2022-12-27

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http://hdl.handle.net/10026.1/20439

10.3390/agronomy13010089
Agronomy
MDPI AG

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Effects of Artificial Light Spectra and Sucrose on the Leaf Pigments, Growth, and Rooting of Blackberry (*Rubus fruticosus*) Microshoots

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Abstract: Light emitting diodes (LEDs) are potential light sources for in vitro plant cultures. Here, axillary blackberry shoots were grown in MS medium with indole-3-butyric acid (1 mg L^{-1}), naphthalene acetic acid (0.5 mg L^{-1}), and sucrose supplementation (0–60 g L^{-1}) and the cultures were incubated under four light treatments: three LED light treatments (blue + red light (2:1 spectral ratio), blue + red light (1:2), and cool + warm white light (1:1)) and a standard florescent tube white spectrum treatment. Sucrose was indispensable for rooting of blackberry microshoots. Sucrose concentrations up to 45 g L^{-1} increased total root length and root surface area under all light treatments. However, at this sucrose concentration, leaf area and vegetative growth were negatively affected. Plantlets grown in media containing 15–30 g L^{-1} of sucrose exhibited the highest leaf pigments, shoot length, and number of leaves. LED treatments increased leaf pigments as compared with florescent treatment. Plantlets grown under blue + red light (2:1) had the highest stoma aperture length and width, whereas cool + warm white light resulted in the lowest values. Among the LED treatments, blue + red light (2:1) resulted in the highest leaf area, chlorophyll and carotenoid contents, and vegetative growth, whereas fluorescent resulted in the lowest values. A combination of blue and red light at a 2:1 spectral ratio with 30 g L^{-1} of sucrose is recommended for the optimal in vitro rooting and vegetative growth of blackberry microshoots.

Keywords: in vitro; light quality; micropropagation; stomata; tissue culture

Citation: Dewir, Y.H.; Al-Ali, A.M.; Rihan, H.Z.; Alshahrani, T.; Alwahibi, M.S.; Almutairi, K.F.; Naidoo, Y.; Fuller, M.P. Effects of Artificial Light Spectra and Sucrose on the Leaf Pigments, Growth, and Rooting of Blackberry (*Rubus fruticosus*) Microshoots. *Agronomy* **2023**, *13*, 89. https://doi.org/10.3390/agronomy13010089

Academic Editor: Youssef Rouphael

Received: 6 December 2022
Revised: 16 December 2022
Accepted: 26 December 2022
Published: 27 December 2022

1. Introduction

Plant tissue cultures must be illuminated by artificial light sources. Light emitting diodes (LEDs) have attracted substantial attention as potential light sources for plant tissue cultures [1]. Several studies have demonstrated the beneficial effects of LED light on plant growth and the quality of crops [2,3]. Compared with conventional light sources, LEDs serve as cheap, cool, and controllable light sources that provide different spectra in a selective and quantitative manner [4]. When multiple LEDs are combined, monochromatic light with different intensities or a combination of light with different spectral compositions can be emitted. Therefore, LEDs can be used to accurately and flexibly control light spectra to provide optimal light wavelengths that match plant photoreceptors and photosynthetic pigments; hence, LED light can optimize plant growth and metabolism [5]. Blue and red light regions are most efficiently absorbed by chlorophyll (the primary photosynthetic pigments) during the photosynthetic process [6]. Therefore, red and blue light have been studied extensively in the plant photobiology field.

Berry fruits are often grown by small farmers and on larger farms owing to their economic importance and health-promoting properties [7]. Blackberry (*Rubus fruticosus*; Rosaceae) is one of the most popular horticultural berry fruit species. It is conventionally propagated using vegetative methods with hard or softwood cuttings, one-year-old suckers, and layering or root cuttings [8]. In vitro clonal propagation techniques facilitate rapid and highly efficient propagation and the maintenance of many high-quality plant materials in a relatively short period with limited space and without seasonal variation. Blackberry is considered a suitable species for commercial propagation under in vitro conditions [9–16]. A critical step in *Rubus* micropropagation is acclimation to ex vitro conditions [17]. Therefore, the well-developed root system and vigorous growth of in vitro plantlets ensure high survival rates and successful acclimation of micropropagated blackberry plants. The efficacy of in vitro blackberry propagation depends on several factors, including genotype, stock plant physiology, season, light source (type and intensity), photoperiod, gelling agents, carbon sources, medium salt strength and composition, and plant growth regulators [9,10,18–20].

The aim of the present study was to determine the effects of sucrose supplement concentrations and light spectra, including blue-dominant, red-dominant, and white light spectra produced by fluorescent tubes and LED sources, on the rooting and growth of blackberry microshoots. Overall, different LED treatments and sucrose concentrations significantly affected the in vitrorooting and vegetative growth of blackberry microshoots. In particular, blue and red (2:1 spectral ratio) LED light and 30 g L^{-1} of sucrose provided optimal in vitro rooting and vegetative growth.

