Article

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

Adriana Maria Tomazini Scolaro , Mariuccia Schlichting De Martin , Renato Luis Vieira , Bianca Schveitzer , Edson Luiz de Souza , Endler Marcel Borges *

Posted Date: 13 December 2024

doi: 10.20944/preprints202410.1742.v2

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

 doi:10.20944/preprints202410.1742.v2

Article

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

Adriana Maria Tomazini Scolaro [1,2], Mariuccia Schlichting De Martin [3], Renato Luis Vieira [3], Bianca Schveitzer [4], Edson Luiz de Souza [3] and Endler Marcel Borges [5,*]

[1] Universidade do Oeste de Santa Catarina, Campus de Videira. Rua Paese, 198, Bairro Universitári, o89560000 - Videira, SC – Brazil
[2A] Epagri - Estação Experimental de Caçador, Rua Abílio Franco, 1500, Bom Sucesso, CEP 89501-032 Caçador, SC. Brazil
[3] Epagri - Estação Experimental de São Joaquim Rua João Araújo Lima, 102, Jardim Caiçara, CEP 88600-000 São Joaquim, SC. Bazil
[4] Instituto do Meio Ambiente de Santa Catarina, Rodovia SC 401, km 4, n°4230, Saco Grande, CEP 88032-005 Florianópolis, SC. Brazil
[5] Departamento de Química, Fundação Universidade Regional de Blumenau, FURB, Campus 1, Rua Antônio da Veiga, 140, Victor Konder, 89012-900 Blumenau-SC, Brazil.
* Correspondence: embsouza@furb.br or marcelborgesb@gmail.com; Tel. +55-47-98421-5666

Abstract: This study presents a novel investigation into the use of Light Emitting Diodes (LEDs) for the in vitro rooting of 'Marubakaido' apple tree rootstocks, marking the first time this approach has been reported in the literature. The research compares four different 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 & Skoog (MS) medium with reduced nutrient concentrations and supplemented with indoleacetic acid (IAA) and sucrose. The explants were incubated under controlled conditions for 30 days, allowing for a thorough evaluation of the effects of different light sources on various growth metric. The study found that blue LEDs significantly increased dry mass accumulation seedlings compared to both red LEDs and fluorescent lamps, demonstrating the effectiveness of LEDs in promoting plant growth. Utilizing LEDs not only enhances seedling development but also offers economic benefits over fluorescent lamps. LEDs are known for their high luminous efficiency, low energy consumption, and long lifespan, which can lead to reduced operational costs in plant production systems. This research not only contributes to the understanding of light effects on plant tissue culture but also suggests that the combination of blue and red LEDs can serve as a viable alternative to fluorescent lamps, potentially transforming practices in the field of horticulture and plant propagation

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

1. Introduction

Apples are the most widely grown fruit in temperate climate regions, leading in both cultivated area and consumption volume [1]. Brazil is a major player in the global apple industry, producing approximately 1.38 million tons of apples annually, with 6.9% of this production being exported [2,3]. Apple cultivation ranks among the country's top six fruit crops, predominantly grown in the southern regions, including the municipalities of Vacaria in Rio Grande do Sul, Fraiburgo and São Joaquim in Santa Catarina, and Palmas in Paraná [4,5].

In the realm of apple cultivation, rootstocks play a crucial role [6]. They are employed for various purposes such as reducing plant vigor, providing resistance to pests and diseases, adapting to different soil conditions, inducing early fruiting, and enhancing orchard productivity. In recent

 doi:10.20944/preprints202410.1742.v2

decades, advancements in clonal rootstocks developed through genetic improvement have revolutionized the apple production chain [7–10].

In Brazil, the predominant rootstocks were Marubakaido [11]. 'Marubakaido' rootstock is recognized for its vigor and resistance to crown rot and woolly aphid [12]. Traditionally, apple rootstocks are propagated by stool layering [13,14]. This method is slow, yields low output, is labor-intensive, and requires extensive physical space [13–15]. Additionally, it may inadvertently propagate materials with phytosanitary issues[16,17]

In vitro vegetative propagation has been researched to produce vegetative material for apples [15,18], aiming at mass multiplication of cultivars and production of pathogen-free plants [14]. Also known as micropropagation, this technique involves the true-to-type reproduction of genetically valuable plants through the cultivation of plant segments in an artificial medium under aseptic conditions [19–22].

High mortality rates of seedlings during the acclimatization phase are common in in vitro propagation systems [19–22]. Therefore, strategies that modify the environment, especially during the final stages of micropropagation, are necessary to make the seedlings more robust and improve their survival in subsequent stages [23–25]. To enhance the efficiency of in vitro propagation techniques, factors such as temperature, humidity, ventilation, and light should be optimized [26]. Among these, light is the most important factor, capable of significantly regulating plant growth and development [27].

In vitro plant growth rooms are typically equipped with artificial light sources, mainly fluorescent lamps [28]. However, fluorescent lamps have undesirable characteristics such as high energy consumption and heat generation, along with varied wavelength peaks, some of which are not necessary for seedling development [27].

Light-emitting diodes (LEDs) were introduced in 1960 for plant production in closed systems. From 1960 to 2024, significant advancements were made in the architecture, construction, and enhancement of these artificial light sources. In 1961, the first infrared LEDs were patented. LEDs are known for their high efficiency, high luminous intensity, low-intensity discharge of far-red and red light, and broad wavelength spectrum, which includes ultraviolet (250–380 nm), visible light (380–760 nm), and infrared light (760–1000 nm) [29].

Light Emitting Diodes (LEDs) are being evaluated and have shown satisfactory results in plant tissue culture [27,30]. LEDs provide specific peaks within the range most favorable to plants [31], and allow for the precise selection of desired wavelengths [32]. They also offer other advantages, such as high luminous efficiency with minimal heating, long lifespan, absence of heavy metals, and low energy consumption [27,33].

Several studies have been conducted to investigate the effects of monochromatic lights, either alone or in combination (two or more colors), on the growth and morphogenesis of a wide variety of in vitro-cultivated seedling species [27]. According to these studies, various characteristics are affected, such as vegetative seedling growth [34], the formation of photosynthetic pigments [26,35], and stomatal development, among others [36,37].

This study aimed to evaluate the effect of using LEDs as light sources on the in vitro rooting of apple rootstocks of the Marubakaido cultivars.

2. Materials and Methods

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

The experiments were conducted in a completely randomized design with four treatments. The treatments consisted of four lighting sources: blue LED (450 nm), red LED (660 nm), red + blue LED (10 diodes of 660 nm and 4 diodes of 450 nm), and fluorescent lamp (control). For the Marubakaido cultivar seedlings, variables such as height, number of leaves and roots, fresh and dry mass of the

 doi:10.20944/preprints202410.1742.v2

aerial part and roots, and chlorophyll and carotenoid content were evaluated. Five replications of eight seedlings each were used.

The culture medium used was MS [38] with half the concentration of macronutrients and micronutrients, and iron chelate (FeEDTA) and ethylenediaminetetraacetic acid (EDTA), supplemented with 1.0 mg/L indoleacetic acid (IAA) and 30.0 g/L sucrose.

The culture medium was distributed in 6.0 mL aliquots into 50 cm^3 test tubes. The tubes were sealed with 7.0 x 7.0 cm aluminum foil and sterilized by autoclaving at 121°C and 1.05 kg/cm^2 pressure for 15 minutes.

After disinfecting the Laminar Flow Hood with 70% ethanol and sterilizing it with UV light (100 to 280 nm) for 20 minutes, the explant inoculation process began. Using tweezers and scissors, the mini-cuttings were isolated and inoculated into the culture medium vertically in the tubes, ensuring each tube contained one explant.

In the growth room, the shelves were equipped with two tubular lamps each. The first shelf was equipped with blue LED lamps (Tecnal®, Tec-Lamp, 14 diodes of 450 nm, 28 W, 99.6 ± 20.4 μmol m^{-2} s^{-1}, 900 mm), the second with red LED lamps (Tecnal®, Tec-Lamp, 14 diodes of 660 nm, 28 W, 82.2 ± 13.0 μmol m^{-2} s^{-1}, 900 mm), the third with red and blue LED lamps (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), and the last with fluorescent lamps (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 to prevent interference from other light sources.

