Effects of Light Treatments on Callus Induction and Morphogenesis in Caladium bicolor

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Abstract

Caladium bicolor is widely used as an ornamental plant outdoors and indoors due to its rich colors, diverse combinations, and strange patterns. In the commercial production of *Caladium bicolor*, tissue culture technology can quickly reproduce seedlings with consistent genetic properties. In practice, there are still aspects that can be improved in terms of energy dissipation and ornamental value when using fluorescent lamps. In this experiment, the light intensity had no significant effect on the induction of callus and the germination rate, but from the number of buds per explant increased. Different light waves affect the occurrence of seedlings of *Caladium bicolor*. Among them, a single light source, LED-Red, is not conducive to callus differentiation, plant height, and carotenoid accumulation compared to other treatments; LED-Blue is beneficial for the formation of relative anthocyanin content and plant height.LED-White is beneficial for leaf size. Fluorescence is not conducive to increasing the total number of seedlings, which is important for production. Compared with fluorescence, the transplanting rate of LED-Blue increased by 94.92%. The results of this experiment suggest that LED light can replace florescent lamps in tissue culture for achieving low energy consumption and high efficiency.

Introduction

Caladium bicolor belongs to the Araceae family. Its diverse leaf shapes and colors are pleasing to the eye, making it very suitable for garden landscapes and indoor pot decoration. Its leaves are poisonous and inedible[1], but its extract has antidepressant medicinal value[2] and the potential for synthetic biology components [3]. In commercial production in Florida, the world's main producing area, *Caladium* mainly relies on tuber division for reproduction[4], which will cause serious losses to producers when the root system is infected and the plant condition is poor[5]. For some species, low seed germination rates or difficulty in cutting propagation make large-scale commercial development difficult [6]. *Caladium bicolor* is a dioecious flower and requires manual pollination[7]. Plant tissue culture has the unique ability to regenerate intact plants through organogenesis or embryogenesis, without considering their source and ploidy level, at the same time, it reproduces evenly, does not rely on seasons and is free from seed-borne diseases[8]. Tissue culture is used to meet the high demand(high-yield, high-quality, and disease-free planting resources) for these high-quality plants, as it can rapidly reproduce at any given time and season[9]. Therefore, using plant tissue culture technology for rapid and large-scale reproduction of *Caladium bicolor* is an inevitable means of its commercialization.

Plant tissue in vitro culture first occurs on the roots of tomatoes and is used as a research tool for plant morphogenesis[10]. The explants used for propagation contain almost all the organs of the plant [11]. A lower degree of differentiation of the organization usually results in less variation[12]. Among the root tip, stem tip meristem, tender leaves, unexpanded leaves, unfolded leaves, and petioles in tissue culture of *Caladium bicolor*, the fully unfolded leaves have the highest number of seedlings, while the stem tip meristem has the lowest degree of variation[13]. However, tissue culture itself is a rich and innovative source of genetic variation, and genetic or epigenetic changes occur in the original traits of characteristic varieties under this regeneration method[14]. Changes in the morphology and color of mutant plants may not necessarily cause harm to

production, as *Caladium* is more likely to obtain mutant individual plants through tissue culture and clonal reproduction than other plants[15]. Some variants of new colors can be used as new ornamental products.

LED lamps have lower heat dissipation and more advanced radiation wavelength configuration capabilities than fluorescent lamps and are applied to optimize the biological processes of each analyzed species [16]. Light quality overcomes or adapts to field stress by changing the morphological and biological characteristics of *Podocarpus macrophyllus* seedlings[17]. The light environment has important effects on the morphology, physiology, and quality of plant seedlings propagated in vitro[18–23]. Under high light conditions (42 μmol/m^2/s PPFD), the volume of calli was significantly increased in *Phoenix dactylifera*[24]; light duration influences leaf color, seedling height growth, and rooting speed of tissue culture seedlings of Catalpa [25]. Plants have different responses to the same type of light, and a high proportion of BL (30%) can induce stress and growth inhibition or improve growth and development, depending on the species[26]. Red light is beneficial for improving the induction rate of calli in *Anthurium andraeanum*[27] and promoting the growth rate and soluble sugar content of tissue-cultured seedlings of *Hydrilla verticillata*, while blue light promotes the synthesis of chlorophyll a[28]. Reducing the number and height of roots in banana plants cultured in vitro improves the survival rate of long-term storage and achieves in vitro slow growth storage (SGS)[29]. *Caladium* is a semidiurnal plant, insufficient light can cause the leaves to turn green and dark, while excessive light can cause burns to the leaves[15]. Different light intensities can affect the horticultural traits and photosynthetic pigments of *Caladium hortulanum* [30]. Under long-term cultivation, the conditions of photoperiod culture and pigment molecules have an impact on the incidence of hyperhydricity and albinism in tissue culture in *Caladium* [31].

In vitro rapid propagation seedlings of *Caladium bicolor* cultivated under fluorescent lamps consume a large amount of electrical energy. Furthermore, the wavelength of the fluorescent lamp is difficult to provide effective light for the growth 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 *Caladium bicolor* with low energy consumption and high efficiency.

Result

Callus induction and differentiation in different leaf parts

Figure 2. The effect of different parts of leaves on callus induction and differentiation. A. The induction rate of callus in different parts of leaves; B. Budding rate of callus in different parts of leaves

Different parts of the leaf tissue used as explants have an impact on callus induction. As shown in Figure 2A, the three types of explant set up were fully unfolded leaves, unexpanded leaves, and fully unfolded leaf margins. Among them, there was no significant difference in the induction rate of callus between fully unfolded leaves and unexpanded leaves, which were 92.41% and 90.77%, respectively, which is significantly higher than the induction rate of callus with fully unfolded leaf margins (76.79%). There was no significant difference among the three different parts of the germination rate, but the highest germination rate was found in the unexpanded leaves, which was 92.71%. The germination rates of fully unfolded leaves and leaf edges were 84.25% and 88.74%, respectively. The experiment showed that leaves with different growth times and

maturities had little effect on the induction rate of calluses of *Caladium bicolor* in vitro, while the germination rate had an impact, but there was also no significant difference.

Callus induction and differentiation under different light intensities

Figure 3. The effect of different light intensities on callus induction and differentiation. A. Callus induction rate under different light intensities; B. Budding rate of calli under different light intensities.

The induction rate and germination rate of calli under different light intensities are shown in **Figure 3**. Under darkness and 0.5 $\mu mols^{-1}m^{-2}$, 10 $\mu mols^{-1}m^{-2}$, 15 $\mu mols^{-1}m^{-2}$, and 20 $\mu mols^{-1}m^{-2}$ light intensities, there were no significant differences in the callus induction rate or germination rate. The highest callus induction rates were found at 0.5 $\mu mols^{-1}m^{-2}$ and 10 $\mu mols^{-1}m^{-2}$, both of which were 94.84%, while the other three treatments were 93.75%, 86.9%, and 90.87%, respectively. The highest germination rate was 92.79% for the 15 $\mu mols^{-1}m^{-2}$ treatment, followed by 92.36% for the 20 $\mu mols^{-1}m^{-2}$ treatment and 85.42%, 86.33%, and 84.52% for the other three treatments. The results showed that light intensity had a relatively small impact on the induction and differentiation of calluses in *Caladium bicolor*.

Table 1. Analysis of variance (ANOVA) of explant site, light intensity, and their combination (explant site×light intensity) on the number of buds per explant.

Parameter	Source of variation					
	explant site		light intensity		explant site × light intensity	
	F	Sig.	F	Sig.	F	Sig.
Number of buds	8.883	0.0003	11.04	0.0001	0.37	0.9342

Table 2 A simple effect analysis of the effects of different explant locations and light intensities on the number of buds per explant.