2. Materials and Methods

2.1. Plant Material

This study was conducted at the laboratory of plant tissue culture, College of Food and Agricultural Sciences, King Saud University. The axillary shoots of blackberry (*R. fruticosus* 'P45') were multiplied in vitro onto Murashige and Skoog's medium (MS) [21] supplemented with 6-benzylaminopurine (1 mg L^{-1}) and sucrose (30 g L^{-1}) according to Dziedzic and Jagła [8]. The medium was solidified using 8.0 g·L^{-1} agar–agar (Dephyte), and the pH of the medium was adjusted to 5.8 before autoclaving at 121 °C and 118 kPa pressure for 15 min. The cultures were incubated for 8 weeks at an air temperature of 25 °C ± 2 °C and a photosynthetic photon flux density (PPFD) of 25 µmol·m^{-2}·s^{-1} provided by cool white fluorescent tubes under a 16:8 h (light:dark) photoperiod. The regenerated axillary shoots were used as the initial explants in this study.

2.2. LED and Sucrose Treatments

The axillary shoots of blackberry (1.5–2.0 cm in length; 9 explants per culture vessel) were cultured in Magenta GA-7 culture vessels (77 × 77 × 97 mm; Sigma Chemical Co., St. Louis, MO, USA) containing 60 mL of MS medium supplemented with indole-3-butyric acid (1 mg L^{-1}), naphthalene acetic acid (0.5 mg L^{-1}), and different concentrations of sucrose (0, 15, 30, 45, and 60 g L^{-1}) for 8 weeks. The MS medium was gelled using 0.8% (*w*/*v*) agar–agar, and the pH of the medium was adjusted to 5.8 before autoclaving at 121 °C and 1.2 kg cm^{-2} for 15 min. Four different light treatments were also applied as follows: three light treatments from LEDs (Shenzhen Lumini Technology Co., Ltd., Shenzhen, China) and a standard florescent tube white spectrum. Each treatment was represented by four Magenta vessels. LED light was provided under a 16:8 h (light:dark) photoperiod with a light intensity of 50 µmol·m^{-2}·s^{-1} PPFD. The LED treatments were as follows: a mixture of blue and red light with a 2:1 spectral ratio, a mixture of blue and red light with a 1:2 spectral ratio, and white light (cool white + warm white; 1:1 ratio). Light emitted from a fluorescent light was considered the control. The spectral energy distribution of the light treatments is shown in Figure 1.

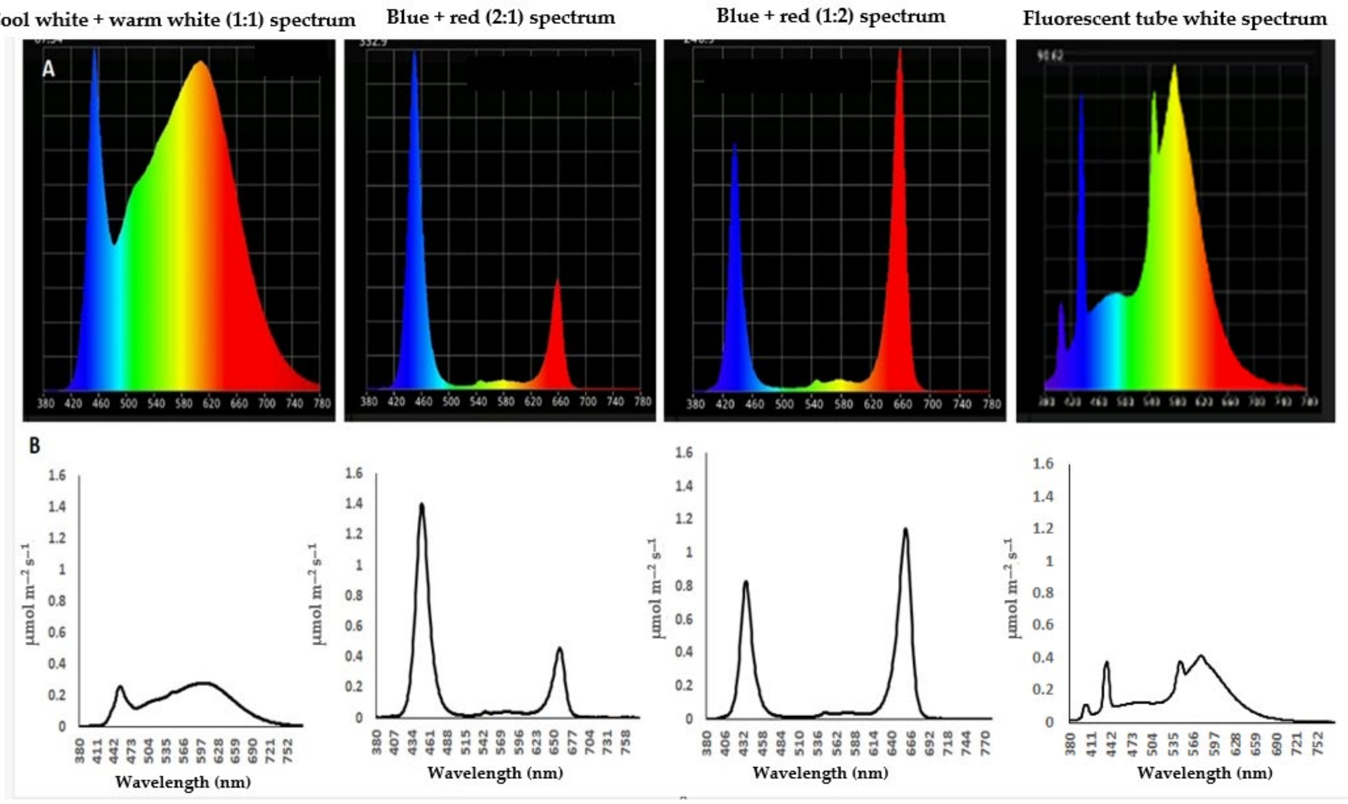

Figure 1. Measured spectra of the light treatments using a UPRtek spectrophotometer. (**A**) Relative light intensity. (**B**) Radiant density of the light spectrum intensity.

