International Journal of
Plant Biology

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Use of Light-Emitting Diodes on the In Vitro Rooting of Apple Tree Rootstocks

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Abstract: This study presents a pioneering investigation into the use of Light Emitting Diodes (LEDs) for in vitro rooting of 'Marubakaido' apple tree rootstocks, marking the first report of this approach in the literature. The research evaluates the effects of four distinct light sources: blue LED (450 nm), red LED (660 nm), a combination of red and blue LEDs, and traditional fluorescent lamps as a control. Mini-cuttings were inoculated in Murashige and Skoog (MS) medium with reduced nutrient concentrations, supplemented with indoleacetic acid (IAA) and sucrose. The explants were incubated under controlled conditions for 30 days, enabling a comprehensive assessment of the impact of different light sources on various growth metrics. The results revealed that blue LEDs significantly enhanced dry mass accumulation in seedlings compared to both red LEDs and fluorescent lamps, demonstrating their superior effectiveness in promoting plant growth. The use of LEDs not only improves seedling development but also offers economic advantages over fluorescent lamps. LEDs are characterized by high luminous efficiency, low energy consumption, and a long operational lifespan, which collectively reduce costs in plant production systems. This research advances the understanding of light-mediated effects on plant tissue culture and highlights the potential of combining blue and red LEDs as a viable alternative to fluorescent lighting. These findings could revolutionize practices in horticulture and plant propagation, providing a more efficient and sustainable approach to in vitro cultivation.

Keywords: *Malus domestica* Borkh; Marubakaido; plants tissue culture; wavelength; LEDs

Academic Editor: Mohammad Anwar Hossain

Received: 19 December 2024
Revised: 8 January 2025
Accepted: 9 January 2025
Published: 14 January 2025

Citation: Tomazini Scolaro, A.M.; De Martin, M.S.; Vieira, R.L.; Schveitzer, B.; de Souza, E.L.; Borges, E.M. Use of Light-Emitting Diodes on the In Vitro Rooting of Apple Tree Rootstocks. *Int. J. Plant Biol.* **2025**, *16*, 12. https://doi.org/10.3390/ijpb16010012

1. Introduction

Apples are the most widely cultivated fruit in temperate climates, leading in both planted areas and consumption volume worldwide [1]. Brazil is a significant player in the global apple industry, producing approximately 1.38 million tons of apples annually, of which 6.9% is exported [2,3]. Apple cultivation ranks among the top six fruit crops in the country, primarily concentrated in the southern regions, particularly in municipalities such

as Vacaria (Rio Grande do Sul), Fraiburgo and São Joaquim (Santa Catarina), and Palmas (Paraná) [4,5].

Rootstocks play an essential role in apple cultivation [6]. They are utilized for various purposes, including reducing plant vigor, providing resistance to pests and diseases, adapting to diverse soil conditions, inducing early fruiting, and enhancing orchard productivity. In recent decades, advances in clonal rootstocks developed through genetic improvement have transformed the apple production chain [7–10].

In Brazil, the predominant rootstock is 'Marubakaido' [11]. Known for its vigor and resistance to crown rot and wooly aphid, 'Marubakaido' is a widely used choice for apple growers [12]. Traditionally, apple rootstocks are propagated through stool layering, a method that, while effective, has significant limitations [13,14]. It is time-consuming, labor-intensive, yields low output, and requires extensive physical space [13–15]. Additionally, this propagation method carries the risk of perpetuating materials with phytosanitary issues, potentially affecting orchard health [16,17].

In vitro vegetative propagation has been extensively studied for the production of apple propagules [14], aiming at the mass multiplication of cultivars and the generation of pathogen-free plants [15,18]. Also referred to as micropropagation, this technique facilitates the true-to-type reproduction of genetically valuable plants by cultivating plant segments in an artificial medium under aseptic conditions [19–22].

A common challenge in in vitro propagation systems is the high mortality rate of seedlings during the acclimatization phase [19–22]. This highlights the need for strategies that modify the environment, particularly during the final stages of micropropagation, to produce more robust seedlings and enhance their survival in subsequent stages [23–25]. To improve the efficiency of in vitro propagation, environmental factors such as temperature, humidity, ventilation, and light must be carefully optimized [26]. Among these, light is the most critical factor, playing a significant role in regulating plant growth and development [27].

Typically, in vitro plant growth rooms are equipped with artificial light sources, predominantly fluorescent lamps [28]. However, fluorescent lamps exhibit several drawbacks, including high energy consumption, excessive heat generation, and wavelength peaks that are not essential for seedling development [27].

Light-emitting diodes (LEDs) were introduced in the 1960s for plant production in controlled environments. Since then, substantial advancements have been made in their architecture, construction, and functionality. In 1961, the first infrared LEDs were patented, paving the way for a broad spectrum of applications. LEDs are characterized by their high efficiency, luminous intensity, low discharge of far-red and red light, and broad wavelength range, encompassing ultraviolet (250–380 nm), visible light (380–760 nm), and infrared light (760–1000 nm) [29].

LEDs have demonstrated promising results in plant tissue culture applications [27,30]. They offer specific peaks within the wavelength range most beneficial for plant growth [31] and enable precise control over light quality by allowing the selection of specific wavelengths [32]. Additional advantages include high luminous efficiency with minimal heat emission, long lifespan, absence of heavy metals, and low energy consumption [27,33].

Numerous studies have investigated the effects of monochromatic light, either individually or in combination (e.g., two or more colors), on the growth and morphogenesis of various in vitro-cultivated plant species [27]. These studies have shown that LED lighting influences several plant characteristics, including vegetative growth [34], the formation of photosynthetic pigments [26,35], and stomatal development, among others [36,37].

Ptak et al. [38] demonstrated that LED light significantly influenced various physiological and biochemical parameters in stevia shoots, including stomatal appearance and

density, photosynthetic pigment levels, soluble sugar content, and antioxidant enzyme activities. Similarly, Saeedi et al. [39] reported that a combination of blue and red LED spectra was particularly effective in promoting the growth and vegetative characteristics of walnut explants in vitro, while also enhancing carotenoid production.

This study aimed to evaluate the effects of LED light sources on the in vitro rooting of apple rootstocks from the 'Marubakaido' cultivar.