Light intensity	Number of buds per explant		
	Fully unfolded leaf	Unexpanded leaf	Fully unfolded leaf edge
Darkness	5.98 ± 0.84 b B	9.05 ± 0.43 ab A	6.08 ± 0.29 b B
0.5 $\mu mol/s/m^2$	5.04 ± 0.45 b B	9.05 ± 0.79 b A	6.54 ± 0.87 b B
10 $\mu mol/s/m^2$	9.67 ± 0.81 a A	11.6 ± 1.07 a A	9.72 ± 0.95 a A
15 $\mu mol/s/m^2$	8.85 ± 0.85 a B	12.55 ± 1.22 a A	10.26 ± 1.09 a AB
20 $\mu mol/s/m^2$	10.44 ± 0.49 a A	11.88 ± 1.16 a A	10.1 ± 0.85 a A

Note: The figure contains different capital letters indicating significant differences in the number of sprouts at different levels of light intensity at different parts of the explant ($P<0.05$). The figure contains different lowercase letters indicating significant differences in the number of sprouts at different levels of explants under different light intensity levels ($P<0.05$).

As shown in **Table 1**, the analysis of variance for the explant site and light intensity showed that the main effect of "explant site" was significant, and the main effect of "light intensity" was also significant. There was an interaction effect between "explant site × light intensity", but it did not reach significance. The light intensity and explant location have a significant impact on the number of buds per explant. Furthermore,, we conducted a simple effect analysis on the experimental results to gain a detailed understanding of the impact of the two on the number of sprouts. The results in **Table 2** indicate that the growth of three different explants under five different light intensities of LED light sources has an impact. For fully unfolded leaves, the number of buds per explant under 10 $\mu mols^{-1}m^{-2}$, 15 $\mu mols^{-1}m^{-2}$, and 20 $\mu mols^{-1}m^{-2}$ light intensities was significantly higher than that under dark and 0.5 $\mu mols^{-1}m^{-2}$ light intensities. The response of fully unfolded leaf edges to light intensity was similar to that of fully unfolded leaves. The number of buds per explant under 10 $\mu mols^{-1}m^{-2}$, 15 $\mu mols^{-1}m^{-2}$, and 20 $\mu mols^{-1}m^{-2}$ light intensities was significantly higher than that under darkness and 0.5 $\mu mols^{-1}m^{-2}$ light intensities. For unexpanded leaves, the number of buds per explant under 10 $\mu mols^{-1}m^{-2}$, 15 $\mu mols^{-1}m^{-2}$, and 20 $\mu mols^{-1}m^{-2}$ light intensities was significantly higher than that under 0.5 $\mu mols^{-1}m^{-2}$ light intensities, but there was no significant difference in the number of buds per explant under dark conditions compared to other treatments.

There are differences in the effects of lighting intensities of LED light sources on three different parts (**Table 2**). Under dark conditions, the number of buds per explant of unexpanded leaves was significantly higher than that of fully unfolded leaves and fully unfolded leaf margins, reaching 9.05 buds; at 0.5 $\mu mols^{-1}m^{-2}$ light intensity, the performance was similar to that under dark conditions. Under 10 $\mu mols^{-1}m^{-2}$conditions, there was no significant difference in the number of sprouts between different explant parts, but the highest number of sprouts was still unexpanded leaves. Under 15 $\mu mols^{-1}m^{-2}$ conditions, the number of buds from unexpanded leaves was significantly higher than that of fully unfolded leaves, reaching 12.55 buds, with an average of 3.7 buds more than fully unfolded leaves. However, there was no significant difference in the number of buds from each explant of fully unfolded leaf edges compared to both fully unfolded and unexpanded leaves. Under 20 $\mu mols^{-1}m^{-2}$ conditions, there was no significant difference in the number of buds per explant among the three leaf explants. Therefore, the number of buds per explant of *Caladium bicolor* in different parts is affected by light intensity. When the light intensity reaches 20 $\mu mols^{-1}m^{-2}$, the differences in low light intensity between different leaf parts lose their effect.

Callus induction and differentiation at different light exposure times

Figure 4. The effect of different light exposure times on callus induction, differentiation, and the number of buds per explant. A. Callus induction rates at different light exposure times; B. The germination rate of calli at different light exposure times; C. The number of buds per explant at different light exposure times.

The growth differences at different light exposure times are shown in **Figure 4A**. On the 0th, 15th, 30th, 45th, 60th, 75th, and 90th days, the explant bottle seedlings were placed under light intensity. There was no significant difference in callus induction rates among the 7 treatments, all reaching over 80%, with the highest being 75 days, reaching 95.83%. There was no significant difference in the germination rate of calli among the 7 treatments (**Figure 4B**), all reaching over 80%, with a 15 day growth rate of over 90%. There were differences in the number of buds per explant among the 7 treatments, but they were not significant (**Figure 4C**), reaching

an average of over 9 buds, with the highest being 90 days, reaching an average of 10.87 buds. Therefore, under the conditions of this experiment, different light exposure times had little effect on the tissue culture of *Caladium bicolor*.

Callus differentiation with different light wavelengths

Figure 5. Differentiation of calli under different wavelengths of light. A. LED White; B. LED Red; C. LED Blue; D. LED-Complex 1; E. LED-Complex 2; F. LED-Complex 3; G. Fluorescence; H. Darkness..

Table 3 The effect of different wavelengths of light on callus differentiation.

Light wave	Callus differentiation rate
LED-White	94.44% ± 5.56 a
LED-Red	86.11% ± 6.05 a
LED-Blue	90.3% ± 5.8 a
LED-Complex 1	100% ± 0 a
LED-Complex 2	94.44% ± 5.56 a
LED-Complex 3	94.44% ± 5.56 a
Fluorescent	96.88% ± 2.76 a

The different wavelengths of light affected the differentiation of *Caladium bicolor* calli (**Figure 5**). Compared with dark conditions, all seven light treatments can cause pigment accumulation in the callus. According to **Table 3**, different light waves have different effects on the germination rate. Among them, the highest germination rate was observed for LED-Complex 1, which reached 100%. The lowest germination rate was observed for LED-Red, which was 86.11%. The germination rate of other wavelengths of light was above 90%, but there was no significant difference between the seven treatments. Therefore, different light qualities have little effect on the differentiation of callus tissue in *Caladium bicolor*.

Growth and development of different light wavelengths

Figure 6. Appearance of tissue-cultured seedlings under different wavelengths of light. A. LED-White; B. LED-Red; C. LED-Blue; D. LED-Complex 1; E. LED-Complex 2; F. LED-Complex 3; G. Fluorescence; Scale bar=5 cm.

Figure 7. The effect of different wavelengths of light on the growth of tissue-cultured seedlings. A. Plant height with different light wavelengths; B. Root length of different light wavelengths; C. The number of elements with different light wavelengths.

Different wavelengths of light have an impact on the appearance and morphology **(Figure 6**). The impact on plant height is shown in **Figure 7A**, and there were differences among different treatments, ranging from large to small LED-White > LED-Blue > LED-Complex 1 > LED-Complex 3 > Fluorescent > LED-Complex 2 > LED-Red. The LED-White treatment had the highest plant height of 6.1 cm, which was significantly higher than the LED-Red, LED-Complex 1, LED-Complex 2, LED-Complex 3, and Florescent treatments. There was no significant difference compared to the LED-Blue treatment, and the LED-Red with the lowest plant height was only 4.28

cm. Pure red light cultivation significantly reduced the plant height of *Caladium bicolor* compared to white light.

The root growth of tissue-cultured seedlings is influenced by different light wavelengths. As shown in **Figure 7B**, the experimental results show that the root lengths of the LED-White, LED-Complex 1 and Fluorescent treatments are significantly higher than those of the LED-Blue. The root lengths of LED-Red, LED-Complex 2, and LED-Complex 3 are greater than those of LED-Blue, but there is no significant difference. The largest root length is Florescent, which is 7.48 cm, and the smallest is LED-Blue, which is only 4.94 cm, with an average root length of 2.54 cm less than that of the Florescent treatment. As shown in **Figure 7C**, there was no significant difference in root length between LED-White, LED-Red, LED-Complex 1, LED-Complex 2, LED-Complex 3, and Florescent, and the root numbers of the six treatments were significantly higher than those of LED-Blue. The LED-White had the highest number of roots, with an average of 22.5, while the LED-Blue had the lowest number, at only 16.63. Therefore, LED-Blue is not conducive to the growth of root systems in tissue culture seedlings.