2.3. Measurement of the Root System

Three root replicates for three plants from each treatment were prepared by extracting the roots from magenta boxes (Figure 2a), washing off the agar, and rinsing the roots with tap water. The roots were stained with toluidine red for approximately 8 h before scanning (Figure 2b). Scanning was performed using a flatbed HP scanner (Scanjet, G2410, 1200 dpi), and the photos were analyzed using WinRHIZO software (V5.0, Regent Instruments, Quebec, QC, Canada). Selected root system traits, i.e., root fresh weight, total root length, root diameter, and surface area, were determined.

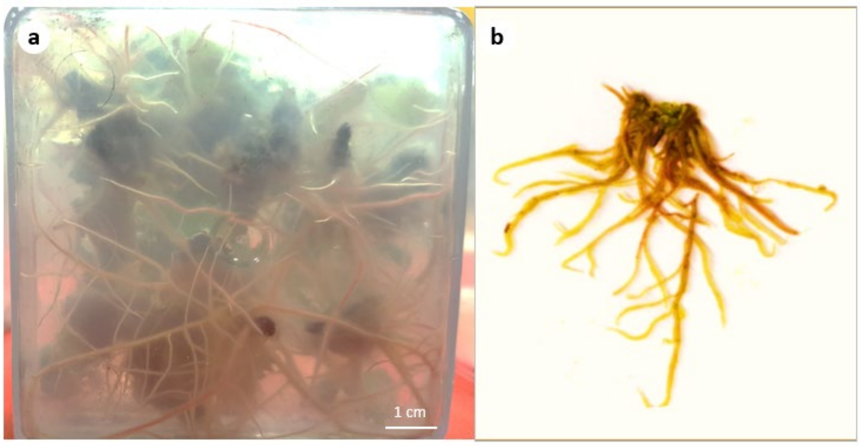

Figure 2. In vitro rooting of blackberry microshoots (**a**) and staining of the adventitious roots (**b**) after 8 weeks in culture.

2.4. Measurements of Vegetative Parameters and Chlorophyll and Carotenoid Contents

Growth responses in terms of fresh weight, plant height, and the number of leaves and leaf area per plantlet were measured after 8 weeks of culture. The leaf area was measured using a portable area meter (CI-202; CID, Inc., Vancouver, WA, USA). All measurements were obtained from 15 randomly chosen plantlets. To measure leaf pigments, three replicates of young leaves (0.5 g each) from each treatment were extracted using 80% cold acetone for 48 h, and the absorbance was measured at 663.2, 646.8, and 470.0 nm with calculations made following Lichtenthaler [22].

2.5. Microscopic Observations of Stomata

The method of Cotton [23] was used to prepare strips from the cuticle of the leaves of blackberry plantlets grown at different LEDs and sucrose levels of 0 and 30 g L^{-1}. The dry leaves were soaked for 24 h and the transparent thin layer of the surface cells of the epidermal layer of the leaf was carefully removed using pointed forceps, placed on a glass slide, stained with a light-green dye (A mixture solution of 0.1 g Triarylmethane dye, 2 mL of glacial acetic acid in a 100 mL distilled water) for several seconds, and covered with a slide cover. The glass slides were examined to study stomata types, stomata size (measured with an ocular ruler), and stomatal density (number of stomata per unit area) using an optical microscope with a SwiftCam 20 Megapixel camera for microscopes (DeltaPix, Smørum, Denmark), which was used to capture microscopic images of the leaf surfaces at 40× magnification.

2.6. Experimental Design and Statistical Analysis

The experiment followed a factorial completely randomized design. Significant differences among the means were determined using Tukey's range and ANOVA tests via SAS (version 6.12; SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Effects of Light Spectra and Sucrose Treatments and Their Interactions on In Vitro Rooting of Blackberry Microshoots

Light and sucrose treatments and their interactions significantly ($p \leq 0.001$) influenced root growth and development in terms of total root length, root diameter, root surface area, and root fresh weight (Table 1). The highest total root length, root diameter, and root surface area were recorded at blue + red light (1:2) and the control treatment. The highest root fresh weight was recorded at blue + red light (1:2) compared with other LED treatments. Cool white + warm white (1:1) treatment recorded the lowest rooting values and negatively affected the rooting of blackberry microshoots compared with other LED treatments. The presence of sucrose in auxin-medium was indispensable for the in vitro rooting of blackberry. Microshoots grown onto medium devoid sucrose did not root under all light treatments. Sucrose supplementation at 15–60 g L^{-1} induced a 100% in vitro rooting. Increased sucrose concentrations from 0–45 g L^{-1} enhanced total root length, root diameter, root surface area, and root fresh weight. The highest values were recorded at a combination of 45 g L^{-1} sucrose under the blue + red light (1:2) treatment.

