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Influence of White, Red, Blue, and Combination of LED Lights on In Vitro Multiplication of Shoots, Rooting, and Acclimatization of *Gerbera jamesonii* cv. 'Shy Pink' Plants

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Abstract: Light-emitting diodes (LEDs) are currently being used as a light source for in vitro regeneration or the growth of plants in a controlled environment. However, it is crucial to define the target system's sensitivity to light quality before LEDs may be employed as the exclusive source of light. The objective of the present work was to investigate the influence of red (R), blue (B), white (W), and a combination of red plus blue (1:1) and red, blue, and green (1:1:1) LEDs on *Gerbera jamesonii* cv. 'Shy Pink' during in vitro regeneration. It was observed that LED lighting had a substantial impact on the process of shoot regeneration, shoot growth, and rooting of the shoots. When compared to other light treatments, red plus blue (1:1) LED had the greatest impact on the regeneration of shoots, growth of shoots, and root regeneration from shoot and root growth. Length of shoots, height of plantlets, and petiole length were affected by a red LED, and leaf length, width, and area were affected by a blue LED. The content of photosynthetic pigments (Chl *a*, Chl *b*, total chlorophyll, and carotenoids) was higher with acclimatized plants upon transplantation, which were regenerated under blue LEDs. In acclimatized plants, photosynthetic efficiency was calculated. Higher internal CO_2 concentrations (*Ci*), photosynthetic rates (*Pn*), stomatal conductance (*Gs*), and transpiration rates (*Tr*) were seen in plants that were grown under red plus blue (1:1) LED. However, the plants that were grown under white LEDs had higher chlorophyll fluorescence (Fv/Fm). Collectively, the current results suggest that red and blue LED is suitable for in vitro regeneration of *Gerbera jamesonii* cv. 'Shy Pink' plants compared to red, blue, and white LEDs.

Keywords: *Gerbera*; in vitro regeneration; light-emitting diodes; plant growth; photosynthetic characteristics

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Citation: Lim, M.-J.; Murthy, H.N.; Song, H.-Y.; Lee, S.-Y.; Park, S.-Y. Influence of White, Red, Blue, and Combination of LED Lights on In Vitro Multiplication of Shoots, Rooting, and Acclimatization of *Gerbera jamesonii* cv. 'Shy Pink' Plants. *Agronomy* **2023**, *13*, 2216. https://doi.org/10.3390/agronomy13092216

Received: 25 July 2023
Revised: 15 August 2023
Accepted: 23 August 2023
Published: 24 August 2023

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1. Introduction

Light is one of the most important environmental factors that affect the plant's growth, development, and physiological processes. The amount, quality, and duration of the light (photoperiod) all have an impact on how the plants respond physiologically and how they grow [1]. Light-emitting diodes (LEDs) have recently become more advanced light sources, especially in protected agriculture, enabling the production of high-quality agricultural and horticultural products [2,3]. The fact that LED light sources emit a narrow spectrum of light and are accessible in a variety of wavelengths, from ultraviolet to infrared, is an additional benefit. Compared to conventional light sources, they have a longer lifetime of up to 100,000 h. In horticulture and plant tissue culture, LED lighting can be used in a variety of ways. Numerous studies have shown that LED light sources have an impact on plant morphology (form, color, and texture), physiology, and metabolism (photosynthesis, as

well as other metabolic processes including the accumulation of secondary metabolites) [4]. Additionally, numerous tissue culture studies have shown that the application of LED technology in plant tissue culture had an impact on morphogenic responses, growth, and yield of high-quality plants. LED lights are replacing traditional light sources like fluorescent, halide metal, high-pressure solid, and incandescent light sources in research and commercial laboratories due to their wavelength specificity, durability, small size, long operation time, relatively cool emitting surfaces, and spectral composition [5].

Gerbera jamesonii Bolus ex Hooker f. is a significant ornamental plant that is grown all over the world and used for cut flowers as well as potted plants. Numerous, distinctive forms and hues are available, as well as floret patterning. In terms of the market for cut flowers worldwide, *Gerbera* ranks fifth [6]. The National Institute of Horticulture and Herbal Research (NIHHS), Korea, introduced the hybrid, essential, and well-liked *Gerbera* cultivar 'Shy Pink' in the Republic of Korea. With a vase life of 12.1 days and a bloom yield of 52.8 stems per plant per year, 'Shy Pink' features a semi-double flower with light pink petals and brown core disk florets [7]. It is a well-liked cultivar with considerable market demand. *Gerbera*, which is propagated from seed, has a high level of heterozygosity and possess issues for the cut flower industry. Therefore, for the propagation of these plants, micropropagation techniques are used, and plants are grown under protected cultivation until they reach the flowering stage [8].

In vitro plant processes like seed germination, shoot multiplication, rooting of shoots, somatic embryogenesis, protocorm, and bulblet regeneration of many plant species have all been used with LED lighting because of its special quality features [9–15]. Only a few studies on *Gerbera* in vitro propagation utilizing LED lighting systems have been conducted [16–20]. *Gerbera* in vitro propagation is highly genotype-dependent; plant responses to various circumstances must be assessed separately for each distinct species, variety, growth stage, and tissue type [8,21]. In this study, we investigated how *Gerbera jamesonii* cv. 'Shy Pink' plants responded to different LED light sources, including white, red, blue, blue + red (1:1), and blue + red + green (1:1:1), on the regeneration of shoots from shoot explants, rooting of shoots, and acclimatization of plants upon transplantation. During the acclimatization of the regenerated plants, we also measured the stomatal conductance, internal carbon dioxide level, photosynthetic rate, transpiration rate, chlorophyll fluorescence, and carotenoid and chlorophyll content.

2. Materials and Methods

2.1. Plant Material and Experimental Conditions

The donor shoot cultures (in vitro regenerated shoots) of *Gerbera jemesonii* cv. 'Shy Pink' were procured from the National Institute of Horticultural and Herbal Research (NIHHS), Korea. For shoot multiplication, shoot tips (30–40 mm in height) with two to three leaf primordia were used as explants, and they were cultured on Murashige and Skoog (MS) [22] medium supplemented with 0.1 mg L^{-1} benzyl adenine (BA), 30 g L^{-1} sucrose, and 7.5 of agar, pH 5.7. The cultures were maintained for four weeks, and data on shoot regeneration were collected at the end of four weeks.

For root induction from shoots (2–4 cm in height with two or three leaves), the shoot explants were cultured on MS medium with 0.1 mg L^{-1} indole-butyric acid (IBA), 30 g L^{-1} sucrose, and 7.5 of agar, pH 5.7. The cultures were maintained for four weeks, and data on root regeneration were collected at the end of four weeks.

Fifty milliliters (mL) of the medium was added in 400 mL capacity magenta boxes (Magenta GA-7-3 Plant Culture Box, 350 mL, 75 × 75 × 100 mm, Magenta Corp., Chicago, IL, USA) for both shoot multiplication and rooting of shoots studies. Ten replicates for each treatment were maintained, with five explants being cultivated in each culture vessel.

All the cultures were maintained under different qualities of light-emitting diodes (LED), and there were five different treatments, i.e., W: white (100%, control), R: red (100%), B: blue (100%), RB: red plus blue (1:1 red and blue), and RGB (1 red:1 green:1 blue). The LED light source was obtained from PLCC 5450 6pin; Itswell Co., Incheon, Republic of Korea.

