tierno et al., 2021). *Scutellaria baicalensis* calluses exposed to blue light show increased flavone content, particularly baicalin (Costine et al., 2022). *Eclipta alba* callus cultures under red light exhibit maximum dry weight and enhanced phenolics and flavonoids content, crucial for therapeutic potential (Khurshid et al., 2020). Overall, different light spectra play a significant role in modulating callus formation and the accumulation of bioactive compounds in various plant species.

Red-blue light and darkness were found to be optimal for biomass, protein content, and cell viability in *Hyoscyamus reticulatus* callus, while red and blue lights induced oxidative damage and altered cell morphology (Hassanpour, 2021). In *Hyptis marrubioides* callus, blue light negatively impacted on phenolic compound synthesis, while red light stimulated specific metabolite production, and darkness led to increased accumulation of certain bioactive compounds (Dantas et al., 2021). *Eutrema salsugineum* callus lines showed lower oxidative stress under red light than blue light, which induced higher antioxidant enzyme activities (Pashkovskiy et al., 2018). Blue light was crucial for enhancing flavone content in *Scutellaria baicalensis* callus tissue (Stepanova et al., 2020). Kazemi et al. (2023) demonstrated that using solely 100% blue light was unsuitable for optimizing the growth and biophysical

characteristics of the electron transport chain in begonias. For all three tested begonia cultivars, a combination of red, blue, and white light proved beneficial, promoting better growth and improving chlorophyll fluorescence parameters in the plants.

This study aimed to determine the optimal proportions of blue, red, blue + red LED, and white fluorescent lights suitable for callus induction, regeneration, and plant growth of two cultivars of *Caladium bicolor*. In this study, morphological and physiological changes were evaluated under different lighting conditions. We aim to achieve a more environmentally friendly production method for *C. bicolor* with low energy consumption and high efficiency.

MATERIALS AND METHODS

Plant material and culture establishment

This research was conducted 2023 at Ardakan University, Ardakan, Iran. In this research, vigorous, healthy young leaves from two distinct *C. bicolor* plant cultivars, by two different color 'White' and 'Red', were used as the initial explant. 'Red' cultivars typically feature vibrant red or pink variegated leaves, often with deep green margins. The leaves may exhibit a striking pattern of veins and spots, enhancing their decorative appeal. 'White' cultivars are characterized by striking white or cream-colored leaves, often with green margins or veins. Similar to 'red' cultivars, 'white' varieties may have unique patterns, including speckles or splashes of green, which add visual interest. The leaves are also heart-shaped, but the overall appearance may appear more delicate due to the lighter colors. To eliminate any potential contaminants, a thorough surface sterilization protocol was performed. The procedure began with triple washing of the leaves in sterile distilled water, followed by rinsing the leaves in a disinfectant solution containing 0.05% citric acid and 0.1% mercuric chloride for 3 minutes. To finalize the sterilization, the leaves were subjected to three additional washes in a solution with 0.05% citric acid to ensure contaminant-free explants for subsequent experimental procedures (Rezaie et al., 2018). Three explants were meticulously placed into a glass jar, measuring a height of 10 cm and a diameter of 6 cm. The explants underwent cultivation in MS (Murashige & Skoog, 1962) medium, which was supplemented with the addition of Indole-3-butyric acid and 6-benzyladenine (IBA at 1.5 mg L^{-1} and BA at 1 mg L^{-1}), to induce callus formation. Each individual jar was filled with 25 ml of MS medium and contained three leaf explants. Agar was added into the MS basal culture media at a density of 7 g L^{-1}, while sucrose was supplemented at a rate of 30 g L^{-1}. Explants were cultured under different light treatments in the controlled conditions in the growth chamber, each culture underwent a cyclical exposure to 16 hours of light and 8 hours of darkness. During the dark period, the temperature inside the chamber was regulated around 18 ± 2 °C, while it was adjusted to approximately 23 ± 2 °C during the light period. Furthermore, the photosynthetic photon flux density (PPFD) ranged from 34 to 40 µmol m^{-2} s^{-1}.

Light treatments

Explants were grown in growth chambers with various light treatments including 100% red LED (660 nm) lights (R), 100% blue (450 nm) LED lights (B), 50% red (660 nm) + 50% blue (450 nm) LED lights (RB), with 40 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and 100% white fluorescent lamps (W) with 100 µmol m^{-2} s^{-1} PPFD (measured with LI-250A; LI-COR Biosciences, Lincoln, NE, USA). These spectra were selected because of their relevance to leaf photosynthesis. Wavelengths of R and B lights were considered the main absorption spectra of chlorophyll (Aalifar et al., 2020), and the W light (WL, containing all light spectra in the range of photosynthetic active radiation) was used. The spectral distributions were estimated using Sekonic C7000 SpectroMaster spectrometer (Sekonic

Corp., Japan) in the wavelength range of 300–800 nm. LEDs were fixed in the number of aluminum boxes (100 × 110 × 50 cm^3) (Iranian Grow Light company, Iran). The distance between the source of light and plant jars were 50 cm. Adjustment of the light intensity in all mentioned growth chambers to the PPFD of 250 ± 10 µmol m^{-2} s^{-1} was performed. Three glass jars (each containing three explants) from each caladium cultivar were grown under each light spectrum, following a light/dark cycle of 16/8 hours, at temperatures of 23 and 20 ± 2 °C, and relative humidity of 20 ± 2 %. Plants were grown at five different light spectra and evaluated after 16 weeks in regard to their growth and biophysical parameters.

