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Regeneration of African Violet in Response to Light Quality

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Abstract: Light-emitting diode (LED) technology is a form of artificial lighting that offers precise control over spectral composition, creating specific conditions for plant growth and development. However, the influence of various LED wavelengths on the regeneration characteristics in African violet (AV) has not been extensively explored. This study aims to investigate the changes in the regeneration traits of AV when exposed to different LED light colors within controlled conditions. In this study, AV leaf cuttings were prepared and subjected to white, red, blue, and red + blue light colors for a period of three months in a growth chamber. Afterward, they were transferred to the laboratory for further analysis. The results indicated that the AVs treated with red + blue colors exhibited the most significant improvement in several morpho-physiological traits of both the roots and shoots. The highest total biomass (2.96 g), shoot fresh weight (1.76 g), root dry weight (0.14 g), root volume (3.10 cm^3), and shoot length (1.60 cm) were observed in this treatment group. Furthermore, the highest levels of photosynthetic pigments, such as chlorophyll a, chlorophyll b, and carotenoids (0.14, 0.12, and 3.80 mg g^{-1} f.w., respectively), were predominantly observed in the red + blue treatment group. In conclusion, this study introduces a novel methodology for optimizing lighting conditions to enhance the regeneration of African violets, shedding light on the potential for improving AV regeneration practices.

Keywords: light-emitting diodes; propagation; light quality; photosynthesis; root growth

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1. Introduction

The African violet (AV), *Saintpaulia ionantha* H. Wendl., is a prevalent houseplant native to East Africa, characterized by its appealing flowers and attractive foliage, belonging to the *Gesneriaceae* family [1]. This species was initially named "ionantha", signifying "having violet-like flowers" in Greek, by Hermann Wendland in 1883 [2]. Over the years, AV has established itself as a significant ornamental plant in the floral industry, thanks to its visual appeal, shade tolerance, and the ability to bloom under artificial lighting conditions [1,2]. More importantly, AV has seen a significant rise in economic status, making it one of the best-selling plants in Europe and the United States [3]. However, certain crucial attributes need improvement, such as enhancing its quality concerning the ability to withstand transportation, tolerate cold temperatures, resist diseases and pests, and develop vibrant flower colors [3]. It features relatively short fleshy stems, roughly rounded leaves with scalloped edges, and small clusters of flowers surrounded by foliage [2]. Under suitable conditions, AV can bloom nearly year-round [2]. Among its specific requirements, adequate lighting is of paramount importance in the growth stages of AV [1]. Although AV is typically considered a low-light-friendly houseplant, insufficient light can disrupt its flowering patterns [2]. Therefore, it is crucial to consider the application of various colors of light with specific wavelengths, as they can impact the quality and health of AV from its vegetative phase to maturity [1].

AV can be propagated by seeds, leaf cuttings, and crown division, but most cultivars are propagated by leaf cuttings in commercial production. For this purpose, rooting hormones are not needed [4]. In vegetative propagation with leaf cuttings, a new shoot system must be initiated from an adventitious bud and new adventitious roots [5]. In order for the cells to form adventitious roots and buds, they must undergo development and differentiation. Given that certain cells and plant organs exhibit a more pronounced expression of new meristem growth points, the propagator must establish optimal conditions to foster the regeneration of the root or stem systems [5]. In addition to managing mother plants, the treatment of cuttings and manipulation of environmental conditions are effective for its success [5]. Light is an influential factor in forming adventitious roots and buds on cuttings [6].

Light serves as a crucial energy source that significantly affects various physiological and biochemical processes in plants. In essence, it is evident that light plays a pivotal role in all aspects of plant growth and development, spanning from seed germination to the ripening stages [7]. According to the quality, intensity, and duration of light, some signaling pathways can be activated or deactivated in plants, thereby influencing biological processes related to their morphogenesis [8]. Light quality affects photosynthesis, chlorophyll formation, root and stem length, flower bud formation, seed germination, and the rooting of cuttings [9,10]. In the commercial propagation of plants, it is necessary to use artificial light sources, especially in controlled conditions, due to the inherent limitations of natural light, including seasonal variations, weather fluctuations, and geographical latitude, which can impede plants' access to the optimal wavelengths necessary for their efficient growth [7].

Light-emitting diodes (LEDs) are new artificial lights, which have made it possible to control the quality of light [11]. LED technology has been increasingly used in protected agriculture nowadays, due to its capacity for precise control over light spectrum, intensity, and timing [12]. LED lighting encompasses the visible light ranges between 400 and 700 nm, i.e., photosynthetically active radiation (PAR), including blue (B)-violet light (400–490 nm), yellow-green (G) light (490–550 nm), and red (R) light (660 nm). Among these wavelengths, R and B wavelengths play a central role in the photomorphogenesis of plants, and achieving an optimal ratio between them (R:B ratio) is important. R light affects all stages of the plant life cycle, largely through a complex network of phytochrome photoreceptors [7]. B light promotes plant growth and development owing to two types of photoreceptors, namely cryptochromes and phototropins. Regardless of R and B, chloroplasts display the specific absorption of other colors by exclusive photoreceptors contributing to PAR [13].

