agronomy

MDPI

Article

Morpho-Metric and Specialized Metabolites Modulation of Parsley Microgreens through Selective LED Wavebands

Petronia Carillo [1,†], Christophe El-Nakhel [2,†], Veronica De Micco [2], Maria Giordano [2], Antonio Pannico [2], Stefania De Pascale [2], Giulia Graziani [3], Alberto Ritieni [3], Georgios A. Soteriou [4], Marios C. Kyriacou [4,*] and Youssef Rouphael [2]

1 Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Via Vivaldi 43, 81100 Caserta, Italy; petronia.carillo@unicampania.it
2 Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; christophe.elnakhel@unina.it (C.E.-N.); demicco@unina.it (V.D.M.); maria.giordano@unina.it (M.G.); antonio.pannico@unina.it (A.P.); depascal@unina.it (S.D.P.); youssef.rouphael@unina.it (Y.R.)
3 Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy; giulia.graziani@unina.it (G.G.); alberto.ritieni@unina.it (A.R.)
4 Department of Vegetable Crops, Agricultural Research Institute, Nicosia 1516, Cyprus; soteriou@ari.gov.cy
* Correspondence: m.kyriacou@ari.gov.cy
† These authors contributed equally to this work.

Abstract: Plant factories and high-tech greenhouses offer the opportunity to modulate plant growth, morphology and qualitative content through the management of artificial light (intensity, photoperiod and spectrum). In this study, three Light Emitting Diode (LED) lighting systems, with blue (B, 460 nm), red (R, 650 nm) and mixed red + green-yellow + blue (RGB) light were used to grow parsley microgreens to understand how light quality could change the phenotype and the profile of secondary metabolites. Plants showed altered morphological characteristics and higher amounts of secondary metabolites under RGB LEDs treatment. The results demonstrated that microgreens under red light showed the highest fresh yield, petiole length, coumaric acid content but also the highest nitrate content. Plants under RGB light showed the highest dry matter percentage and highest content of total and single polyphenols content, while blue light showed the highest ascorbic acid and ABTS antioxidant activity. Moreover, microgreens under red light showed more compact leaves with less intercellular spaces, while under blue and RGB light, the leaves displayed ticker spongy mesophyll with higher percentage of intercellular spaces. Therefore, the specific spectral band was able to modify not only the metabolic profile, but also it could modulate the differentiation of mesophyll cells. Light quality as a preharvest factor helps to shape the final parsley microgreens product as a whole, not only in terms of yield and quality, but also from a morpho-anatomical point of view.

Keywords: monochromatic light; *Petroselinum crispum*; growth chamber; light-emitting diodes; controlled environment; HPLC; ion chromatography; light microscopy

check for updates

Citation: Carillo, P.; El-Nakhel, C.; De Micco, V.; Giordano, M.; Pannico, A.; De Pascale, S.; Graziani, G.; Ritieni, A.; Soteriou, G.A.; Kyriacou, M.C.; et al. Morpho-Metric and Specialized Metabolites Modulation of Parsley Microgreens through Selective LED Wavebands. *Agronomy* **2022**, *12*, 1502. https://doi.org/10.3390/agronomy12071502

Academic Editor: Dimitrios Savvas

Received: 31 May 2022
Accepted: 21 June 2022
Published: 23 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Public interest in healthy eating habits has increased over the years, along with consumers' awareness of the nutritional and nutraceutical properties of plants, and of the capacity of plant-rich diets to improve health, delay aging and increase expectation of life [1,2]. This is certainly due to the development of policies addressing food and nutrition in recent decades, and to the related information and communication campaigns to promote a healthy lifestyle. The International Conference on Nutrition in 1992 and the World Food Summit in 1996 stressed, for the first time, the important concept that accessing a safe and healthy variety of food is a fundamental human right. However, it was only in 2001 that the first action plan for food and nutrition policy was proposed, whose ambition was for people across the society to adopt healthier patterns of living before 2015 [3]. Recently, the Italian