Following eight weeks, each jar was individually checked to record "the percentage of callus induction", the percentage of indirect regeneration, and finally "the percentage of shoot/bud regeneration (i.e., direct regeneration). Furthermore, "the percentage of hyperhydric micro shoots formation" was also documented.

Proliferation and rooting

For the proliferation phase, individual shoot explants, which were cultivated in the preceding stage and measured about 2-3 cm in height, were employed. In this context, each individual shoot explant was cultivated using MS basal salts and vitamins, supplemented with 30 g L^{-1} sucrose, 7.0 g L^{-1} agar, and 0.05 mg L^{-1} Gibberellic acid (GA3; specifically for proliferation). These conditions were maintained for a growth period of four weeks. In the following, the resultant 4-week-old shoots were transferred into the plant growth regulator-free MS medium for about 2 weeks. Lastly, various parameters such as plantlet height, leaf number, shoot number, root number, root length, were documented for all the 10-week shoots. The length of each shoot and root was measured from the agar starting point to the tip using a ruler in centimeters. The number of shoots and roots was counted for each jar.

Statistical analysis

All statistical analyses were carried out using a factorial experiment with two main factors including two individual cultivars ('White' and 'Red') and four different light treatments (Red, blue, red+blue, and white spectra). In this research, it was used of the factorial test based on a completely random design. The means was analyzed with ANOVA test using SPSS (version 26.0, SPSS Inc., Chicago, IL, USA) software. The significant variation between the treatments data was computed using Duncan's multiple-range tests (DMRT) at $p \leq 0.05$. To cluster all the treatment(s), hierarchical cluster analysis (HCA) combined with heatmap visualization was applied using the CIMMiner web tool. CIMMiner generates color-coded Clustered Image Maps (CIMs) ("heat maps") to represent "high-dimensional" data sets. The principal component analysis (PCA) was also applied to recognize the associations among the variables using the Paleontological Statistics Software (PAST version 4.03).

RESULTS AND DISCUSSION

Callus induction and regeneration

All treatments successfully stimulated callus induction in both cultivars. Significantly, the R spectrum demonstrated the greatest efficacy in callus induction across both cultivars, achieving a complete success rate of 100% (Fig. 1A). Also, both W and B+R spectra effectively stimulated callus formation in the 'White' and 'Red' cultivars. The lowest percentage of callus induction (30.33 and 20.00%) was observed in the B spectrum applied to both cultivars (Fig. 1A).

Regeneration rates displayed considerable variation across all four spectra, ranging from 20% to 80%, as depicted in Figure 1B. The 'Red' caladium showed maximum callus regeneration

under the B+R spectrum, while its minimal regeneration was noted in the B spectrum (Fig. 1B). Inducing callus is a crucial phase in reverting differentiated plant tissues to undifferentiated, facilitating *in vitro* morphogenesis to generate plantlets (Tůmová et al., 2010). Nonetheless, the process of callogenesis *in vitro* is influenced by multiple variables, including the nature and concentration of plant growth regulators, the age and kind of the explant, the composition of the growth media, and physical factors such as pH, temperature, and lighting conditions within the growth chamber (Khan et al., 2020). Light signaling pathways play a crucial role in controlling growth, differentiation, and metabolic processes in callus cultures. Distinct wavelengths of light elicit varied responses that promote biomass production in *in vitro* callus cultures (Ali & Abbasi, 2014; Fazal et al., 2016; Khan et al., 2019). The percentage of callus induction under constant white light can be attributed to the heightened levels of energy (100 µmol m^{-2} s^{-1} PPFD), which significantly influence the physiological activities, morphogenesis, and biochemical pathways in plants (Bajwa et al., 2023). This includes the synthesis of both primary and secondary metabolites. Comparable outcomes were noted in callus cultures of *Moringa oleifera* when subjected to continuous white light (24 hours) succeeded by exposure to yellow light (Bajwa et al., 2023). Additionally, comparable results were noted in cell cultures of *Withania somnifera* when exposed to continuous white light, which led to a significant increase in biomass accumulation (Adil et al., 2019). Previous research has demonstrated that *Artemisia absinthium* L. callus cultures exhibit significantly higher biomass accumulation under continuous white light compared to other lighting conditions. Similarly, callus cultures of *Lepidium sativum* L. exposed to continuous white light (24 hours) also showed enhanced biomass growth (Ali & Abbasi, 2014; Ullah et al., 2019).

Proliferation and elongation of the adventitious shoots

The percentage of proliferation varied among four spectra, ranging from 15% (B light treatment on 'Red' caladium) to 100% (B+R treatment on 'Red' cultivar, Fig. 2B). During the proliferation phase, which involves an increase in plant and internode length, additional explants are collected from the plantlet. This practice enhances productivity in commercial tissue culture operations, as was also observed in the current study.