Photomorphogenesis is a developmental response of plants to light correlated to photoreceptor proteins and signaling pathways [14]. It takes part in a myriad of morphological, physiological, and molecular activities of plants. Light shapes plant architecture, including the elongation of stem and coleoptile cells, the branching of shoots, and the expansion and enlargement of leaves [15]. Regarding the effect of light quality on root and shoot regeneration, studies have been conducted on some plants and different results have been obtained. The use of R light increased in vitro *Tripterospermum* rooting, but B light prevented it [16]. Kurilčik et al. [17] observed that B light added to R and far red (FR) light affected the rhizogenesis of *Chrysanthemum* micro-cuttings. The B light component was found to inhibit the rooting rate, but it increased the ratio of the fresh and dry weight of the explants [17]. Cavallaro et al. [18] reported the highest pineapple shoot proliferation under R light and the lowest under white (W) LED light. Kwon et al. [19] demonstrated that a combination of R + B LED yielded an optimal rate of in vitro regeneration in *Populus euramericana*, compared to B LED, R LED, or fluorescent light. Bello-Bello et al. [20] investigated the effect of five light treatments, including fluorescent, W, B, R, and R + B LEDs, on the shoot proliferation and growth of the vanilla plant (*Vanilla planifolia* Andrews) under in vitro conditions. Shoot proliferation was the highest in fluorescent light, W LED, and R + B combination. Dewir et al. [21] recorded that the cultivation of *Spathiphyllum cannifolium* under R LED yielded a higher shoot multiplication rate than under B or R + B LEDs. It is also noteworthy that, under R LED, the plant height was maximal in *Gerbera*

jamesonii [22]. However, Kostadinova et al. [23] recorded that the shoot length in *Pyrus communis* 'OHF333' was greater under white and B LED. Lotfi [24], investigating the effect of R, B, and equal R + B LED lights on the growth of *Pyrus communis* 'Arbi' explants under in vitro conditions, showed that the shoot height was better under R light. According to the studies mentioned above, obtaining information on using LEDs as a light source for plants is essential, especially in the propagation stage. Furthermore, due to the short size of AVs growing on multi-story shelves, determining the optimal artificial light quality for such conditions becomes inevitable. However, according to our information, no report has been published about the effect of light quality on the regeneration and growth of adventitious shoots/buds in the leaf cuttings of any plant. Therefore, this experiment investigated the effect of different LED light spectra on root and bud regeneration in African violet leaf cuttings. In this investigation, we hypothesized that each light color of the LEDs would significantly enhance the specific regeneration and relevant morpho-physiological traits of AV. The objectives were to (i) investigate the role of LEDs in enhancing the regeneration parameters of AV, particularly in the roots, shoots, and adventitious buds, (ii) compare the effects of white, red, blue, and red + blue light colors on AV regeneration, (iii) assess the accumulation of photosynthetic pigments in AV, and (iv) establish an optimal lighting condition for regenerating AV.

2. Materials and Methods

2.1. Plant Materials and Conditions

2.1.1. Specimen Species

The common propagation method of AV is leaf cutting. Several steps were carried out before transferring the cuttings into specific lighting conditions. In the initial step, a number of micro-propagated AVs were purchased from the Agricultural Biotechnology Research Institute, Isfahan, Iran. In the second step, these micro-propagated AVs were acclimated to the Research Greenhouse of the Department of Horticultural Sciences, Shahrekord University, Shahrekord, Iran (50°49′ E and 32°21′ N—altitude 2125 m a.s.l.). They were planted in plastic pots (i.e., a 1:1 mix of coco coir and perlite) and grown for nearly four months, with daytime and nighttime temperatures averaging 25 ± 2 and 18 ± 2 °C, respectively, and a relative humidity range of 60 ± 5%. In the third step, leaf-cutting materials of AV (approximately 4.5 × 3 cm, with 2 cm of petiole) were prepared from the mature leaves of the stock plants in the middle of winter, on January 21st. In the fourth step, before placing the leaf cuttings in the growing media (i.e., a 1:1 mix of coco coir and perlite) of the growth chambers, they were treated with a systemic benzimidazole fungicide, known as 'Benomyl' (Zagro Europe GmbH, Rheinfelden, Germany) at a concentration of 1 mg L^{-1} in distilled water for 10 min. This treatment was employed to protect the cuttings from pathogens.

2.1.2. Lighting Treatments

After disinfecting the AV leaf cuttings of the mature leaves with the aforesaid fungicide, they were transferred into four plant growth chambers. The experiment was carried out in two two-story cabinets, with each floor containing a chamber measuring 120 × 80 cm. Each chamber had a light panel at the top and a planting box equipped with a heating cable at the bottom. All three spectra of light included (1) white (W) [a green (G) (480–660 nm): blue (B) (410–460 nm) ratio of 0.33, i.e., 25 and 75%, respectively], considered the control, (2) red (R) (600–650 nm), (3) B (430–500 nm), and (4) R + B (a R:B ratio of 1) wavelengths (Figure 1). The lights were installed in the chambers and their design was such that it was possible to turn on and off and adjust the intensity of each color of light separately. Therefore, it was possible to use each of the white, blue, and red colors individually and any desired combination or intensity of them.