The wavelength of the light was as follows: red (645–675 nm), blue (440–460 nm), green (530–550 nm), and white (430–640 nm). Both experiments (multiplication and rooting) were carried out under a 16 h photoperiod, 25/23 ± 1 °C (day/night) temperature, and 70% relative humidity in the plant growth chambers. The photosynthetic photon flux density (PPFD) in all treatments was adjusted to 40 ± 2 µmol m^{-2} s^{-1}. The light intensity and spectral parameters were adjusted using an LI-250A light meter with a Q50604 sensor (LI-COR, Lincoln, NE, USA).

2.2. Data Collection

Data on shoot regeneration/proliferation were assessed after the four weeks of culture, the data on the total number of shoots regenerated per explant, the average length of the shoots (cm), fresh and dry weight of shoots (mg), leaf length (mm), leaf width (mm), and leaf area (mm^2) were measured.

The data on the rooting of shoots were evaluated four weeks after transfer to the rooting medium. The total length of the shoots (cm), length of root (cm), number of roots (per plantlet), number of leaves (per plantlet), plantlet height (cm), petiole length (cm), leaf length (mm), leaf width (mm), leaf index (length/width), leaf area (mm^2), fresh weight and dry weight of plantlets (mg), were measured.

The dry weight of the shoots (shoots without roots after the shoot regeneration cycle) and dry weights of the plantlets (after rooting of shoots or regenerated plantlets with roots) were determined. The samples were dried in an oven (Sanyo, MoV-112V, Aichi, Japan) at 65 °C until a constant weight was attained and then the dry weights of the samples were assessed.

2.3. Acclimatization

Plants grown under different light treatments in vitro were collected, and roots were carefully washed with distilled water to remove the adhering medium, then plants were transplanted into plastic trays containing garden soil (Cocopeat 51%, Peat moss 10%, Vermiculite 13%, Humic acid 0.1%, Perlite 15%, Zeolite 10%, Fertilizer 0.4%; Shinsung Mineral Co., Ltd., Dunchon-aero, Seongnam, Republic of Korea). The plants were maintained in a growth chamber of 80% humidity, 300 µmol m^{-2} s^{-1} PPFD, and at a temperature of 25 ± 2 °C. Four weeks after transplantation, data on total plant fresh weight (g) and dry weight (mg), plant height (mg), length of the shoot (cm), length of the root (cm), number of roots (per plant), number of leaves (per plant), petiole length (cm), leaf length (cm), leaf width (cm), leaf index (length/width), and leaf area (cm^2) were evaluated. The dry weight of the plantlets (regenerated plantlets with roots) was measured after drying the plant material as explained above.

2.4. Estimation of Chlorophyll and Carotenoid Content

The content of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids of in vitro regenerated plants after acclimatization was analyzed. A total of 200 mg fresh-weight tissue samples were collected from the third leaf from the top of plantlets and were subjected to extraction using 80% acetone [23]. The absorbance was measured using a spectrophotometer (Libra S22, Biochrome Ltd., Cambridge, UK) at the following wavelengths maxima (Amax): chlorophyll *a* at 663 nm, chlorophyll *b* at 645 nm, and total carotenoids at 470 nm. The amount of chlorophyll *a*, chlorophyll *b*, and carotenoids was calculated using the following equations.

$$\text{Chl } a \text{ (mg g}^{-1}\text{)} = (12.72 \times \text{OD}_{663} - 2.5 \times \text{OD}_{645})\ \text{V}/1000\ \text{W}$$

$$\text{Chl } b \text{ (mg g}^{-1}\text{)} = (22.88 \times \text{OD}_{646} - 4.67 \times \text{OD}_{663})\ \text{V}/1000\ \text{W}$$

$$\text{Car (mg g}^{-1}\text{)} = [(1000 \times \text{OD}_{470} - 3.27 \times \text{Chl } a - 104 \times \text{Chl } b)/229]\ \text{V}/1000\ \text{W}$$

where V is the total volume of acetone extract (mL), and W is the FW (G) of the sample.

2.5. Measurement of Photosynthetic Characteristics and Chlorophyll Fluorescence

Photosynthetic characteristics were measured using fully expanded third leaves of six different plants and photosynthetic activity was analyzed using the Li-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). The leaves were exposed to light under saturated CO_2 conditions at 25 °C and 70% relative humidity. The conditions within the leaf chamber of the portable photosynthesis measurement system were as follows: temperature 25 °C, 200 µmol m^{-2} s^{-1} PPFD, CO_2 1000 µmol m^{-2} s^{-1}, and airflow rate 700 µmol s^{-1} The data on the net photosynthetic rate (*Pn*), transpiration rate (*Tr*), internal CO_2 concentration (*Ci*), and stomatal conductance (*Gs*) were measured.

The chlorophyll fluorescence parameters were measured on the third leaves of plants using a potable FluorPen FP100 (Photon Systems Instruments, Drasov, Czech Republic). The ground state fluorescence (Fo) was measured on 30 dark-adapted leaves of plants moved into a dark room. The maximum fluorescence level in the dark-adapted state was triggered by a 1 s saturating light pulse of 3000 µmol m^{-2} s^{-1} PPFD (Fm). The maximum quantum efficiency of PSII photochemistry was calculated as Fv/Fm, where Fv = Fm − Fo.

2.6. Statistical Analysis

The data obtained were subjected to a one-way analysis of variance (ANOVA). The statistical significance of the differences between mean values was assessed using Duncan's multiple range test at $p < 0.05$. All the statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

3. Results

The *Gerbera jamesonii* cv. 'Shy Pink' shoot regeneration and growth were significantly influenced by the types of LED light conditions to which the cultures were exposed (Figures 1 and 2). The number of shoots regenerated from shoot tip explants was optimum with a red plus blue LED combination when compared to white, red, blue, and RGB light sources. The highest number of 6.56 shoots were regenerated with the explants cultured under RB LED (Figures 1 and 2A). The fresh weight (FW) and dry weight (DW) of shoots were all maximum with cultures grown under RB LED light (Figure 2B). However, the shoot length (3.90 cm) and petiole length (2.10 cm) were the highest among the plants cultivated under red LED (Figure 2C). The length (10.4 mm) and width (6.1 mm) of leaves and leaf area (43.9 mm^2) were the highest with plants grown under RB LED (Figure 2D).

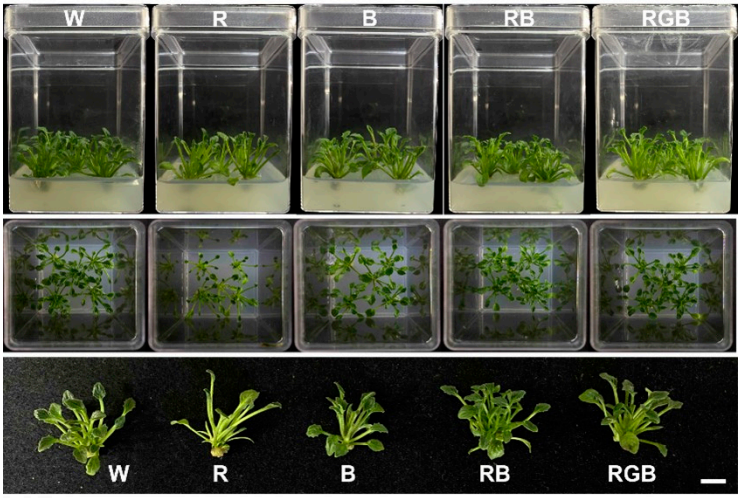


Figure 1. Effect of light quality on in vitro shoot multiplication and growth of *G. jamesonii* cv. 'Shy Pink' after 4 weeks of culture. W: white LED, R: 100% red LED, B: 100% blue LED, RB: red 1 and blue 1, and RGB: red 1, green 1, and blue 1. Scale bar = 1 cm.