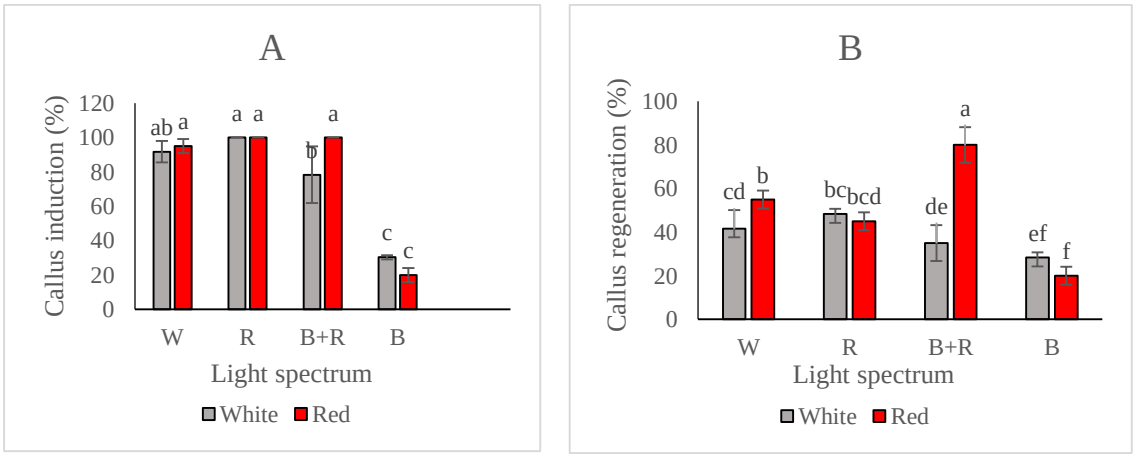

Fig. 1. Interaction effect of cultivars and different light spectra on A) callus induction and B) regeneration of two cultivars of *Caladium bicolor* on the MS medium. The distinct letter(s) indicated a significant difference ($p \leq 0.05$) as determined by Duncans multiple range test.

In this research, the application of combined blue and red LED on tissue-cultured leaf explants of 'White' and 'Red' caladium varieties resulted in an increase in proliferation rates (134.80% and 81.81%, respectively), when compared to traditional white fluorescent light. This finding aligns with similar outcomes observed in studies conducted on Vanilla (Bello-Bello et al., 2016). They discovered that the growth of *Vanilla planifolia* was hindered when exposed solely to blue and red LED. Conversely, the most effective propagation was achieved under white LED, and combine of blue and red LEDs, as well as under fluorescent light. The growth rate of *Panax vietnamensis* was found to be twice as high under red: blue LED lights at a 60:40 ratio, with an observed value of 11.21. In contrast, fluorescent lights resulted in a growth rate of 5.8. (Nhut et al., 2015). The effectiveness of using combined LEDs for the growth and development of plants, as opposed to monochromatic lighting, has been documented for various species including *Prunus domestica* (Nacheva et al., 2023), *Chrysanthemum* sp. (Kim et al., 2004), and *Lycium barbarum* L. (de Oliveira Prudente et al., 2019).

Plantlet height

The maximum plantlet height (10.33, 11.66, and 11.00 cm) was achieved in both cultivars in W treatment and 'White' caladium in R light spectrum. Other treatments did not show a significant difference (Fig. 6B).

In this research, it was found that the spectrum of light significantly affects the shoot length produced. It has been observed that plantlets of *Prunus domestica* subsp. *insititia* cultivated under red LED exhibited the longest average stem length, a finding that diverges from the outcomes presented in our study (Nacheva et al., 2023). Throughout the propagation stage of *Pyrus communis* 'Arbi', red LED displayed important advantages: it produced optimal shoot height and leaf surface (Lotfi, 2022). Red light typically promotes the elongation of stems and the lengthening of the spaces between nodes (Poudel et al., 2008; Li et al., 2010). Furthermore, blue light impedes cellular proliferation and modulates gene expression to restrict the elongation of stems (Lin et al., 2013). Enhanced internode observed under red and white light spectra can be attributed to augmented cell elongation and cell proliferation. These processes, which involve both the division and elongation of cells, correlate with elevated levels of gibberellic acid (GA) (Nacheva et al., 2023). The findings suggest that GA signaling is essential for the elongation of shoots in caladium *in vitro* explants under red and white light conditions.

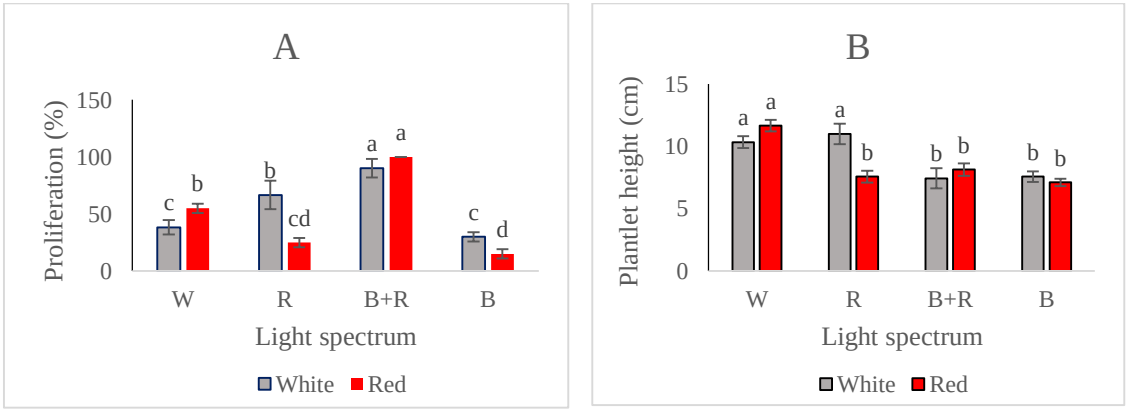

Fig. 2. Interaction effect of cultivars and different light spectra on (A) proliferation and (B) plantlet height of two *Caladium bicolor* cultivars on MS medium. The distinct letter(s) indicated a significant difference ($p \leq 0.05$) as determined by Duncans multiple range test.