3.2. Effects of Light Spectra and Sucrose Treatments and Their Interactions on Shoot Growth, Leaf Area, Pigments and Stomata of Blackberry Microshoots

Both sucrose concentrations and light treatments, as well as their interaction effects, had significant effects ($p \leq 0.05$) on the shoot length, shoot fresh weight, and number of leaves of blackberry plantlets (Table 2). Among the light treatments, blue + red light (2:1) resulted in the highest vegetative growth. Compared with plantlets grown in medium without sucrose or with a high-concentration sucrose supplement ($\geq$45 g L^{-1}), plantlets grown in medium containing 15–30 g L^{-1} of sucrose exhibited the highest shoot length and number of leaves (Figure 3). However, blackberry plantlets grown at 30 g L^{-1} sucrose and blue + red light (2:1) treatment presented the highest shoot growth.

Light spectra and sucrose concentrations and their interaction significantly ($p \leq 0.01$ and $p \leq 0.001$) influenced the leaf area and content of chlorophyll and carotenoids in blackberry plantlets (Table 3). Regardless of sucrose concentration, blue + red light (2:1) resulted in the highest leaf area as compared with LED treatments. Sucrose supplementation at 15–30 g L^{-1} resulted in the highest leaf area, whereas higher sucrose concentrations (45 and 60 g L^{-1}) negatively affected leaf area. Blackberry plantlets grown at 30 g L^{-1} sucrose and blue + red light (2:1) recorded the highest value of leaf area.

Table 1. Effect of light spectra and sucrose concentration treatments on total root length, root surface area, root diameter, and root fresh weight of in vitro blackberry plantlets.

Treatments		Root Length/Plantlet (cm)	Root Diameter/Plantlet (mm)	Root Surface Area/Plantlet (cm^2)	Root Fresh Weight/Plantlet (g)
Light treatments					
Fluorescent (control)		21.03 a	1.49 ab	12.15 a	0.415 b
Cool white + Warm white (1:1)		15.13 b	1.07 b	6.50 b	0.490 b
Blue + Red (2:1)		14.53 b	1.58 ab	10.05 ab	0.549 b
Blue + Red (1:2)		20.71 a	1.87 a	12.10 a	0.8741 a
F-value		90.15 ***	11.76 ***	45.42 ***	90.15 ***
p-value		<0.001	<0.001	<0.001	<0.001
Sucrose concentrations (g L^{-1})					
	0	0.00 d	0.00 d	0.00 d	0.000 e
	15	15.63 c	1.55 b	8.534 c	0.556 c
	30	16.55 c	2.16 a	14.27 b	0.745 b
	45	35.65 a	2.49 a	18.76 a	1.214 a
	60	21.41 b	1.30 b	9.44 c	0.376 d
F-value		290.92 ***	79.83 ***	254.79 ***	290.92 ***
p-value		<0.001	<0.001	<0.001	<0.001
Light treatments × Sucrose concentrations (g L^{-1})					
Fluorescent (control)	0	0.00 i	0.00 j	0.00 k	0.000 g
	15	22.37 d	1.44 e–h	10.43 g	0.473 ef
	30	16.14 f	2.65 bc	13.99 def	0.435 f
	45	36.36 b	1.84 def	21.21 b	1.040 c
	60	30.26 c	1.53 e–h	15.11 cde	0.126 g
Cool white + Warm white (1:1)	0	0.00 i	0.00 j	0.00 k	0.000 g
	15	9.59 h	0.75 i	2.33 jk	0.503 ef
	30	16.87 ef	2.33 cd	12.54 efg	0.611 e
	45	35.26 b	1.15 f–i	13.15 ef	1.187 b
	60	13.90 fg	1.10 ghi	4.49 ij	0.147 g
Blue + Red(2:1)	0	0.00 i	0.00 j	0.00 k	0.000 g
	15	10.64 h	2.03 cde	7.59 h	0.391 f
	30	12.30 gh	1.84def	14.05 def	0.772 d
	45	27.78 c	3.12 b	16.92 c	1.159 bc
	60	21.91 d	0.91 hi	11.71 fg	0.425 f
Blue + Red(1:2)	0	0.00 i	0.00 j	0.00 k	0.000 g
	15	19.91 d	1.97 de	13.80 def	0.856 d
	30	20.90 d	1.84 def	16.52 cd	1.240 b
	45	43.17 a	3.85 a	23.75 a	1.469 a
	60	19.56 de	1.67 d–g	6.45 hi	0.806 d
F-value		30.58 ***	8.46 ***	11.81 ***	30.58 ***
p-value		<0.001	<0.001	<0.001	<0.001

Values followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level, according to Tukey's range test. *** = significant at $p \leq 0.001$.

Table 2. Effects of light spectra and sucrose concentration treatments on the shoot length, shoot fresh weight, and number of leaves of in vitro blackberry plantlets.