The tubes containing the inoculated explants were transferred to the Growth Room, where they remained for 30 days under controlled environmental conditions: a temperature of 25 ± 2°C, a 16-hour photoperiod, and different lighting conditions. The environmental factors were monitored throughout the rooting period of the seedlings.

After removing the plant material from the test tubes, evaluations were conducted for height, number of leaves and roots, and fresh and dry mass of shoots and roots. Height was measured using a caliper, recording the distance between the collar region and the insertion of the last leaf. The number of leaves and roots was then counted. The shoots and roots were separated and weighed to obtain the fresh mass, then placed separately in paper bags and transferred to an oven for drying at 60°C. After 96 hours, the dry mass was measured.

Samples (approximately 150 mg of fresh mass) were randomly collected and ground in a mortar with a pestle. The macerate was filtered, and ethanol (NEON®, 95%) was added to make up 50 mL in a volumetric flask, previously wrapped with aluminum foil. The extract was analyzed using a UV-VIS spectrophotometer (Varian Cary 50) with readings at 664 nm for chlorophyll a, 648 nm for chlorophyll b, and 470 nm for carotenoids. The absorbance values obtained were substituted into LICHTENTHALER's equations (1987), and the final values were expressed in milligrams per gram of fresh mass (mg/g).

2.1. Statistical Analysis

The data obtained from the four independent treatments were assessed for normality and homogeneity using the Shapiro-Wilk test and Bartlett's test, respectively. When the data were both homogeneous and normally distributed, the four treatments were compared using a one-way ANOVA (a parametric test). If the data were not normally distributed, the treatments were compared using the Kruskal-Wallis test (a nonparametric test) [39]. Hypothesis testing (one-way ANOVA and Kruskal-Wallis) was carried out at a 95% confidence level (p-value < 0.05). The Tukey test was used as the post hoc test following the one-way ANOVA, while the Dwass-Steel-Critchlow-Fligner pairwise comparisons were used with the Kruskal-Wallis test. All post hoc tests were conducted with a 95% confidence interval. These tests were performed using JAMOVI (version 2.3.28) [40]. Boxplots with violin plots, created using JASP (version 0.18.3.0), were used to represent the data from the four independent treatments [41–45]. Principal component analysis (PCA) was conducted using JAMOVI's MEDA plugin [44,45].

 doi:10.20944/preprints202410.1742.v2

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
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

 doi:10.20944/preprints202410.1742.v2

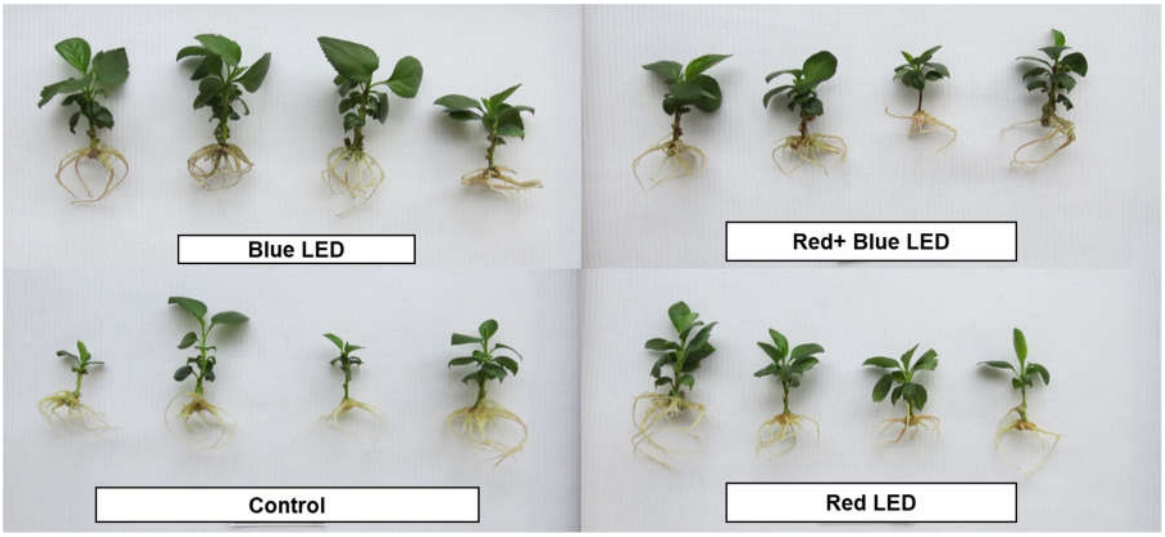

Figure 1. Seedlings of the Marubakaido apple rootstock cultivar obtained after 30 days of in vitro rooting under different light sources.

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 was 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).

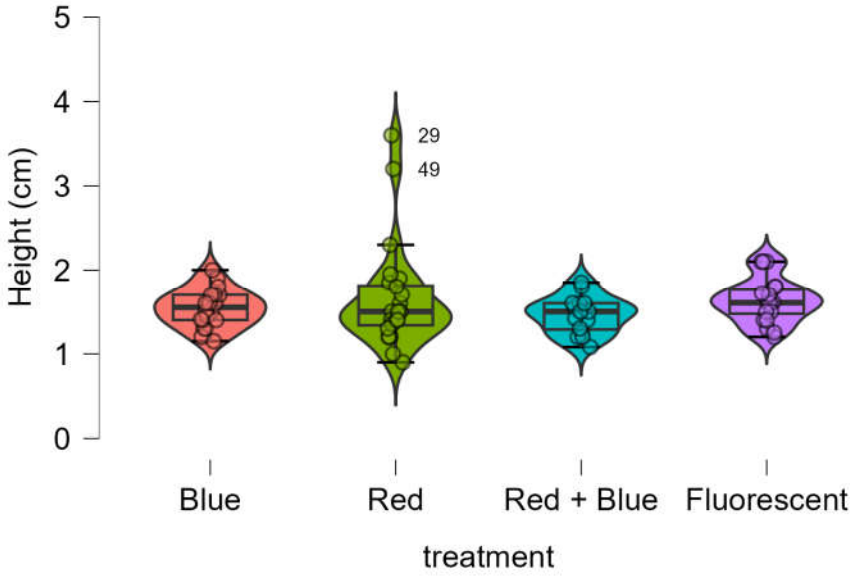

Figure 2. Boxplot of height of Marubakaido apple rootstock seedlings obtained using four independent treatments.

3.2. Dry and Fresh Mass of the Aerial Parts

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

The fresh mass of the aerial parts was notably higher in seedlings treated with LEDs than in those exposed to the control (fluorescent lamp) (Figure 3). The Shapiro-Wilk test confirmed that the data was normally distributed (p-value = 0.600), while Bartlett's test indicated that variances were

 doi:10.20944/preprints202410.1742.v2

equivalent (p-value = 0.904). One-way ANOVA revealed significant differences in fresh mass across the four treatments (p-value < 0.001).

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

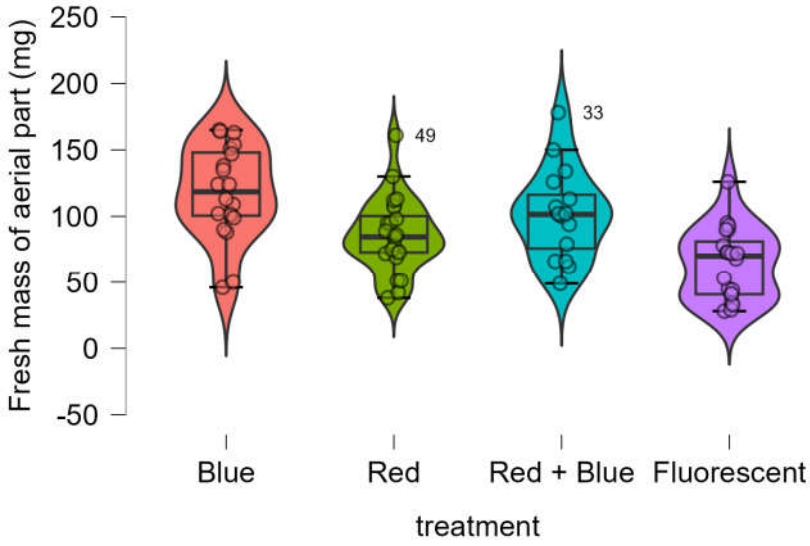

Figure 3. Boxplot of fresh mass of aerial part of Marubakaido apple rootstock seedlings obtained using four independent treatments.