Shoot and leaf number of regenerated plantlets

There was a significant difference in shoot proliferation among the various light spectra (Fig. 3A). The maximum of average shoot number of 6.33 shoots per jar was observed in R light treatment in 'White' cultivar (Fig. 3A). The optimum light spectrum for leaf production was evident in W treatment in both cultivars (Fig. 3B). In the W light spectrum used, 80 and 85 leaves were observed per jar in both 'White' and 'Red' caladium cultivars, respectively (Fig. 3B). However, B light for both cultivars was not successful in leaf production, resulting in the lowest average number of leaves (6.66 and 5.66 leaves per jar, respectively).

In the commercial propagation of caladium via tissue culture, the quantity of lateral shoots produced from a single explant is crucial. Our study demonstrated that utilizing red LED lighting enhanced the shoot multiplication in *in vitro*. This augmentation in shoot numbers facilitated a greater yield of explants during the proliferation phase and boosted the rate of proliferation. In 'White' caladium, the proliferation rate of lateral shoots experienced a 26.6% increase under red LED when compared to fluorescent lighting. Red light resulted in an 88.66% reduction in the number of shoots for 'Red' caladium when compared to white fluorescent light. The findings indicate that red LED light has a beneficial impact on the development of lateral shoots in tissue-cultured nodal shoots of the 'White' caladium cultivar. Seminally, research performed on Stevia (Ramirez-Mosqueda et al., 2017) and plum plantlets demonstrated that exposure to red LED light spectra enhanced the shoot proliferation per explant (Nacheva et al., 2023). Conversely, studies have shown that employing a mix of blue and red LED light spectra can lead to a significant increase in the number of shoots per explant in both Canola and Dendrobium (Lin et al., 2011; Lin et al., 2013).

The impact of differential light spectra on plants varies considerably depending on the intensity and quality of the light used, the duration of exposure, and the specific type of plant involved (Fazal et al., 2016). Nacheva et al. (2023) observed that the maximum leaf count occurred under red light, a finding that diverges from the results presented in this study.

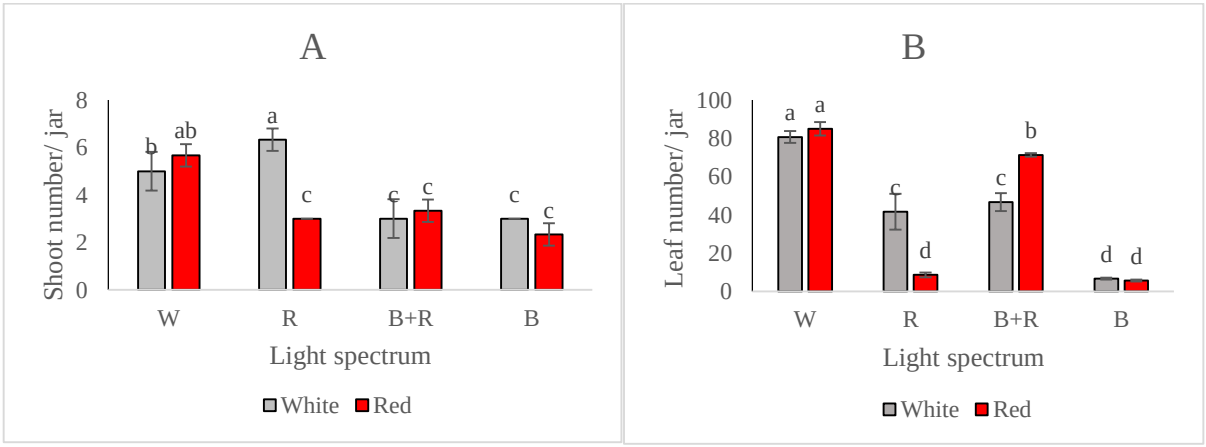

Fig. 3 Interaction effect of cultivars and different light spectra on (A) shoot and (B) leaf number of two cultivars of *Caladium bicolor* on the MS medium. The distinct letter(s) indicated a significant difference ($p \leq 0.05$) as determined by Duncans multiple range test.