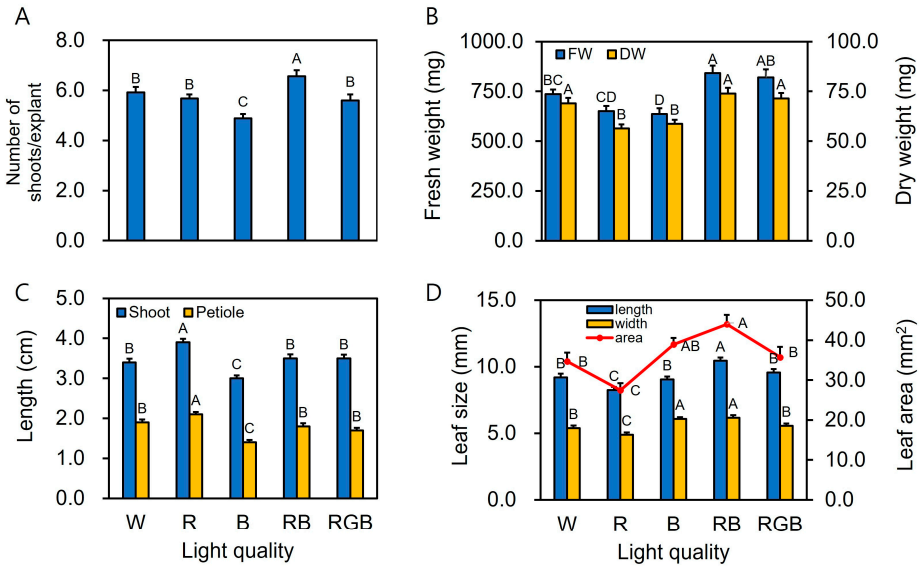


Figure 2. Number of shoots/explants (**A**); fresh and dry weight of shoots (**B**); length of shoot and petiole (**C**); leaf length, width, and area (**D**) of *G. jamesonii* cv. 'Shy Pink' grown in vitro under different LED lighting for 4 weeks. W: white LED, R: 100% red LED, B: 100% blue LED, RB: red 1 and blue 1, and RGB: red 1, green 1, and blue 1. Scale bar = 1 cm. Bars with different letters differ significantly from each other using Duncan's multiple range test (DMRT; $p \leq 0.05$).

Regeneration of roots from the shoots was also influenced by light regimes under which cultures have been grown (Figure 3). Red and white LED triggered rooting in 75% of shoots in the first week, and 100% rooting was achieved after the second week onwards with the shoots cultured from all light sources. Further, the growth of plantlets was also determined by light sources (Tables 1 and 2). The FW (607.89 mg) and DW (56.29 mg) and the number of roots (7.77) and leaves (7.29) per plant were all optimum with plants grown under RB LED compared to other light sources, whereas plant height (8.35 cm), length of shoot (7.01 cm), and length of root (2.10 cm) were excellent with the plants grown under red LED (Table 1). Similarly, petiole length (4.24 cm) and leaf index were maximum with plants cultivated under red LED. In contrast, the plants grown under blue LED depicted the highest leaf length (16.52 mm), leaf width (11.32 mm), and leaf area (127.41 mm^2) (Table 2).

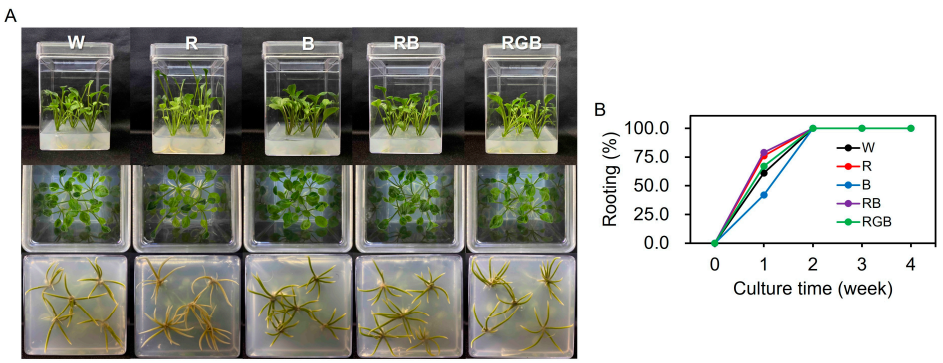


Figure 3. Rooting of shoots and growth of plantlets (**A**) and percentage of rooting (**B**) of *G. jamesonii* cv. 'Shy Pink' shoots grown in vitro under different LED lighting for 4 weeks. W: white LED, R: 100% red LED, B: 100% blue LED, RB: red 1 and blue 1, RGB: red 1, green 1, and blue 1. Scale bar = 1 cm.

The plants which were regenerated under different LED light sources were harvested and transplanted into soil, and growth parameters were measured again after 4 weeks of transplantation and acclimatization (Figure 4, Tables 3 and 4). The plants which were regenerated under RB LED again showed better growth characteristics in terms of FW (1.39 g),

DW (144.13 mg), number of roots (9.63 per plant), and number of leaves (6.63 per plant). Nevertheless, the plants which have regenerated under blue LED exhibited the highest plant height (13.46 cm), shoot length (9.08 cm), and root length (5.48 cm) (Table 3). Likewise, petiole length (5.98 cm), leaf length (3.72 cm), leaf width (3.07 cm), and leaf area (8.57 cm^2) were all excellent with transplanted plants that were regenerated under blue LED (Table 4).

Table 1. Effect of light quality on rooting of shoots and growth of *G. jamesonii* cv. 'Shy Pink' plantlets after 4 weeks of culture on rooting medium.

Light Quality	Total Fresh Weight (mg)	Total Dry Weight (mg)	Plantlet Height (cm)	Length (cm)		Number (per Plant)	
				Shoot	Root	Roots	Leaves
W [z]	506.37 b [y]	47.89 c	6.27 c	5.01 c	1.83 b	6.60 bc	7.26 a
R	581.26 a	52.66 b	8.35 a	7.01 a	2.10 a	6.11 c	6.77 b
B	542.77 b	53.09 b	6.05 c	4.79 c	1.77 b	7.00 b	7.26 a
RB	607.89 a	56.29 a	6.68 b	5.41 b	1.80 b	7.77 a	7.29 a
RGB	515.40 b	47.83 c	6.23 c	4.83 c	1.83 b	7.00 b	7.40 a

[z] W: white LED, R: 100% red LED, B: 100% blue LED, RB: red 1 and blue 1 LED, RGB: red 1, green 1, and blue 1 LED. [y] Different lowercase letters within each column indicate significant differences ($p \leq 0.05$; Duncan's multiple range test).

Table 2. Effect of light quality on leaf growth characteristics of *G. jamesonii* cv. 'Shy Pink' plantlets after 4 weeks of culturing on rooting medium.