Ministry of Health published the new edition of the "Guidelines for healthy eating, Revision 2018" of the Council for Agricultural Research and Economics (CREA); it highlighted the impact of the food choices on environmental, economic and social sustainability, and explained how directing food choices towards plant foods over animal foods may save money, help the environment and boost people's health [4]. Accordingly, the "Farm to Fork Strategy" [5], which is at the heart of the European Green Deal, has the main aim to create a favourable food environment that encourages healthy and sustainable diets to improve consumers' health and quality of life, and reduce health-related costs for society. Therefore, fostering healthy eating habits is one of the main goals of modern societies [6]. In light of this, nutrient-dense foods such as microgreens (i.e., tender immature vegetable greens harvested just after the cotyledon leaves have developed with one set of true leaves) are attaining popularity for the high content of nutraceutical plant secondary metabolites present in their first true leaves compared to their mature leaf counterparts [7–9]. It has been demonstrated that when microgreens are introduced in plant rich diets, their antioxidant metabolites can help to protect cells from oxidative damage by direct scavenging of ROS, thus delaying and/or inhibiting oxidative damage and preventing chronic degenerative diseases, such as diabetes, cardiovascular disease, gastrointestinal and kidney disorders, and even cancer [7,9,10].

Indeed, the plant metabolic profile is mostly genetically determined; however, the secondary metabolites pattern in microgreens may be modulated by developmental and environmental factors, but also by crop practices and cultivation conditions [11,12]. In particular, light is a pivotal environmental factor able to modify the concentration of secondary metabolites, influencing the colour, taste and nutraceutical quality of microgreens [13,14]. Light not only provides plants with energy to power the light-dependent reaction of photosynthesis and produce ATP and NADPH, which are needed for the reductive pentose phosphate pathway (Calvin cycle), but it represents a fundamental source of information on the surrounding environment able to reprogram growth, tissue differentiation and metabolism [15,16]. In fact, plants constantly adapt to changing light environments to maximise energy conversion through photosynthesis while limiting photodamage by means of chlorophylls and carotenoids. In addition, plants have a series of photoreceptors able to finely detect changes in the spectral composition over a broad light spectrum spanning from UV-B to far-red wavelength (280–750 nm), and trigger dynamic adaptative responses [17].

Nowadays, in growth chambers, high-tech greenhouses and plant factories, light quantity (intensity and photoperiod) and quality (spectral composition) may be modulated in order to manipulate plant development and yield [18]. In particular, by using light-emitting diodes (LEDs), the spectral composition of artificial lighting can be adapted in order to promote the synthesis of health-promoting phytochemicals and increase the functional quality of microgreens [19,20]. Recently, the effects of selected spectral narrow-band LEDs lighting (red, blue, blue-red) on the phytochemical profile and antioxidant properties of several microgreens species have been investigated [21–27]. However, morphological alterations together with metabolic profile changes in response to LEDs are poorly characterized in microgreens. Therefore, in our study, we used optical LEDs bandwidth that were red (R), blue (B) and blue, red and green-yellow (control, RGB) to evaluate their modulating effect on morphological and metabolic traits of parsley microgreens.

2. Materials and Methods

2.1. Climate Chamber and Experimental Design

A climate chamber experiment was carried out at the Department of Agricultural Sciences, University of Naples Federico II, Portici (NA), Italy, in order to grow parsley microgreens under diverse light spectrum. Smooth-leaf parsley *Petroselinum crispum* cv. Comune seeds (Semiorto Sementi, Sarno (SA), Italy) were sown (5 seeds cm^{-2}) in plastic trays (204 cm^2), adopting a peat-based substrate (pH 5.48 and EC 0.282 mS cm^{-1}; Special Mixture, Floragard Vertriebs-GmbH, Oldenburg, Germany). The germination occurred in darkness in the climate chamber (KBP-6395F, Termaks, Bergen, Norway) at 24 °C and