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

		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	< .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	< .001

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

Tukey's test (Table 3) showed that the red LED treatment and the red + blue LED treatment resulted in statistically equivalent dry masses (p-value = 0.255). However, all LED treatments produced significantly larger dry masses than the control.

 doi:10.20944/preprints202410.1742.v2

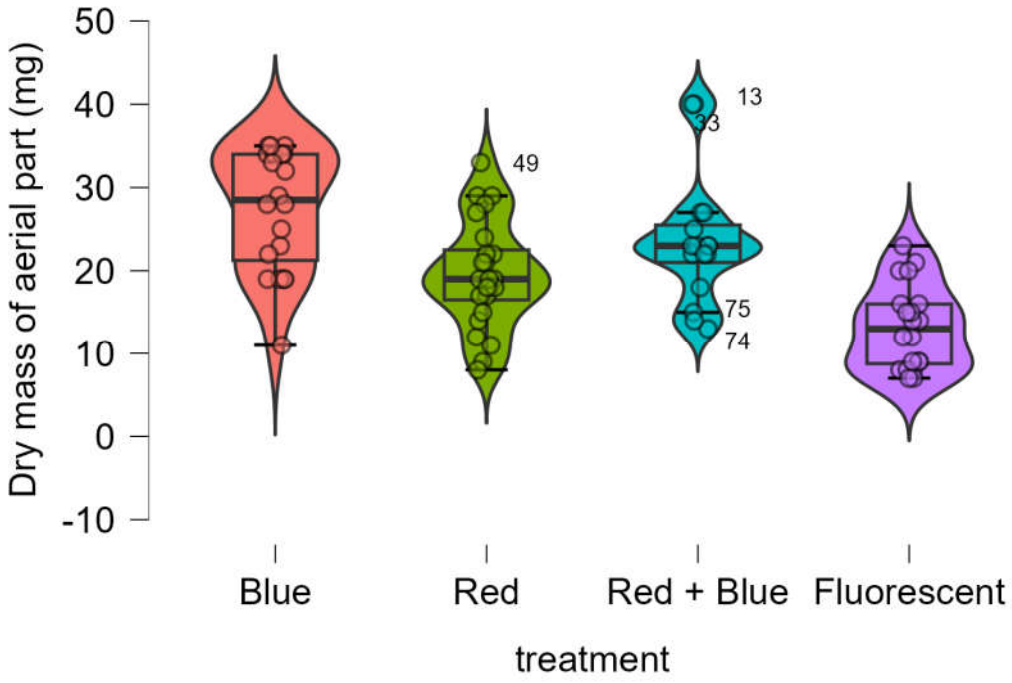

Figure 4. Boxplots of dry mass of aerial part (mg) of Marubakaido apple rootstock seedlings obtained using four independent treatments**.**

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

		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	< .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	< .001

The light emission peaks in the blue and red wavelengths coincide with the maximum absorption of chlorophylls, thereby allowing photosynthesis to occur with maximum efficiency [46,47]. This effect may be related to the greater accumulation of dry mass in the seedlings exposed to blue LED and red + blue LED compared to those exposed to fluorescent lamps.

The results of the current experiment indicate that blue light (450 nm wavelength) positively influences the development of the aerial parts of Marubakaido apple rootstock seedlings. Beyond its role in photosynthesis, light is essential for regulating growth and morphogenesis processes. Plant responses to blue light are attributed to the presence of pigments such as phytochromes, cryptochromes, and phototropins. When stimulated, these pigments control the expression of certain genes, stomatal opening, flowering, and other processes [48]. Blue light-induced stomatal opening enhances gas exchange during photosynthesis, directly impacting crop productivity [47].

A similar effect was found by Shin et al [48]. when cultivating orchids in vitro. They observed that seedlings grown under a combination of red and blue LEDs exhibited higher fresh and dry leaf mass compared to those grown under fluorescent lamps. In studies on Gossypium hirsutum L., Li et al [49]. found that the red and blue light combination also resulted in greater fresh and dry seedling mass than fluorescent lamps. The increase in fresh and dry mass can help seedlings survive the

 doi:10.20944/preprints202410.1742.v2

acclimatization phase [50], which is the most critical stage in micropropagation systems due to the high mortality rate of seedlings.

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 mass of the aerial part (). This indicates that red LEDs at 660 nm can also be used for in vitro cultivation of Marubakaido apple rootstock.

Similarly, Lin et al [35]. revealed that fluorescent lamps and red LEDs were less efficient than blue LEDs, leading to lower shoot formation and dry mass in Dendrobium officinale explants cultivated in vitro. Liu et al [51]. observed that Platycodon gradiflorum seedlings showed greater dry mass increase when grown under blue LEDs compared to red LEDs. Red light may induce starch accumulation in chloroplasts, which can inhibit photosynthesis in plants [37]. According to SÆBØ et al [52]. one response induced by exposure to red light is the reduction of photoassimilate translocation from leaves to other parts of the plant. This can lead to starch accumulation in chloroplasts and indirectly reduce the photosynthetic rate, potentially explaining the lower dry mass production 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 equivalent to that obtained with the control (fluorescent lamp) (Figure 5). The Shapiro-Wilk test indicated that the data was not normally distributed (p-value = 0.001), and Bartlett's test revealed that variances were not homogeneous (p-value = 0.01). Consequently, the Kruskal-Wallis test was used, showing a significant difference among the treatments (p-value < 0.014). However, post hoc analysis using the Dwass-Steel-Critchlow-Fligner pairwise comparisons (Table 4) confirmed that the root dry mass results from LED treatments were statistically equivalent to those of the control.

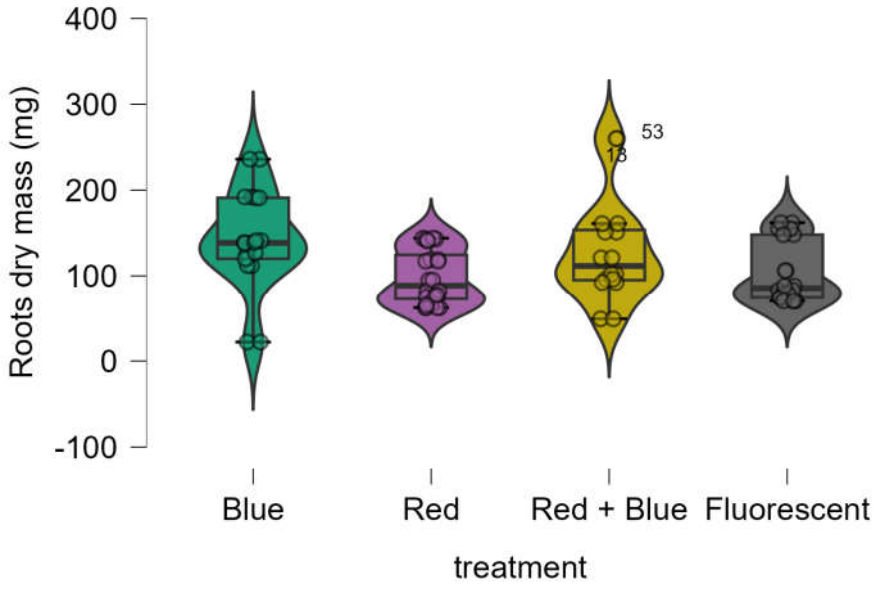

Figure 5. Boxplot of roots dry mass (mg) of Marubakaido apple rootstock seedlings obtained using four independent treatments.