Light Quality	Petiole Length (cm)	Leaf Length (mm)	Leaf Width (mm)	Leaf Index (length/width)	Leaf Area (mm^2)
W [z]	2.54 b [y]	14.60 b	9.47 b	1.55 b	94.35 b
R	4.24 a	13.79 c	7.51 c	1.83 a	67.40 c
B	2.15 c	16.52 a	11.32 a	1.46 d	127.41 a
RB	2.61 b	16.44 a	11.05 a	1.49 c	124.11 a
RGB	2.47 b	14.65 b	9.91 b	1.48 cd	99.39 b

[z] W: white LED, R: 100% red LED, B: 100% blue LED, RB: red 1 and blue 1 LED, RGB: red 1, green 1, and blue 1 LED. [y] Different lowercase letters within each column indicate significant differences ($p \leq 0.05$; Duncan's multiple range test).

Table 3. Effect of light quality on growth of *G. jamesonii* cv. 'Shy Pink' plantlets after 4 weeks of transplantation into soil.

Light Quality	Total Fresh Weight (g)	Total Dry Weight (mg)	Plantlet Height (cm)	Length (cm)		Number (per Plant)	
				Shoot	Root	Roots	Leaves
W [z]	1.36 ab [y]	144.13 a	12.37 ab	8.95 a	5.27 a	9.88 a	4.88 b
R	1.14 b	115.25 b	12.47 ab	9.11 a	4.36 b	7.00 b	5.13 b
B	1.39 a	149.25 a	13.46 a	9.08 a	5.48 a	10.13 a	5.13 b
RB	1.39 a	144.13 a	12.95 ab	9.56 a	4.47 b	9.63 a	6.63 a
RGB	1.25 ab	128.25 ab	12.25 b	8.72 a	4.95 ab	8.38 ab	5.00 b

[z] W: white LED, R: 100% red LED, B: 100% blue LED, RB: red 1 and blue 1 LED, RGB: red 1, green 1, and blue 1 LED. [y] Different lowercase letters within each column indicate significant differences ($p \leq 0.05$; Duncan's multiple range test).

The chlorophyll *a* (1.33 mg g^{-1} FW), chlorophyll *b* (0.73 mg g^{-1} FW), total chlorophyll (2.05 mg g^{-1} FW), and carotenoid (0.35 mg g^{-1} FW) content were highest with plants which were regenerated under blue LED when compared to the plants that were grown under W, R, RB, and RGB LED sources (Table 5).

Analysis of photosynthetic characteristics with regenerated Gerbera plants under different LED light sources revealed that there were differences related to light quality (Table 6). The *Ci* value was optimum in the plants grown under RB LED, and it was comparable with the plants grown under B and RGB LED. However, the *Ci* value was lowest with the plants grown under R and W LED sources. A similar trend was also

recorded concerning the photosynthetic rate (*Pn*) and transpiration rate (*Tr*). Again, plants grown under RB LED depicted the highest *Pn* and *Tr* values compared to other light sources (Table 6). Stomatal conductance (*Gs*) values were also highest with RB-grown plants in comparison with other LED light sources. The *Gs* values were lowest with the plants grown under W and R light regimes (Table 6).

Table 4. Effect of light quality on leaf growth characteristics of *G. jamesonii* cv. 'Shy Pink' plantlets after 4 weeks of transplantation into soil.

Light Quality	Petiole Length (cm)	Leaf Length (cm)	Leaf Width (cm)	Leaf Index (Length/Width)	Leaf Area (cm^2)
W [z]	5.78 a [y]	3.60 ab	2.91 a	1.25 b	7.22 ab
R	6.14 a	2.87 c	2.17 b	1.35 ab	4.67 c
B	5.98 a	3.72 a	3.07 a	1.23 b	8.57 a
RB	5.48 ab	3.54 ab	2.87 a	1.25 b	7.54 a
RGB	5.04 b	3.19 bc	2.29 b	1.41 a	5.23 bc

[z] W: white LED, R: 100% red LED, B: 100% blue LED, RB: red 1 and blue 1 LED, RGB: red 1, green 1, and blue 1 LED. [y] Different lowercase letters within each column indicate significant differences ($p \leq 0.05$; Duncan's multiple range test).

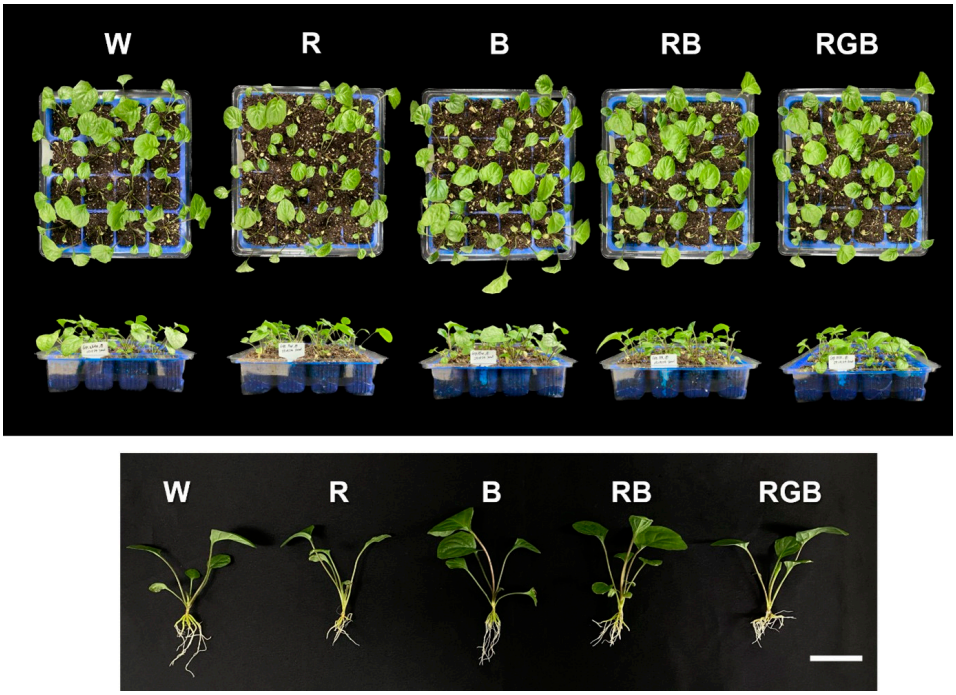


Figure 4. Acclimatization of in vitro grown *G. jamesonii* cv. 'Shy Pink' plants after 4 weeks of transplantation. W: plants regenerated under white LED; R: plants regenerated under 100% red LED; B: plants regenerated under 100% blue LED; RB: plants regenerated under red 1 and blue 1; RGB: plants regenerated under red 1, green 1, and blue 1. Scale bar = 1 cm.

Minimal fluorescence (Fo), maximal fluorescence (Fm), maximum variable fluorescence (Fv), and photosynthetic efficiency of PSII (Fv/Fm) values of plants grown under different LED sources were measured in in vitro grown plants (before transplantation) and after transplantation into the soil (acclimatized plants), and the data are presented in Figure 5. Even though high variation was not observed in Fo, Fm, and Fv values of plants grown under varied LED treatments, the highest values were recorded with W LED grown plants and then with RB and RGB grown plants. The fluorescence indices were comparatively lower with R and B when compared to RB and RGB treatments.

Table 5. Effect of light quality on chlorophyll content (mg·g^{-1} FW) of *G. jamesonii* cv. 'Shy Pink' plantlets after 4 weeks of transplantation into soil.