100% relative humidity (RH) for nine days. Then, Light-Emitting Diode LED panels (K5 Series XL750, Kind LED, Santa Rosa, CA, USA) were turned on, and the climate chamber was set up at 24/18 °C ± 2 and 70/80% ± 5 day/night, respectively. The intensity of the panels was set at 300 ± 15 μmol m^{-2} s^{-1} at canopy level with a photoperiod of 12/12 h. Parsley microgreens were grown under three different light spectra (treatments) described as follows: Control (RGB): 45% R, 10% G, 45% B, Blue: 0% R, 10% G, 90% B and Red: 90% R, 10% G, 0% B. Where R represents the customizable channel red of the panels (600–700 nm) peaking at 650 nm, G represents the channel green-yellow (500–600 nm), and B represents the blue channel (400–500 nm) peaking at 460 nm. The photosynthetic photon flux density (PPFD) percentage contributions of R, G, and B were determined from bandwidth integration. The light spectrum of the three treatments are illustrated in Kyriacou et al. [11] study. The PPFD and spectral composition were regulated by twelve individual spectral scans per treatment using a spectral radiometer (MSC15, Gigahertz-Optik, Türkenfeld, Germany). Manual fertigation was carried out on a daily basis, using a quarter-strength modified Hoagland solution with the following elements added to the osmotic water: 2.0 mM NO_3-N, 0.25 mM S, 0.20 mM P, 0.62 mM K, 0.75 mM Ca, 0.17 mM Mg, 0.25 mM NH_4-N, 20 μM Fe, 9 μM Mn, 0.3 μM Cu, 1.6 μM Zn, 20 μM B, and 0.3 μM Mo, resulting a 0.4 ± 0.05 dS m^{-1} electrical conductivity (EC) and a pH of 6.0 ± 0.2. All the treatments were replicated three times and randomly distributed along the shelf of the growth chamber. In order to procure homogenous light, RH and temperature distribution, the trays were rotated on a daily basis.

2.2. Parsley Microgreens Harvest, Yield, Sampling and Macro-Mineral Analysis

Twenty-one days after sowing, parsley microgreens were harvested at the first true-leaf phonological stage. Via sterilized scissors, all the microgreens were harvested just above the substrate level, and then weighed to assess the fresh weight that was converted in kg m^{-2}. Aliquots of harvested fresh material were stored at −80 °C for the biochemical analysis, whereas the remaining part was placed in a forced-air oven at 70 °C for three days in order to determine the dry weight and subsequently calculate the dry matter content (DM) percentage, and eventually to be used for macro-mineral analysis.

Dry microgreens tissues were ground in a Wiley Mill (841 μm screen) and then processed for macro-mineral analysis via ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) and electrical conductivity detection [28]. In brief, 250 mg of dried sample ground at 0.5 mm in a Wiley Mill (IKA, MF 10.1, Staufen, Germany) were suspended in 50 mL of ultrapure water (Milli-Q, Merck Millipore, Darmstadt, Germany) and stirred in a shaking water bath (ShakeTemp SW22, Julabo, Seelbach, Germany) at 80 °C for 10 min. The mixture was centrifuged at 6000 rpm for 10 min (R-10M, Remi Elektrotechnik Limited, Mumbai, India), then filtered through a 0.45 μm syringe filter (Phenomenex, Torrance, CA, USA). Chromatographic separation of K, Ca, Mg and Na was achieved in isocratic mode (20 mM methanesulphonic acid) on an IonPac CS12A analytical column (4 × 250 mm, Dionex) equipped with an IonPac CG12A precolumn (4 × 250 mm, Dionex) and a self-regenerating suppressor CERS500 (4 mm, Dionex Sunnyvale, CA, USA). Nitrate, P, and S were detected in gradient mode (1 mM–50 mM KOH) on an IonPac ATC-HC anion trap (9 × 75 mm, Dionex), and an AS11-HC analytical column (4 × 250 mm, Dionex) equipped with an AG11-HC precolumn (4 × 50 mm, Dionex) and a self-regenerating suppressor AERS500 (4 mm, Dionex). Ions were expressed as g kg^{-1} dry weight (DW) and nitrate was expressed as mg kg^{-1} fresh weight (FW) on the basis of each sample's original DW.