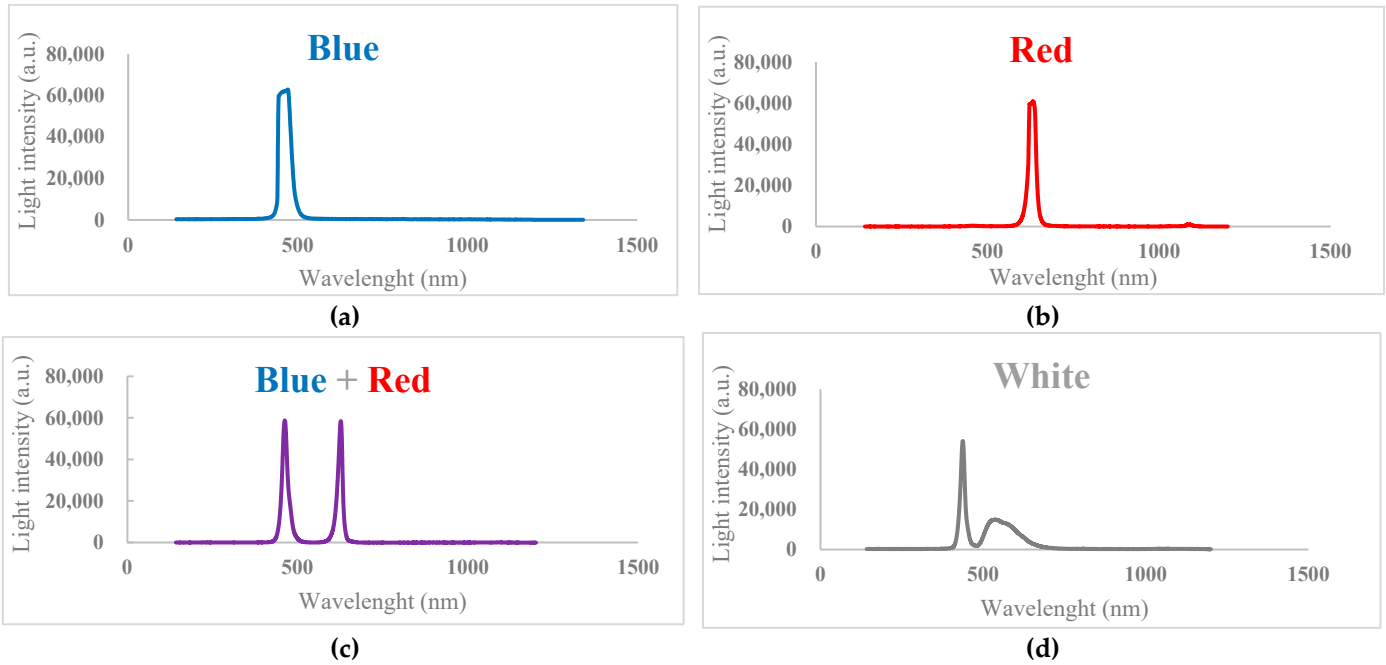

Figure 1. Light spectrum of different LED colors used in the experiment, measured using a spectrometer (Spectrometer V900, Optical Physics Technologists Co., Kashan, Iran).

This study was conducted based on a completely randomized experiment with four light treatments: 100% white, 100% red, 100% blue, and a 50% red + 50% blue combination. Each treatment had its own chamber where the desired light intensity and qualities were adjusted (Figure 2). The light intensity in each chamber/treatment was set at 80 µmol m^{-2} s^{-1}, measured by a PAR quantum sensor (MQ-500: Full-Spectrum Quantum Meter, Apogee Instruments, Inc., Logan, UT, USA). The reason why this light intensity was selected is that the optimal light intensity for micro-propagating of AV is just 70–100 µmol m^{-2}, in accordance with the previous studies [25]. Each treatment consisted of three replicates with 10 leaf cuttings in each one, which were planted in rows with a spacing of 5 cm between leaf cuttings and 10 cm between rows. The propagation substrate consisted of a mixture of coir and perlite in a 1:1 (v/v) ratio, which had been autoclaved for 20 min.

The light panels of the growth chambers were equipped with 3-Watt high-power 252 LEDs (Epistar Group, Xiamen, China), including 84 W, 84 R, and 84 B LEDs, evenly distributed at a distance of 50 cm from the planting beds. The regulation of light intensity, photoperiod, temperature, humidity, and irrigation for each chamber was as follows: (1) the intensity of each lighting treatment was adjusted using a dimmer and a PAR meter; (2) the photoperiod was set to 12 h light from 7 a.m. to 7 p.m. using a timer; (3) the internal chamber temperature was maintained at 21 ± 3 °C; (4) the root-zone temperature was controlled by a thermostat and maintained at 20 ± 2 °C; (5) the relative humidity range was regulated to 80 ± 5% by a humidity meter; (6) the irrigation system applied to the cuttings was an intermittent mist control system with nozzles sized at 0.5 mm and running timer of 1 min per every 2.5 h. The growing media were watered up to field capacity (FC).