Light Quality	Total Chlorophyll	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
W [z]	1.77 b [y]	1.18 b	0.60 ab	0.29 b
R	1.75 b	1.18 b	0.57 b	0.26 b
B	2.05 a	1.33 a	0.73 a	0.35 a
RB	1.80 ab	1.21 ab	0.60 ab	0.28 b
RGB	1.73 b	1.16 b	0.57 b	0.29 b

[z] W: white LED, R: 100% red LED, B: 100% blue LED, RB: red 1 and blue 1 LED, RGB: red 1, green 1, and blue 1 LED. [y] Different lowercase letters within each column indicate significant differences ($p \leq 0.05$; Duncan's multiple range test).

Table 6. Effect of light quality on photosynthetic efficiency of *G. jamesonii* cv. 'Shy Pink' after 4 weeks of acclimatization.

Light Quality	Transpiration Rate (*Tr*) ($mol \cdot H_2O \cdot m^{-2} \cdot s^{-1}$)	Photosynthetic Rate (*Pn*) ($\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$)	Internal CO_2 Concentration (*Ci*) ($\mu mol \cdot CO_2 \cdot mol^{-1}$)	Stomatal Conductance (*Gs*) ($mol \cdot H_2O \cdot m^{-2} \cdot s^{-1}$)
W [z]	0.0005 b [y]	3.86 b	276.74 c	0.037 b
R	0.0005 b	3.40 c	297.71 b	0.040 b
B	0.0010 a	5.31 a	318.12 a	0.077 a
RB	0.0010 a	5.44 a	325.78 a	0.080 a
RGB	0.0009 a	5.40 a	313.98 a	0.072 a

[z] W: white LED, R: 100% red LED, B: 100% blue LED, RB: red 1 and blue 1 LED, RGB: red 1, green 1, and blue 1 LED. [y] Different lowercase letters within each column indicate significant differences ($p \leq 0.05$; Duncan's multiple range test).

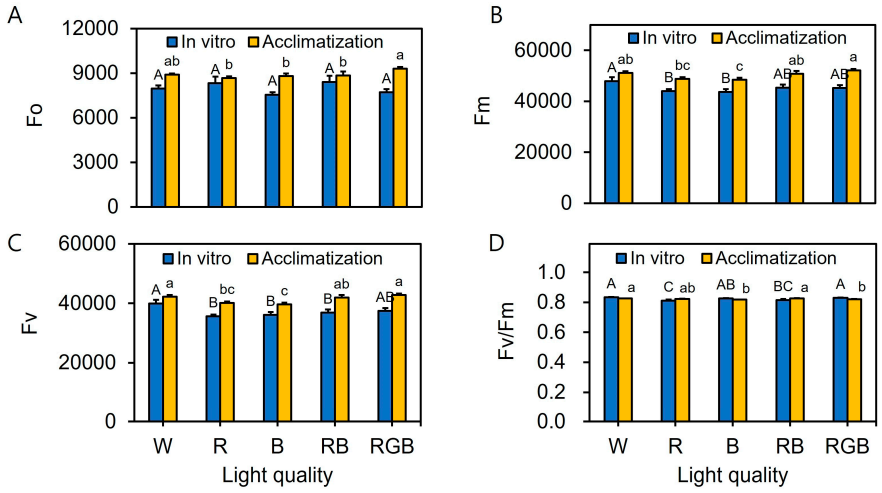


Figure 5. Chlorophyll fluorescence parameters in *G. jamesonii* cv. 'Shy Pink' plants are grown in vitro under different LED treatments. F_0 (**A**); Fm (**B**); Fv (**C**); Fv/Fm (**D**). W: white LED, R: 100% red LED, B: 100% blue LED, RB: red 1 and blue 1, RGB: red 1, green 1, and blue 1. Scale bar = 1 cm. Bars with different letters differ significantly from each other by Duncan's multiple range test (DMRT; $p \leq 0.05$).

4. Discussion

Light is one of the prominent factors that influence plant growth, development, and morphological, and physiological characteristics [24–26]. The success of a plant tissue culture system is directly impacted by light quality. Therefore, in the recent past, artificial light sources such as light-emitting diodes (LEDs) have been tested and used efficiently for manipulating morphogenic responses in plant tissue cultures as well as for the cultivation of plants in greenhouse horticulture and protected agriculture [3,27,28]. Several studies have described the effect of different LEDs on in vitro plant regeneration of *Gerbera jamesonii* [16–20]; however,

a specific protocol cannot be used successfully for different gerbera cultivars because in vitro regeneration of gerbera is highly genotype and season dependent [8]. *Gerbera jamesonii* 'Shy Pink' is a cultivar that was released in Korea by the National Institute of Horticulture and Herbal Research (NIHHS), and it is a popular cultivar grown and utilized in Korea. In the current study, we attempted the micropropagation of *Gerbera jamesonii* 'Shy Pink' under different LED treatments and assessed the multiplication of shoots and growth parameters during the shoot regeneration, rooting of shoots, and acclimatization.

Plant morphogenesis is controlled by light quality through multiple photoreceptors including blue light photoreceptors, the cryptochromes (CRY1 and CRY2), red/far-red light photoreactors, and the phytochromes (phy A to phy E) [29,30]. The synergism between R and B requires the coaction of phytochrome B and cryptochromes [31]. In the current study, red plus blue light treatments were responsible for the highest number of shoot regeneration (6.56 shoots per explant). R alone or B alone was responsible for lesser regeneration response, and we hypothesize that the synergism of both R and B lights is responsible for enhanced morphogenetic response in *Gerbera jamesonii* 'Shy pink'. Similar responses on shoot regeneration were observed in other plant species. For example, Hung et al. [10] and Smith et al. [26] have reported improved shoot regeneration and shoot growth in *Vaccinium corymbosum* and *Chrysanthemum* under R (50%) and B (50%) combination of LEDs.

Red LED alone was responsible for shoot elongation and petiole length in *Gerbera jamesonii* 'Shy pink' when compared to blue or RGB LED treatments during regeneration of shoots and roots. Similar results were reported in *Gerbera jamesonii* cv. Dura [16] and *Vitis ficifolia* [32] and *Fragaria* × *ananassa* [28] and showed red light-stimulated shoot and petiole elongation, respectively. Interestingly, after the transplantation of plants in soil, enhanced plant height was recorded with the plants that were regenerated under blue LED. Fukuda et al. [33] have demonstrated the antagonistic action of blue and red light on shoot elongation reported in *Petunia* plants. Red light inhibited shoot elongation, but the opposite was true with blue light treatments. Through several experimental evidences, they showed the negative effect of R light on gibberellic acid signaling. Therefore, variations in plant responses to LED treatments in *Gerbera jamesonii* cv. 'Shy pink' may be caused by variations in gibberellic acid concentration levels as a result of the use of particular LEDs. Leaf length, leaf width, and leaf area were all triggered by monochromatic blue LED treatment during the rooting and acclimatization stage, whereas red LED comparatively inhibited leaf growth parameters. Similar to current results, blue LEDs induced the largest leaf area in *Alternanthera brasiliana* and *Gossypium hirsutum* [34,35]. However, Shin et al. [15] reported an increment in the leaf area of *Doritaenopsis* with 50%/50% red and blue LEDs in comparison to monochromatic red and blue LEDs and fluorescent light treatments.