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

Treatment	Treatment	W	p
Blue	Fluorescent	-3.371	0.120
Blue	Red	-3.805	0.055
Blue	Red + Blue	-3.515	0.094
Blue	Red + Blue	-0.221	1.000
Fluorescent	Red	-1.202	0.915

 doi:10.20944/preprints202410.1742.v2

Fluorescent	Red + Blue	-1.760	0.725
Fluorescent	Red + Blue	3.531	0.091
Red	Red + Blue	-1.863	0.681
Red	Red + Blue	4.183	0.026
Red + Blue	Red + Blue	2.760	0.290

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

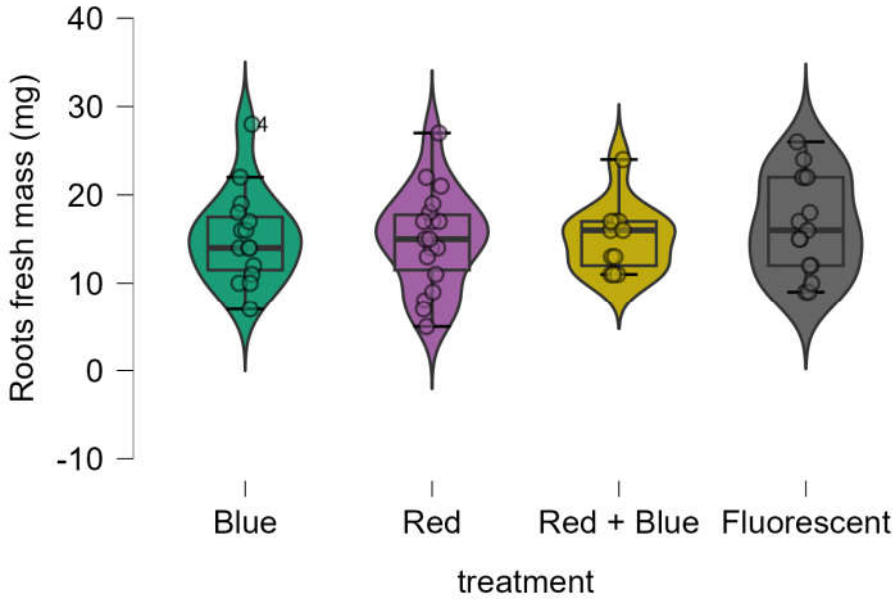

Figure 6. Boxplot of roots fresh mass (mg) of Marubakaido apple rootstock seedlings obtained using four independent treatments.

3.4. Number of Leaves and Roots

The number of leaves obtained using LED treatments were equivalent to those obtained using the control (Figure 7). The Shapiro-Wik test showed that the data was not normally distributed (p-value = 0.001), the Bartlett's test showed that variances were not equivalent (p-value = 0.012), the Kruskal-Wallis test showed that the four treatments provided equivalent results (p-value < 0.213).

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 December 2024 doi:10.20944/preprints202410.1742.v2

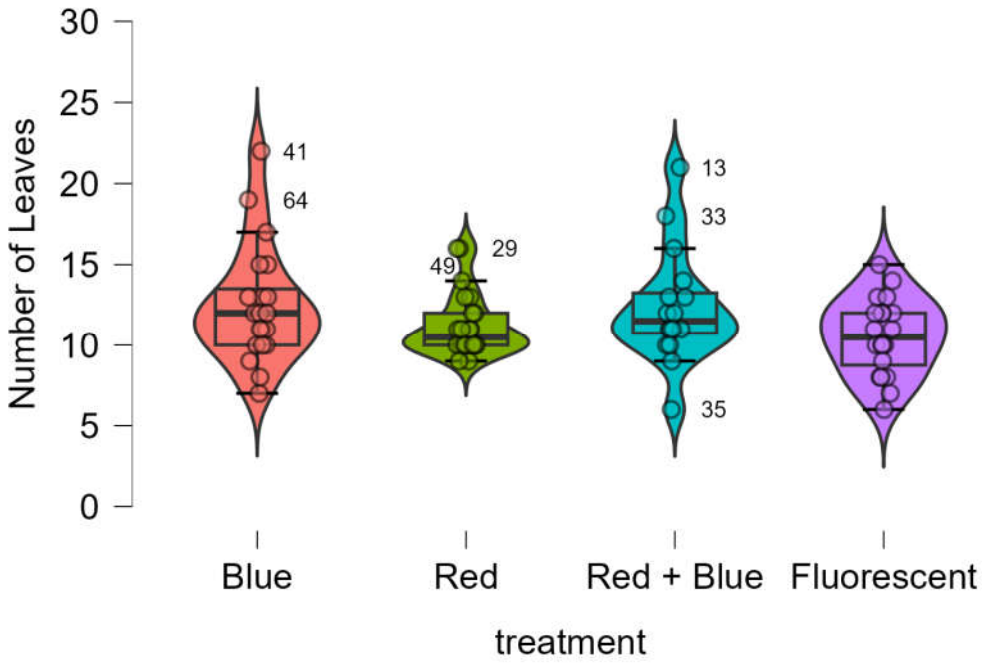

Figure 7. Boxplots of number of leaves in Marubakaido apple rootstock seedlings obtained using four independent treatments.

The number of roots obtained using LED treatments were equivalent to those obtained using the control (Figure 8). The Shapiro-Wik test showed that the data was normally distributed (p-value = 0.134), the Bartlett's test confirmed data homogeneity (p-value = 0.999), the one-way ANOVA test showed that the four treatments provided equivalent results (p-value < 0.127).

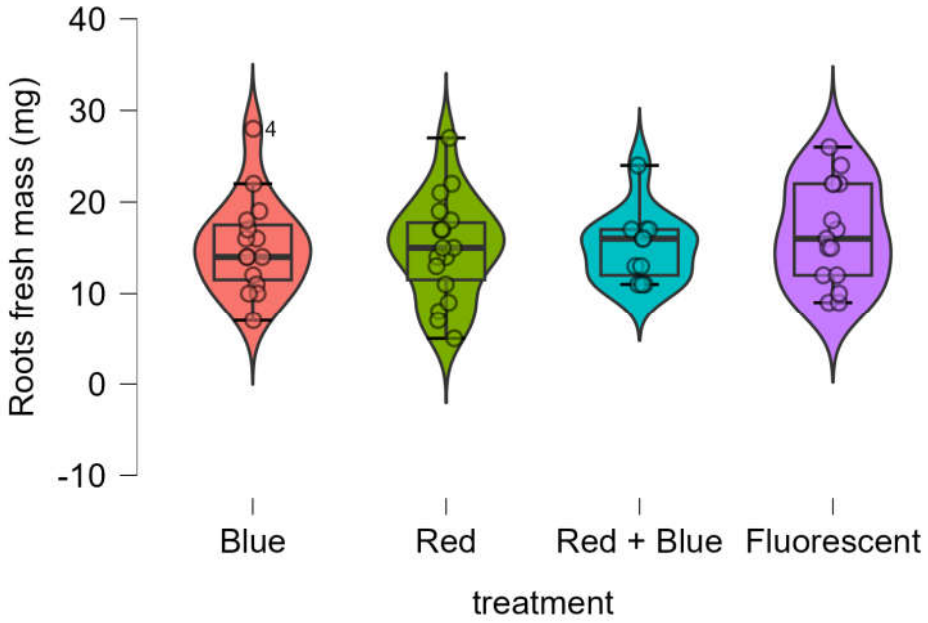

Figure 8. Boxplot of the number of roots fresh mass (mg) of Marubakaido apple rootstock seedlings obtained using four independent treatments.

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 affected by the different light sources. According to Moon et al [26]. light quality can influence plant morphology. For example, plant height can be promoted

 doi:10.20944/preprints202410.1742.v2

or inhibited depending on the different interactions between blue and red-light receptors and phytochromes [53].

A similar result was obtained by Li et al [49]. when they cultivated Brassica napus L. in vitro. The researchers observed that the stem length of seedlings treated with LEDs was comparable to those treated with fluorescent lamps.

The wavelength of light can affect the rooting of in vitro seedlings, varying according to the cultivated species [26]. A study by Chée[54] showed that blue LEDs had more promising effects on the rooting of grapevine seedlings compared to red LEDs. On the other hand, Moon et al [26]. observed that the number of roots in Tripterospermum japonicum was induced by fluorescent lamps and the red + blue LED combination but inhibited by isolated red and blue LEDs. Shin et al [48]. reported that the fresh and dry root masses of in vitro orchids increased when grown under the red + blue LED combination. However, Jao et al [55]. found that Zantedeschia jucunda seedlings subjected to fluorescent lamps had greater dry root mass formation than those treated with LEDs.