2.3. Morpho-Metric Measurements and Anatomical Determination of Microgreens

At harvest, 5 microgreens per treatment were cut at the base and fixed in F.A.A. (38% formaldehyde, glacial acetic acid, 50% ethanol solution, 5/5/90 by volume). After a one-week fixation, microgreens were dissected under a light microscope and portions of hypocotyl, petiole and first true-leaf (in the median part) and subjected to dehydration in an alcohol series up to embedding in the acrylic resin JB4 (Polysciences, Hirschberg,

Germany). Cross sections (5 µm-thick) of the three organs were obtained through a rotative microtome and sections were stained with Toluidine blue. Details of the procedures are reported in De Micco et al. [29].

All sections were analysed through light microscopy (BX51, Olympus, Hamburg, Germany) and microphotographs were captured (EP50; Olympus). In the case of leaves, anatomical traits were quantified with the software Olympus CellSens 2.3 as follows: thickness of epidermis, palisade and spongy tissues (in five regions of the lamina); spongy parenchyma density (as percentage of tissue occupied by intercellular spaces over a given surface, in three regions of the parenchyma); stomata frequency on the lower epidermis (as number of stomata per mm of epidermis along three epidermal transects).

2.4. Assessment of Chlorophylls, Total Ascorbic Acid and Antioxidant Activities

Chlorophylls a and b and total chlorophylls content were assessed based on the protocol detailed by Lichtenthaler and Wellburn [30]. In brief, 0.5 g of fresh material was extracted with 90% acetone (10 mL) and the centrifuged. The supernatant absorbance was measured at 662 nm for chlorophyll a and 645 nm for chlorophyll b through spectrophotometry (Hach DR 4000; Hach Co, Loveland, CO, USA). Total chlorophylls content was calculated as the sum of chlorophyll a and b and expressed in mg g^{-1} fresh weight (fw).

Based on the method described by Kampfenkel et al. [31], 0.4 g of fresh material was extracted for the determination of total ascorbic acid. The absorbance was measured at 525 nm through an UV-Vis spectrophotometer (Hach DR 4000; Hach Co, Loveland, CO, USA).

As for the hydrophilic antioxidant activity [32], it was quantified with the *N*,*N*-dimethyl-p-phenylenediamine (DMPD) method through UV-Vis spectrophotometry, by extracting 0.2 g of lyophilized microgreens material in distilled water. The absorbance was measured at 505 nm. The hydrophilic antioxidant activity was expressed in mmol ascorbic ac. eq. 100 g^{-1} DW.

Determination of the antioxidant activity in lipophilic extracts employed the 2,20-azinobis 3-ethylbenzothiazoline-6-sulfonic acid ABTS method [33]. Quantification relied on UV-Vis spectrophotometry, with the absorbance of lipophilic extracts measured at 734 nm. The ABTS antioxidant activity was expressed in mmol Trolox equivalent (6-hydroxy-2,5,7,8-tetramethylchro man-2-carboxylic acid) 100 g^{-1} DW.

2.5. Carotenoids Pigments and Polyphenols Quantification

Carotenoids pigments (Lutein and β-carotene) were quantified in a reverse Phase-HPLC separation through a Shimadzu HPLC LC 10 (Shimadzu, Osaka, Japan). Following the method described in detail by Kyriacou et al. [24]. An aliquot of 0.1 g of lyophilized microgreens material was necessary for the extraction in 6 mL ethanol containing 0.1% butylated hydroxytoluene.

As for polyphenols [24], 0.1 g of lyophilized microgreens material was necessary for the extraction in 5 mL methanol/water (60:40, *v*/*v*). Polyphenols were separated and quantified in an UHPLC system (UHPLC, Thermo Fisher Scientific, Waltham, MA, USA); the mass spectrometry analysis was facilitated by a Q Exactive Orbitrap LC-MS/MS (Thermo Fisher Scientific, Waltham, MA, USA).