The results of the current study showed that a 50% blue and 50% red LED combination is effective in the accumulation of both fresh and dry biomass during the multiplication, rooting, and acclimatization stages. These data are consistent with several studies that the combination of R and B is significant for plant productivity [36–38]. For instance, a mixture of R- and B-augmented plant biomass in the plants such as *Oryza sativa* [39], *Lactuca sativa* [40], and *Cucumis sativus* [41]. Our results suggest a reduction in plant biomass for the plants grown under R LED alone. Thus, the photoreceptor cryptochrome activation by B LED may play a prominent role in the biomass accumulation of *Gerbera jamesonii* cv. 'Shy pink' plants.

The results of our experiments showed that red (50%) plus blue (50%) LED treatment resulted in the highest induction of roots from shoot cultures in the first week of culture initiation itself. These results were similar to *Gossypium hirsutum* wherein the greatest root induction and growth was reported in 50%/50% red and blue light [35]. In contrast to these results, red LED treatment resulted in the formation of roots in *Gerbera jamesonii* cv. Dura [16]; however, a higher percentage of rooting was reported under 30%/70% red and blue LEDs. In plants such as *Jatropha curcus* and *Vitis ficifolia*, rooting percentage was higher in red LEDs; however, the results were genotype dependent [32,42]. It was

reported by Xu et al. that a combination of light sources is beneficial in the accumulation of soluble sugar (SSC) and soluble protein content (SPC) as well as increase antioxidants including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), and lower malonaldehyde (MDA) and polyphenol activities (PPO) in *Cunninghamia lanceolata* [43]. Xu et al. [43] also demonstrated that increased activity of SPC, SOD, and CAT was positively correlated with root growth parameters in *Cunninghamia lanceolata*. Therefore, we believe that an effect of blue plus red LED might be responsible for an increase in soluble proteins and antioxidant enzymes, which might be the responsible promotive effect on root induction and growth in *Gerbera jamesonii* cv. 'Shy pink' plants; however, further studies are needed to test such hypotheses.

The lighting system is the most important factor for chlorophyll synthesis. Light sources with different wavelengths affect different photoreceptors of plants to control pigment synthesis [44]. It was reported that blue light is prominent in the synthesis of chlorophyll [45], and monochromatic red light may trigger the process of chlorophyll synthesis at specific intensities [46]. We observed the highest concentration of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids in the plants grown under blue LEDs, followed by red (50%) and blue (50%) LEDs. Previous results obtained by Poudel et al. [32] in *Vitis ficifolia* plants grown under blue light compared to that in plants grown under either red or fluorescent light. Moreover, Li et al. [35] and Kim et al. [47] reported the highest chlorophyll contents in *Gossypium hirsutum* and *Chrysanthemum* plantlets cultured under blue LEDs. Likewise, a study by Mengxi et al. [48] reported the accumulation of the highest chlorophyll *a*, chlorophyll *b*, and carotenoids in *Oncididum* plants grown under blue LEDs. In contrast, Dong et al. [49] recorded an optimum accumulation of chlorophyll *a* and chlorophyll *b* in *Triticum aestivum* plants grown under red LEDs than those grown under blue LEDs. This suggests that light quality has different effects on the cytochrome accumulation of different plant species.

The measurement of leaf internal CO_2 concentration (*Ci*), transpiration rate (*Tr*), stomatal conductance (*Gs*), and photosynthetic rate (*Pn*) of in vitro regenerated plants upon ex vitro transplantation are useful parameters to assess their photosynthetic performance. Our results showed that plants regenerated under blue LED depicted higher *Pn,* which exactly corresponded to the *Gs*, *Ci*, and *Tr*. However, plants grown under red or white LEDs did not show a higher *Pn* as compared to other light sources. The lower *Pn* with the plants grown under red and white LEDs could be due to the lower internal CO_2 concentration (*Ci*), lower transpiration rate (*Tr*), and stomatal conductance (*Gs*). Nevertheless, the highest *Pn* was recorded with plants grown under red (50%) plus blue (50%) LED treatments. Similarly, when plants such as *Chrysanthemum*, *Withania somnifera*, *Lactuca sativa* var. *capitata*, and *Nicotiana tabacum* were grown under red light combined with blue light, they showed higher *Pn* as compared to those under monochromatic lights [47,50–52]. Moreover, Shang et al. [53] recorded that the *Pn* of *Lilium* plants was lower under red light than blue light, but the *Pn* increased when red and blue lights were supplied together. The main functions of stomata are the exchange of water and gases with the external environment. In the present study, the *Gs* were higher in the plants grown under the B LED treatment than in the R LED treatment. Inoue et al. [54] reported that blue light promotes stomatal opening by activating phototropins; therefore, we speculate that the enhanced photosynthetic rate in *Gerbera jamesonii* cv. 'Shy pink' plants that were grown under B LED are due to higher stomatal conductance (*Gs*), which is also responsible for a higher intercellular CO_2 concentration (*Ci*).

Chlorophyll fluorescence parameters, which reflect the photosynthetic capacity of the plants, are influenced by several parameters such as light quality and quantity [55–57]. In the present study, red LED induced a large depletion of Fv/Fm; thus, we speculate that red LED is an adverse spectrum for photosynthetic capabilities of *Gerbera* plantlets. Similar observations were recorded for *Cucumis sativus* plants grown under red light [57]. However, white or blue LEDs and red (50%) plus blue (50%) LEDs were responsible for higher Fv/Fm in the current study, and the higher Fv/Fm indicated higher photosynthetic efficiency [52].

5. Conclusions

In conclusion, our study demonstrates that a mixture of blue and red (1:1) LEDs was found suitable for in vitro regeneration of *Gerbera jamesonii* cv. 'Shy Pink' plants. Higher shoot regeneration rates, rooting of shoots, and vegetative growth of plants were observed under blue plus red light treatments. However, the red LED was responsible for the increment in shoot height and petiole length. Chlorophyll *a*, chlorophyll *b*, and carotenoid contents were maximum with the plants grown under blue LED. Photosynthetic characteristics such as internal CO_2 concentration (*Ci*), photosynthetic rate (*Pn*), stomatal conductance (*Gs*), and transpiration rate (*Tr*) were all higher with plants grown under red plus blue LEDs after their acclimatization. These findings help in utilizing LED as light sources for plant regeneration of *Gerbera jamesonii* cv. 'Shy Pink' for the production of quality plant material.

Author Contributions: M.-J.L., H.-Y.S. and S.-Y.L. contributed to data acquisition and experiments. H.N.M. participated in the writing and editing of the manuscript. S.-Y.P. was involved in the conception and design of the study and made a substantial contribution to data interpretation and revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the development of technology to maintain and manage the quality of superior and standard seedlings in floral crops (Project No. PJ017080), Rural Development Administration, Republic of Korea.

Data Availability Statement: Data are available on request.