2. Materials and Methods

Mini-cuttings approximately 15 mm in length, derived from in vitro pre-established seedlings of the 'Marubakaido' apple rootstock cultivar, were used as explants. These explants were obtained from in vitro culture stocks, representing five generations of clonal rootstock propagation originating from a living collection.

The experiments followed a completely randomized design with four treatments, corresponding to four light sources: blue LED (450 nm), red LED (660 nm), a combination of red and blue LEDs (10 diodes of 660 nm and 4 diodes of 450 nm), and fluorescent lamps (control). Variables evaluated in the 'Marubakaido' seedlings included plant height, number of leaves and roots, fresh and dry mass of shoots and roots, and chlorophyll and carotenoid content. Five replicates, each consisting of four seedlings, were used for the analysis.

The culture medium consisted of Murashige and Skoog (MS) medium [40], modified to half the concentration of macronutrients and micronutrients, supplemented with iron chelate (FeEDTA), ethylenediaminetetraacetic acid (EDTA), 1.0 mg/L indoleacetic acid (IAA), and 30.0 g/L sucrose. The test tubes, with a capacity of 50 cm^3, were sealed using 7.0 × 7.0 cm pieces of aluminum foil and sterilized in an autoclave at 121 °C under 1.05 kg/cm^2 pressure for 15 min. Following sterilization, 6.0 mL aliquots of the medium were dispensed into the test tubes.

Prior to inoculation, the Laminar Flow Hood was disinfected with 70% ethanol and sterilized with UV light (100–280 nm) for 20 min. Using sterilized tweezers and scissors, mini-cuttings were isolated and vertically inoculated into the culture medium, with one explant per tube.

The growth room was equipped with shelves featuring distinct light sources. Each shelf was fitted with two tubular lamps:

Blue LEDs: Tecnal®, Tec-Lamp (450 nm, 14 diodes, 28 W, 99.6 ± 20.4 μmol m^{-2} s^{-1}, 900 mm).

Red LEDs: Tecnal®, Tec-Lamp (660 nm, 14 diodes, 28 W, 82.2 ± 13.0 μmol m^{-2} s^{-1}, 900 mm).

Red and Blue LEDs: Tecnal®, Tec-Lamp (10 diodes of 660 nm and 4 diodes of 450 nm, 28 W, 81.8 ± 14.3 μmol m^{-2} s^{-1}, 900 mm).

Fluorescent Lamps (control): Osram®, T8 FO 32W/640 (24.4 ± 4.4 μmol m^{-2} s^{-1}, 1200 mm).

Light-blocking curtains (Blackout®) were installed at the ends of the shelves to prevent light interference from adjacent treatments.

The inoculated tubes were transferred to the growth room, where they were maintained for 30 days under controlled conditions: temperature of 25 ± 2 °C, 16 h photoperiod, and specific lighting treatments. Environmental factors were monitored throughout the rooting period.

At the end of the 30-day period, plant material was carefully removed from the test tubes for evaluation. The parameters assessed included the following:

Height: Measured with a caliper, recording the distance between the collar region and the insertion of the last leaf.

Number of Leaves and Roots: Counted manually.

Fresh and Dry Mass: Shoots and roots were separated and weighed for fresh mass. They were then dried in paper bags at 60 °C for 96 h, after which the dry mass was measured.

For pigment analysis, approximately 150 mg of fresh plant material was randomly collected and ground in a mortar with a pestle. The macerate was filtered, and ethanol (95%, NEON®) was added to a final volume of 50 mL in a volumetric flask wrapped in aluminum foil. The extract was analyzed using a UV–VIS spectrophotometer (Varian Cary 50) with absorbance readings at the following:

664 nm for chlorophyll *a*.

648 nm for chlorophyll *b*.

470 nm for carotenoids.

The absorbance values were substituted into Lichtenthaler's equations (to calculate the concentrations of pigments, which were expressed in milligrams per gram of fresh mass (mg/g) [41–43].

Statistical Analysis

The data obtained from the four independent treatments were assessed for normality and homogeneity using a Shapiro–Wilk test and Bartlett's test, respectively. When the data met the assumptions of normality and homogeneity, the treatments were compared using a one-way ANOVA (parametric test). If the data were not normally distributed, a Kruskal–Wallis test (nonparametric test) was applied [44].

Hypothesis testing, including the one-way ANOVA and Kruskal–Wallis, was performed at a 95% confidence level ($p < 0.05$). For post hoc analysis, a Tukey test was used following the one-way ANOVA, while the Dwass–Steel–Critchlow–Fligner pairwise comparisons were employed after the Kruskal–Wallis test. All post hoc tests were conducted with a 95% confidence interval.

Data analysis was performed using JAMOVI (version 2.3.28) [45]. Visual representation of the data was achieved through boxplots with violin plots, created using JASP (version 0.18.3.0) [46–48]. Principal component analysis (PCA) was conducted using the MEDA plugin in JAMOVI [48,49].

3. Results and Discussion

The vegetative growth parameters measured for Marubakaido apple rootstock seedlings, which were grown using LEDs and a florescent lamp, were provided in Table 1. Figure 1 shows the seedlings of the Marubakaido apple rootstock cultivar obtained using LEDs and a fluorescent lamp (control).

Table 1. Vegetative growth of seedlings of 'Marubakaido' apple tree rootstocks rooted in vitro under different sources of light. IQR is the interquartile range.

		Mean	Std. Deviation	IQR
Dry mass of aerial part (mg)	Blue	27.400	7.287	12.750
Dry mass of aerial part (mg)	Red	19.583	6.494	6.000
Dry mass of aerial part (mg)	Red + Blue	23.563	7.668	4.500
Dry mass of aerial part (mg)	Fluorescent	13.150	5.060	7.250
Height (cm)	Blue	1.540	0.224	0.300
Height (cm)	Red	1.656	0.624	0.475
Height (cm)	Red + Blue	1.459	0.215	0.313
Height (cm)	Fluorescent	1.632	0.264	0.287
Number of Leaves	Blue	12.500	3.663	3.500
Number of Leaves	Red	11.208	1.933	2.000
Number of Leaves	Red + Blue	12.375	3.594	2.500