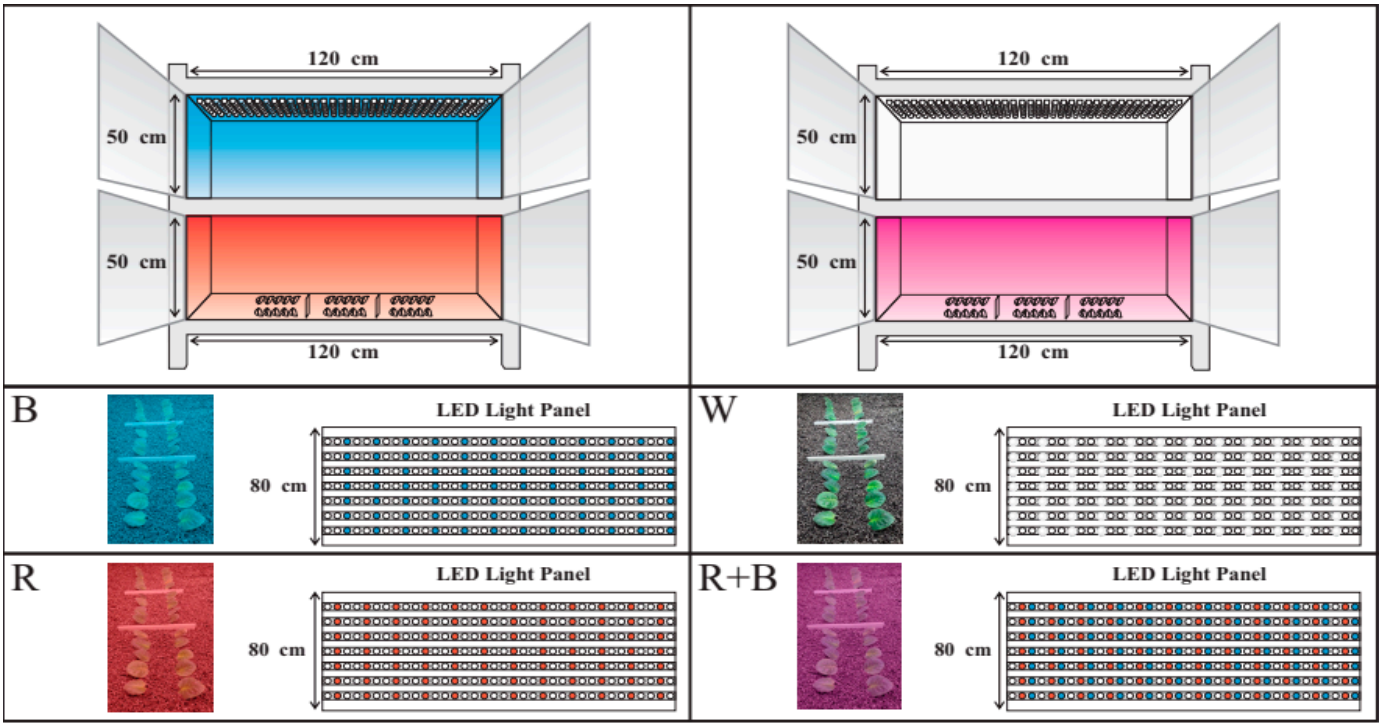

Figure 2. The schematic image of the chambers used to apply different LED light treatments and the layout of African violet leaf cuttings.

2.2. Measurements

2.2.1. Growth Parameters

Three months after planting, which took place in the middle of winter, all the leaf cutting materials subjected to the light treatments were removed from their growing media for the analysis of AV regeneration characteristics. By doing this, the length of roots (RL), the volume of roots (RV), the fresh weight of roots (RFW), the dry weight of roots (RDW), the length of shoots (SL), the number of adventitious buds/shoots (SN), the number of leaves (LN), the fresh weight of shoots (SFW), and the dry weight of shoots (SDW) were measured separately. The adventitious buds/shoots and leaves were counted for each treatment. To determine the length of roots and shoots, an electronic digital vernier caliper (Model: 0–150 mm, Guanglu Instruments Co., Ltd., Guilin, China) was used. The root volume was determined based on Archimedes' principle using the water displacement method, immersing each root in water in a graduated cylinder and measuring the volume [26]. The fresh and dry weights of roots and shoots were measured by an electronic weighing scale (Model: GR-200, A&D Co., Ltd., Tokyo, Japan). The roots and shoots were dried in an oven (FD 56, Binder GmbH, Tuttlingen, Germany) at 70 °C for 48 h. Furthermore, it is worth noting that plants typically produce thin and elongated stems with low dry weight in low-light conditions [27]. Therefore, the following parameters were calculated using measurement ratios: total biomass (TB), shoot length to shoot dry wight ratio (SL:SDW ratio), and root to shoot ratio (Ro:Sh ratio).

2.2.2. Photosynthetic Pigments Content

The chlorophyll content (Chl) (including *a*, *b*, and total) and the carotenoid content (Car) were measured based on the method described by Lichtenthaler [28], with slight modifications. To extract the photosynthetic pigments, 500 mg of fresh leaf blade was ground with a mortar and pestle with 5 mL of 80% acetone. Then, each homogenous treatment was transferred to a 10 mL falcon, and the volume of each falcon was increased to 10 mL with acetone. The extracted solution was centrifuged at 4000 rpm for 5 min. After that, the absorbance of the separated supernatant was read spectrophotometrically (T60

UV-Visible Spectrophotometer, PG Instruments Ltd., Lutterworth, UK) at 663 nm for Chl *a*, 646 nm for Chl *b*, and 470 nm for Car. The equations for determining them quantitatively (μg mL^{-1}) are as follows:

$$\text{Chl } a \text{ (μg mL}^{-1}\text{)} = (12.25 \times A_{663}) - (2.79 \times A_{646})$$

$$\text{Chl } b \text{ (μg mL}^{-1}\text{)} = (21.50 \times A_{646}) - (5.10 \times A_{663})$$

$$\text{Chl } a{+}b_{\text{ (total)}} \text{ (μg mL}^{-1}\text{)} = \text{Chl } a + \text{Chl } b$$

$$\text{Car (μg mL}^{-1}\text{)} = [(1000 \times A_{470}) - (1.82 \times \text{Chl } a) - (85.02 \times \text{Chl } b)] \div 198$$

where A is optical density, and their formulations were converted from μg mL^{-1} to mg g^{-1} by multiplying each equation yield and V/1000 W, V and W here were the final solution volume and the fresh leaf weight, respectively.

2.3. Experimental Design and Statistical Analysis

The research was laid out in a completely randomized design (CRD) experiment with three replicates and ten leaf cuttings per each replication. Data were analyzed using the SAS® software (version 9.3; SAS Institute Inc., Cary, NC, USA) employing the one-way analysis of variance (ANOVA). The *p*-value was considered in accordance with the least significant difference (LSD) test at the level of 5%. Furthermore, the loading plot and score plot for the parameters under analysis were generated using the Principal Component Analysis (PCA) utilizing Minitab® software (version 19.2020.1; Minitab LLC., State College, PA, USA).

3. Results

The results of the statistical analyses are reported in the following paragraphs, excluding the total chlorophyll (Chl *t*) content and the root to shoot ratio (Ro:Sh ratio) as they had no differences among the LED light treatments at the 5% level.