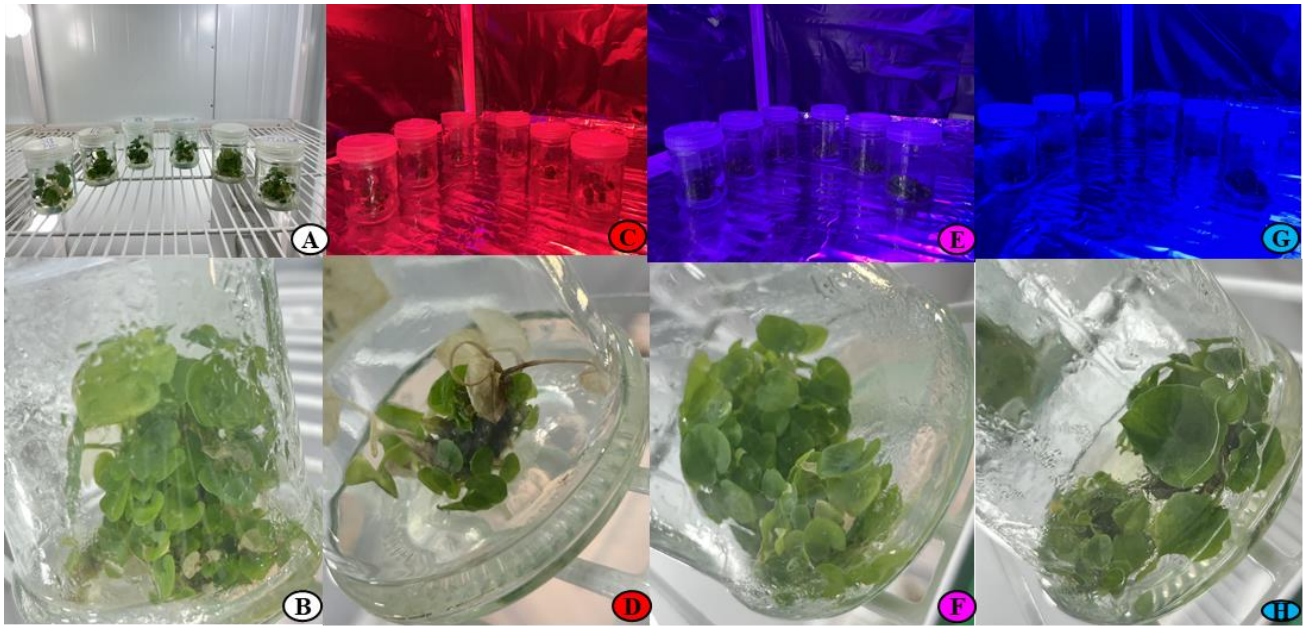

Fig. 4. Effects of different light spectra on the shoot regeneration and proliferation of *Caladium bicolor* cv. 'Red'. (A) and (B) 100% White fluorescent lamp, (C) and (D) 100% Red LED light spectrum, (E) and (F) 50% Blue+ 50% Red LED and (G) and (H) 100% Blue LED spectrum.

Rooting of shoot and plantlet development

Eight weeks into the experiment, the initiation of root induction was started, with the associated data being documented after a period of 10 weeks. Next, the following parameters were measured and recorded.

Number of roots and root length

Figure 4 illustrates that the number and length of root systems are significantly influenced by various lighting conditions. The greatest root count observed was in the 'Red' caladium under the W light spectrum, averaging 7.00 roots per jar as shown in Figure 6A. The minimum root number was recorded for both varieties under B treatment ('White' and 'Red' 7.00 and 6.00 roots/jar, respectively) and for the 'Red' variety under R light was recorded 8.33 roots per jar (Fig. 6A).

The 'Red' cultivar exposed to W light (5.53 cm), as well as both varieties under the B+R light spectrum ('White' and 'Red' 5.83, and 6.00 cm, respectively), exhibited the greatest root lengths, measuring. In contrast, the shortest root lengths were recorded in the B light spectrum for both cultivars, with measurements of 3.66 and 3.33 cm, as illustrated in Figure 5B. The root system exhibited growth under all lighting conditions, as illustrated in Figure 4. The greatest quantity of roots was observed under W light, surpassing those grown under combined B+R light. Similarly, the longest average root lengths were achieved with the B+R light, notably exceeding those under W light in 'Red' caladium. These results diverge from the findings of Elsabaa et al. (2022) regarding potato *in vitro* culture, where an increase in both root number and length was noted predominantly under red light, followed by blue light.

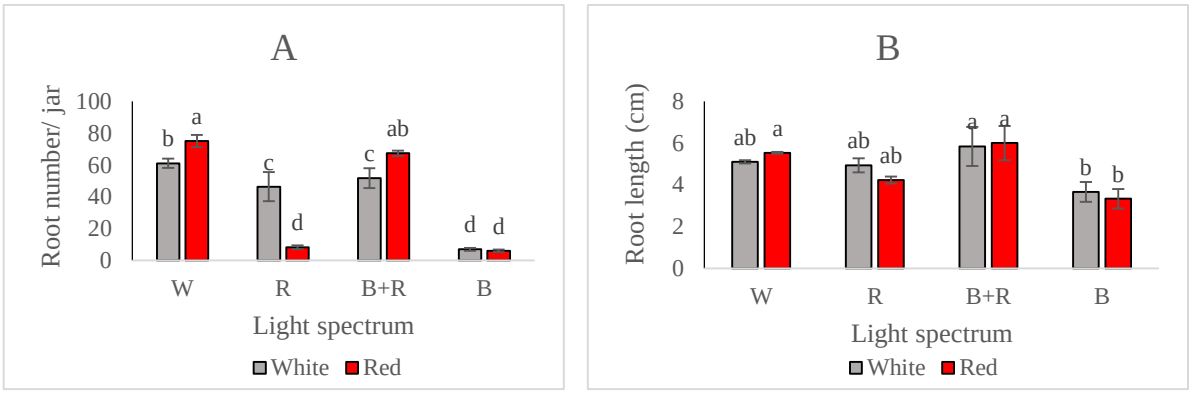

Fig. 5. Interaction effect of cultivars and different light spectra on (A) root number and (B) length of two cultivars of *Caladium bicolor* on the MS medium. The distinct letter(s) indicated a significant difference ($p \leq 0.05$) as determined by Duncans multiple range test.