3.5. Principal Component Analysis (PCA)

An analysis of vegetative growth was conducted using PCA [56,57], The score plot would be used to observe correlations and trends of the data [39,58–60]. The score plot (Figure 9) illustrates that the number of roots, root fresh mass, and height were positively correlated variables, with samples located at the top-right quadrant showing higher values for these parameters. Additionally, fresh mass of the aerial parts, dry mass of aerial parts, and root dry mass were correlated variables, with samples positioned in the bottom-left quadrant displaying higher values for these variables.

The loading plot (Figure 10) reveals that *Marubakaido* apple rootstock samples exhibiting greater vegetative growth were primarily located on the right side of the plot. Most of these samples were grown under blue LED light, indicating that this light source provided the best vegetative growth. Conversely, samples with smaller vegetative growth were predominantly located on the left side of the plot, representing control samples grown under fluorescent lamps. These results demonstrate that LED lights, particularly blue LEDs, were more efficient than fluorescent lamps in promoting vegetative growth.

 doi:10.20944/preprints202410.1742.v2

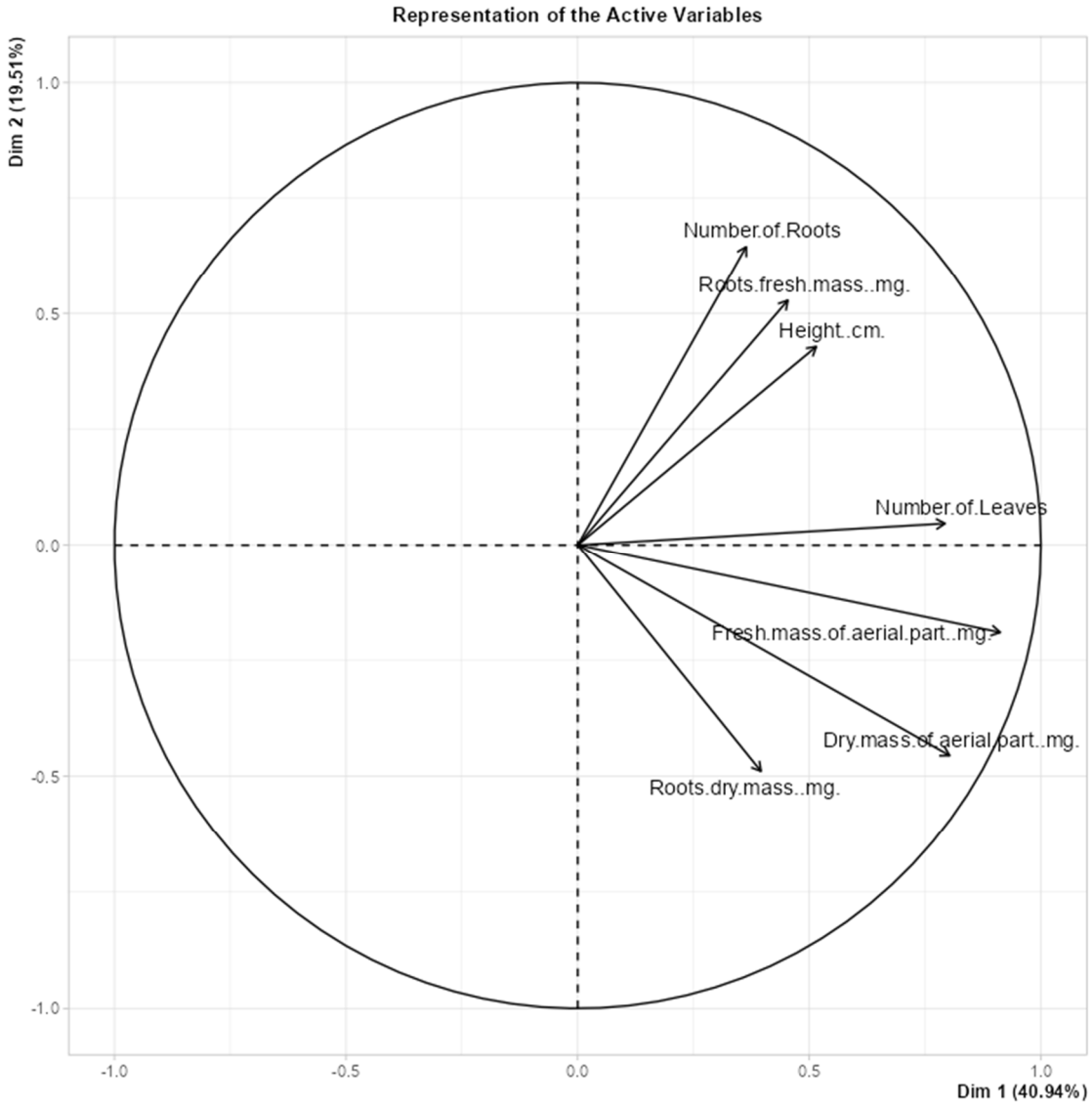

Figure 9. Loading plot of the vegetative growth of seedlings of 'Marubakaido' apple tree rootstocks rooted in vitro under different sources of light.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 December 2024 doi:10.20944/preprints202410.1742.v2

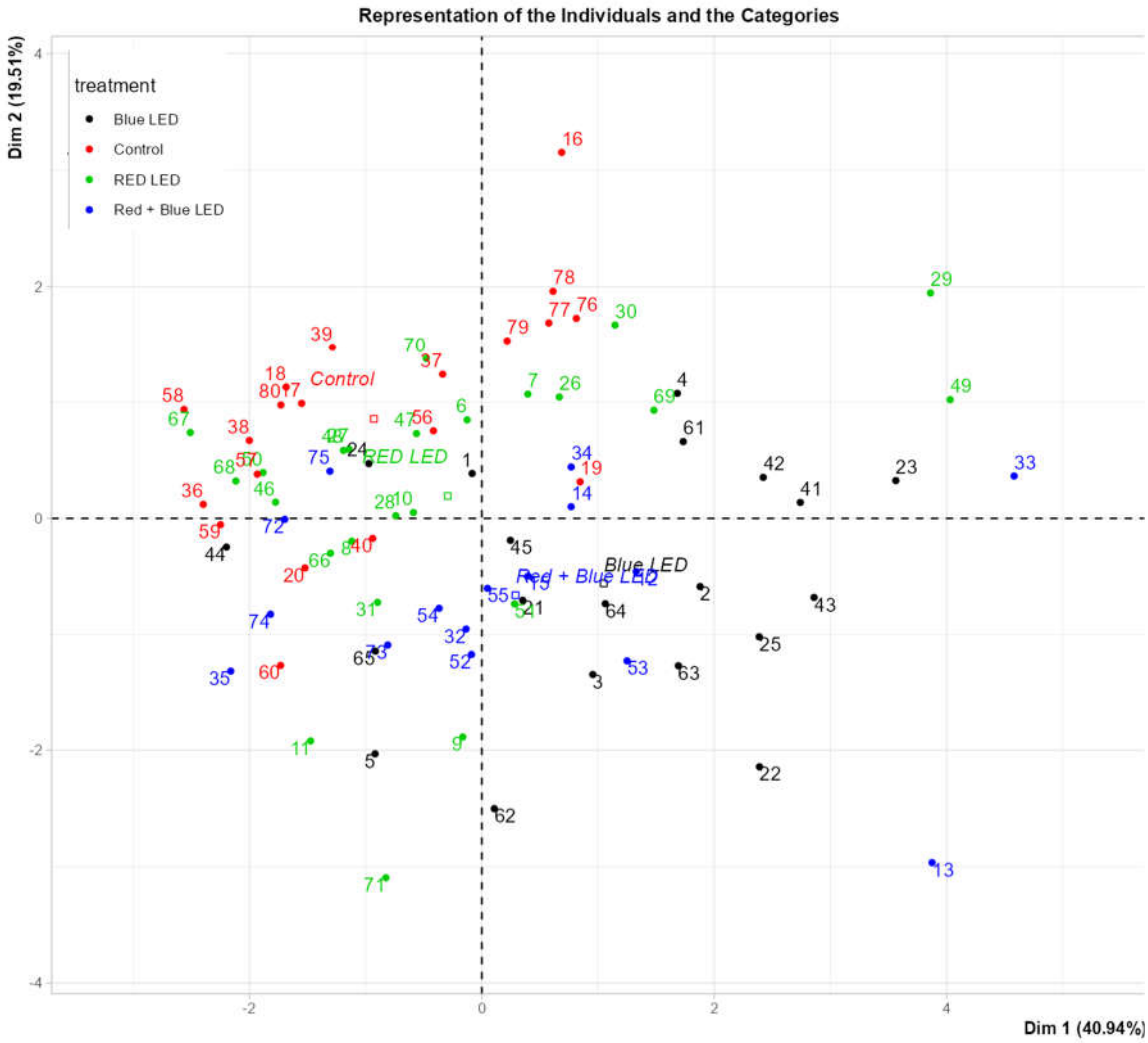

Figure 10. Score plot of the vegetative growth of seedlings of 'Marubakaido' apple tree rootstocks rooted in vitro under different sources of light.