Table 1. *Cont.*

		Mean	Std. Deviation	IQR
Number of Leaves	Fluorescent	10.550	2.395	3.250
Number of Roots	Blue	7.450	3.268	5.000
Number of Roots	Red	6.250	3.193	4.250
Number of Roots	Red + Blue	5.188	3.209	5.000
Number of Roots	Fluorescent	7.500	3.269	4.250
Fresh mass of aerial part (mg)	Blue	118.250	34.865	47.500
Fresh mass of aerial part (mg)	Red	85.833	27.998	27.500
Fresh mass of aerial part (mg)	Red + Blue	101.813	34.083	40.500
Fresh mass of aerial part (mg)	Fluorescent	63.850	27.017	40.250
Roots dry mass (mg)	Blue	141.800	55.981	71.000
Roots dry mass (mg)	Red	98.500	31.903	52.000
Roots dry mass (mg)	Red + Blue	129.000	61.449	58.500
Roots dry mass (mg)	Fluorescent	103.500	36.182	74.000
Roots fresh mass (mg)	Blue	15.200	5.281	6.000
Roots fresh mass (mg)	Red	14.944	5.620	6.250
Roots fresh mass (mg)	Red + Blue	15.091	3.885	5.000
Roots fresh mass (mg)	Fluorescent	16.600	5.604	10.000

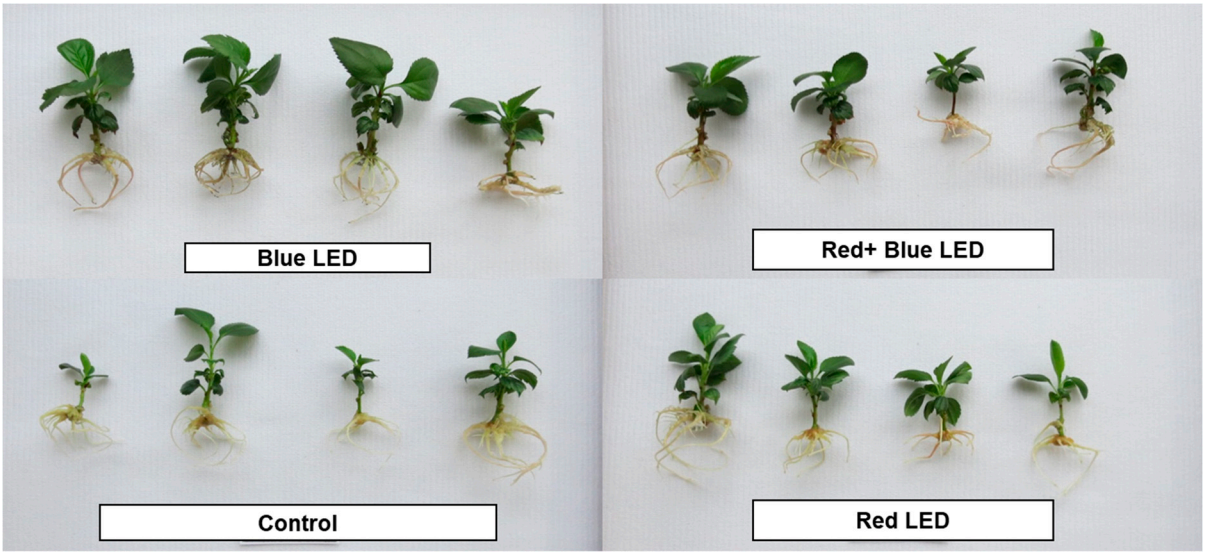

Figure 1. Seedlings of the Marubakaido apple rootstock cultivar obtained after 30 days of in vitro rooting under different light sources.

3.1. Height

No significant differences were observed in the height of *Marubakaido* apple rootstock seedlings among the four treatments, as all provided equivalent results (Figure 2). The Shapiro–Wilk test indicated that the data were not normally distributed (p-value < 0.001). Consequently, the Kruskal–Wallis test was used and confirmed that the heights obtained from the four treatments were statistically equivalent (p-value = 0.304).

3.2. Dry and Fresh Mass of the Aerial Parts

Treatments with blue LED and the combination of red + blue LED significantly increased both the fresh and dry mass of the aerial parts compared to the fluorescent lamp treatment (Table 1).

Seedlings exposed to LEDs exhibited notably higher fresh mass in the aerial parts compared to those under the control (fluorescent lamp) (Figure 3). The Shapiro–Wilk test confirmed that the data were normally distributed (p = 0.600), and the Bartlett's test

indicated equivalent variances (p = 0.904). The one-way ANOVA revealed significant differences in fresh mass among the four treatments (p < 0.001).

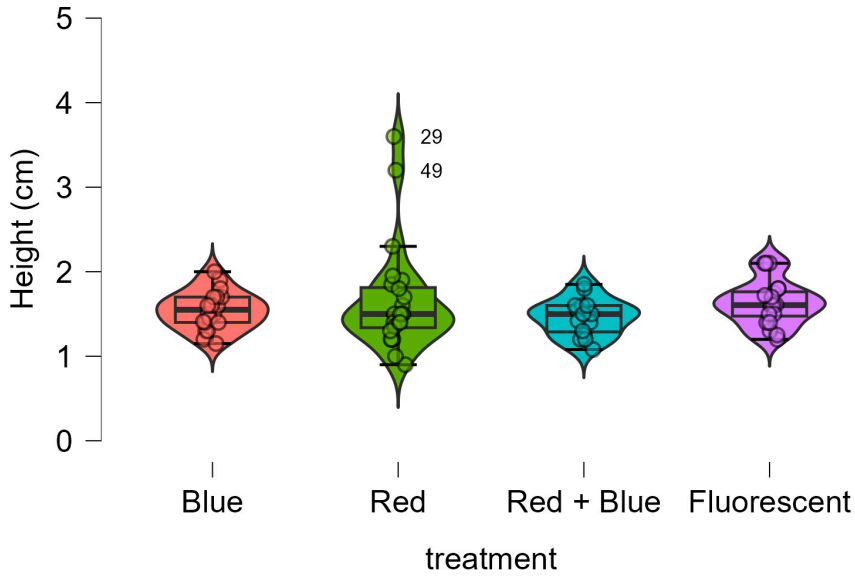

Figure 2. Boxplot of height of Marubakaido apple rootstock seedlings obtained using four independent treatments.