Figure 8. Effect of different wavelengths of light on leaf growth in tissue culture seedlings. A. Petiole thickness under different light wavelengths; B. Leaf thickness with different light wavelengths; C. Leaf area of different light wavelengths; D. Leaf width with different light wavelengths; E. Leaf length with different light wavelengths; F. Leaf aspect ratio of different light wavelengths.

Different light qualities have different effects on the leaves of tissue-cultured seedlings. As shown in **Figure 8A**, the petiole thickness of the LED-Red treatment was significantly higher than that of the LED-White, LED-Complex 1, LED-Complex 2, LED-Complex 3, and Florescent treatments and was higher than that of the LED-Blue treatment but did not reach significance. The petiole thickness of the LED-Red treatment reached 1.7 mm, with the lowest petiole thickness being only 1.1 mm for the LED-Complex 3 and Florescent treatments. There was no significant difference between LED-Blue and the other treatments. From the perspective of leaf thickness, as shown in **Figure 8B**, the arrangement of leaf thickness from large to small is LED-Complex 3 > LED-Complex 1 > Florescent > LED-Red > LED-Blue > LED-Complex 2 > LED-White. The leaf thickness of the LED-Complex 3 treatment was significantly higher than that of the other treatments, with a value of 1.8 μm. The blade thickness treated with LED-White is the lowest, at 1.3 μm.

From the perspective of leaf area (**Figure 8C**), tissue-cultured seedlings showed significant differences, with LED-White significantly higher than LED-Red, LED-Blue, LED-Complex 2, LED-Complex 3, and Florescent, reaching 5.0 cm^2, followed by LED-Complex 1 at 4.3 cm^2. Among them, the LED-Blue treatment had the smallest leaf area, only 2.2 cm^2, which was more than half smaller than that of LED-White. As shown in **Figure 8D-E**, the trends of leaf length and width among the different treatments were similar. The leaf width and length of the LED-Blue treatment were significantly lower than those of the other treatments, at 1.68 cm and 1.65 cm, respectively. The leaf length and width of the LED-White treatment were greater than those of the other treatments, at 2.50 cm and 2.50 cm, respectively. From the aspect ratio perspective (**Figure 8F**), there was no significant difference among the treatments.

Pigment content of different light wavelengths

Figure 9. The effect of different wavelengths of light on the pigment content in the leaves of tissue cultured seedlings. A. The effect of different light waves on chlorophyll a; B. The effect of different light wavelengths on chlorophyll b; C. The effect of different light wavelengths on total chlorophyll content; D. The effect of different light wavelengths on carotenoids; E. The effect of different light wavelengths on relative anthocyanin content.

Different wavelengths of light treatment affect the accumulation of pigments in tissue-cultured seedlings. As shown in **Figure 9A**, the chlorophyll a content is arranged in descending order: Fluorescent > LED-Blue > LED - White > LED-complex 3 > LED-complex 1 > LED-complex 2 > LED-Red. Compared with Fluorescent, there was no significant difference in the chlorophyll a content of LED-Blue, while the other five treatments significantly decreased. Among them, LED-Red had the lowest chlorophyll a content, only 1.41 mg/g FW. From the perspective of chlorophyll b (**Figure 9B**), the results are similar to chlorophyll a, with the exception of LED-Blue, the chlorophyll b content of all treatments being significantly lower than that of Fluorescent. The results of total chlorophyll content were similar to those of chlorophyll a and chlorophyll b (**Figure 9C**). The total chlorophyll content of Fluorescent and LED-Blue was higher than that of the other treatments, at 2.82 mg/g FW and 2.46 mg/g FW, respectively. The lowest was LED-Red, at 1.87 mg/g FW. From the perspective of carotenoid content (**Figure 9D**), the order of carotenoids from large to small is Fluorescent > LED-White > LED-Complex 3 >LED-Blue > LED-Complex 2 > LED-Complex 1 > LED-Red. Compared with Fluorescent, there was no significant difference in carotenoid content between LED-White, LED-Blue, LED-complex 1, LED-complex 2, and LED-complex 3. The carotenoid content of LED-Red was significantly lower than that of Fluorescent, at only 0.72 mg/g FW.

From the relative content of anthocyanins, LED-Blue was significantly higher than the other treatments, reaching 1.6 U/g FW. Next were LED-Complex 2, LED-Complex 3, and Fluorescent, with a relative anthocyanin content of 0.6 U/g FW, 0.7 U/g FW, and 0.7 U/g FW, respectively. The relative anthocyanin content of these three treatments was significantly higher than that of LED-White and LED-Red; the lowest was that of LED-Red, which was only 0.3 U/g FW, which is 81.25% less than LED-Blue. Therefore, LED-Blue is beneficial for the accumulation of anthocyanins in tissue culture seedlings of *Caladium bicolor*, while LED-Red is not conducive to the accumulation of anthocyanins in tissue culture seedlings.

Number of seedlings with different light wavelengths

Figure 10. The effect of different wavelengths of light on the number of tissue-cultured seedlings. Different lowercase letters represent differences in the total number of seedlings, and different capital letters represent differences in the number of transplantable seedlings ($P<0.05$).

Table 4. The Effect of Different Wavelengths of Light on the Number of Transplantable Tissue Culture Seedlings.

Treatments	Proportion of transplantable seedlings (%)	Increased ratio of transplantable seedlings (%)	coefficient of variation (%)	Transplantation survival rate (%)
LED-White	23.17	84.75	43.29	100
LED-Red	10.64	-23.73	41.83	100
LED-Blue	25.19	94.92	44.88	100
LED-Complex 1	19.46	48.31	35.36	100
LED-Complex 2	15.77	36.44	84.71	100
LED-Complex 3	19.96	68.64	78.38	100
Fluorescent	15.33	0	64.32	100

The effect of different wavelengths of light on the total number of seedlings and the number of transplantable seedlings in tissue culture is shown in **Figure 10**. The average number of seedlings per callus of LED-White, LED-Red, LED-Blue, LED-Complex 1, LED-Complex 2, and LED-Complex 3 was higher than that of Fluorescent, with 13.1, 11.7, 12.7, 12.5, 14.1, and 13.8 plants, respectively. Among them, the average number of seedlings per callus of LED-Complex 2 and LED-Complex 3 was significantly higher than that of Fluorescent's 10.7 plants. From the number of seedlings reaching 3 cm, that of LED-White, LED-Blue and LED-Complex 3 was significantly higher than that of LED-Red. Among them, LED-Blue had the highest number of seedlings above 3 cm and was significantly higher than the LED-Red and Fluorescent treatments, with 3.19 plants, accounting for 25.16% of the total number of seedlings. The lowest among them was LED-Red, with an average of only 1.25 plants reaching 3 cm, accounting for only 10.65% of the total number of seedlings. The coefficient of variation of LED-Red, LED-Blue, and LED-Complex 1 is lower than that of Florescent (**Table 4**), and the number of seedlings reaching transplanting height is more stable. The survival rate of transplanted tissue culture seedlings treated with different wavelengths was 100%. Therefore, compared to fluorescence, LED-Blue is beneficial for increasing the number of *Caladium bicolor* seedlings reaching the transplanting level, and the number is more stable, while red light is not conducive to reaching the transplanting level, even if the number is equally stable.

Discussion

In *Caladium bicolor*, the number of seedlings that can be induced using unexpanded and fully unfolded leaves as explants is similar[13]. There was no significant difference in callus induction and differentiation rates between fully unfolded and unexpanded leaves (Fig. 2), except for the edge of the leaves. This result is similar to the above results. Therefore, the results of this experiment suggest that to obtain more explant materials and increase the clonal variation of *Caladium bicolor* during tissue culture seedling production, fully unfolded and unexpanded leaves are both ideal materials.