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 [55]. 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 [48]. 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.

 doi:10.20944/preprints202410.1742.v2

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 [61,62], both carotenoids and chlorophylls are involved in energy capture by plants [63–65]. 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 [66,67].

Alvarenga et al [68]. 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 dry and fresh mass of the aerial parts than fluorescent lamps, may be a promising alternative to fluorescent lamps for in vitro rooting of Marubakaido apple rootstocks. This would lead to more developed seedlings with a higher likelihood of survival during the acclimatization phase. Although red LEDs inhibit dry mass production compared to blue LEDs, they can still be used, as they produced similar dry mass levels to fluorescent lamps. Additionally, LEDs offer several advantages beyond their specific peaks within the favorable range for plants, such as high luminous efficiency, minimal heating, long lifespan, absence of heavy metals, and low energy consumption, enhancing profitability in in vitro plant propagation.

Fluorescent lamps induce a higher accumulation of chlorophylls and carotenoids compared to LEDs but result in lower dry mass production compared to blue LEDs and the red + blue LED combination in the in vitro rooting of Marubakaido apple rootstock seedlings.

Blue LEDs are more favorable than red LEDs and fluorescent lamps for dry mass accumulation in the in vitro rooting of Marubakaido apple rootstock seedlings.

Blue LEDs, red LEDs, and the red + blue LED combination can be used as alternatives to fluorescent lamps in the in vitro rooting of Marubakaido apple rootstock seedlings.

Author Contributions: EMB and MSM conceived and coordinated this project; AMTS performed the experiments with the help of RLV, BS, ELS. EMB and MSM analyzed data and wrote the manuscript. All authors have read and approved the final 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).

Institutional Review Board Statement: The study did not require ethical approval.

Informed Consent Statement: Not applicable. This study did not involve humans.

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.

References

1. Stanger, M.C.; Steffens, C.A.; Soethe, C.; Moreira, M.A.; do Amarante, C.V.T. Phenolic Content and Antioxidant Activity during the Development of 'Brookfield' and 'Mishima' Apples. *J Agric Food Chem* **2017**, *65*, 3453–3459, doi:10.1021/acs.jafc.6b04695.
2. Bueno, M.P.; Silva, A.C.; Nunes, A.L. de P.F.; Sardinha, A.C.; Lima, P.T. dos S.; Silva, J.F. Análise Da Comercialização Da Cadeia Produtiva Da Maçã Brasileira: Produção, Importação e Exportação No Período 2015 a 2019/ Analysis of the Commercialization of the Brazilian Apple Production Chain: Production, Import and Export in the Year 2015 to 2019. *Brazilian Journal of Development* **2021**, *7*, 34061–34078, doi:10.34117/bjdv7n4-048.
3. FAO FAOSTAT Crops and Livestock Production. FAOSTAT Analytical Brief 60 Agricultural production statistics 2000-2021 **2021**, 60.
4. Purin, S.; Filho, O.K.; Stürmer, S.L. Mycorrhizae Activity and Diversity in Conventional and Organic Apple Orchards from Brazil. *Soil Biol Biochem* **2006**, *38*, 1831–1839, doi:10.1016/j.soilbio.2005.12.008.
5. Pertille, R.H.; Citadin, I.; Oliveira, L. de S. de; Broch, J. de C.; Kvitschal, M.V.; Araujo, L. The Influence of Temperature on the Phenology of Apple Trees Grown in Mild Winter Regions of Brazil, Based on Long-Term Records. *Sci Hortic* **2022**, *305*, 111354, doi:10.1016/j.scienta.2022.111354.
6. Marini, R.P. Experimental Designs and Statistical Analyses for Rootstock Trials. *Agronomy* **2024**, *14*, 2312, doi:10.3390/agronomy14102312.
7. Mahmud, I.; Kousik, C.; Hassell, R.; Chowdhury, K.; Boroujerdi, A.F. NMR Spectroscopy Identifies Metabolites Translocated from Powdery Mildew Resistant Rootstocks to Susceptible Watermelon Scions. *J Agric Food Chem* **2015**, *63*, 8083–8091, doi:10.1021/acs.jafc.5b02108.
8. Orazem, P.; Stampar, F.; Hudina, M. Fruit Quality of Redhaven and Royal Glory Peach Cultivars on Seven Different Rootstocks. *J Agric Food Chem* **2011**, *59*, 9394–9401, doi:10.1021/jf2009588.
9. Jin, Z.; Sun, H.; Sun, T.; Wang, Q.; Yao, Y. Modifications of 'Gold Finger' Grape Berry Quality as Affected by the Different Rootstocks. *J Agric Food Chem* **2016**, *64*, 4189–4197, doi:10.1021/acs.jafc.6b00361.
10. Liu, X.; Li, J.; Huang, M.; Chen, J. Mechanisms for the Influence of Citrus Rootstocks on Fruit Size. *J Agric Food Chem* **2015**, *63*, 2618–2627, doi:10.1021/jf505843n.
11. de Macedo, T.A.; da Silva, P.S.; Sander, G.F.; Welter, J.F.; Rufato, L.; de Rossi, A. Productivity and Quality of "Fuji Suprema" Apple Fruit in Different Rootstocks and Growing Conditions. *Sci Hortic* **2019**, *256*, 108651, doi:10.1016/j.scienta.2019.108651.
12. Santos da Silva, P.; de Lima, J.M.; dos Santos, M.F.S.; Petry, D.; Rufato, L.; Nerbass, F.R.; Bogo, A. Performance of 'Gala Select' and 'Fuji Suprema' Grafted on Geneva Series Rootstocks under Fallow Land and Replanting Conditions in Southern Brazil. *Heliyon* **2023**, *9*, e22125, doi:10.1016/j.heliyon.2023.e22125.
13. Marini, R.P.; Fazio, G. Apple Rootstocks. In *Horticultural Reviews*; Wiley, 2018; pp. 197–312.
14. Jones, O.P.; Hopgood, M.E.; O'Farrell, D. Propagation in Vitro of M.26 Apple Rootstocks. *Journal of Horticultural Science* **1977**, *52*, 235–238, doi:10.1080/00221589.1977.11514751.
15. Dobránszki, J.; Teixeira da Silva, J.A. Micropropagation of Apple — A Review. *Biotechnol Adv* **2010**, *28*, 462–488, doi:10.1016/j.biotechadv.2010.02.008.
16. Luiz de Oliveira, J.; Ramos Campos, E.V.; Fraceto, L.F. Recent Developments and Challenges for Nanoscale Formulation of Botanical Pesticides for Use in Sustainable Agriculture. *J Agric Food Chem* **2018**, *66*, 8898–8913, doi:10.1021/acs.jafc.8b03183.
17. Di Serio, M.G.; D'Ascenzo, D.; Giansante, L.; Del Re, P.; Bendini, A.; Casadei, E.; Gallina Toschi, T.; Mori, N.; Di Giacinto, L. Effect of *Halyomorpha Halys* Infestation on the Physicochemical Composition and Sensory Characteristics of Olive Fruits and Olive Oils. *ACS Food Science & Technology* **2024**, *4*, 821–832, doi:10.1021/acsfoodscitech.3c00438.
18. Naz, B.; Afzal, A.; Ali, H.; Ahmad, N.; Fazal, H.; Ullah, R.; Ali, E.A.; Ali, M.; Ullah, Z.; Ali, A.; et al. Melatonin-Induced Stress Enhanced Biomass and Production of High-Value Secondary Cell Products in Submerged Adventitious Root Cultures of *Stevia Rebaudiana* (Bert.). *ACS Omega* **2024**, doi:10.1021/acsomega.3c07404.
19. Clemente, I.; Menicucci, F.; Colzi, I.; Sbraci, L.; Benelli, C.; Giordano, C.; Gonnelli, C.; Ristori, S.; Petruccelli, R. Unconventional and Sustainable Nanovectors for Phytohormone Delivery: Insights on *Olea Europaea*. *ACS Sustain Chem Eng* **2018**, *6*, 15022–15031, doi:10.1021/acssuschemeng.8b03489.
20. Hussain, N.; Ali, H.; Mustafa, G.; Sarwar Khan, M.; Ali, B.; Ameer, S.; Zamir, S.; Iqbal, R.; Ali, B.; Khan, M.N.; et al. In Vitro Plant Regeneration from Petioles of Spinach (*Spinacia Oleracea* L.). *ACS Agricultural Science & Technology* **2024**, *4*, 57–62, doi:10.1021/acsagscitech.3c00283.