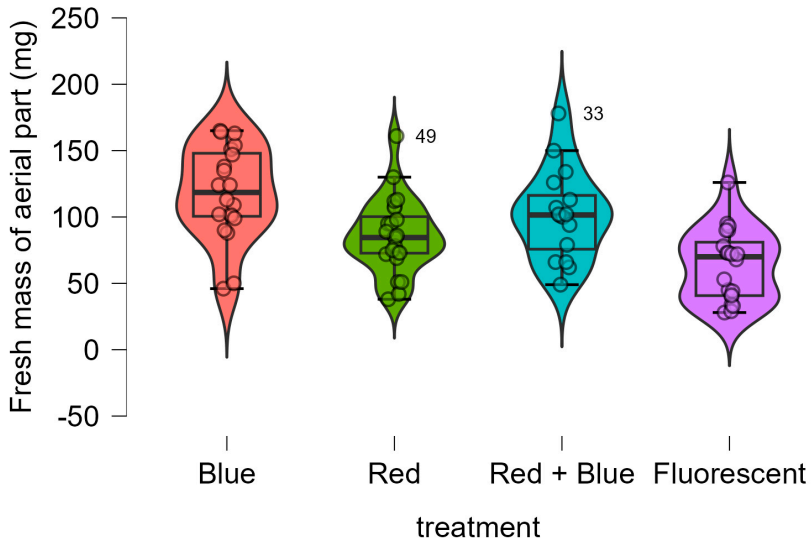

Figure 3. Boxplot of fresh mass of aerial part of Marubakaido apple rootstock seedlings obtained using four independent treatments.

Post hoc analysis using Tukey's test (Table 2) indicated that the red LED and red + blue LED treatments produced statistically similar dry masses in the aerial parts (p = 0.255). However, the blue LED treatment resulted in a significantly greater dry mass compared to the red LED treatment (p = 0.319).

The dry mass of the aerial parts was significantly higher in seedlings treated with LEDs compared to those under the control treatment (fluorescent lamp) (Figure 4). The Shapiro–Wilk test confirmed that the data followed a normal distribution (p = 0.615), and the Bartlett's test indicated homogeneity of variances (p = 0.432). The one-way ANOVA identified significant differences in dry mass among the four treatments (p < 0.001).

Table 2. Tukey post hoc test for the fresh mass of aerial part (mg) obtained for the four treatments.

Treatment	Treatment	Mean Difference	SE	t	p_{tukey}
Blue	Red	7.817	2.009	3.890	0.001
	(Red + Blue)	3.838	2.226	1.724	0.319
	Fluorescent	14.250	2.099	6.790	<0.001
Red	(Red + Blue)	−3.979	2.142	−1.858	0.255
	Fluorescent	6.433	2.009	3.202	0.011
(Red + Blue)	Fluorescent	10.413	2.226	4.678	<0.001

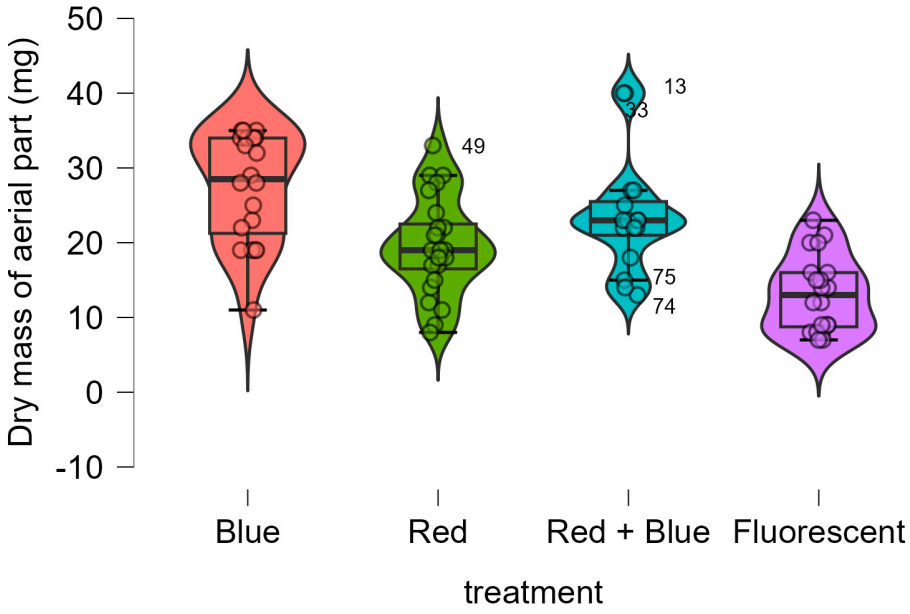

Figure 4. Boxplots of dry mass of aerial part (mg) of Marubakaido apple rootstock seedlings obtained using four independent treatments.

The post hoc analysis with the Tukey's test (Table 3) revealed that the red LED and red + blue LED treatments produced statistically equivalent dry masses ($p = 0.255$). However, all LED treatments yielded significantly higher dry masses compared to the control.

Table 3. Tukey post hoc test for the dry mass of aerial part (mg) obtained for the four treatments.

		Mean Difference	SE		p_{tukey}
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	(Red + Blue)	3.838	2.226	1.724	0.319
	Fluorescent	14.250	2.099	6.790	<0.001
Red	(Red + Blue)	−3.979	2.142	−1.858	0.255
	Fluorescent	6.433	2.009	3.202	0.011
(Red + Blue)	Fluorescent	10.413	2.226	4.678	<0.001

The emission peaks of blue and red light coincide with the maximum absorption wavelengths of chlorophyll, enabling photosynthesis to occur with maximum efficiency [50,51]. This phenomenon likely explains the greater accumulation of dry mass observed in seedlings exposed to blue LEDs and red + blue LEDs compared to those grown under fluorescent lamps.

The results of this experiment demonstrate that blue light (450 nm wavelength) positively influences the development of the aerial parts of 'Marubakaido' apple rootstock seedlings. In addition to its role in photosynthesis, light is essential for regulating growth and morphogenesis processes. Plant responses to blue light are mediated by pigments such

as phytochromes, cryptochromes, and phototropins. When stimulated, these pigments regulate various physiological processes, including gene expression, stomatal opening, and flowering [52].

Blue-light-induced stomatal opening enhances gas exchange during photosynthesis, which has a direct impact on plant growth and crop productivity [51].