3.1. Growth Responses of Roots to Light Colors

The analysis indicated that the greatest amount of root length (RL) was in the AVs treated with B light, measuring 14 cm, followed by R + B lights at 12.70 cm, with no significant difference between them. In contrast, the lowest RL was observed in the AVs treated with R light, measuring 7.80 cm, which did not significantly differ from the W treatments at 9.10 cm (Table 1). Regarding the root volume (RV), the highest (3.10 cm^3) and lowest (1.50 cm^3) RVs were observed in the R + B and R treatments, respectively. The latter did not significantly differ from the B and W treatments (Table 1). The greatest root fresh weight (RFW) was obtained in the R + B (1.20 g), followed by the W treatments (1.03 g), showing no significant difference. Conversely, the lowest RFW was in the AV plants treated under R light (0.65 g), and then under B light (0.79 g), with no significant difference observed between these two treatments (Table 1). The greatest RDW, measuring 0.14 g, was observed in the plants treated with R + B light, significantly higher that all the other treatments (Table 1). Overall, the data analysis regarding the root attributes reveals that the combination of R and B lights could enhance the roots growth of AV in most cases; however, R light alone did not significantly impact the roots of the AVs.

Table 1. Effect of different LED light spectra on root growth indices in African violets.

Light Spectra	RL (cm)	RV (cm^3)	RFW (g)	RDW (g)
White	9.10 ± 0.62 ^{b†}	2.20 ± 0.25 ^{b}	1.03 ± 0.12 ^{ab}	0.09 ± 0.01 ^{b}
Red	7.80 ± 0.34 ^{b}	1.50 ± 0.18 ^{b}	0.65 ± 0.05 ^{c}	0.06 ± 0.03 ^{b}
Blue	14.00 ± 0.82 ^{a}	2.00 ± 0.15 ^{b}	0.79 ± 0.13 ^{bc}	0.09 ± 0.03 ^{b}
Red + Blue	12.70 ± 091 ^{ab}	3.10 ± 0.35 ^{a}	1.20 ± 0.13 ^{a}	0.14 ± 0.01 ^{a}
Significance	***	**	*	*

Abbreviations: RL, root length; RV, root volume; RFW, root fresh weight; RDW, root dry weight. † Means having different letters are significantly different at 5% level from LSD test. Data are presented as the mean ± standard error. Not significant (ns), *, **, *** indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

3.2. Leaf and Shoot Growth Dynamics

The results showed that the highest adventitious bud/shoot number (SN) was counted in AV plants grown under R + B or W light spectra, both recording 5.50. The lowest SN was noted in AV plants grown under R light spectrum (3.80), and B light (3.90), with no significant difference observed (Table 2, Figure 3). Additionally, the analysis of the leaf number (LN) indicated that treatments with B light resulted in 3.40 leaves, significantly more than all the other treatments. Specifically, 1.60 leaves were observed for R + B light, 1.30 for W light, and merely 0.50 leaves for R light, all statistically different from one another (Table 2). Regarding the shoot length (SL), considering the AV's typical rosette growth, part of its total length comprises petioles. There was a notable increase in the SL in the AVs treated with R + B light, measuring 1.60 cm, while the shortest SL was seen in the AVs treated with W and B light at 1.30 cm, and R light at 1.40 cm, with no significant difference (Table 2).

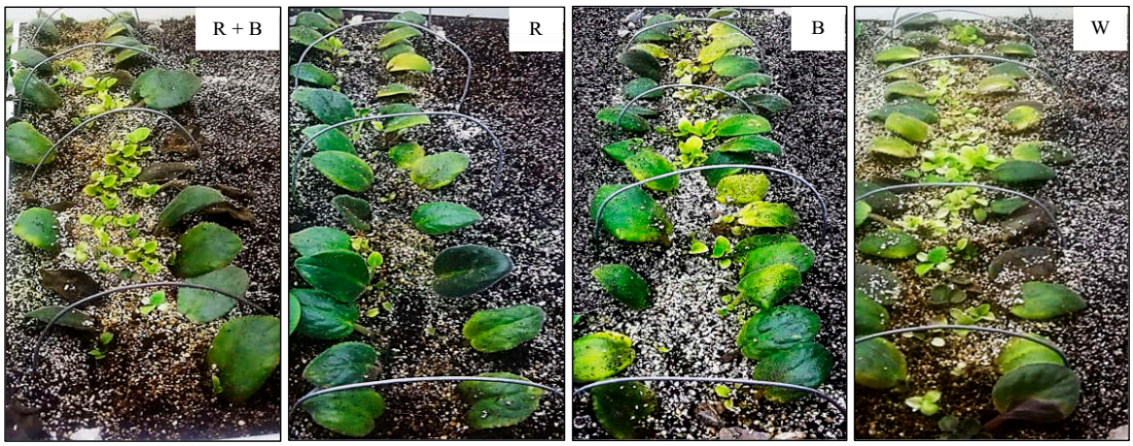

Figure 3. The effect of different LED light spectrums on African violet adventitious bud formation.

For the shoot fresh weight (SFW), the R + B treatments exhibited the highest (1.76 g), and R light the lowest (0.97 g), which were significantly different, while the B and W light treatments fell in between, differing from both the highest and lowest value. No significant difference was observed between the W and B treatments (Table 2). A consequent similar trend was observed for the shoot dry weight (SFW), with the highest (0.13 g) and lowest (0.04 g) recorded in the R + B and R treatments, respectively, while B and W fell in the middle, not significantly different from each other (Table 2). Hence, applying R + B spectra might enhance the shoot characteristics and root attributes compared to other LED treatments, while it appears that the R light alone does not positively impact the shoot growth and developmental traits in AVs.