Treatments		Shoot Length (cm)	Shoot Fresh Weight (g)	Number of Leaves
Light treatments				
Fluorescent (control)		5.0 b	0.558 b	8.3 b
Cool white + Warm white (1:1)		6.3 a	0.474 b	8.6 b
Blue + Red(2:1)		6.7 a	0.710 a	10.5 a
Blue + Red(1:2)		5.2 b	0.713 a	8.4 b
F-value		26.76 ***	22.73 ***	26.91 ***
p-value		< 0.001	< 0.001	< 0.001
Sucrose concentrations (g L^{-1})				
	0	4.9 bc	0.379 c	7.5 b
	15	6.7 ab	0.566 ab	9.0 a
	30	7.2 a	0.623 a	9.3 a
	45	5.6 c	0.677 ab	7.4 c
	60	4.5 d	0.430 bc	5.4 d
F-value		63.36 ***	14.75 ***	88.17 ***
p-value		<0.001	<0.001	<0.001
Light treatments × Sucrose concentrations (g L^{-1})				
	0	5.3 efg	0.454 ef	8.5 fgh
	15	6.4 cde	0.503 ef	10.0 cde
Fluorescent (control)	30	6.2 cde	0.605 de	11.0 abc
	45	4.3 gh	0.740 bcd	7.3 hi
	60	3.0 i	0.487 ef	4.7 k
	0	6.3 cde	0.240 g	9.0 efg
	15	7.0 bc	0.394 fg	9.8 c–f
Cool white + Warm white (1:1)	30	7.7 ab	0.623 cde	11.0 abc
	45	6.3 cde	0.739 fg	7.7 ghi
	60	4.2 h	0.372 abc	5.3 k
	0	6.8 bc	0.495 ef	10.7 bcd
	15	7.3 bc	0.854 ab	12.3 a
Blue + Red(2:1)	30	8.4 a	0.918 ab	12.0 a
	45	6.8 bc	0.863 a	10.5 cd
	60	4.3 gh	0.419 f	7.0 ij
	0	6.3 cde	0.706 bcd	9.5 def
	15	6.6 bcd	0.832 ab	10.7 bcd
Blue + Red(1:2)	30	5.5 def	0.788 abc	9.0 efg
	45	4.8 fgh	0.741 bcd	7.0 ij
	60	3.0 i	0.499 ef	5.7 jk
F-value		2.31 *	10.49 ***	2.15 *
p-value		0.0167	<0.001	0.0260

Values followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level, according to Tukey's range test. * and *** = significant at $p \leq 0.05$ and $p \leq 0.001$, respectively.

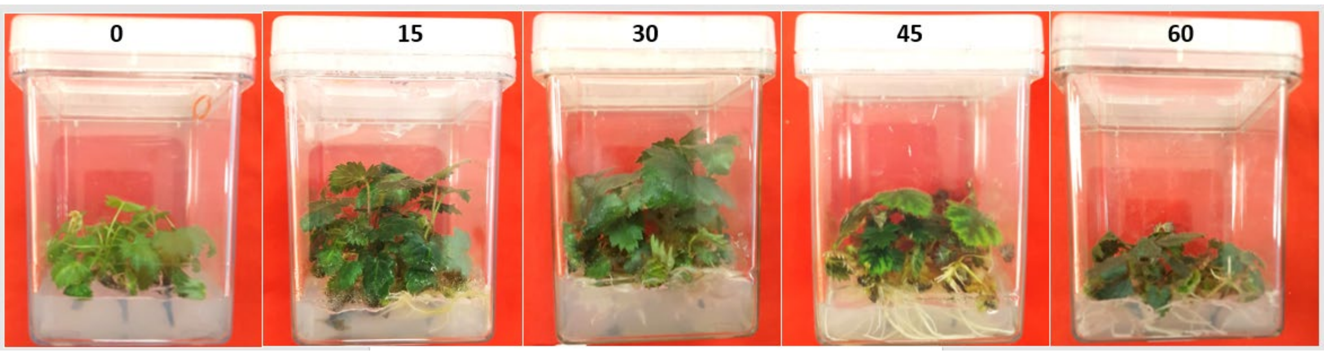

Figure 3. In vitro blackberry plantlets grown under blue + red light (2:1) and different sucrose concentrations (0–60 g L^{-1}).

Table 3. Effect of light spectra and sucrose concentration treatments on leaf area and pigments of in vitro blackberry plantlets.