Artificial control of environmental factors to shorten crop growth cycles is an effective means of promoting plant growth. Under full blue light conditions, the growth of wheat seedling roots, chlorophyll accumulation,

and crop growth are all unfavorable, but they are beneficial for the accumulation of carotenoids[32]. For fir seedlings, high blue light ratio spectra can promote their growth and biomass but reduce the length of their roots[26]. In this experiment, blue light was not conducive to the growth of the root system of *Caladium bicolor* seedlings (Fig. 7B, C), manifested as having the least number of roots and the shortest length, similar to the growth of wheat and fir seedlings under blue light mentioned above. Compared with fluorescent lamps, blue LED lamps exhibit better biomass accumulation and reproduction rate in vivo in *Dracocephalum forrestii* shoot culture but lower photosynthetic pigment content[33]. Compared with fluorescent lamps, all blue LED lamps showed no significant decrease in chlorophyll a, chlorophyll b, total chlorophyll content and carotenoids (Fig. 9A-D) and were higher than other LED lamp treatments. The results were similar to those mentioned above, but there were differences, which may be due to differences in plant species. In contrast, red light is not conducive to the accumulation of photosynthetic pigments. Chlorophyll a, chlorophyll b, total chlorophyll content, and carotenoids were significantly lower than those in the other treatments. For the reproduction rate (Fig. 10), compared with fluorescent light, LED light treatment increased the number of seedlings, but there was no significant difference compared to white light, Different from Aggarwal's [34] works, which suggests that compared to white light, blue light can differentiate more buds of *Bacopa monnieri*, which may be due to different proportions of wavelengths in white light. Red and blue light can significantly increase the height of Subalpine Fir[35]. Although blue light can also promote the height of seedlings in this experiment to meet the standards for transplantation (Fig. 10), and the number of transplantable seedlings is steadily higher than other treatments (Table 4), the increase in seedling growth and height does not necessarily mean the robustness of the seedlings, and evaluation still needs to be combined with factors such as petiole or stem thickness.

Studies have shown that red light can promote an increase in the stem diameter of *Brosimum gaudichaudi* Trécul compared to blue and white light, while the anthocyanin content of blue light is higher than that of white light, red light, and red blue light[19]. In this experiment, red light promoted the thickening of petioles (Fig. 8A), while blue light significantly promoted the increase in relative anthocyanin content (Fig. 9E). The experimental results of *Caladium bicolor* seedlings are similar to the growth of *Brosimum gaudichaudi* Trécul, and an increase in petioles diameter is beneficial for the health of the seedlings. However, the differences in petiole and stem thickness still need further investigation. In saffron, 100% blue light can promote root growth but is not conducive to leaf growth[36]. Here, blue light did not promote root elongation but rather inhibited root growth. Therefore, the performance of plant species varies greatly under different light qualities. However, in terms of leaf growth, the above results are similar to the results of this experiment, and blue light is not conducive to the growth of *Caladium bicolor* leaves (Fig. 8C-E).

Unlike the white, pink, and yellow colors observed in the other light quality treatments, the color of the callus induced by blue light was purple[37]. *Peucedanum japonicum* Thunb. Pigment accumulation has already occurred during the callus induction stage, which is similar to our experimental results (Fig. 5). However, for *Vaccinium corymbosum* L. cv. Sun Blue Giant, compared to blue light, red light can promote the accumulation of anthocyanins in callus culture[38]. Under full blue light, the relative content of anthocyanins in the seedlings of *Caladium bicolor* was significantly higher than that in the other treatments (Fig. 9E). This experiment suggests that blue light is beneficial for the accumulation of anthocyanin in tissue cultures of *Caladium bicolor* seedlings. Therefore, different types of plants may have significant differences in response to light.

Therefore, from the perspective of single wavelength light, blue light is more conducive to the accumulation of photosynthetic pigments and anthocyanins and stable transplantable plant height and is not conducive to the growth of leaves and roots. Red light is more conducive to increasing petiole thickness but is not conducive to leaf growth, accumulation of photosynthetic pigments, or plant height. For *Anthurium andraeanum* in vitro seedlings, the combination of red and blue light treatment has significant advantages over a single red and blue light treatment[39]. From the perspective of combined lighting (Figs. 7, 8, and 9), compared to fluorescent lamps, LED-Complex 2 balances the short board situation caused by single wavelength blue and red light in photosynthetic pigments, leaf growth, root growth, and plant height. However, it has no advantage in specific parts of growth. Therefore, different stages of using different light wave research can be carried out on *Caladium bicolor* tissue culture seedlings to meet and promote the growth needs of a certain stage.

Materials and Methods

Plant materials. This study is based on *Caladium bicolor* preserved at the Zhanjiang Research Center, Institute of Nanfan & Seed Industry, Guangdong Academy of Sciences, Zhanjiang, Guangdong, China. At the same time, this experiment was conducted at that location.

Induction of callus. Unopened and fully unfolded leaves were collected from the germplasm resource bank and placed in two clean preservation bags. Disinfect them with 75% alcohol and place them on a clean bench. Disinfect them with 0.1 g/L $HgCl_2$ for approximately 7 minutes. Afterwards, the unexpanded blades were cut into 3 mm rolls, 0.5 * 0.5 cm small pieces from the fully unfolded leaf edge, and 0.5 * 0.5 cm small pieces from the leaves except for the leaf edge. All explants were inoculated in the starter medium, which was formulated as MS + 6-BA 2.0 mg/L + NAA 0.2 mg/L + 30 g/L sucrose + 7.0 g/L agar, with a pH of 5.8. Each culture bottle was inoculated with 4 explant materials, and after inoculation, they were placed in incubators with different LED light intensities in the dark, 0.5 μmol $s^{-1}m^{-2}$, 10 μmol $s^{-1}m^{-2}$, 15 μmol $s^{-1}m^{-2}$, and 20 μmol $s^{-1}m^{-2}$, at a culture temperature of 26 ± 2 °C. All treatments had at least 3 biological replicates; on the 0th, 15th, 30th, 45th, 60th, 75th, and 90th days (represented by 0 d, 15 d, 30 d, 45 d, 60 d, 75 d, and 90 d, respectively), they were placed in a light intensity 20 μmol $s^{-1}m^{-2}$ incubator with at least 3 biological replicates for all treatments. On the 100th day, the callus induction of explants under different light intensities was counted[38]:

$$\mathrm{callusformation}\,(\%) = 100 \times \frac{\mathrm{Noofexplantsformingcallus}}{\mathrm{Totalcultivatedexplants}}$$

1

Differentiation of callus tissue. The budding rate was calculated on the 100th day in an incubator with the different light intensities mentioned above:

$$\mathrm{BuddingRate}\,(\%) = 100 \times \frac{\mathrm{Numberofexplantsinducedtosprout}}{\mathrm{Totalcultivatedexplants}}$$

2

The unopened leaf material was inoculated into the initiation medium for dark culture until the 50th day. The induced callus tissue was cut into small pieces of 0.5 * 0.5 mm and placed in proliferation medium. The medium formula was MS + 6-BA 2.0 mg/L + NAA 0.2 mg/L + 30 g/L sucrose + 7.0 g/L agar, with a pH of 5.8.

Each culture bottle was inoculated with 4 callus tissue pieces, which were then placed in an incubator with different light waves. Each incubator is set with 9 points to measure the average Photosynthetic photo flux density (PPFD) of 35 ± 1 μ mol $s^{-1}m^{-2}$, The light wave is, respectively white light, red light, blue light, red : blue : white = 1:1:1, red : blue : green = 1:1:1, red : blue : white : green = 1:1:1:1 of LED lamps, and white fluorescent light(**Figure 1**), epresented by LED-White, LED-Red, LED-Blue, LED-Complex 1, LED-Complex 2, LED-Complex 3, and Fluorescent, the culture temperature was 26 ± 2 °C. All treatments have at least 3 biological replicates;Counting the differentiation of callus which is budding rate 90 days after light wave treatments. After 180 days, the total number of seedlings and the number of seedlings above 3 cm (reaching the height of the transplanted plant) were counted:

$$\mathrm{Transplantablerate}\,(\%) = 100 \times \frac{\mathrm{numberofseedlingsabove3cm}}{\mathrm{totalnumberofseedlings}}$$

3

The ratio of transplantable seedlings increased compared to fluorescence was calculated as follows:

$$\mathrm{Increasingoftransplantableseedlings}\,(\%) = 100 \times \frac{\mathrm{LEDlightabove3cm} - \mathrm{fluorescenceabove3cm}}{\mathrm{fluorescenceabove3cm}}$$

4

Plant growth morphology. Rootless seedlings were transferred to 2 cm into a new rooting medium with a formula of 1/2 MS + IBA 0.2 mg/L + 30 g/L sucrose + 7.0 g/L agar, pH 5.8. Four seedlings from each bottle were transplanted and placed in the 7 different light wavelengths mentioned above, with each treatment repeating biology at least three times. After 90 days, the height of the tissue-cultured seedlings was measured, and the number of roots was counted. The diameter of the petiole was measured using a Vernier caliper, the root length, leaf area, leaf length and width were measured using IMAGE Pro 6.0 software, and the leaf thickness was measured using a multifunctional plant measuring instrument Multispe Q[40,41].