A similar effect was observed by Shin et al. [52] in their study on in vitro orchid cultivation. They reported that seedlings grown under a combination of red and blue LEDs exhibited higher fresh and dry leaf masses compared to those grown under fluorescent lamps. Similarly, Li et al. [53] found that the combination of red and blue light resulted in greater fresh and dry seedling masses in *Gossypium hirsutum* L. compared to fluorescent lamps. This increase in fresh and dry mass can enhance the survival of seedlings during the acclimatization phase, which is the most critical stage in micropropagation systems due to the high mortality rates typically observed [54].

When used alone, red LED light (660 nm) produced results inferior to blue light but comparable to fluorescent lamps and the red + blue LED combination in terms of fresh and dry aerial mass. This suggests that red LEDs at 660 nm can also be effectively used for the in vitro cultivation of 'Marubakaido' apple rootstock.

Similarly, Lin et al. [35] demonstrated that fluorescent lamps and red LEDs were less efficient than blue LEDs, resulting in lower shoot formation and dry mass in Dendrobium officinale explants cultivated in vitro. Liu et al. [55] also observed that Platycodon grandiflorum seedlings exhibited greater increases in dry mass under blue LEDs compared to red LEDs.

The reduced effectiveness of red light may be explained by its impact on starch accumulation in chloroplasts, which can inhibit photosynthesis [37]. According to Sæbø et al. [56], red light exposure can induce a reduction in the translocation of photoassimilates from leaves to other parts of the plant, leading to starch accumulation in chloroplasts. This accumulation can indirectly reduce the photosynthetic rate, potentially explaining the lower dry mass production observed in seedlings exposed to red light compared to blue light.

3.3. Roots Dry and Fresh Mass

The root dry mass obtained using LED treatments was statistically equivalent to that obtained with the control (fluorescent lamp) (Figure 5). The Shapiro–Wilk test indicated that the data were not normally distributed ($p = 0.001$), and the Bartlett's test revealed that variances were not homogeneous ($p = 0.01$). As a result, the Kruskal–Wallis test was applied, showing a significant difference among treatments ($p < 0.014$). However, the post hoc analysis using Dwass–Steel–Critchlow–Fligner pairwise comparisons (Table 4) confirmed that the root dry mass results from LED treatments were statistically comparable to those of the control.

The fresh mass of roots obtained under LED treatments was comparable to that obtained with the control (fluorescent lamp) (Figure 6). The Shapiro–Wilk test confirmed the normality of the data (p-value = 0.501), and Bartlett's test indicated homogeneity of variances (p-value = 0.629). One-way ANOVA further revealed that there were no significant differences in root fresh mass among the four treatments (p-value = 0.835).

3.4. Number of Leaves and Roots

The number of leaves obtained using LED treatments was comparable to that obtained using the control (Figure 7). The Shapiro–Wilk test indicated that the data were not normally distributed (p-value = 0.001), and the Bartlett's test revealed unequal variances (p-value = 0.012). However, the Kruskal–Wallis test demonstrated that the four treatments yielded equivalent results (p-value < 0.213).

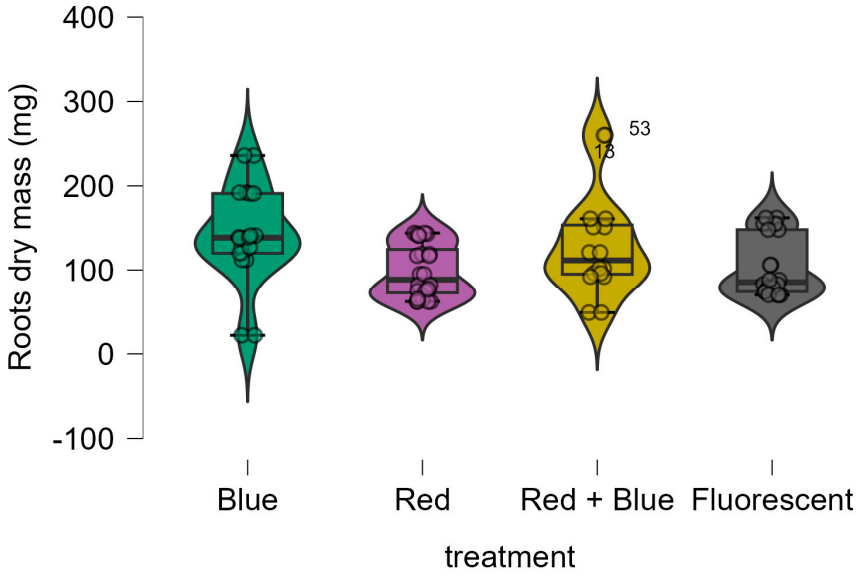

Figure 5. Boxplot of roots dry mass (mg) of Marubakaido apple rootstock seedlings obtained using four independent treatments.

Table 4. Pairwise comparisons of roots dry masses (mg) obtained using the four treatments.

Treatment	Treatment	*p*-Values
Blue	Fluorescent	0.120
Blue	Red	0.055
Blue	Red + Blue	0.094
Blue	Red + Blue	1.000
Fluorescent	Red	0.915
Fluorescent	Red + Blue	0.725
Fluorescent	Red + Blue	0.091
Red	Red + Blue	0.681
Red	Red + Blue	0.026
Red + Blue	Red + Blue	0.290

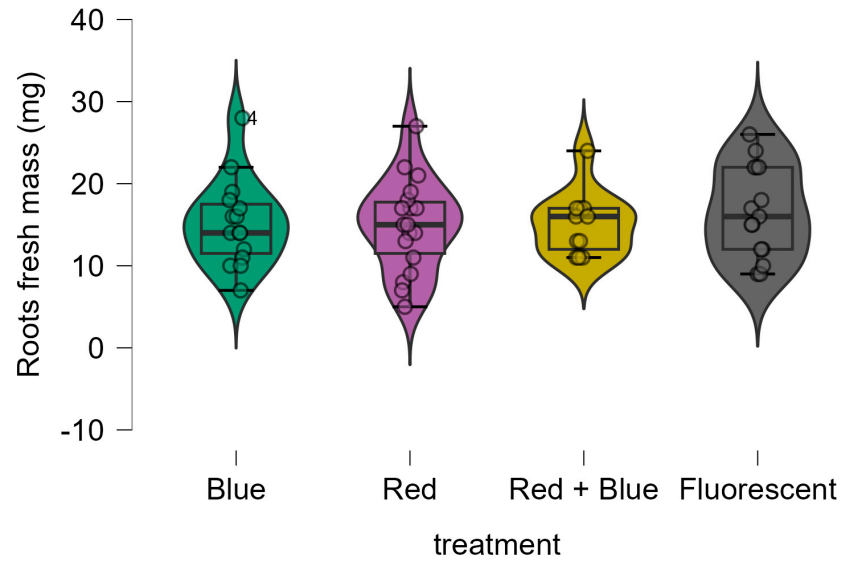

Figure 6. Boxplot of roots fresh mass (mg) of Marubakaido apple rootstock seedlings obtained using four independent treatments.