Acknowledgments: Hosakatte Niranjana Murthy is thankful to the "Brain Pool" (BP) program, Grant No. 415 2022H1D3A2A02056665.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ouzounis, T.; Rosenqvist, E.; Ottosen, C.O. Spectral effects of artificial light on plant physiology and secondary metabolism: A review. *HortScience* **2015**, *50*, 1128–1135. [CrossRef]
2. Higuchi, Y.; Hisamatsu, T. Light acts as a signal for regulation of growth and development. In *LED Lighting for Urban Agriculture*; Kozai, T., Fujiwara, K., Runkle, E., Eds.; Springer: Singapore, 2016; pp. 57–73. [CrossRef]
3. Paradiso, R.; Proietti, S. Light-quality manipulation to control plant growth and photomorphogenesis in greenhouse horticulture: The state of the art and the opportunities of modern LED systems. *J. Plant Growth Regul.* **2022**, *41*, 742–780. [CrossRef]
4. Batista, D.S.; Felipe, S.H.S.; Silva, T.D.; de Castro, K.M.; Mamedes-Rodriques, T.; Miranda, N.A.; Rios-Rois, A.M.; Faria, D.V.; Fortini, E.A.; Chagas, K.; et al. Light quality in plant tissue culture: Does it matter? *Vitr. Cell. Dev. Biol.–Plant* **2018**, *54*, 195–2015. [CrossRef]
5. Agarwal, A.; Gupta, S.D. Impact of light-emitting diodes (LEDs) and its potential on growth and development in controlled environment plant production system. *Curr. Biotechnol.* **2016**, *5*, 28–43. [CrossRef]
6. Zhou, Y.; Yin, M.; Abbas, F.; Sun, Y.; Gao, T.; Yan, F.; Li, X.; Yu, Y.; Yue, Y.; Yu, R.; et al. Classification and association analysis of gerbera (*Gerbera hybrida*) flower color traits. *Font. Plant Sci.* **2022**, *12*, 779288. [CrossRef]
7. Song, H.Y.; Park, J.T.; Jeong, J.A.; Lim, S.H.; Kwon, O.K. A high-yielding standard gerbera cultivar 'Shy Pink' with light pink colored petals and semi-double type for cut flower. *Flower Res. J.* **2022**, *30*, 227–231.
8. Cardoso, J.C.; Teixeira da Silva, J.A. Gerbera micropropagation. *Biotechnol. Adv.* **2013**, *31*, 1344–1357. [CrossRef]
9. Hahn, E.J.; Kozai, T.; Paek, K.Y. Blue and red light-emitting diodes with or without sucrose and ventilation affect in vitro growth of *Rehmannia glutinosa* plantlets. *J. Plant Biol.* **2000**, *43*, 247–250. [CrossRef]
10. Hung, C.D.; Hong, C.H.; Kim, S.K.; Lee, K.H.; Park, J.Y.; Nam, M.W.; Choi, D.H.; Lee, H.I. LED light for in vitro and *ex vitro* efficient growth of economically important highbush blueberry (*Vaccinium corymbosum* L.). *Acta Physiol. Plant.* **2016**, *38*, 152. [CrossRef]
11. Kim, Y.W.; Moon, H.K. Enhancement of somatic embryogenesis and plant regeneration in Japanese red pine (*Pinus densiflora*). *Plant Biotechnol. Rep.* **2014**, *8*, 259–266. [CrossRef]
12. Li, H.; Tang, C.; Xu, Z. The effect of different light qualities on rapeseed (*Brassica napus* L.) plantlet growth and morphogenesis *in vitro*. *Sci. Hortic.* **2013**, *150*, 117–124. [CrossRef]
13. Lian, M.L.; Murthy, H.N.; Paek, K.Y. Effects of light emitting diodes (LEDs) on the in vitro induction and growth of bulblets of *Lilium* oriental hybrid 'Pesaro'. *Sci. Hortic.* **2002**, *94*, 365–370. [CrossRef]

14. Park, Y.G.; Oh, H.J.; Jeong, B.R. Growth and anthocyanin concentration of *Perilla frutescence* var. *acuta* Kudo as affected by light source and DIF under controlled environment. *Hortic. Environ. Biotechnol.* **2013**, *54*, 103–108. [CrossRef]
15. Shin, K.S.; Murthy, H.N.; Heo, J.W.; Hahn, E.J.; Paek, K.Y. The effect of light quality on the growth and development of in vitro cultured *Doritaenopsis* plants. *Acta Physiol. Plant.* **2008**, *30*, 339–343. [CrossRef]
16. Pawlowska, B.; Zupnik, M.; Szewczyk-Taranek, B.; Cioc, M. Impact of LED light sources on morphogenesis and levels of photosynthetic pigments in *Gerbera jamesonii* grown in vitro. *Horticul. Environ. Biotechnol.* **2018**, *59*, 115–123. [CrossRef]
17. Cioc, M.; Kalisz, A.; Zupnik, M.; Pawlowska, B. Different LED light intensities and 6-benzyladenine concentration in relation to shoot development, leaf architecture, and photosynthetic pigments of *Gerbera jamesonii* Bolus in vitro. *Agronomy* **2019**, *9*, 358. [CrossRef]
18. Cioc, M.; Pawlowska, B. Leaf response to differential light spectrum compositions during micropropagation of *Gerbera* axillary shoots. *Agronomy* **2020**, *10*, 1832. [CrossRef]
19. Cioc, M.; Dziurka, M.; Pawlowska, B. Changes in endogenous phytohormones of *Gerbera jamesonii* axillary shoots multiplied under different light emitting diodes light quality. *Molecules* **2022**, *27*, 1804. [CrossRef]
20. Meng, X.; Wang, Z.; He, S.; Shi, L.; Song, Y.; Lou, X.; He, D. LED-supplied red and blue light alters the growth, antioxidant status, and photochemical potential of in vitro-grown *Gerbera jamesonii* plantlets. *Horticul. Sci. Technol.* **2019**, *37*, 473–489. [CrossRef]
21. Gupta, S.D.; Jatohu, B. Fundamentals and applications of light emitting diodes (LEDs) in in vitro plant growth and morphogenesis. *Plant Biotechnol. Rep.* **2013**, *7*, 211–220. [CrossRef]
22. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
23. Lichtenthaler, H.K.; Buschmann, C. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectrophotometry. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R.E., Ed.; Wiley: New York, NY, USA, 2001; pp. F4.3-1–F4.3.8. [CrossRef]
24. Faukda, N. Advanced light control technologies in protected horticulture: A review on morphological and physiological responses in plant to light quality and its applications. *J. Dev. Sustain. Agric.* **2013**, *8*, 32–40. [CrossRef]
25. Paradiso, R.; Meinen, E.; Snel, J.F.; De Visser, P.; Van Ieperen, W.; Hogewoning, S.W.; Marcelis, L.F.M. Spectral dependence of photosynthesis and light absorptance in single leaves and canopy in rose. *Sci. Horitc.* **2011**, *127*, 548–554. [CrossRef]
26. Smith, H.L.; McAusland, L.; Murchie, E.H. Don't ignore the green light: Exploring diverse role in plant processes. *J. Exp. Bot.* **2017**, *68*, 2099–2119. [CrossRef] [PubMed]
27. Moon, H.K.; Park, S.Y.; Kim, Y.W.; Kim, C.S. Growth of tsuru-rindo (*Tripoterospermum japanicum*) cultured in vitro under various sources of light-emitting diode (LED) irradiation. *J. Plant Biol.* **2006**, *49*, 174–179. [CrossRef]
28. Nhut, D.T.; Takamura, T.; Watanabe, H.; Okamoto, K.; Tanaka, M. Responses of strawberry plantlets cultured in vitro under super bright red and blue light-emitting diodes (LEDs). *Plant Cell Tissue Organ Cult.* **2003**, *73*, 43–52. [CrossRef]
29. Cashmore, A.R.; Jarillo, J.A.; Wu, Y.J.; Liu, D.M. Cryptochromes: Blue receptors for plants and animals. *Science* **1999**, *284*, 760–765. [CrossRef]
30. Quail, P.H. Phytochrome photosensory signaling networks. *Nat. Rev. Mol. Cell. Biol.* **2002**, *3*, 85. [CrossRef]
31. Sellaro, R.; Hoecker, U.; Yanovsky, M.; Chory, J.; Casal, J.J. Synergism of red and blue light in the control of *Arabidopsis* gene expression and development. *Curr. Biol.* **2009**, *19*, 1216–1220. [CrossRef]
32. Poudel, P.R.; Kataoka, I.; Mochioka, R. Effect of red and blue light-emitting diodes on growth and morphogenesis of grapes. *Plant Cell Tissue Organ Cult.* **2008**, *92*, 147–153. [CrossRef]
33. Fukuda, N.; Ajima, C.; Yukawa, T.; Olsen, J.E. Antagonistic action of blue and red light on shoot elongation in petunia depends on gibberellin, but the effects on flowering are not generally linked to gibberellin. *Environ. Exp. Bot.* **2016**, *121*, 102–111. [CrossRef]
34. Macedo, A.F.; Leal-Costa, M.V.; Tavares, E.S.; Lage, C.L.S.; Esquibel, M.A. The effect of light quality on leaf production and development of in vitro-cultured plants of *Alternanthera brasiliana* Kuntze. *Environ. Exp. Bot.* **2011**, *70*, 43–50. [CrossRef]
35. Li, H.; Xu, Z.; Tang, C. Effect of light-emitting diodes on growth and morphogenesis of upland cotton (*Gossypium hirsutum* L.) plantlets in vitro. *Plant Cell Tissue Organ Cult.* **2010**, *103*, 155–163. [CrossRef]
36. Hogewoning, S.W.; Trouborst, G.; Maljaars, H.; Poorter, H.; Van Ieperen, W.; Harbinson, J. Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *J. Exp. Bot.* **2010**, *61*, 3107–3117. [CrossRef] [PubMed]
37. Johkan, M.; Shoji, K.; Goto, F.; Hashida, S.; Yoshihara, T. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. *HortScience* **2010**, *45*, 414–415. [CrossRef]
38. Lin, Y.; Li, J.; Li, B.; He, T.; Chun, Z. Effects of light quality on growth and development of protocorm-like bodies of *Dendrobium officinale* in vitro. *Plant Cell Tissue Organ Cult.* **2011**, *105*, 329–335. [CrossRef]
39. Ohashi-Kaneko, K.; Matuda, R.; Goto, E.; Fujiwara, K.; Kurata, K. Growth of rice plants under red light with or without supple mental blue light. *Soil Sci. Plant Nutr.* **2006**, *52*, 444–452. [CrossRef]
40. Wang, J.; Lu, W.; Tong, Y.X.; Yang, Q.C. Leaf morphology, photosynthetic performance, chlorophyll fluorescence, stomata devel opment of lettuce (*Lactuca sativa* L.) exposed to different ratios of red light to blue light. *Fornt. Plant Sci.* **2016**, *7*, 250. [CrossRef]
41. Hernandez, R.; Kubota, C. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. *Environ. Exp. Bot.* **2016**, *121*, 66–74. [CrossRef]