Pigment content. The photosynthetic pigment content[42,43] and relative anthocyanin content[44] in the leaves of transplantable seedlings were measured, with at least three biological replicates per treatment. The extraction agent was used as a control to eliminate the background. Leaf material (0.1 g) was placed in 5 mL of a 1:1 mixture of 80% acetone and 95% ethanol. The extract was placed at dark room temperature for 24 hours, and its light absorption values were measured at wavelengths of 663 nm, 645 nm, and 440 nm. Leaf material (1 g) was placed in 10 ml of 0.1 mol/L HCL solution. After sealing, the samples were placed at 32 °C for 4 hours. The sample was filtered every half hour, and the light absorption value of the extract was measured at a wavelength of 530 nm. The concentration of anthocyanins at an optical density of 0.1 is called 1 unit (U). Relative concentration unit of anthocyanins per gram of material U = A_{530} × 10/g FW. This process was repeated 3 times for each sample, and the average value was taken.

$$\mathrm{Chlorophyllacontent}\,(\mathrm{mg/gFW}) = (12.7\mathrm{A}_{663} - 2.69\mathrm{A}_{645}) \times \frac{\mathrm{V}}{1000 \times \mathrm{W}}$$

$$\mathrm{Chlorophyllbcontent}\,(\mathrm{mg/gFW}) = (22.5\mathrm{A}_{645} - 4.68\mathrm{A}_{663}) \times \frac{\mathrm{V}}{1000 \times \mathrm{W}}$$

5

$$\mathrm{Total chlorophyll content\,(mg/gFW)} = (20.21\mathrm{A}_{645} + 8.02\mathrm{A}_{663}) \times \frac{\mathrm{V}}{1000 \times \mathrm{W}}$$

$$\mathrm{Carotenoid content\,(mg/gFW)} = [4.7\mathrm{A}_{440} - 0.27 \times (20.21\mathrm{A}_{645} + 8.02\mathrm{A}_{663})] \times \frac{\mathrm{V}}{1000 \times \mathrm{W}}$$

Note

A_{663}, A_{645}, A_{440}, and A represent the absorbance at wavelengths of 663 nm, 645 nm, and 440 nm, respectively. V represents the volume of the extraction solution (mL), and W represents the fresh weight of the leaves (g).

Transplantation of tissue-cultured seedlings. Tissue culture seedlings with a height over 3 cm were picked out and transplanted into a substrate pot with a diameter of 9 cm. The ratio of the substrate was peat soil: vermiculite = 1:1, and the samples were placed in the cultivation room. Water the nutrient solution Huabao No. 2 once every two weeks. After two weeks, the survival rate was calculated, and each treatment had at least three biological replicates.

Data analysis. The data were organized using Microsoft Excel software and analyzed for variance (ANOVA) using IBM SPSS Statistics 24. The mean values were compared using Duncan's test at a significance level of 0.05.

Declarations

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Data availability statement

Data will be made available on request.Please contact Mengyi Chen (404074368@qq.com) for relevant data and contact Jinyan Guan (guanjinyan1987@163.com) for relevant materials.

Competing Interests Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Plant Collection Declaration

we statement that all methods in regards to plants were carried out in accordance with IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. The plant material variety selection used in this study was sourced from experts Marut

Cittham and Alongkot Haoyton, and can be purchased in two markets in Thailand (Klong 15 and Talay Pahten).

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Figures

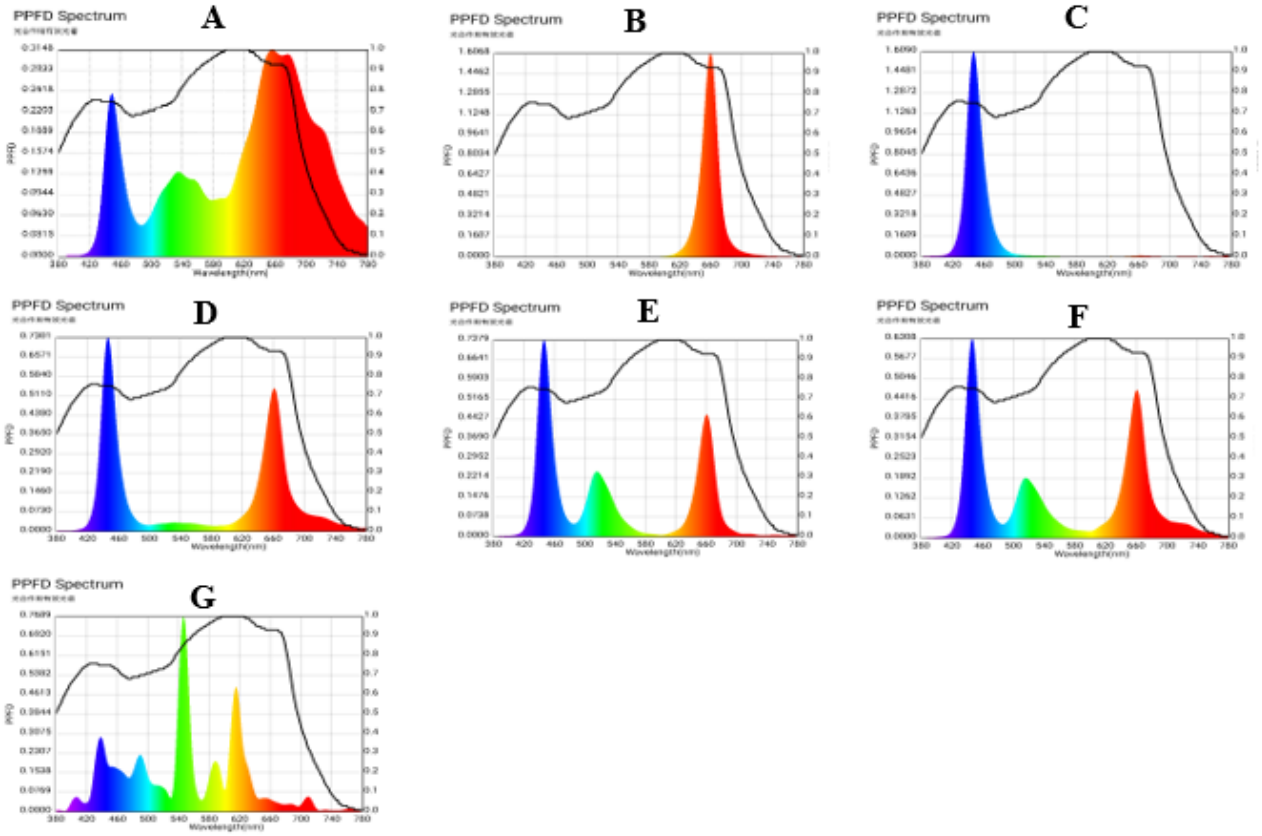

Figure 1

PPFD images of different light wavelength treatments. A. LED-White; B. LED-Red; C. LED-Blue; D. LED-Complex 1; E. LED-Complex 2; F. LED-Complex 3; G. Fluorescence.