Hyperhydric micro shoots formation

Hyperhydricity is a morpho-physiological disorder that manifests as diminished structural integrity in plantlets, stemming from excessive tissue hydration, insufficient lignification, and compromised stomatal functionality. These alterations can decrease the efficiency of *in vitro* regeneration and lower the survival rates of regenerates during the acclimatization process (Cassel & Curry, 2001; Gao et al., 2018). In this research, the preservation of 'White' caladium plantlets under R and B light spectra diminished the occurrence of hyperhydric micro shoots formation (Fig. 6).

In this research, 'White' caladium plantlets exposed to R and B light spectra showed minimal hyperhydric micro shoot formation, with no evidence of the disorder. The greatest proportion of hyperhydrated shoots was recorded in 'Red' plantlets under R light spectrum, reaching a percentage of 30% (Fig. 6). Insufficient chlorophyll and elevated moisture levels can disrupt physiological functions, potentially resulting in the translucency of shoots and leaves characteristic of hyperhydricity (Cassel & Curry, 2001). Elevated relative humidity, reduced lighting, gas build-up within the culture vessel, prolonged subculture periods and frequency, variations in the type and concentration of gelling agents, and hormonal imbalances contribute to hyperhydricity in plantlets. This condition can result in significant losses during the micropropagation process (Rojas-Martinez et al., 2010; Barbosa et al., 2013; Isah, 2015).

Observations indicate a decrease in chlorophyll a and b levels in the hyperhydric leaves of strawberry (Barbosa et al., 2013) and vanilla (Sreedhar et al., 2009) micro shoots. Isah (2019) demonstrated that the incidence of hyperhydricity in *C. bicolor* was higher in liquid medium cultures compared to solid ones, likely because the unrestricted diffusion of the medium into plant tissues induced conditions akin to anoxia. Incorporating 7.5 µM of silver nitrate effectively decreased the occurrence of the condition compared to other tested concentrations. However, alterations in the concentration of the gelling agent and variations in the light exposure duration did not yield effective results.

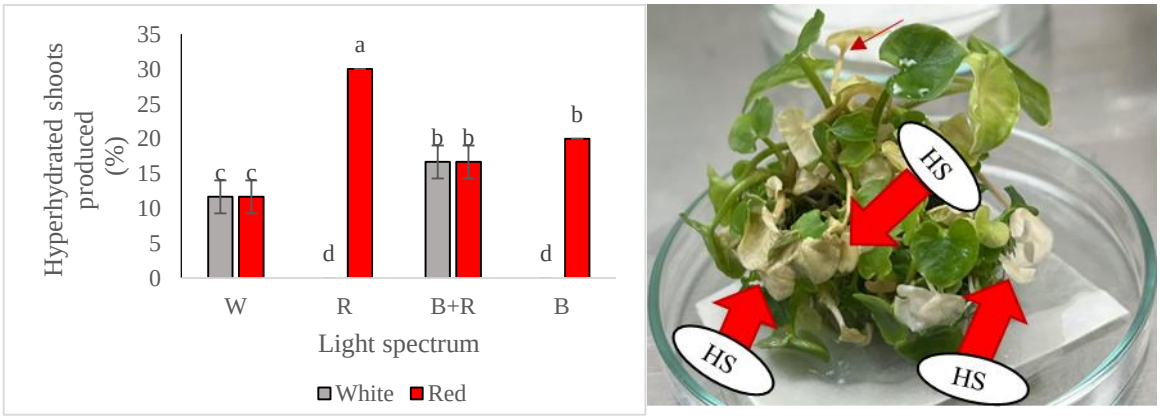

Fig. 6. Interaction effect of cultivars and different light spectra on hyperhydrated shoots (HS) of two cultivars of *C. bicolor* on the MS medium. The distinct letter (s) indicated a significant difference ($p \leq 0.05$) as determined by Duncans multiple range test.

Multivariate analyses

Heatmap-based cluster analysis

To enhance our comprehension of the ways in which traits react to various light spectrum treatments, we employed a heatmap-based clustering analysis (Fig. 7). The dendrogram illustrates the clustering of various light spectra according to their comparable efficacy. This also demonstrates how similar morphological traits are grouped, mirroring their distinct responses to varying degrees of radiation (Fig. 7). The horizontal axis of the heatmap represents different growth indicators, whereas the vertical axis displays varying degrees of light spectra. In the diagram, the deepest blue hue indicates the lowest values, while the brightest red denotes the highest values. Values that fall between these extremes are depicted using a gradient that transitions smoothly from one to the other.

The clustered heatmap revealed that the four distinct treatments formed two separate clusters. The first group divided into two subgroups consisted of the B+R spectrum of 'Red' cultivar and W light of both cultivars and R and B+R light spectra of 'White' caladium in the second subgroup. The B+R light spectrum outperformed the other treatments in terms of the majority of characteristics, demonstrating the significant impact of B+R light on the observed traits. The second major group was also divided into two subgroups consisting of R light in 'Red' caladium as first subgroup and B light spectrum in both caladium cultivars as the second subgroup. Among these treatments, only B light spectrum in both cultivars had the minimum values for most attributes.

In analyzing different growth traits, it became clear that the B+R light spectrum yielded superior results for the 'Red' caladium cultivar. Conversely, the B light spectrum resulted in the least favorable growth across both examined cultivars when compared to other light spectra. Conversely, characteristics including the number of roots and leaves, the percentage of regeneration and proliferation, and the induction of callus were categorized within the primary group. This classification suggests that the plants exhibited uniform reactions across varying light spectra concerning these particular traits. Within the secondary group, two distinct subgroups were established. The first subgroup encompassed measurements related to root length, shoot count, and the height of plantlets. Conversely, the second subgroup specifically grouped instances of hyperhydrated shoots (Fig. 7).