2.6. Statistics

Analysis of variance (one-way ANOVA) of the experimental data was executed using the SPSS 20 software package. To separate treatment means within the single measured parameters, Tukey's HSD test was applied at $p = 0.05$. The loading plot and score plot of all the analyzed parameters were determined by principal component analysis (PCA) by using Minitab® 18 statistical software (Minitab LLC, State College, PA, USA) [34].

3. Results

3.1. Morpho-Metric Data of Parsley Microgreens

Parsley microgreens' response to the different light treatments was evident; particularly, it was asserted by plant height, yield and DM % data presented in Figure 1. Interestingly, parsley microgreens under blue light were unquestionably shorter and more compact as demonstrated by hypocotyl length, where only a staggering 2.9 cm was registered for parsley microgreens under blue light, which is significantly 2.1-fold lower than that registered under red light and 1.4-fold lower than RGB light (Table 1). Cotyledon and petiole lengths were significantly higher under red light (Table 1), whereas leaf length and width were significantly higher under RGB light (Table 1). In line with canopy height and hypocotyl and petiole length, red light engendered a significant 16.9% higher yield compared to the other two light treatments (1.34 and 1.37 kg FW m^{-2} for blue and RGB, respectively; Figure 1). As for parsley microgreens DM%, a clear accumulation was seen under RGB light with a significantly higher DM of 7.37% compared to blue (5.75%) and red (5.57%) light (Figure 1).

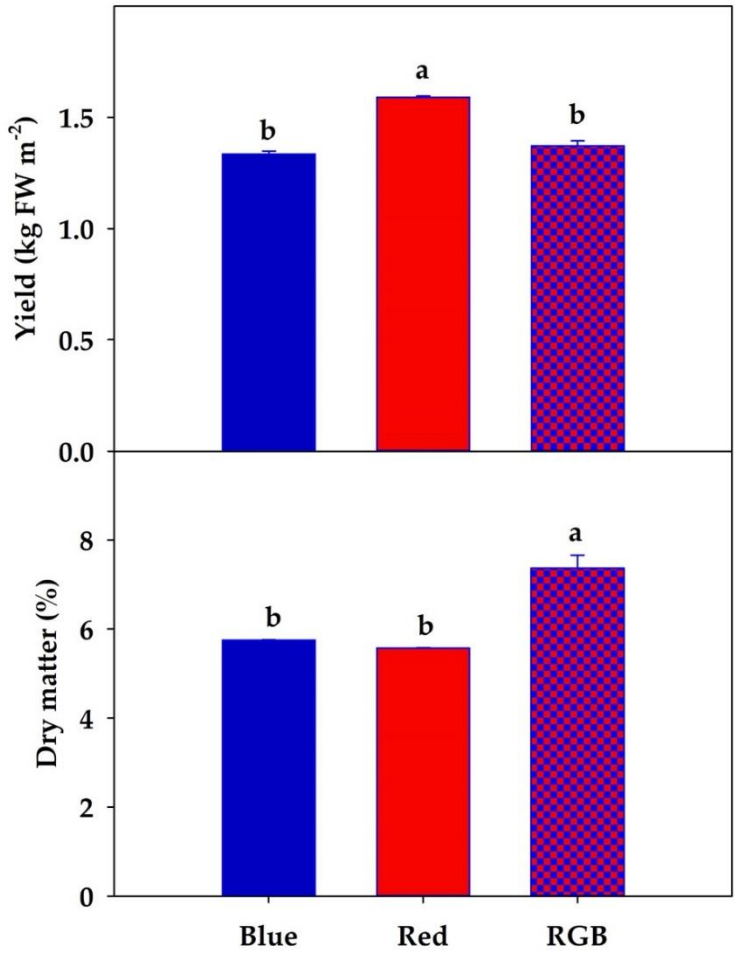

Figure 1. Fresh yield and dry matter percentage of parsley microgreens with reference to the different light treatments (red, blue and RGB). Different letters indicate significant differences according to Tukey's HSD test (p = 0.05). All data are expressed as mean ± standard error, n = 3. RGB: red, green-yellow, blue.