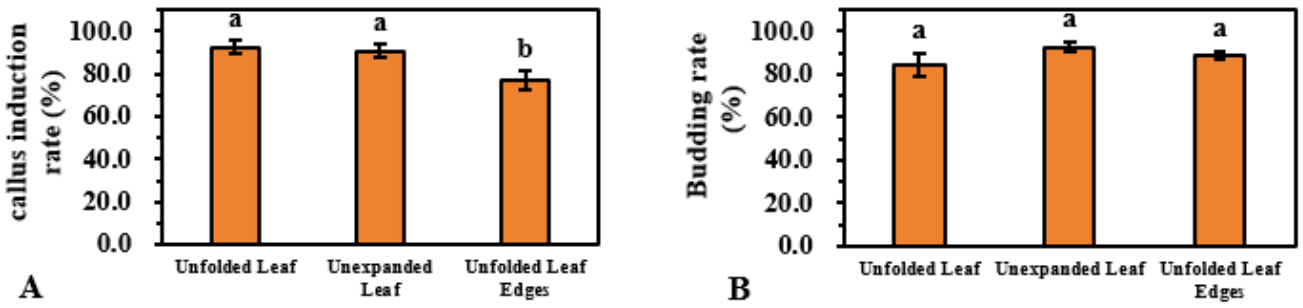

Figure 2

The effect of different parts of leaves on callus induction and differentiation. A. The induction rate of callus in different parts of leaves; B. Budding rate of callus in different parts of leaves

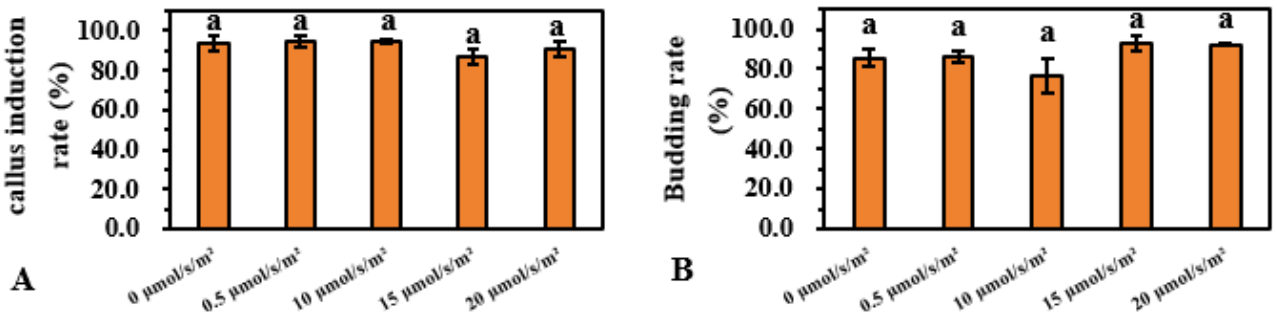

Figure 3

The effect of different light intensities on callus induction and differentiation. A. Callus induction rate under different light intensities; B. Budding rate of calli under different light intensities.

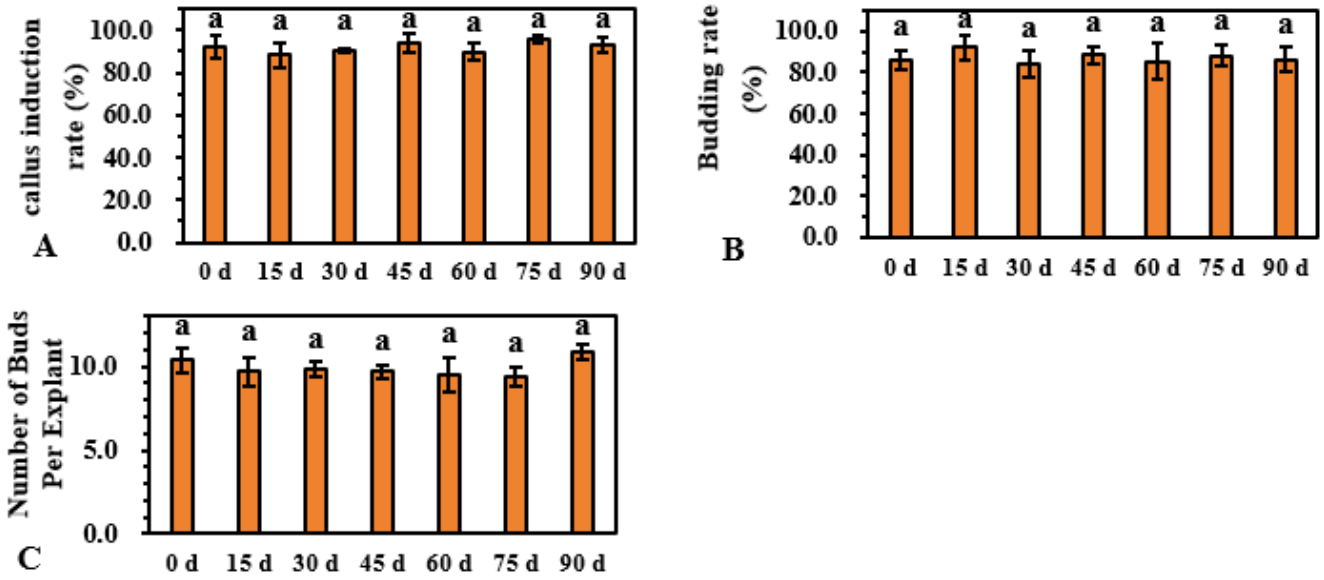

Figure 4

The effect of different light exposure times on callus induction, differentiation, and the number of buds per explant. A. Callus induction rates at different light exposure times; B. The germination rate of calli at different light exposure times; C. The number of buds per explant at different light exposure times.

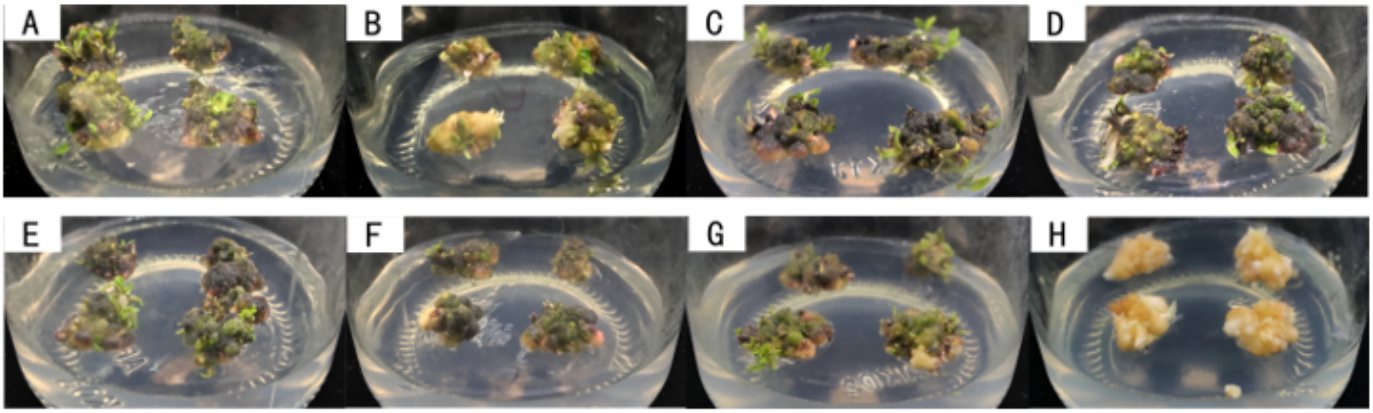

Figure 5

Differentiation of calli under different wavelengths of light. A. LED White; B. LED Red; C. LED Blue; D. LED-Complex 1; E. LED-Complex 2; F. LED-Complex 3; G. Fluorescence; H. Darkness.