Treatments		Leaf Area/Plantlet (cm^2)	Chlorophyll a ($mg\ g^{-1}$ FW)	Chlorophyll b ($mg\ g^{-1}$ FW)	Chlorophyll a + b ($mg\ g^{-1}$ FW)	Total Carotenoids ($mg\ g^{-1}$ FW)
Light treatments						
Fluorescent (control)		14.53 b	1.67 b	0.57 b	2.25 b	0.55 b
Cool white + Warm white (1:1)		12.56 b	2.37 ab	0.78 ab	3.15 ab	0.80 ab
Blue + Red(2:1)		21.42 a	2.74 a	0.84 a	3.57 a	0.86 a
Blue + Red(1:2)		12.86 b	2.36 ab	0.72 ab	3.08 ab	0.74 ab
F-value		49.75 ***	117.31 ***	73.23 ***	74.67 ***	112.79 ***
p-value		<0.001	<0.001	<0.001	<0.001	<0.001
Sucrose concentrations ($g\ L^{-1}$)						
	0	10.87 c	1.78 b	0.54 b	2.32 c	0.59 b
	15	14.38 ab	2.40 a	0.74 a	3.14 a	0.81 a
	30	16.38 a	2.21 ab	0.72 a	2.94 ab	0.70 ab
	45	12.18 bc	1.99 b	0.62 ab	2.61 bc	0.62 b
	60	9.19 c	0.94 c	0.32 c	1.26 d	0.31 c
F-value		27.34 ***	344.90 ***	323.22 ***	294.17 ***	362.96 ***
p-value		<0.001	<0.001	<0.001	<0.001	<0.001
Light treatments × Sucrose concentrations ($g\ L^{-1}$)						
Fluorescent (control)	0	10.25 i–k	1.98 gh	0.60 j	2.59 fg	0.66 g–i
	15	15.44 e–h	2.39 f	0.81 fg	3.20 e	0.81 ef
	30	19.46 b–e	2.18 f	0.88 e–g	3.06 e	0.75 f
	45	16.30 d–g	1.28 i	0.39 k	1.67 h	0.37 j
	60	11.23 j–i	0.55 j	0.17 l	0.72 i	0.16 kl
Cool white + Warm white (1:1)	0	11.42 j–i	2.81 de	0.87 d–f	3.69 cd	0.89 de
	15	13.99 f–i	3.08 b–d	0.96 b–d	4.04 b	1.06 ab
	30	20.61 bc	2.18 fg	0.70 hi	2.88 ef	0.72 f–h
	45	10.19 i–k	2.23 fg	0.75 gh	2.98 e	0.74 fg
	60	6.60 k	1.57 k	0.62 m	2.19 j	0.59 l
Blue + Red(2:1)	0	19.80 b–d	1.87 h	0.58 j	2.45 g	0.63 hi
	15	23.46 ab	3.49 a	1.09 a	4.58 a	1.13 a
	30	25.37 a	3.26 ab	0.98 bc	4.24 b	1.02 bc
	45	19.36 b–e	3.17 bc	0.93 c–e	4.10 b	0.94 cd
	60	19.12 c–e	1.90 h	0.60 j	2.50 g	0.58 i
Blue + Red(1:2)	0	12.88 g–i	2.24 fg	0.65 ij	2.89 ef	0.75 fg
	15	17.21 c–f	2.78 e	0.80 fg	3.57 d	0.94 cd
	30	14.22 f–i	3.20 bc	1.03 ab	4.23 b	0.93 cd
	45	12.45 g–i	2.96 c–e	0.97 bc	3.93 bc	0.92 cd
	60	7.56 jk	0.59 j	0.18 l	0.78 i	0.19 k
F-value		2.87 **	33.17 ***	34.33 ***	22.21 ***	35.35 ***
p-value		0.0035	<0.001	<0.001	<0.001	<0.001

Values followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level, according to Tukey's range test. ** and *** = significant at $p \leq 0.01$ and $p \leq 0.001$, respectively.

For leaf pigments, fluorescent light exhibited lower content of chlorophyll and carotenoid contents compared with other LED treatments. Moderate sucrose concentrations (15–30 g L^{-1}) resulted the highest values of leaf pigments. Compared with all other treatments, higher chlorophyll and carotenoid contents were detected in plantlets grown under blue + red light (2:1) and 15 g L^{-1} sucrose. Higher sucrose levels resulted in negative effects on the leaf pigment content of plantlets.

Light microscopic observations of the leaf stomata of plantlets grown with two sucrose concentrations (0 and 30 g L^{-1}) under different light spectra revealed the existence of significant variation in stomata frequency and aperture length and width (Figures 4 and 5). Compared with blackberry plantlets grown with 30 g L^{-1} of sucrose, those grown in medium devoid of sucrose and incubated under fluorescent light or blue + red light (1:2) exhibited the highest stoma number. Conversely, plantlets grown under blue + red light (2:1) had the highest stoma aperture length and width, indicating stomatal opening. Plantlets grown under cool + warm white light had the lowest values among all light treatments at both sucrose levels (0 and 30 g L^{-1}). The stomata were elliptical without sucrose but almost round with the 30 g L^{-1} sucrose treatment.

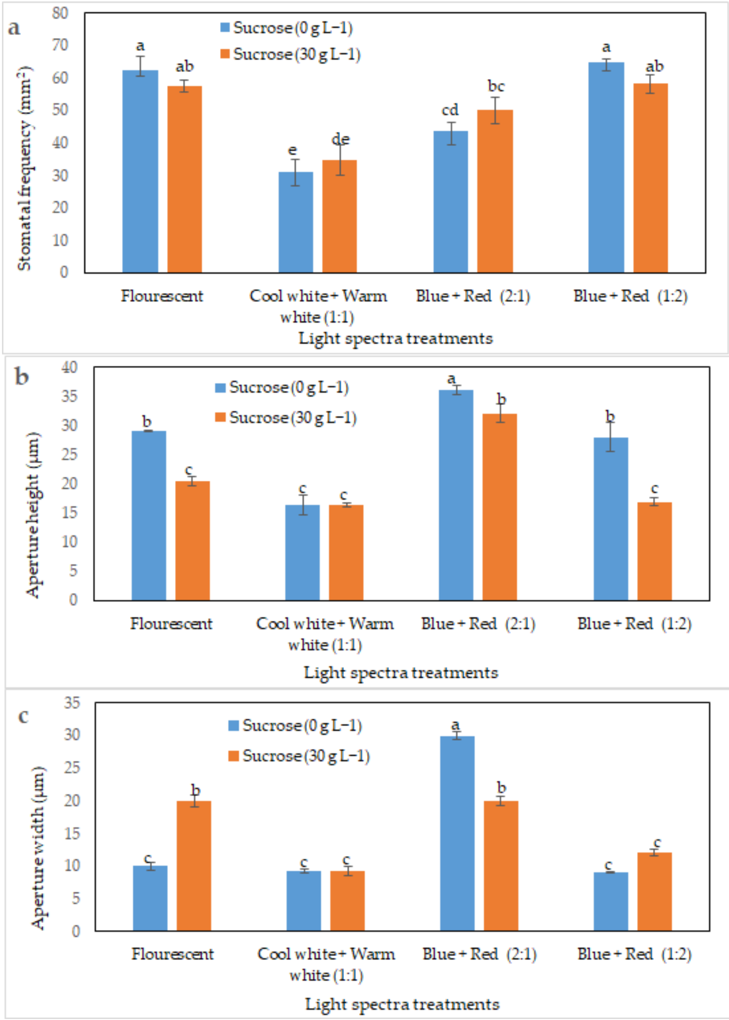

Figure 4. (**a**) Leaf stomatal density and (**b**) aperture length and (**c**) width of in vitro blackberry plantlets according to light spectra and sucrose concentration treatments. Data represent means ± standard errors. Different letters show significant differences at $p \leq 0.05$.