Table 1. Morpho-metric measurements of parsley microgreens with reference to the different light treatments (red, blue and RGB).

Light Treatments	Leaf Length (cm)	Leaf Width (cm)	Petiole Length (cm)	Hypocotyl Length (cm)	Cotyledon Length (cm)	Cotyledon Width (cm)
Blue	0.84 ± 0.01 b	0.88 ± 0.11 b	2.32 ± 0.10 b	2.90 ± 0.18 c	1.35 ± 0.06 b	0.63 ± 0.02
Red	0.81 ± 0.02 b	0.80 ± 0.03 b	3.44 ± 0.21 a	6.10 ± 0.16 a	1.55 ± 0.04 a	0.65 ± 0.02
RGB	1.28 ± 0.12 a	1.37 ± 0.07 a	2.61 ± 0.23 b	4.28 ± 0.24 b	1.36 ± 0.02 b	0.64 ± 0.01
Significance	**	**	*	***	*	ns

Nonsignificant (ns). *, **, *** indicate significance at $p < 0.05$, 0.01 and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$. RGB: red, green-yellow, blue.

3.2. Anatomical Data of Parsley Microgreens

Microscopy observations did not evidence any qualitative difference in tissue organization in all analysed organs. The quantification of leaf anatomical traits evidenced that upper epidermis thickness was significantly higher under blue light than under red light, but it did not significantly differ from that under RGB treatment (Table 2). The spongy parenchyma tissue under RGB light was significantly thicker than under R light, while B treatment induced intermediate values (Table 2). The occurrence of thicker spongy tissue was due to an increased amount of intercellular spaces (lower tissue density), which was significantly higher in microgreens grown under RGB light compared to R light, which in turn sowed higher values than in R treatment. Lower epidermis thickness, palisade tissue thickness and stomata frequency were unaffected by light treatments, although a tendency towards a higher stomata number was found under RGB light (Table 2).

Table 2. Anatomical measurements of parsley microgreens with reference to the different light treatments (red, blue and RGB).

Light Treatments	Upper Epidermis Thickness (μm)	Lower Epidermis Thickness (μm)	Palisade Mesophyll Thickness (μm)	Spongy Mesophyll Thickness (μm)	Intercellular Spaces (%)	Stomatal Frequency (*n*/mm)
Blue	25.31 ± 0.10 a	17.36 ± 0.21	29.50 ± 2.04	55.07 ± 1.96 ab	22.19 ± 0.34 b	4.96 ± 0.85
Red	23.15 ± 0.37 b	17.34 ± 0.40	23.34 ± 1.84	49.48 ± 0.20 b	17.70 ± 0.60 c	4.63 ± 0.85
RGB	24.05 ± 0.45 ab	16.46 ± 1.03	28.45 ± 1.99	58.83 ± 2.26 a	25.70 ± 1.20 a	6.02 ± 0.72
Significance	*	ns	ns	*	***	ns

Nonsignificant (ns). *, *** indicate significance at $p < 0.05$ and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$. RGB: red, green-yellow, blue.

3.3. Nitrate and Macro-Minerals of Parsley Microgreens

As stated in Table 3, nitrate accumulation in microgreens fresh material ranged from 2684 mg kg^{-1} FW under blue light to 3571 mg kg^{-1} FW under red light, with RGB light engendering significantly lower values than that under red light (–12.7%). Phosphorus potassium and calcium concentrations in parsley microgreens under blue light were significantly the highest among all light treatments, opposite to sulfur sodium that were higher under RGB and red light, respectively (Table 3).

Table 3. Nitrate and macro-minerals of parsley microgreens with reference to the different light treatments (red, blue and RGB).