Table 2. Effect of different LED light spectra on shoot growth indices in African violets.

Light Spectra	SN (Count)	LN (Count)	SL (cm)	SFW (g)	SDW (g)
White	$5.50 \pm 0.17^{a\dagger}$	1.30 ± 0.08^{c}	1.30 ± 0.02^{b}	1.24 ± 0.09^{b}	0.08 ± 0.00^{b}
Red	3.80 ± 0.10^{b}	0.50 ± 0.05^{d}	1.40 ± 0.05^{b}	0.97 ± 0.06^{c}	0.04 ± 0.00^{c}
Blue	3.90 ± 0.17^{b}	3.40 ± 0.03^{a}	1.30 ± 0.08^{b}	1.33 ± 0.08^{b}	0.09 ± 0.00^{b}
Red + Blue	5.50 ± 0.35^{a}	1.60 ± 0.06^{b}	1.60 ± 0.03^{a}	1.76 ± 0.07^{a}	0.13 ± 0.00^{a}
Significance	***	***	*	***	***

Abbreviations: SN, adventitious bud/shoot number; LN, leaf number; SL, shoot length; SFW, shoot fresh weight; SDW, shoot dry weight. † Means having different letters are significantly different at 5% level from LSD test. Data are presented as the mean ± standard error. Not significant (ns), *, *** indicate significance at $p \leq 0.05$ and $p \leq 0.001$, respectively.

3.3. Photosynthetic Pigments and Vegetative Characteristics

Under specific LED light color treatments, the chlorophyll (Chl) content exhibited significant variations. Surprisingly, the highest Chl *a* content was observed in the AVs treated with R light (0.15 mg g^{-1} f.w.), which did not significantly differ from the R + B (0.14 mg g^{-1} f.w.) and W (0.12 mg g^{-1} f.w.) treatments. However, the lowest Chl *a* content (0.07 mg g^{-1} f.w.) was measured in the B treatment of the AVs (Table 3). Contrary to Chl *a*, the results indicated that the content of Chl *b* (0.09 mg g^{-1} f.w.) was lowest when the plants were exposed to R light. The highest Chl *b* content (0.13 mg g^{-1} f.w.) was observed in the W and B treatments, with no significant difference between them (Table 3). The Chl *a*/*b* ratio followed a similar pattern to Chl *a*. The AVs treated with the R light showed a ratio at 1.55, while the lowest value was assessed in the AVs treated with the B color, at 0.57 (Table 3).

Table 3. Effect of different LED light spectra on photosynthetic pigments and vegetation indices in African violets.

Light Spectra	Chl *a* (mg g^{-1} f.w.)	Chl *b* (mg g^{-1} f.w.)	Chl *a*/*b* (mg g^{-1} f.w.)	Chl *t* (mg g^{-1} f.w.)	Car (mg g^{-1} f.w.)	TB (g)	Ro:Sh	SL:SDW Ratio
White	$0.12 \pm 0.02^{a\dagger}$	0.13 ± 0.01^{a}	0.98 ± 0.27^{bc}	0.25 ± 0.03	3.20 ± 0.17^{ab}	2.27 ± 0.09^{b}	1.08 ± 0.13	16.50 ± 0.97^{b}
Red	0.15 ± 0.00^{a}	0.09 ± 0.00^{b}	1.55 ± 0.10^{a}	0.24 ± 0.01	2.70 ± 0.19^{b}	1.62 ± 0.09^{c}	1.05 ± 0.13	36.90 ± 4.99^{a}
Blue	0.07 ± 0.00^{b}	0.13 ± 0.01^{a}	0.57 ± 0.06^{c}	0.20 ± 0.02	2.60 ± 0.18^{b}	2.12 ± 0.20^{b}	0.95 ± 0.24	14.92 ± 1.32^{b}
Red + Blue	0.14 ± 0.01^{a}	0.12 ± 0.00^{ab}	1.14 ± 0.52^{ab}	0.26 ± 0.04	3.80 ± 0.44^{a}	2.96 ± 0.08^{a}	1.05 ± 0.05	12.52 ± 0.80^{b}
Significance	*	*	**	ns	*	***	ns	***

Abbreviations: Chl, chlorophyll; Chl *t*, total chlorophyll; Car, carotenoids; TB, total biomass; Ro:Sh, the root to shoot ratio; SL, shoot length; SDW, shoot dry weight. † Means having different letters are significantly different at 5% level from LSD test. Data are presented as the mean ± standard error. Not significant (ns), *, **, *** indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Parallel to Chl, the content of carotenoids (Car) was assayed. The results indicated that the AVs treated with the R + B lights reached a peak of 3.80 mg g^{-1} f.w., and the B and R lights reduced the Car content to 2.60 mg g^{-1} f.w., with no significant difference between them (Table 3). The total biomass (TB) in the AVs treated with the R + B lights (2.96 g) was significantly higher than in the other treatments. With R light, the plants had the lowest TB at 1.62 g (Table 3). Concerning the shoot length to shoot dry wight ratio (SL:SDW ratio), the plants treated with R light exhibited a notably increased ratio at 36.90 cm g^{-1} compared to the other treatments, which had lower values. However, these values were not significantly different from each other, ranging down to 12.52 cm g^{-1} in the plants grown under R + B light (Table 3).

3.4. Principal Component Analysis (PCA) of Root Growth, Shoot Growth, and Pigments

A PCA was performed on the data from all the AV leaf cuttings, taking into account the different LED wavelength treatments. The biplot graph illustrating this analysis is shown in Figure 4. The first two components (PCs) together accounted for over 90% of the total variance, with PC1 and PC2 explaining 59% and 32.4% of the variance, respectively. Therefore, these PCs can be effectively used to evaluate the relationships between the traits and treatments. The percentage of variance explained and the eigenvalues associated with each component (four components in total) are detailed in Table 4.