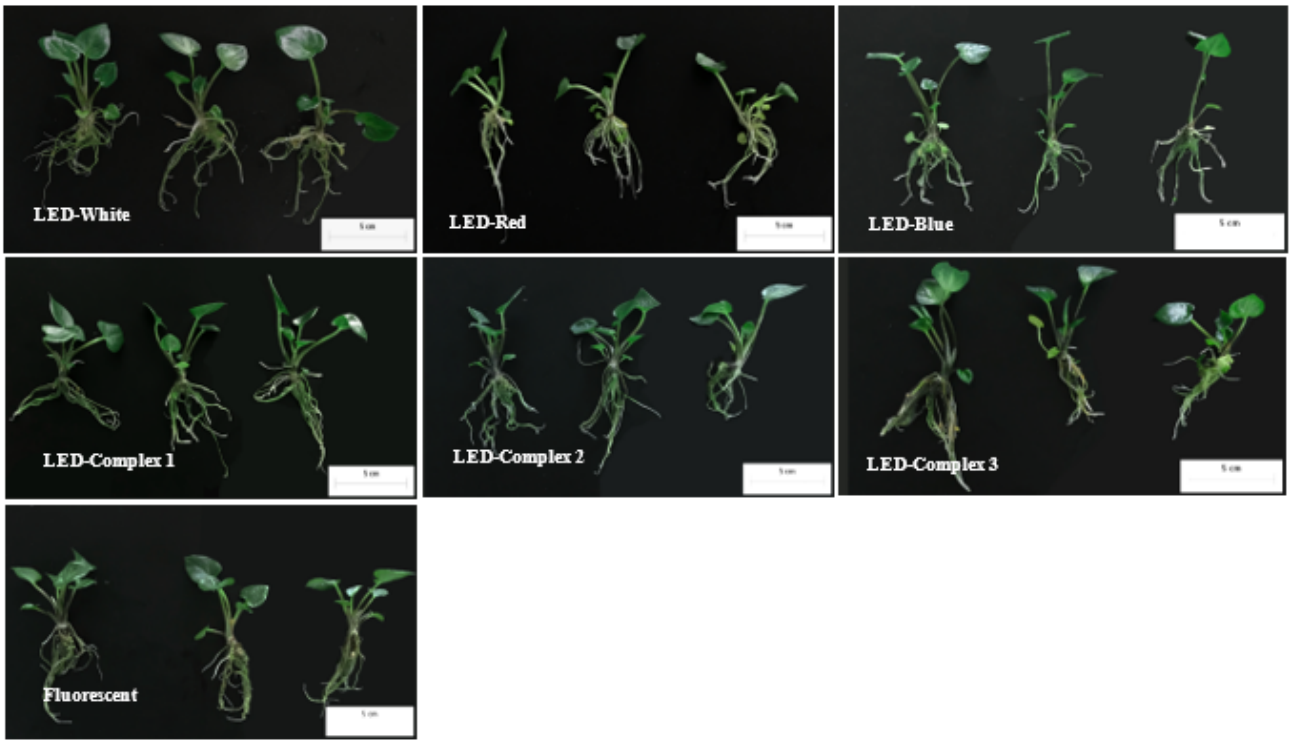

Figure 6

Appearance of tissue-cultured seedlings under different wavelengths of light. A. LED-White; B. LED-Red; C. LED-Blue; D. LED-Complex 1; E. LED-Complex 2; F. LED-Complex 3; G. Fluorescence; Scale bar=5 cm.

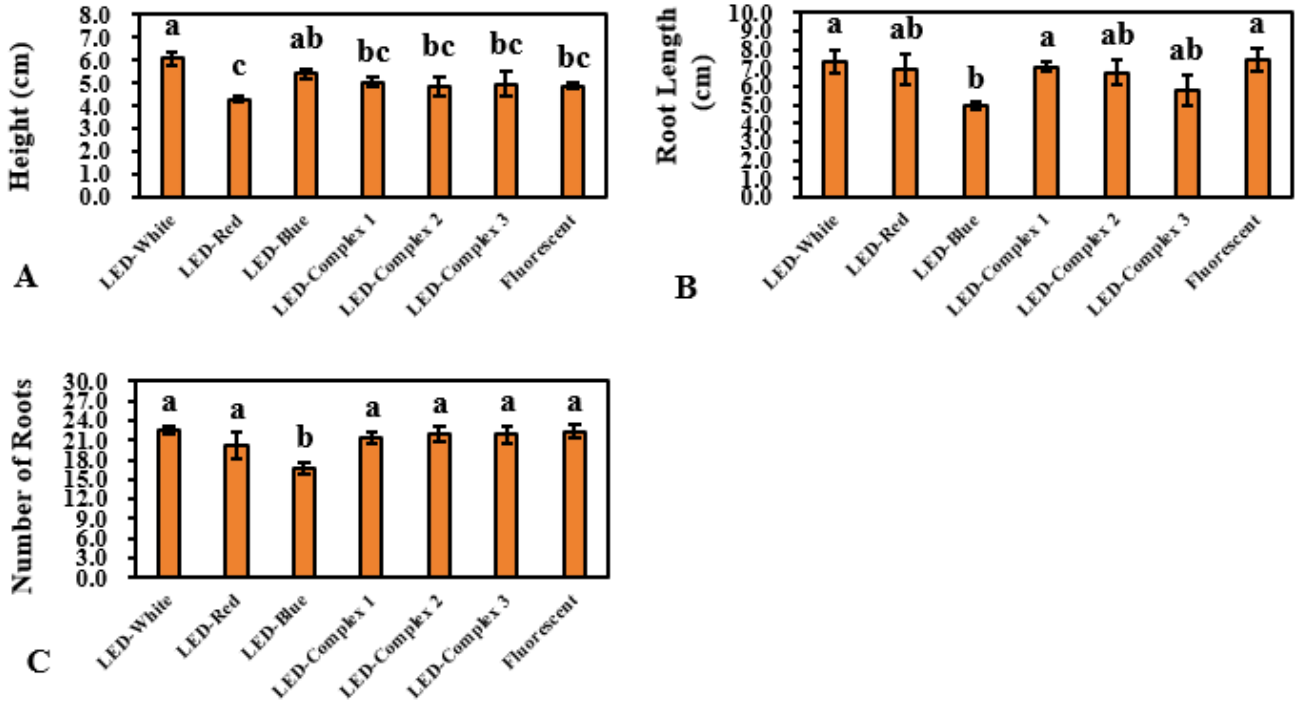

Figure 7

The effect of different wavelengths of light on the growth of tissue-cultured seedlings. A. Plant height with different light wavelengths; B. Root length of different light wavelengths; C. The number of elements with different light wavelengths.