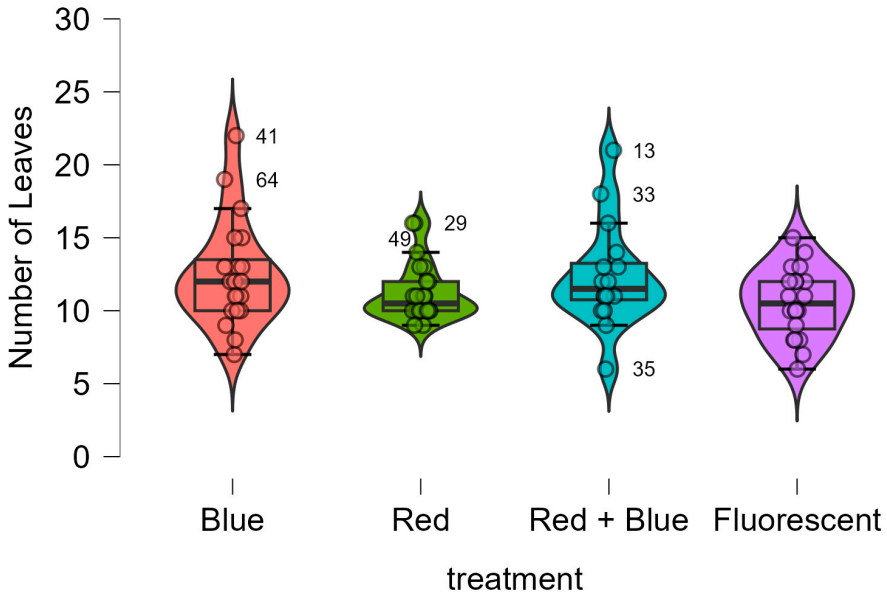

Figure 7. Boxplots of number of leaves in Marubakaido apple rootstock seedlings obtained using four independent treatments.

The number of roots obtained using LED treatments was equivalent to that obtained using the control (Figure 8). The Shapiro–Wilk test indicated that the data were normally distributed (p-value = 0.134), and the Bartlett's test confirmed homogeneity of variances (p-value = 0.999). One-way ANOVA demonstrated that the four treatments yielded equivalent results (p-value < 0.127).

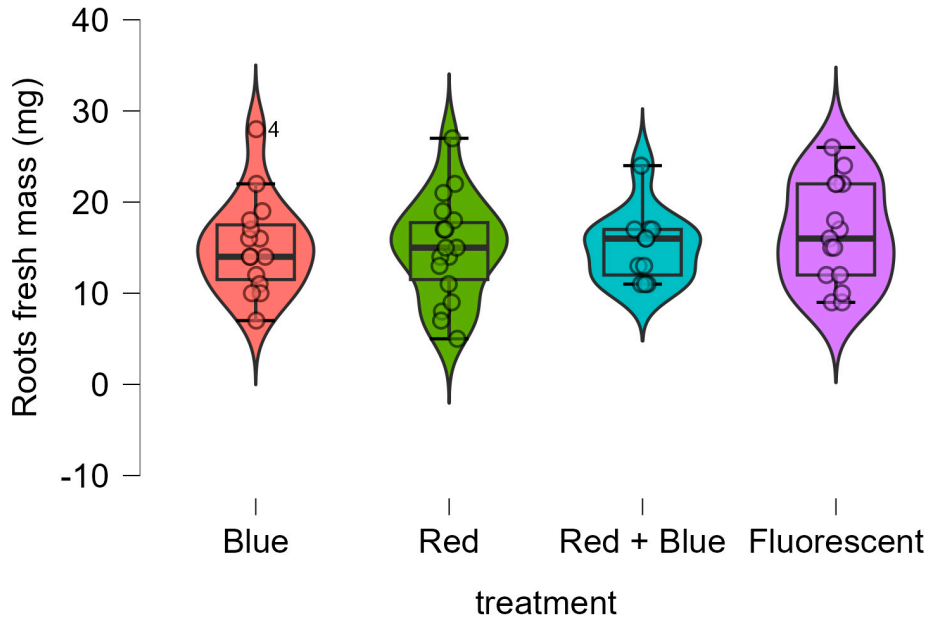

Figure 8. Boxplot of the number of roots fresh mass (mg) of Marubakaido apple rootstock seedlings obtained using four independent treatments.

Plant height (Figure 2), number of leaves (Figure 7), number of roots (Figure 8), and fresh (Figure 5) and dry root mass (Figure 6) were not significantly affected by the different light sources. According to Moon et al. [26], light quality can influence plant morphology. For instance, plant height may be promoted or inhibited depending on the interactions between blue and red light receptors and phytochromes [57]

A similar outcome was observed by Li et al. [53] who cultivated *Brassica napus* L. in vitro. Their study showed that the stem length of seedlings treated with LEDs was comparable to those treated with fluorescent lamps.

The wavelength of light can influence the rooting of in vitro seedlings, with effects varying depending on the species [26]. For example, a study by Chée [58] demonstrated that blue LEDs had a more pronounced effect on the rooting of grapevine seedlings compared to red LEDs. Conversely, Moon et al. [26] observed that the number of roots in *Tripterospermum japonicum* was promoted by fluorescent lamps and the red + blue LED combination but inhibited by individual red or blue LEDs. Similarly, Shin et al. [52] reported that fresh and dry root masses of in vitro orchids increased under the red + blue LED combination. However, Jao et al. [59] found that *Zantedeschia jucunda* seedlings grown under fluorescent lamps exhibited greater dry root mass formation compared to those treated with LEDs.