Table 4. Eigenvalues associated with each component in the principal components analysis.

	PC1	PC2	PC3	PC4
Eigenvalue	10.038	5.510	1.452	0.000
Proportion	0.590	0.324	0.085	0.000
Cumulative	0.590	0.915	1.000	1.000

In this study, several correlations and patterns were observed. PC1 showed positive correlations with the RFW, RDW, RV, SFW, SDW, TB, SN, and Chl *b*. Conversely, PC1 had

negative correlations with the SL:SDW and Ro:Sh. On the other hand, PC2 was found to have positive correlations with the LN, RL, and Chl *b*, and negative correlations with the Chl *a*, Chl *t*, Chl *a/b*, Car, SL, and SN. In terms of the LED light treatments, the R, B, and R + B treatments were clearly differentiated and clustered based on PC1 and PC2. The R + B and W treatments were quite similar and positioned on the positive side of PC1, specifically in the lower right quadrant, and were clustered with the SN, RFW, and Car. On the other hand, the B light treatments were situated on the positive side of PC2, in the upper right quadrant, near the y-axis, and clustered with LN and RL. In contrast, the R treatments appeared on the negative side of PC1, in the lower left quadrant, and were correlated with the SL:SDW, Ro:Sh, and Chl *a/b* (Figure 4).

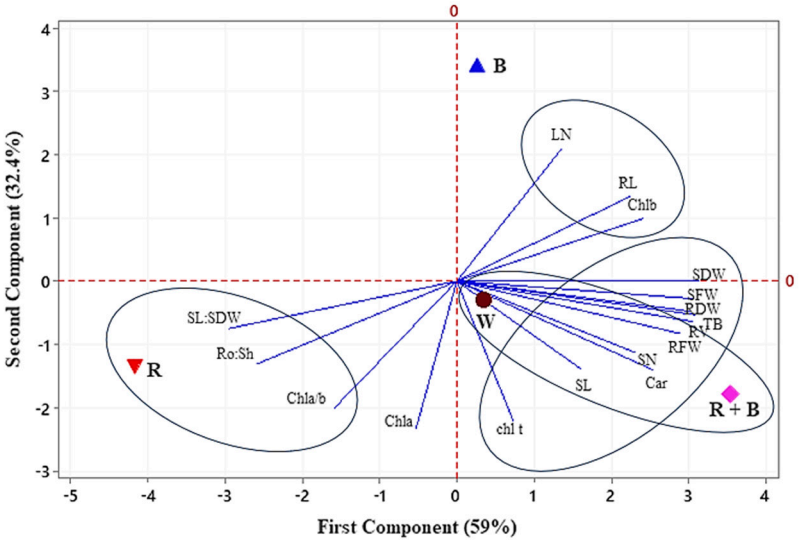

Figure 4. Biplot of principal component analysis for measured traits and treatments of African violets under different LED light conditions. Abbreviations: Chl, chlorophyll; Chl *t*, total chlorophyll; Car, carotenoids; LN, leaf number; RFW, root fresh weight; RDW, root dry weight; SFW, fresh weight of shoots; RL, root length; RV, root volume; SFW, shoot fresh weight; SDW, shoot dry weight; SN, adventitious bud/shoot number; SL, shoot length; TB, total biomass; Ro:Sh, the root to shoot ratio.

4. Discussion

In this study, the highest RL measurements were observed in the cuttings exposed to the B and R + B light colors, while the lowest measurements were recorded for the R and then W light. These findings align with the existing literature suggesting that B light stimulates the RL, while R light inhibits it. Previous studies in other plant species, such as *Symphyotrichum novi-belgii* var. *novi-belgii*, have shown similar results [29]. The primary reason for this phenomenon in roots may be linked to the biosynthesis and transport of auxin and gibberellin and their interaction with photoreceptors in plants [30]. For instance, B light has been found to increase root growth by reducing gibberellin levels and increasing auxin in Norway spruce (*Picea abies* (L.) Karst.) [31]. Conversely, R light facilitates the transfer of auxin from leaves to roots [32]. Studies have revealed that Cryptochrome1 (CRY1) positively regulates primary root elongation but negatively impacts lateral root development, particularly under a higher B light intensity [33]. Additionally, the present investigation found that the R + B LED lights increased levels of the RV, RFW, and RDW in the AVs, while the lowest values were observed in the R treatments. The AVs treated with W light showed a higher RV, RFW, and RDW compared to those treated with R light. Figure 1 indicates that the W LED lamps used in this experiment consisted of blue-violet (400–470 nm with a pick at 440 nm) and blue-green (655–485 nm with a pick at 557 nm) spectrum, resulting in similar impacts on the RV, RFW, and RDW as the B treatments in AV. Similar findings have been reported in *Malus domestica* Borkh., where the B and W lights did not significantly differ in the total RL, RV, and lateral root numbers [34].

The results revealed that the SFW and SDW were higher in the AVs treated with the R + B lights than in those treated with the R light, consistent with numerous previous studies. For instance, Miao et al. [35] in *Cucumis sativus* L. and Lim et al. [36] in *Gerbera jamesonii* cv. 'Shy Pink', reported the highest fresh and dry weight in the combination of R + B. Generally, R light initiates photosynthesis, while B light promotes the process [35,37]. The short-term effectiveness of B light is less than that of R light under limited light conditions [38]. Conversely, long-term exposure to R light can lead to the abnormal functioning of photosystem II (PSII), reducing the rate of photosynthesis [38]. Thus, the accumulation of dry and fresh matters can vary under different light conditions due to these reasons.