42. Daud, N.; Faizal, A.; Grelen, D. Adventitious rooting of *Jatropha curcus* L. is stimulated by phloroglucinol and by red LED light. *Vitr. Cell Dev. Biol.–Plant* **2013**, *31*, 1344–1357. [CrossRef]
43. Xu, Y.; Liang, Y.; Yang, M. Effect of composite LED light on root growth and antioxidant capacity of *Cunnighamia lanceolata* tissue culture seedlings. *Sci. Rep.* **2019**, *9*, 9766. [CrossRef] [PubMed]
44. Stuefer, J.F.; Huber, H. Differential effect of light quantity and spectral light quality on growth, morphology and development of two stoloniferous *Potentilla* species. *Oecologia* **1998**, *117*, 1–8. [CrossRef] [PubMed]
45. Dougher, T.A.; Bugbee, B.G. Is blue light good or bad for plants? *Life Support Biosph. Sci.* **1998**, *5*, 129–136.
46. Tripathy, B.C.; Brwon, C.S. Root-shoot interaction in greening of wheat seedlings grown under red light. *Plant Physiol.* **1995**, *107*, 407–411. [CrossRef] [PubMed]
47. Kim, S.J.; Hahn, E.J.; Heo, J.W.; Paek, K.Y. Effects of LED on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets in vitro. *Sci. Hortic.* **2004**, *101*, 143–151. [CrossRef]
48. Mengxi, L.; Zhigang, X.; Yang, Y.; Yijie, F. Effect of different spectral lights on *Oncidium* PLBs induction, proliferation and plant regeneration. *Plant Cell Tissue Organ Cult.* **2011**, *106*, 1–10. [CrossRef]
49. Dong, C.; Fu, Y.M.K.; Liu, G.H.; Liu, H. Growth, photosynthetic characteristics, antioxidant capacity and biomass yield and quality of wheat (*Triticum aestivum* L.) exposed to LED light sources with different spectra combination. *J. Agron. Crop Sci.* **2014**, *200*, 219–230. [CrossRef]
50. Lee, S.H.; Tewari, R.K.; Hanh, E.J.; Paek, K.Y. Photon flux and light quality induce changes in growth, stomatal development, photosynthesis and transpiration of *Withania somnifera* (L.) Dunal. Plantlets. *Plant Cell Tissue Organ Cult.* **2007**, *90*, 141–151. [CrossRef]
51. Lin, K.H.; Hunag, M.Y.; Huang, W.D.; Hsu, M.H.; Yang, Z.W.; Yang, C.M. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. *capitata*). *Sci. Hortic.* **2013**, *150*, 86–91. [CrossRef]
52. Yang, L.Y.; Wang, L.T.; Ma, E.D.; Li, J.Y.; Gong, M. Effects of light quality on growth and development, photosynthetic character istics and content of carbohydrates in tobacco (*Nicotiana tabacum* L.) plants. *Photosynthetica* **2017**, *55*, 467–477. [CrossRef]
53. Shang, W.; Song, Y.; Zhang, C.; Shi, L.; Shen, Y.; Li, X.; Wang, Z.; He, S. Effects of light quality on growth, photosynthetic charac teristics, and endogenous hormones in in vitro-cultured *Lilium* plantlets. *Hortic. Environ. Biotechnol.* **2023**, *64*, 65–81. [CrossRef]
54. Inoue, S.; Takemiya, A.; Shimazaki, K. Phototropin signaling and stomatal opening as a model case. *Curr. Opin. Plant Biol.* **2010**, *13*, 587–593. [CrossRef] [PubMed]
55. Lichtenthaler, H.K.; Babani, F. Light adaption and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence parameters, and photosynthetic activity. In *Chlorophyll a Fluorescence. Advances in Photosynthesis and Respiration*; Papageorgiou, G.C., Govindjee, Eds.; Springer: Dordrecht, The Netherlands, 2004; Volume 19, pp. 713–736. [CrossRef]
56. Wang, H.; Gu, M.; Cui, J.; Shi, K.; Zhou, Y.; Yu, J. Effects of light quality on CO_2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. *J. Photochem. Photobiol. B* **2009**, *96*, 30–37. [CrossRef] [PubMed]
57. Wang, X.Y.; Xu, X.M.; Cui, J. The importance of blue light for leaf expansion, development of photosynthetic apparatus, and chloroplast ultrastructure of *Cucumis sativus* grown under weak light. *Photosynthetica* **2015**, *53*, 213–222. [CrossRef]

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