3.5. Principal Component Analysis (PCA)

A principal component analysis (PCA) was performed on the normalized data to identify patterns in vegetative growth [60,61]. The score plot (Figure 9) was used to observe correlations and trends within the data [44,62–64]. It illustrates that the number of roots, root fresh mass, and plant height were positively correlated, with samples located in the top-right quadrant exhibiting higher values for these parameters. Similarly, the fresh mass of aerial parts, dry mass of aerial parts, and root dry mass were correlated, with samples positioned in the bottom-left quadrant showing higher values for these variables.

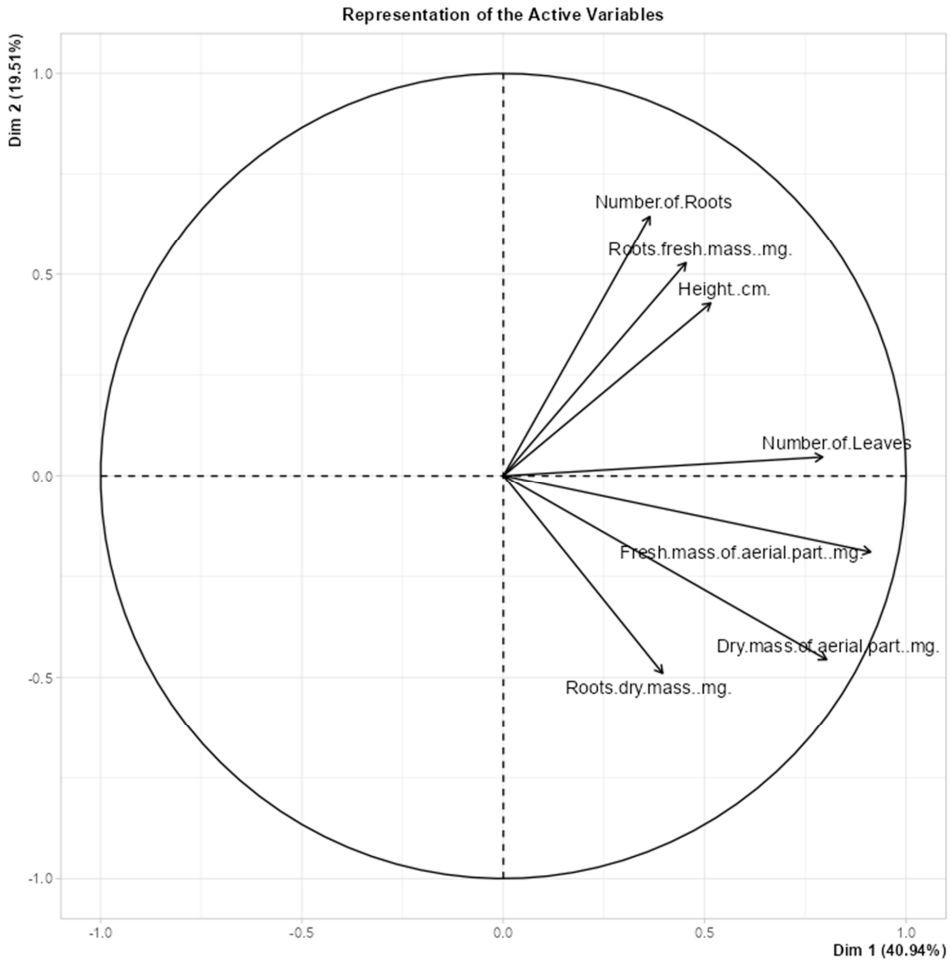

Figure 9. Loading plot of the vegetative growth of seedlings of 'Marubakaido' apple tree rootstocks rooted in vitro under different sources of light.

The loading plot (Figure 10) highlights that Marubakaido apple rootstock samples with greater vegetative growth were primarily situated on the right side of the plot. Most of these samples were grown under blue LED light, indicating that this light source promoted the best vegetative growth. In contrast, samples with less vegetative growth were predominantly located on the left side of the plot, corresponding to control samples grown under fluorescent lamps. These findings demonstrate that LED lights, particularly blue LEDs, were more effective than fluorescent lamps in enhancing vegetative growth.