Light Treatments	Nitrate (mg kg^{-1} FW)	P (g kg^{-1} DW)	K (g kg^{-1} DW)	Ca (g kg^{-1} DW)	Mg (g kg^{-1} DW)	S (g kg^{-1} DW)	Na (g kg^{-1} DW)
Blue	2684 ± 40 c	6.49 ± 0.16 a	61.23 ± 0.39 a	8.53 ± 0.27 a	5.60 ± 0.33	2.25 ± 0.09 b	5.25 ± 0.51 b
Red	3571 ± 152 a	5.74 ± 0.05 b	58.72 ± 0.71 ab	6.45 ± 0.34 b	5.69 ± 0.32	2.21 ± 0.11 b	8.49 ± 0.22 a
RGB	3116 ± 12 b	5.38 ± 0.14 b	53.01 ± 1.37 b	6.45 ± 0.16 b	5.03 ± 0.10	3.20 ± 0.17 a	6.73 ± 0.19 b
Significance	***	**	**	**	ns	**	***

Nonsignificant (ns). **, *** indicate significance at $p < 0.01$ and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$. RGB: red, green-yellow, blue.

3.4. Total Chlorophylls, Carotenoids and Total Ascorbic Acids of Parsley Microgreens

Photosynthetic pigments were clearly influenced by light treatments, as seen in Table 4. Chlorophyll a, b and total chlorophylls were significantly higher under blue and red light. With total chlorophylls being on average 23.5% less under RGB light. As for carotenoids, lutein was significantly the highest under RGB, while β-carotene was not significantly different among blue and RGB, whilst it was lowest under red light. Moreover, total ascorbic acid was more accentuated under blue light, to a lesser extent under RGB and finally under red light.

Table 4. Antioxidant activities, total ascorbic acid and photosynthetic pigments of parsley microgreens with reference to the different light treatments (red, blue and RGB).

Light Treatments	ABTS (mmol Trolox 100 g^{-1} DW)	HAA (mmol Ascorbic ac. eq. 100 g^{-1} DW)	Total Ascorbic Acid (mg 100 g^{-1} FW)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophylls (mg g^{-1} FW)	Lutein (mg kg^{-1} DW)	β-Carotene (mg kg^{-1} DW)
Blue	61.99 ± 2.46 a	10.79 ± 0.48 ab	37.27 ± 0.24 a	13.15 ± 0.25 a	4.59 ± 0.21 a	17.74 ± 0.46 a	35.13 ± 0.53 b	362.4 ± 11.9 a
Red	50.72 ± 0.81 b	10.17 ± 0.58 b	12.90 ± 0.70 c	13.87 ± 0.40 a	5.36 ± 0.29 a	19.23 ± 0.54 a	75.63 ± 4.47 b	158.2 ± 9.04 b
RGB	49.61 ± 1.18 b	13.88 ± 1.23 a	20.26 ± 0.63 b	11.00 ± 0.43 b	3.15 ± 0.23 b	14.15 ± 0.63 b	153.0 ± 20.9 a	412.7 ± 13.4 a
Significance	**	*	***	**	**	**	***	***

*, **, *** indicate significance at $p < 0.05$, 0.01 and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$. HAA: hydrophilic antioxidant activity. RGB: red, green-yellow, blue.

3.5. Polyphenols and Antioxidant Activities of Parsley Microgreens

As mentioned in Table 5, light treatments engendered a significant difference in the polyphenols of parsley microgreens, with the highest significant value of total polyphenols under RGB (10,347 mg kg^{-1} DW) followed by blue and lastly by red light treatment. Nonetheless, RGB light significantly induced more apigenin-7-O-glucoside, apigenin-malonyl-apiosyl-glucoside, chlorogenic acid, chrysoeriol, kaempferol-7-O-glucoside, luteolin-7-O-glucoside and quercetin-3-O-galactoside. In general, apigenin-7-apiosyl-glucoside was the most abundant in parsley microgreens followed by apigenin-malonyl-apiosyl-glucoside and apigenin-7-O-glucoside, both making around 99.0% of all polyphenols, and followed by chlorogenic acid, chrysoeriol and coumaric acid. Moreover, the hydrophilic antioxidant activity was in harmony with total polyphenols findings. It was the highest under RGB light and the lowest under red light (Table 4). As for the ABTS antioxidant activity, it was the highest under blue light, with no significant difference among the rest of the treatments (Table 4).