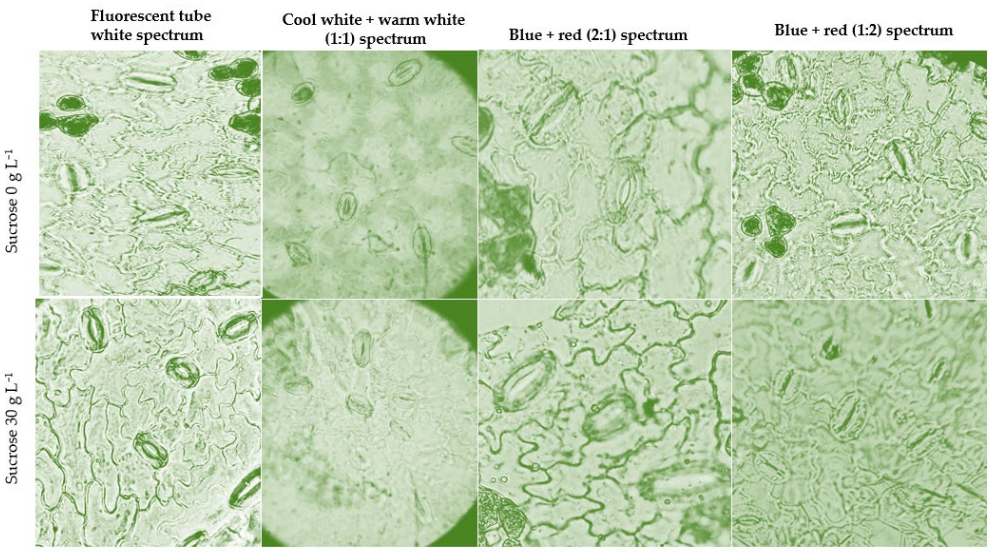

Figure 5. Light microscopic observations (40× magnification) of the leaf stomata of in vitro blackberry plantlets according to light spectra and sucrose concentration treatments.

4. Discussion

4.1. Effects of Light Spectra and Sucrose Treatments and Their Interactions on In Vitro Rooting of Blackberry Microshoots

Few studies on the root growth and physiology of plants under combinations of LED light have been conducted. Light is known to influence root elongation through photomorphogenic action, i.e., root elongation may be controlled by phytochromes [24]. Wu and Lin [25] found that the rooting percentage was higher in in vitro *Protea cynaroides* plantlets cultured under red LED light (67%) than under conventional white fluorescent light (7%). Red LED light has also been found to stimulate root formation in *Anthurium andreanum* [26] and *Chrysanthemum morifolium* [27]. Ren et al. [28] showed that the root length and root activity of *Phalaenopsis* were improved with an LED light combination of red/blue/far red light (3:6:1). In another study, compared with a white LED light treatment, an LED light combination of red/blue/purple/green light (8:1:1:1) resulted in a higher rooting rate, root activity, and root growth in tissue-cultured *Cunninghamia lanceolata* seedlings [29]. A combination of blue and red light has been shown to increase blueberry (*Vaccinium corymbosum*) shoot and root biomass [30]. In the present study, blue + red light (1:2) recorded the highest values of rooting indicating that red light favors the root growth and development as compared with fluorescent light. Thus, according to the present results and previous studies, light spectra significantly affect the growth and morphology of the rooting system in a species-dependent manner.

Carbohydrates have been shown to promote adventitious root formation in many species, mainly by acting as an energy source [31]. Sugars regulate root initiation by a coordinated modulation of gene expression and enzyme activities in the meristematic cells [32]. Varying sucrose concentrations in a rooting medium can positively affect root induction and development. For example, the in vitro shoots of *Astragalus chrysochlorus* failed to root in MS medium containing 30 g L^{-1} of sucrose, but rooting was strongly stimulated (93% of shoots) when the sucrose concentration was reduced to 20 g L^{-1} [33]. In our study, no rooting occurred on medium without sucrose under all different LED treatments. The absence of carbon sources in the rooting medium has been reported to hinder the rooting of micropropagated plants [34,35].