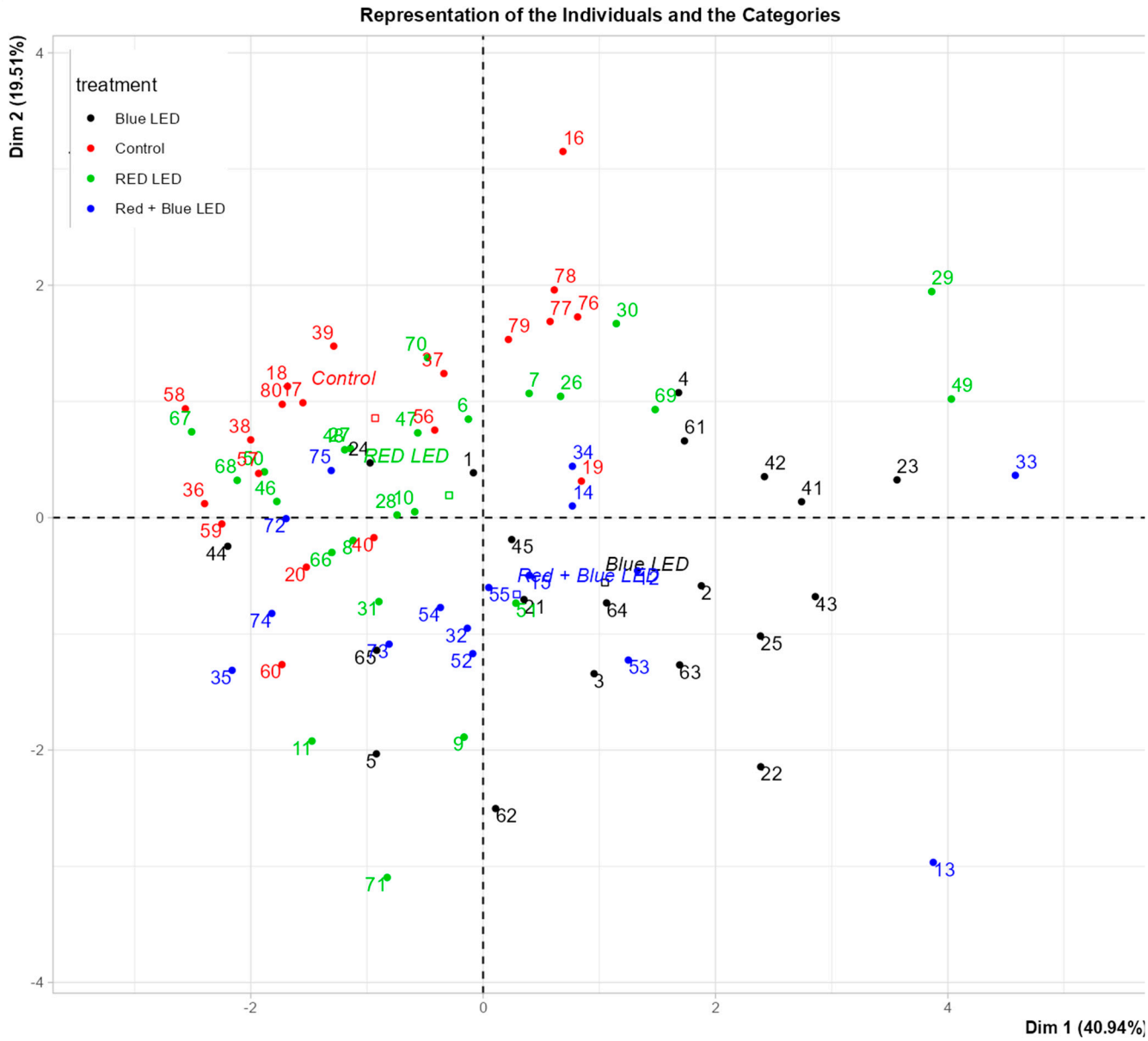

Figure 10. Score plot of the vegetative growth of seedlings of 'Marubakaido' apple tree rootstocks rooted in vitro under different sources of light.

3.6. Chlorophyll a, b, Total (a + b), and Carotenoids Content

Treatment with fluorescent lamps resulted in higher concentrations of chlorophylls a, b, total chlorophyll (a + b), and carotenoids in leaf samples compared to LED treatments. The different LED wavelengths did not show significant differences among themselves for pigment formation (Table 5). Jao et al. [59] cultivated Zantedeschia jucunda in vitro and reported that fluorescent lamps yielded more promising results for chlorophyll formation than LED treatments. Another study by Moon et al. [26] showed that chlorophyll content in

Tripterospermum japonicum was higher when seedlings were treated with fluorescent lamps and red + blue LED combinations but inhibited under isolated red and blue LEDs. However, Shin et al. [52] observed that in vitro-cultivated Doritaenopsis plants under the red + blue LED combination had higher chlorophyll and carotenoid content than those grown under fluorescent lamps. These studies indicate that the synthesis of chlorophylls and carotenoids in plants exposed to different light sources may vary depending on the species.

Table 5. Chlorophyll a, b, total (a + b), and carotenoids content in leaf samples of 'Marubakaido' apple tree rootstock seedlings rooted in vitro under different light sources.

Treatments	Chlorophyll *a* (mg g^{-1})	Chlorophyll *b* (mg g^{-1})	Total Chlorophyll (*a* + *b*) (mg g^{-1})	Carotenoids (mg g^{-1})
Blue LED	2.37 [b]	0.54 [b]	2.9 [b]	0.74 [b]
RED LED	2.49 [b]	0.63 [b]	3.12 [b]	0.78 [b]
Red+ Blue LED vermelho + azul	2.59 [b]	0.62 [b]	3.21 [b]	0.79 [b]
Control	3.17 [a]	0.83 [a]	3.99 [a]	0.94 [a]
RSD (%)	7.7	8.1	7.7	6.9

Means followed by the same letter in the column do not differ statistically according to Tukey's test at 5%.

Although carotenoids are known for their important role in protecting organisms from light-induced damage [65,66], both carotenoids and chlorophylls are involved in energy capture by plants [67–69]. Light wavelengths play a crucial role in regulating photosynthesis, with blue and red LEDs being the most used for seedling growth. Their wavelengths, approximately 460 nm and 660 nm, respectively, represent the ranges of highest photosynthetic efficiency [27].

Fluorescent lamps have wavelength peaks ranging from 350 to 750 nm in the electromagnetic spectrum, emitting light in a broad range of colors, many of which are unnecessary for seedling development [27]. Plants exposed to white light preferentially absorb light in the blue, red, and part of the green spectra [70].

Alvarenga et al. [71] showed that green LEDs induced greater synthesis of chlorophylls a, b, and total (a + b), and carotenoids in Achillea millefolium seedlings compared to blue and red LEDs. According to the same authors, the increase in pigment levels in plants when exposed to green light may be associated with stress in response to a lack of photosynthetically active light.

4. Conclusions

Blue LEDs and the red + blue LED combination, which resulted in greater fresh and dry mass of the aerial parts compared to fluorescent lamps, may serve as promising alternatives for in vitro rooting of Marubakaido apple rootstocks. These light sources promote the development of more robust seedlings with an increased likelihood of survival during the acclimatization phase. While red LEDs inhibited dry mass production relative to blue LEDs, they still produced dry mass levels comparable to those achieved with fluorescent lamps, making them a viable option.

In addition to their specific spectral peaks favorable for plant growth, LEDs offer several practical advantages, including high luminous efficiency, minimal heat generation, long lifespan, absence of heavy metals, and low energy consumption. These features improve the cost-effectiveness of in vitro plant propagation.

Although fluorescent lamps promote higher accumulation of chlorophylls and carotenoids compared to LEDs, they yield lower dry mass production than blue LEDs

and the red + blue LED combination during the in vitro rooting of Marubakaido apple rootstock seedlings.

Overall, blue LEDs are the most effective light source for enhancing dry mass accumulation in Marubakaido apple rootstock seedlings. However, blue LEDs, red LEDs, and the red + blue LED combination all represent viable alternatives to fluorescent lamps for in vitro rooting of these seedlings.

Author Contributions: E.M.B. and M.S.D.M. conceived and coordinated this project; A.M.T.S. performed the experiments with the help of R.L.V., B.S., E.L.d.S., E.M.B. and M.S.D.M. analyzed data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The authors acknowledge financial support and fellowships from the Brazilian agencies FAPESC (Fundação de Amparo a Pesquisa do Estado de Santa Catarina), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

Data Availability Statement: The data that support the findings of this study are available upon request from the corresponding author.

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

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