21. Ma, X.; Gang, D.R. Metabolic Profiling of Turmeric (*Curcuma Longa* L.) Plants Derived from in Vitro Micropropagation and Conventional Greenhouse Cultivation. *J Agric Food Chem* **2006**, *54*, 9573–9583, doi:10.1021/jf061658k.
22. Ashrafi, K.; Iqrar, S.; Saifi, M.; Khan, S.; Qamar, F.; Quadri, S.N.; Mishra, A.; Abdin, M.Z. Influence of Plant Growth Regulators on Glandular Trichome Density and Steviol Glycosides Accumulation in *Stevia Rebaudiana*. *ACS Omega* **2022**, *7*, 30967–30977, doi:10.1021/acsomega.2c02957.
23. Gulzar, B.; Mujib, A.; Qadir Malik, M.; Mamgain, J.; Syeed, R.; Zafar, N. Plant Tissue Culture: Agriculture and Industrial Applications. In *Transgenic Technology Based Value Addition in Plant Biotechnology*; Elsevier, 2020; pp. 25–49.
24. Abdalla, N.; El-Ramady, H.; Seliem, M.K.; El-Mahrouk, M.E.; Taha, N.; Bayoumi, Y.; Shalaby, T.A.; Dobránszki, J. An Academic and Technical Overview on Plant Micropropagation Challenges. *Horticulturae* **2022**, *8*, 677, doi:10.3390/horticulturae8080677.
25. Gutiérrez-Gamboa, G.; Alañón-Sánchez, N.; Mateluna-Cuadra, R.; Verdugo-Vásquez, N. An Overview about the Impacts of Agricultural Practices on Grape Nitrogen Composition: Current Research Approaches. *Food Research International* **2020**, *136*, 109477, doi:10.1016/j.foodres.2020.109477.
26. Moon, H.K.; Park, S.-Y.; Kim, Y.W.; Kim, C.S. Growth of Tsuru-Rindo (Tripterospermum Japonicum) Culturedin Vitro under Various Sources of Light-Emitting Diode (LED) Irradiation. *Journal of Plant Biology* **2006**, *49*, 174–179, doi:10.1007/BF03031014.
27. Dutta Gupta, S.; Jatothu, B. Fundamentals and Applications of Light-Emitting Diodes (LEDs) in in Vitro Plant Growth and Morphogenesis. *Plant Biotechnol Rep* **2013**, *7*, 211–220, doi:10.1007/s11816-013-0277-0.
28. Sharma, A.; Hazarika, M.; Heisnam, P.; Pandey, H.; Devadas, V.S.; Wangsu, M.; Kartha, B.D. Factors Affecting Production, Nutrient Translocation Mechanisms, and LED Emitted Light in Growth of Microgreen Plants in Soilless Culture. *ACS Agricultural Science & Technology* **2023**, *3*, 701–719, doi:10.1021/acsagscitech.3c00260.
29. Sharma, A.; Pandey, H.; Nampoothiri Devadas, V.A.S.; Singh, D.; Kartha, B.D.; Wangsu, M. Regulations and Factors Affecting of Light Emitting Diodes Artificial Light in Crop Production under Soilless Culture. *ACS Agricultural Science & Technology* **2023**, *3*, 616–630, doi:10.1021/acsagscitech.3c00127.
30. Soltani, S.; Arouiee, H.; Salehi, R.; Nemati, S.H.; Moosavi-Nezhad, M.; Gruda, N.S.; Aliniaeifard, S. Morphological, Phytochemical, and Photosynthetic Performance of Grafted Tomato Seedlings in Response to Different LED Light Qualities under Protected Cultivation. *Horticulturae* **2023**, *9*, 471, doi:10.3390/horticulturae9040471.
31. Yeh, N.; Chung, J.-P. High-Brightness LEDs—Energy Efficient Lighting Sources and Their Potential in Indoor Plant Cultivation. *Renewable and Sustainable Energy Reviews* **2009**, *13*, 2175–2180, doi:10.1016/j.rser.2009.01.027.
32. SCHUERGER, A. Anatomical Features of Pepper Plants (Capsicum AnnuumL.) Grown under Red Light-Emitting Diodes Supplemented with Blue or Far-Red Light. *Ann Bot* **1997**, *79*, 273–282, doi:10.1006/anbo.1996.0341.
33. Nacheva, L.; Dimitrova, N.; Koleva-Valkova, L.; Stefanova, M.; Ganeva, T.; Nesheva, M.; Tarakanov, I.; Vassilev, A. In Vitro Multiplication and Rooting of Plum Rootstock 'Saint Julien' (Prunus Domestica Subsp. Insititia) under Fluorescent Light and Different LED Spectra. *Plants* **2023**, *12*, 2125, doi:10.3390/plants12112125.
34. Geng, F.; Moran, R.; Day, M.; Halteman, W.; Zhang, D. In Vitro Shoot Proliferation of Apple Rootstocks 'B.9', 'G.30', and 'G.41' Grown under Red and Blue Light. *HortScience* **2015**, *50*, 430–433, doi:10.21273/HORTSCI.50.3.430.
35. 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 and Organ Culture (PCTOC)* **2011**, *105*, 329–335, doi:10.1007/s11240-010-9871-9.
36. Senger, H. THE EFFECT OF BLUE LIGHT ON PLANTS AND MICROORGANISMS. *Photochem Photobiol* **1982**, *35*, 911–920, doi:10.1111/j.1751-1097.1982.tb02668.x.
37. 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 and Organ Culture (PCTOC)* **2010**, *103*, 155–163, doi:10.1007/s11240-010-9763-z.
38. Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol Plant* **1962**, *15*, 473–497, doi:10.1111/j.1399-3054.1962.tb08052.x.
39. Borges, E.M. Hypothesis Tests and Exploratory Analysis Using R Commander and Factoshiny. *J Chem Educ* **2023**, *100*, 267–278, doi:10.1021/acs.jchemed.2c00155.
40. Navarro, D.J.; Foxcroft, D.R. Learning Statistics with Jamovi. Learning Statistics with Jamovi: A Tutorial for Psychology Students and Other Beginners **2022**.
41. Silva de Souza, R.; Borges, E.M. Teaching Descriptive Statistics and Hypothesis Tests Measuring Water Density. *J Chem Educ* **2023**, *100*, 4438–4448, doi:10.1021/acs.jchemed.3c00402.