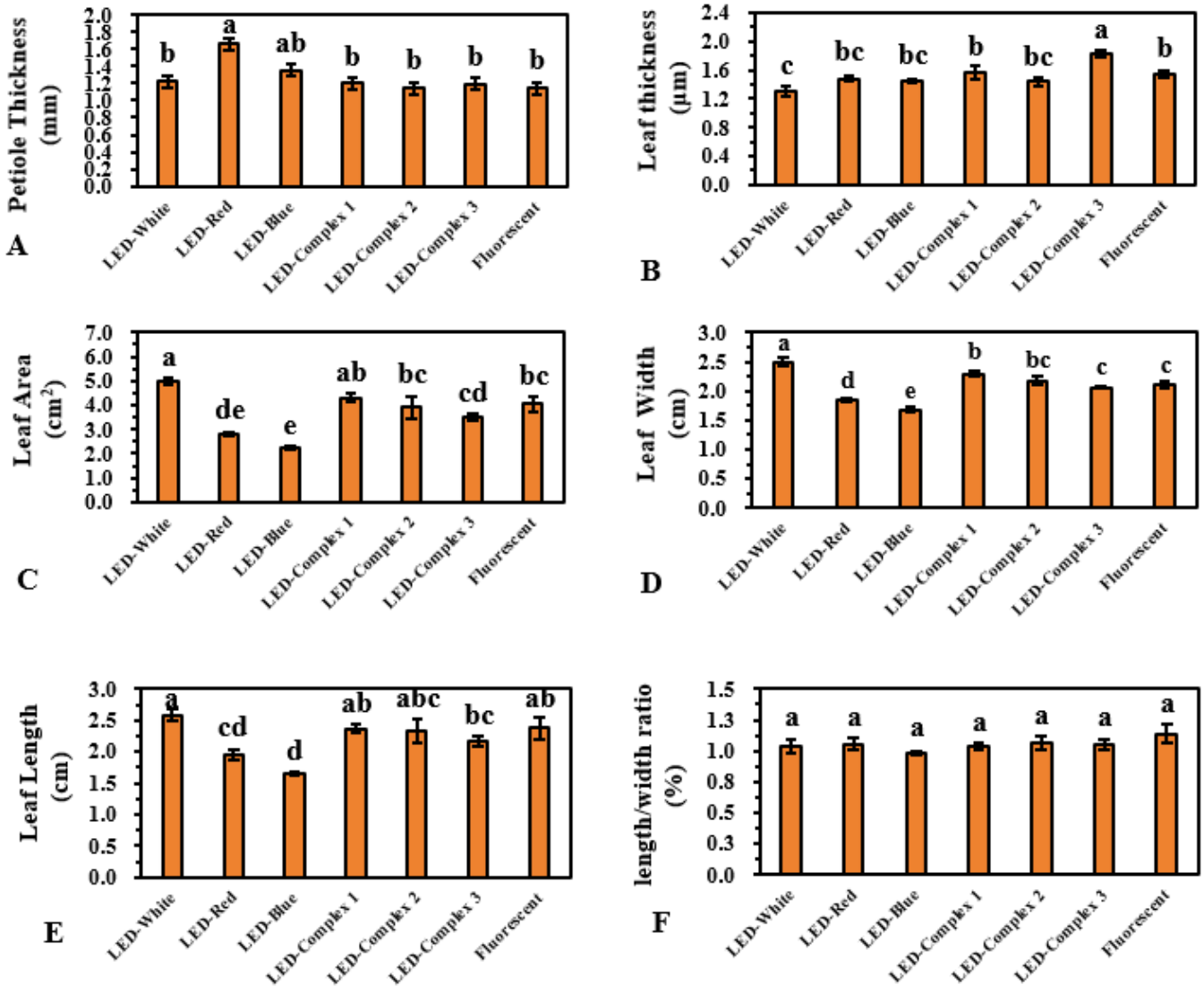

Figure 8

Effect of different wavelengths of light on leaf growth in tissue culture seedlings. A. Petiole thickness under different light wavelengths; B. Leaf thickness with different light wavelengths; C. Leaf area of different light wavelengths; D. Leaf width with different light wavelengths; E. Leaf length with different light wavelengths; F. Leaf aspect ratio of different light wavelengths.

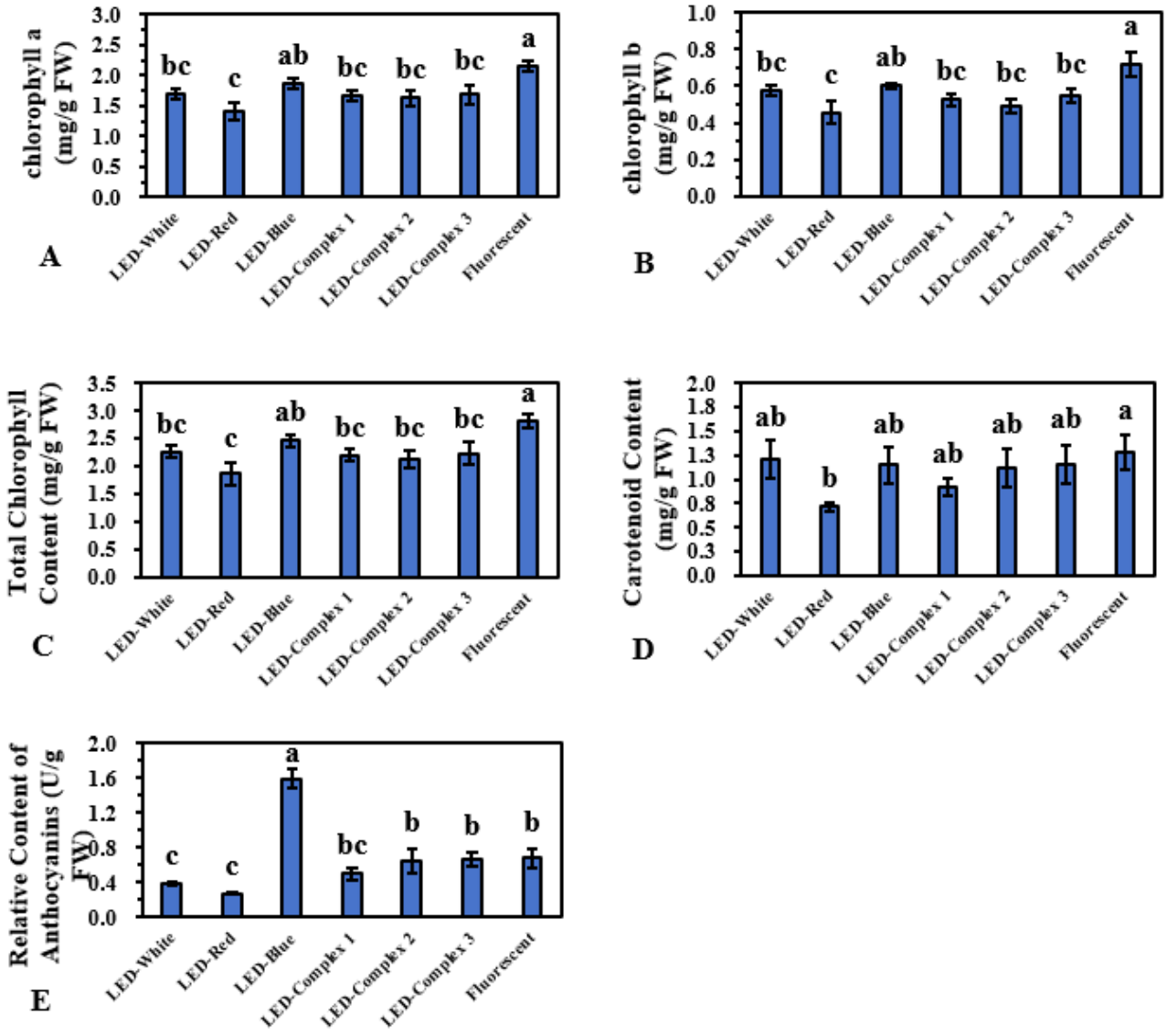

Figure 9

The effect of different wavelengths of light on the pigment content in the leaves of tissue cultured seedlings. A. The effect of different light waves on chlorophyll a; B. The effect of different light wavelengths on chlorophyll b; C. The effect of different light wavelengths on total chlorophyll content; D. The effect of different light wavelengths on carotenoids; E. The effect of different light wavelengths on relative anthocyanin content.

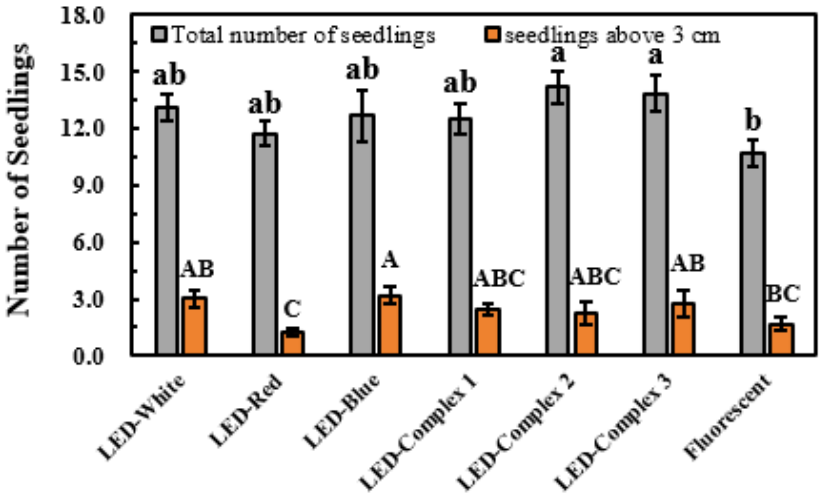

Figure 10

The effect of different wavelengths of light on the number of tissue-cultured seedlings. Different lowercase letters represent differences in the total number of seedlings, and different capital letters represent differences in the number of transplantable seedlings (*P<0.05*).