4.2. Effects of Light Spectra and Sucrose Treatments and Their Interactions on Shoot Growth, Leaf Area, Pigments and Stomata of Blackberry Microshoots

Changes in light spectra are known to strongly influence plant morphogenesis and growth [36]. The role of blue and red light in stomata opening and the importance of blue light in stomata opening has been emphasized [37,38]. In the present study, the stomata aperture length and width were increased in blackberry microshoots grown under blue + red light (2:1). Kim et al. [39] reported larger leaf stomata in *Chrysanthemum* plants grown under blue and red LED light. Terfa et al. [40] showed that a high proportion of blue light (20%) combined with high-pressure sodium light markedly increased the number of stomata, chlorophyll content, photosynthesis performance per unit leaf area, growth, and morphological changes of *Rosa* × *hybrida*. In plants, stomata maximize homeostasis by controlling the extent of physical exchange between the plant and its surroundings through stomatal control of pore apertures [41]. Therefore, micropropagated plantlets use their stomata as a means of adapting to environmental change and stress. In the present study, blue and red LED light treatment increased the leaf area and leaf pigment content of blackberry microshoots. LEDs provide photons that can activate discrete developmental pathways to enhance plant growth in terms of leaf area and stem length through photoreceptors such as phytochromes and cryptochromes [42,43]. Both red and blue light are effective for enhancing plant growth because they are more efficiently absorbed by photosynthetic pigments than other regions of the light spectrum. Stem elongation can be promoted or inhibited by different synergistic interactions between blue/red light receptors and phytochrome in a species-dependent manner [39]. Blue light, which is strongly absorbed by carotenoid pigments, was reported to increase chlorophyll content, promote stomatal opening, and control the integrity of chloroplast proteins [44]. Carotenoids play fundamental roles in photosynthetic organisms. They act as accessory light-harvesting pigments but also perform photoprotective roles by quenching triplet state chlorophyll molecules and scavenging singlet oxygen and other toxic oxygen species formed within the chloroplast [45]. Blue light was reported to promote photosynthesis and vegetative growth by increasing chlorophyll content, promoting the formation of the photosynthetic apparatus, and potentially inducing stomatal opening [46,47]. Under in vitro conditions, blue light resulted in the highest chlorophyll and carotenoid content in *Spathiphyllum cannifolium* and the highest fresh weight, dry weight, and leaf number in *Euphorbia milii* microshoots [48]. Similarly, in the present study, blue and red (2:1) treatment resulted in a high content of chlorophyll and carotenoids, indicating the enhanced photosynthetic capacity of blackberry microshoots as compared with fluorescent light treatment.

Poudel et al. [49] found that red light might be effective for increasing shoot height, internode length, and rooting frequency, whereas blue light might be required for the chlorophyll synthesis and stomatal development of grape plants. Kim et al. [39] revealed that the combination of blue and red light increased the plant fresh weight, leaf area, and chlorophyll content of *Chrysanthemum* plantlets and resulted in the highest net photosynthetic rate compared with that achieved under fluorescent light or red or blue light treatments alone. A combination of red and blue light irradiation increased the number of leaves in lettuce (*Lactuca sativa*) plants [50,51]. The stimulatory effect of red + blue LED light on in vitro leaf growth has been reported in *Chrysanthemum* [39], *Doritaenopsis* [52], and *Fragaria* × *ananassa* cv. Akihime [53]. An optimized red:blue light ratio may be more beneficial for photosynthesis. For instance, the net photosynthetic rate increased as the red:blue light ratio was decreased [54], and the red light-induced impairment of photosynthetic parameters and chloroplast development was alleviated by adding blue light [55]. In the present study, blackberry microshoots grown onto medium either with high sucrose supplement or without sucrose recorded the lowest values of leaf area, pigments and vegetative growth. Sucrose provides energy to in vitro plants, supports the maintenance of osmotic potential, and acts as a carbon precursor and signaling metabolite [56–58]. In vitro cultures grown on medium without sucrose supplement are unable to fix sufficient CO_2 to maintain their growth due to the limited CO_2 inside the culture vessels [59]. Conversely, high sucrose supplementation reduces the leaf pigments and negatively restricts the efficiency the photosynthetic system [60].

5. Conclusions

In conclusion, different LED treatments and sucrose concentrations significantly influenced the root formation and shoot growth of blackberry microshoots in the present study. Regardless of sucrose concentrations, blue and red light at a 1:2 spectral ratio favored root growth while a ratio of 2:1 favored leaf pigments and shoot growth. Although a 45 g L^{-1} sucrose supplement favored the growth and development of the root system, the shoot growth of blackberry was negatively affected by this sucrose concentration. Therefore, the combination of blue and red light at a 2:1 spectral ratio with 30 g L^{-1} of sucrose is recommended for the optimal in vitro rooting and growth of blackberry microshoots.

Author Contributions: Conceptualization, Y.H.D. and H.Z.R.; methodology, Y.H.D., A.M.A.-A., T.A., H.Z.R. and M.S.A.; investigation, Y.H.D., A.M.A.-A., T.A., H.Z.R. and M.S.A.; formal analysis and data curation, Y.H.D., A.M.A.-A., T.A., H.Z.R., M.S.A. and Y.N.; writing—original draft preparation, Y.H.D., T.A. and H.Z.R.; validation, M.S.A., K.F.A., Y.N. and M.P.F.; visualization, M.S.A., K.F.A., Y.N. and M.P.F. writing—review and editing, Y.H.D., T.A., H.Z.R., K.F.A., Y.N. and M.P.F. All authors have read and agreed to the published version of the manuscript.

Funding: Authors extend their appreciation to the International Scientific Partnership Program (ISPP) at King Saud University for funding this research work through (ISPP# 0064).

Data Availability Statement: All data are presented in the article.

Acknowledgments: Authors extend their appreciation to the International Scientific Partnership Program (ISPP) at King Saud University for funding this research work through (ISPP# 0064).

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

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