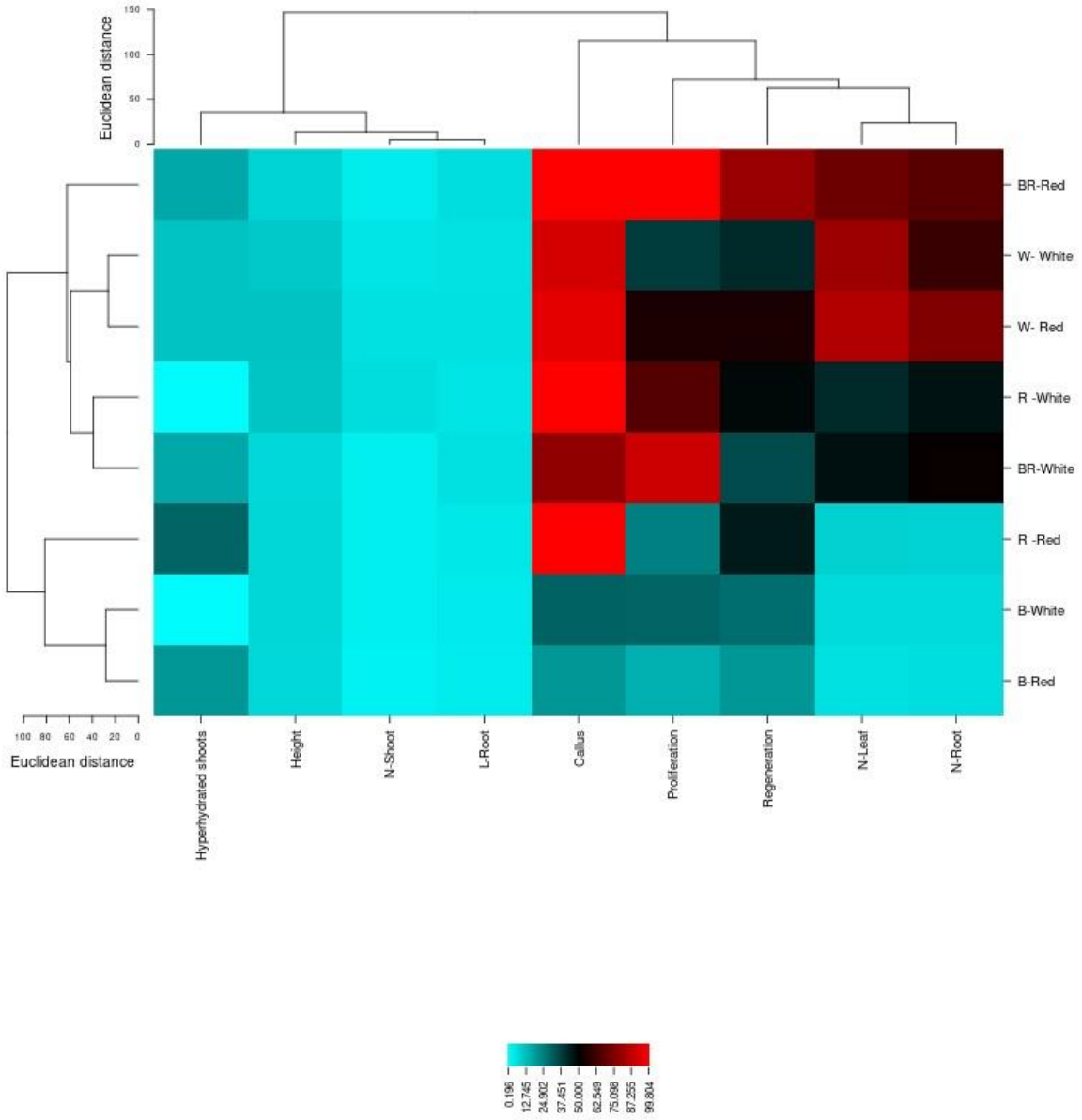

Fig. 7. Clustered heatmap for grouping of all 4 light spectra treatments (W, B, BR and R) in terms of the studied traits obtained from the *in vitro*-raised plantlets of both *Caladium bicolor* cultivars. Rows were clustered using *Euclidean distance* and average linkage. Columns were clustered using *maximum distance* and *McQuitty linkage*.

Principal Component Analysis (PCA)

PCA, or Principal Component Analysis, is a technique used in unsupervised learning to reduce the dimensionality of large data sets, thereby simplifying the complexity of the data. This method simplifies the comprehension of data while maintaining its informational integrity, thereby facilitating the graphical depiction of complex data sets. In this study, PCA was employed to explore the association and influence of various spectral light treatments on two varieties of *C. bicolor* (Fig. 8). According to the PCA, the first component accounted for 73.92% of the overall variance, while the second component represented 11.28%. Consequently, two distinct groups emerged from this analysis. Considering the first component, it was shown that the variables related to the traits are mainly includes length of

root, number of shoots and plantlet height. So, B+R light spectrum in 'Red' caladium had the highest values for these traits (Fig. 8). Number of leaf, root and proliferation percentage is grouped together. So, B+R light spectrum in 'White' caladium and W light spectrum in both cultivars had the highest values for these traits (Fig. 8). Callus induction, regeneration percentage and hyperhydrated shoots were clustered together and R light spectrum in 'White' cultivar showed the highest values for these traits (Fig. 8). The orientation of the vectors indicates the degree of association between the pertinent characteristics. In this study, exposure to the B light spectrum resulted in the lowest attribute values for both caladium cultivars compared to other treatments. Additionally, the results from the hierarchical clustering heat map corroborated the principal component analysis (PCA) findings. In both analyses demonstrated the B+R light spectrum in 'Red' caladium had the highest values. Also B light spectrum in both cultivars showed the lowest value.