42. Marcel Borges, E. Data Visualization Using Boxplots: Comparison of Metalloid, Metal, and Nonmetal Chemical and Physical Properties. *J Chem Educ* **2023**, *100*, 2809–2817, doi:10.1021/acs.jchemed.3c00207.
43. Love, J.; Selker, R.; Marsman, M.; Jamil, T.; Dropmann, D.; Verhagen, J.; Ly, A.; Gronau, Q.F.; Smíra, M.; Epskamp, S.; et al. JASP: Graphical Statistical Software for Common Statistical Designs. *J Stat Softw* **2019**, *88*, doi:10.18637/jss.v088.i02.
44. de Souza, R.S.; Sequeira, C.A.; Borges, E.M. Enhancing Statistical Education in Chemistry and STEAM Using JAMOVI. Part 1: Descriptive Statistics and Comparing Independent Groups. *J Chem Educ* **2024**, doi:10.1021/acs.jchemed.4c00563.
45. Sequeira, C.A.; Borges, E.M. Enhancing Statistical Education in Chemistry and STEAM Using JAMOVI. Part 2. Comparing Dependent Groups and Principal Component Analysis (PCA). *J Chem Educ* **2024**, *101*, 5040–5049, doi:10.1021/acs.jchemed.4c00342.
46. Tanaka, M.; Takamura, T.; Watanabe, H.; Endo, M.; Yanagi, T.; Okamoto, K. In Vitro Growth of *Cymbidium* Plantlets Cultured under Superbright Red and Blue Light-Emitting Diodes (LEDs). *J Hortic Sci Biotechnol* **1998**, *73*, 39–44, doi:10.1080/14620316.1998.11510941.
47. Hiyama, A.; Takemiya, A.; Munemasa, S.; Okuma, E.; Sugiyama, N.; Tada, Y.; Murata, Y.; Shimazaki, K. Blue Light and CO2 Signals Converge to Regulate Light-Induced Stomatal Opening. *Nat Commun* **2017**, *8*, 1284, doi:10.1038/s41467-017-01237-5.
48. 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, doi:10.1007/s11738-007-0128-0.
49. Li, H.; Tang, C.; Xu, Z. The Effects of Different Light Qualities on Rapeseed (Brassica Napus L.) Plantlet Growth and Morphogenesis in Vitro. *Sci Hortic* **2013**, *150*, 117–124, doi:10.1016/j.scienta.2012.10.009.
50. Murashige, T. Plant Propagation Through Tissue Cultures. *Annu Rev Plant Physiol* **1974**, *25*, 135–166, doi:10.1146/annurev.pp.25.060174.001031.
51. Liu, M.; Xu, Z.; Guo, S.; Tang, C.; Liu, X.; Jao, X. Evaluation of Leaf Morphology, Structure and Biochemical Substance of Balloon Flower (Platycodon Grandiflorum (Jacq.) A. DC.) Plantlets in Vitro under Different Light Spectra. *Sci Hortic* **2014**, *174*, 112–118, doi:10.1016/j.scienta.2014.05.006.
52. SÆBØ, A.; Krekling, T.; Appelgren, M. Light Quality Affects Photosynthesis and Leaf Anatomy of Birch Plantlets in Vitro. *Plant Cell Tissue Organ Cult* **1995**, *41*, 177–185, doi:10.1007/BF00051588.
53. Kim, S.-J.; Hahn, E.-J.; Heo, J.-W.; Paek, K.-Y. Effects of LEDs on Net Photosynthetic Rate, Growth and Leaf Stomata of Chrysanthemum Plantlets in Vitro. *Sci Hortic* **2004**, *101*, 143–151, doi:10.1016/j.scienta.2003.10.003.
54. Chée, R. In Vitro Culture of Vitis: The Effects of Light Spectrum, Manganese Sulfate and Potassium Iodide on Morphogenesis. *Plant Cell Tissue Organ Cult* **1986**, *7*, 121–134, doi:10.1007/BF00043036.
55. Jao, R.-C.; Lai, C.-C.; Fang, W.; Chang, S.-F. Effects of Red Light on the Growth of Zantedeschia Plantlets in Vitro and Tuber Formation Using Light-Emitting Diodes. *HortScience* **2005**, *40*, 436–438, doi:10.21273/HORTSCI.40.2.436.
56. Lindemann, I. da S.; Lang, G.H.; Dittgen, C.L.; Rombaldi, C.V.; Elias, M.C.; Vanier, N.L. Effects of Preharvest Desiccation Using Glufosinate-Ammonium on Quality Attributes of Freshly Harvested and Long-Term Stored Soybeans. *ACS Agricultural Science & Technology* **2021**, *1*, 312–321, doi:10.1021/acsagscitech.1c00004.
57. Righini, H.; Tedeschi, P.; Maietti, A.; Francioso, O.; Quintana, A.M.; Zuffi, V.; Ciurli, A.; Roberti, R. Enhanced Growth and Nutritional Properties of Radish Sprouts Using Extracts from *Anabaena Minutissima* and *Sargassum Vulgare*. *ACS Agricultural Science & Technology* **2024**, *4*, 700–710, doi:10.1021/acsagscitech.4c00102.
58. Borges, E.M.; Gelinski, J.M.L.N.; de Oliveira Souza, V.C.; Barbosa Jr., F.; Batista, B.L. Monitoring the Authenticity of Organic Rice via Chemometric Analysis of Elemental Data. *Food Research International* **2015**, *77*, 299–309, doi:10.1016/j.foodres.2015.06.008.
59. Sidou, L.F.; Borges, E.M. Teaching Principal Component Analysis Using a Free and Open Source Software Program and Exercises Applying PCA to Real-World Examples. *J Chem Educ* **2020**, *97*, 1666–1676, doi:10.1021/acs.jchemed.9b00924.
60. Borges, E.M. How to Select Equivalent and Complimentary Reversed Phase Liquid Chromatography Columns from Column Characterization Databases. *Anal Chim Acta* **2014**, *807*, 143–152, doi:10.1016/j.aca.2013.11.010.
61. Zhou, J.; Wang, Y.; Zuverza-Mena, N.; Dimkpa, C.O.; White, J.C. Copper-Based Materials as an Effective Strategy for Improving Drought Resistance in Soybean (*Glycine Max*) at the Reproductive Stage. *ACS Agricultural Science & Technology* **2024**, *4*, 735–746, doi:10.1021/acsagscitech.4c00193.
62. Datta Majumdar, T.; Ghosh, C.K.; Mukherjee, A. Dual Role of Copper Nanoparticles in Bacterial Leaf Blight-Infected Rice: A Therapeutic and Metabolic Approach. *ACS Agricultural Science & Technology* **2021**, *1*, 160–172, doi:10.1021/acsagscitech.0c00064.

63. Neo, D.C.J.; Ong, M.M.X.; Lee, Y.Y.; Teo, E.J.; Ong, Q.; Tanoto, H.; Xu, J.; Ong, K.S.; Suresh, V. Shaping and Tuning Lighting Conditions in Controlled Environment Agriculture: A Review. *ACS Agricultural Science & Technology* **2022**, *2*, 3–16, doi:10.1021/acsagscitech.1c00241.
64. Perea-Brenes, A.; Garcia, J.L.; Cantos, M.; Cotrino, J.; Gonzalez-Elipe, A.R.; Gomez-Ramirez, A.; Lopez-Santos, C. Germination and First Stages of Growth in Drought, Salinity, and Cold Stress Conditions of Plasma-Treated Barley Seeds. *ACS Agricultural Science & Technology* **2023**, *3*, 760–770, doi:10.1021/acsagscitech.3c00121.
65. Tang, C.; Zhang, X.; Zhao, Y.; Li, L.; Wei, J.; Yang, Z.; Chen, X.; Zheng, H.; Zhou, Z.; Tang, W.; et al. Response of Chlorosis and Growth to Light Intensity in Rice Seedlings. *ACS Agricultural Science & Technology* **2024**, *4*, 234–243, doi:10.1021/acsagscitech.3c00461.
66. Garg, V.K.; Gupta, R.; Kumar, R.; Gupta, R.K. Adsorption of Chromium from Aqueous Solution on Treated Sawdust. *Bioresour Technol* **2004**, *92*, 79–81, doi:10.1016/j.biortech.2003.07.004.
67. Shrivastava, A.; Gupta, V. Methods for the Determination of Limit of Detection and Limit of Quantitation of the Analytical Methods. *Chronicles of Young Scientists* **2011**, *2*, 21, doi:10.4103/2229-5186.79345.
68. Alvarenga, I.C.A.; Pacheco, F.V.; Silva, S.T.; Bertolucci, S.K.V.; Pinto, J.E.B.P. In Vitro Culture of Achillea Millefolium L.: Quality and Intensity of Light on Growth and Production of Volatiles. *Plant Cell, Tissue and Organ Culture (PCTOC)* **2015**, *122*, 299–308, doi:10.1007/s11240-015-0766-7.