This investigation revealed that the SN in the AVs treated with W and R + B lights was significantly higher than those treated with B and R alone. Firstly, various pieces of literature support this phenomenon, highlighting the increased SN in many propagated plants when exposed to R + B wavelengths in comparison to other pure colors [19,20,36,39]. Bello-Bello et al. [20] in vanilla (*Vanilla planifolia* Andrews), Hung et al. [39] in *Vaccinium corymbosum*, Kwon et al. [19] in *Populus euramericana*., and Lim et al. [36] in *Gerbera jamesonii* cv. 'Shy Pink' found that the combination of B and R lights could enhance the regeneration of shoots compared to using B or R LED lights under in vitro conditions. Secondly, as depicted in Figure 1, the W LED light here comprised two spectra: 75% blue-violet (400–470 nm) and 25% blue-green (655–485 nm). This underlines the significance of green (G) wavelengths in the regeneration of AV shoots. In this context, Kaewjampa and Shimasaki [40] demonstrated that the use of interval lighting with G LEDs could stimulate shoot proliferation and formation in *Cymbidium waltz 'Idol'* under in vitro conditions. The experiment clearly demonstrated the R + B treatment consistently produced the highest SL measurements in the AVs, while no significant differences were observed between the W, R, and B wavelengths. This aligns with previous research in blueberry [39], banana [41], and vanilla [20], where the increasing effect of R + B light on the shoot length was also observed. Conflicting reports exist on the effects of light quality on the SL, with R light generally increasing elongation and B light inhibiting growth [36,42]. However, there are exceptions, such as lettuce [43] and petunia [44], where the positive and negative effects of B and R lights on SL were observed, respectively. R light can individually enhance the SL by causing the excessive elongation of internodes in stems, known as red light syndrome [45], which is not considered a desirable trait [46]. On the other hand, the promotion of shoot elongation due to the shade-avoidance responses in plants often occurs when B light is combined with lower PHY activity (e.g., pure B and impure B created by adding low-level far-red light) [47,48]. However, in the present study, where the AVs treated with B showed the highest measurements of the LN, SFW, and SDW compared to the R-treated AVs, it appears that the increase in the SL of the AVs is more closely related to photosynthesis and less to shade-avoidance responses. Nonetheless, the highest ratio of SL:SDW was related to the R treatment, which indicates the effect of an unfavorable increase in the shoot length due to the individual R light (red light syndrome). Therefore, maintaining a balance between B and R wavelengths is crucial for the optimal growth and development of plants, as emphasized in previous studies [42,46].

Regarding photosynthetic pigments, the research revealed that R light (including both R and R + B colors) increased the content of Chl *a*, while B light (comprising W, B, and R + B colors) enhanced the content of Chl *b* in AV. This suggests that AVs, like many other plant species, have the ability to adjust their Chl content in response to different light conditions, specifically in the presence of R or B wavelengths, in order to maintain their health [49]. The highest and lowest measurements of Chl *a*/*b* in the AVs were associated with the R and B lights, respectively. In natural lighting, the Chl *a*/*b* ratio is typically falls within the range of 2 to 4. Various factors, such as ambient light, biotic or abiotic stresses, and plant growth stages, greatly influence the levels of Chl *a*, Chl *b*, total Chl, and Chl *a*/*b* in plants [50,51]. Furthermore, under different light conditions, Chl *a* and Chl *b* can convert into each other [51,52]. For instance, Chl *a* can be converted to Chl *b* when the

rate of Chl synthesis is low in darkness [51], or Chl *b* can be converted back to Chl *a* in the chloroplast [50,52]. Apart from Chl, the increase in the Car levels in the AVs exposed to R + B colors compared to B and R colors individually aligns with the findings reported by Shin et al. [53], who observed enhanced Car and Chl synthesis in *Doritaenopsis* hort. (*Orchidaceae*) plants exposed to R + B LEDs. The AVs treated with W light showed the highest accumulation of Car, consistent with the general understanding that B wavelengths tend to increase the Car content in many plants. Previous research has also demonstrated that light supplementation (R:B = 7:2) in the morning improved Chl and Car contents in the leaves of *Solanum lycopersicum* L., further supporting the findings of the present study [54].

Ultimately, the PCA revealed the impact of the different lighting treatments on the root and shoot regeneration and growth. Consistent with the findings of the mean comparisons, the R + B treatment demonstrated a positive influence on various root and shoot growth indices, such as the RL, RV, RFW, RDW, SN, SL, SFW, SDW, TB, and Car content. Additionally, R light positively affected the SL:SDW, Chl *a*, Chl *a/b*, but negatively affected several growth indices, particularly the LN and RL. Furthermore, both the R + B and W treatments exhibited similar effects on root and shoot regeneration, growth, and the biosynthesis of Car and Chl *a*.

5. Conclusions

In conclusion, the light quality significantly influences photosynthesis and various aspects of plant growth and development. Therefore, determining the optimal light spectrum for each plant species is crucial. LED lighting, especially that emitting blue, red, and white light wavelengths, offers the potential to tailor wavelengths for individual species. The present study on African violet leaf cuttings unveiled that a blend of blue and red light had a more positive effect on root and shoot regeneration and growth compared to other lights. Furthermore, plants treated separately with white and blue light showed better performance than those exposed to red light. This suggests that blue light might have a more significant role in stimulating African violets' regeneration than red light, contrasting with reports suggesting a higher ratio of red to blue light for overall plant growth. The outcomes of this research are anticipated to shed more light on how different light wavelengths impact plant-regenerative processes.

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