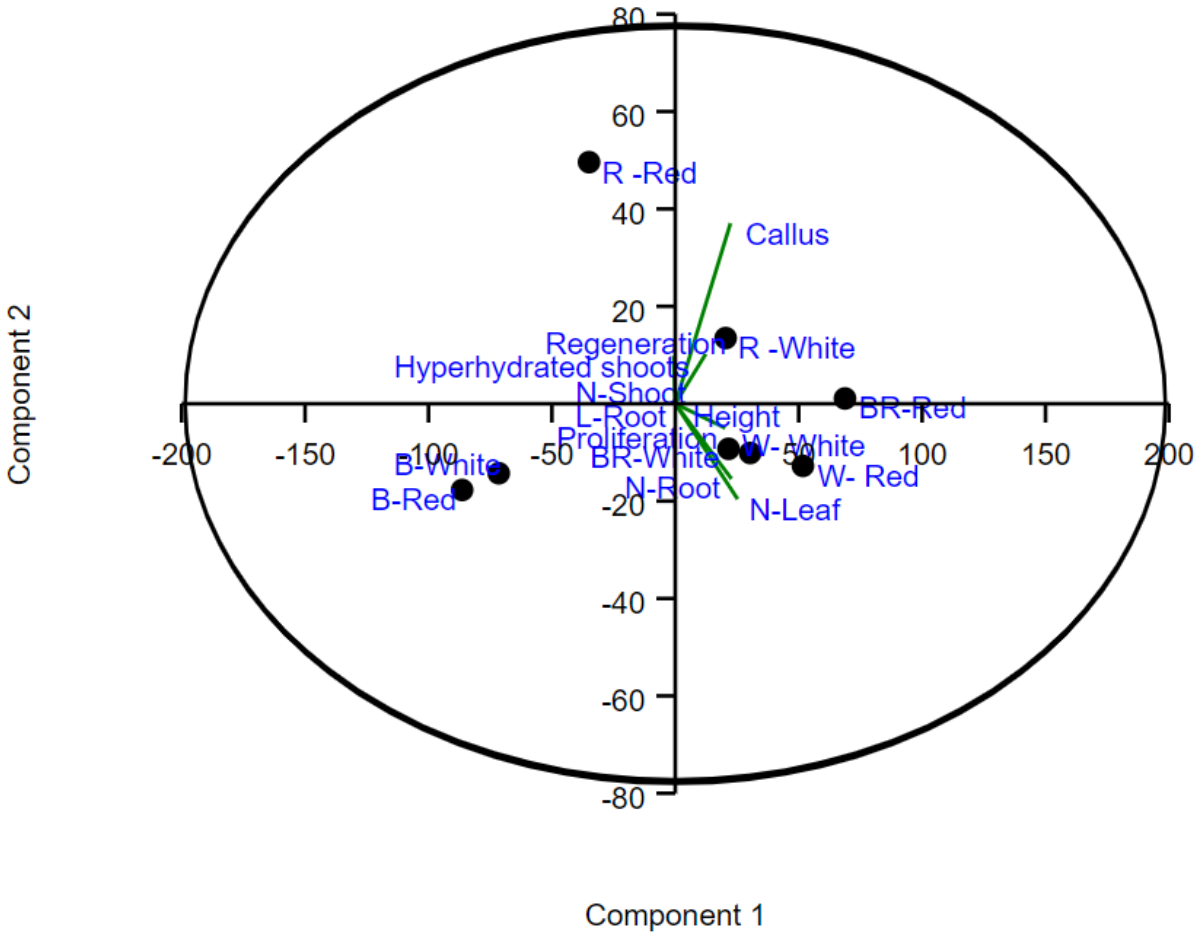

Fig. 8. Principal component analysis (PCA) of all 4 light spectra treatments (W, B, BR and R) in terms of the studied traits obtained from the *in vitro*-raised plantlets of both *Caladium bicolor* cultivars.

CONCLUSION

The current research showed that varying light spectra influenced all measured variables in the course of *in vitro* cultivation. Employing light-emitting diodes (LEDs) may offer significant benefits in addressing common issues encountered in *in vitro* cultures under fluorescent lighting, such as shoot and root elongation and elevated levels of photosynthetic pigments. So, it could be concluded that, W light were the best for maximum callus induction, leaf number, and plantlet height in both cultivars. Red + blue LED light spectrum motivated the proliferation percentage of callus in both cultivars as compared to other light spectra. Conservation of 'White' caladium plantlets in R and B light spectra resulted in no hyperhydric micro shoot formation incidences. It was shown that the B+R light spectrum yielded the most favorable results for the 'Red' caladium, whereas the B light spectrum resulted in the least favorable performance across both cultivars when compared to other light spectra.

Conflict of interest

The authors declare that there is no conflict of interest.

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