Table 5. Total polyphenols of parsley microgreens with reference to the different light treatments (red, blue and RGB).

Polyphenols (mg kg^{-1} DW)	Light Treatments			Significance
	Blue	Red	RGB	
Apigenin	4.60 ± 0.23 a	3.95 ± 0.06 b	2.12 ± 0.05 c	***
Apigenin-7-apiosyl-glucoside	5068 ± 45.4	5056 ± 26.3	5095 ± 47.0	ns
Apigenin-7-O-glucoside	65.8 ± 1.73 b	49.1 ± 1.08 c	118.1 ± 2.85 a	***
Apigenin-malonyl-apiosyl-glucoside	4408 ± 63.3 b	3380 ± 212 c	5014 ± 46.9 a	***
Caffeic acid	3.92 ± 0.01	3.94 ± 0.06	3.82 ± 0.03	ns
Chlorogenic acid	11.55 ± 0.27 b	7.83 ± 0.52 c	13.89 ± 0.06 a	***
Chrysoeriol	25.30 ± 3.81 b	7.43 ± 0.48 c	47.80 ± 3.26 a	***
Coumaric acid	28.6 ± 0.12 b	42.3 ± 1.09 a	31.3 ± 0.15 b	***
Ferulic acid	3.47 ± 0.00 c	4.22 ± 0.05 a	3.59 ± 0.00 b	***
Kaempferol-7-O-glucoside	2.61 ± 0.04 a	0.39 ± 0.08 b	2.66 ± 0.06 a	***
Luteolin-7-O-glucoside	5.08 ± 0.14 b	2.88 ± 0.22 c	7.29 ± 0.18 a	***
Quercetin-3-O-galactoside	5.66 ± 0.06 b	0.64 ± 0.03 c	7.20 ± 0.36 a	***
Total polyphenols	9632 ± 113 b	8559 ± 208 c	10347 ± 26.1 a	***

Nonsignificant (ns). *** indicates significance at $p < 0.001$. Different letters within each column indicate significant differences according to Tukey's HSD test ($p = 0.05$). All data are expressed as mean ± standard error, $n = 3$. RGB: red, green-yellow, blue.

3.6. Principal Component Analysis (PCA) of Morpho-Metric and Qualitative Attributes of Parsley Microgreens

A principal component analysis was performed on all parsley microgreens data with reference to the different LEDs treatments, and the loading plot and scores are reported in Figure 2. The variables in the first two principal components (PCs) were highly correlated, with eigen values greater than 1, and explained for 100.0% of the total variance, with PC1 and PC2 accounting for 65.9% and 34.1%, respectively. PC1 was positively correlated to intercellular spaces, luteolin-7-Oglucoside, thickness of spongy mesophyll, total and single polyphenols, β-Carotene, ascorbic acid, dry matter and S. PC1 was negatively correlated to Chl b and Chl a, total Chls, caffeic acid, Mg, thickness of abaxial epidermis, fresh yield, ferulic and coumaric acid and petiole length. Moreover, PC2 was positively correlated to Ca, ABTS antioxidant activity, P, ascorbic acid, thickness of adaxial epidermis, K and apigenin; while it was negatively correlated to dry weight, nitrate, hypocotyl length and lutein. The three LEDs light treatments were well separated and univocally clustered in respect to PC1 and PC2. In fact, RGB was in the positive side of PC1 in the lower right quadrant clustered with dry matter and polyphenols, blue treatment was in the positive side of PC2 in the upper right quadrant close to the y-axis and clustered to ABTS antioxidant activity and ascorbic acid, while red treatment was in the negative side of PC1 in the lower left quadrant, correlated to fresh yield, coumaric and ferulic acids and petiole length (Figure 2).

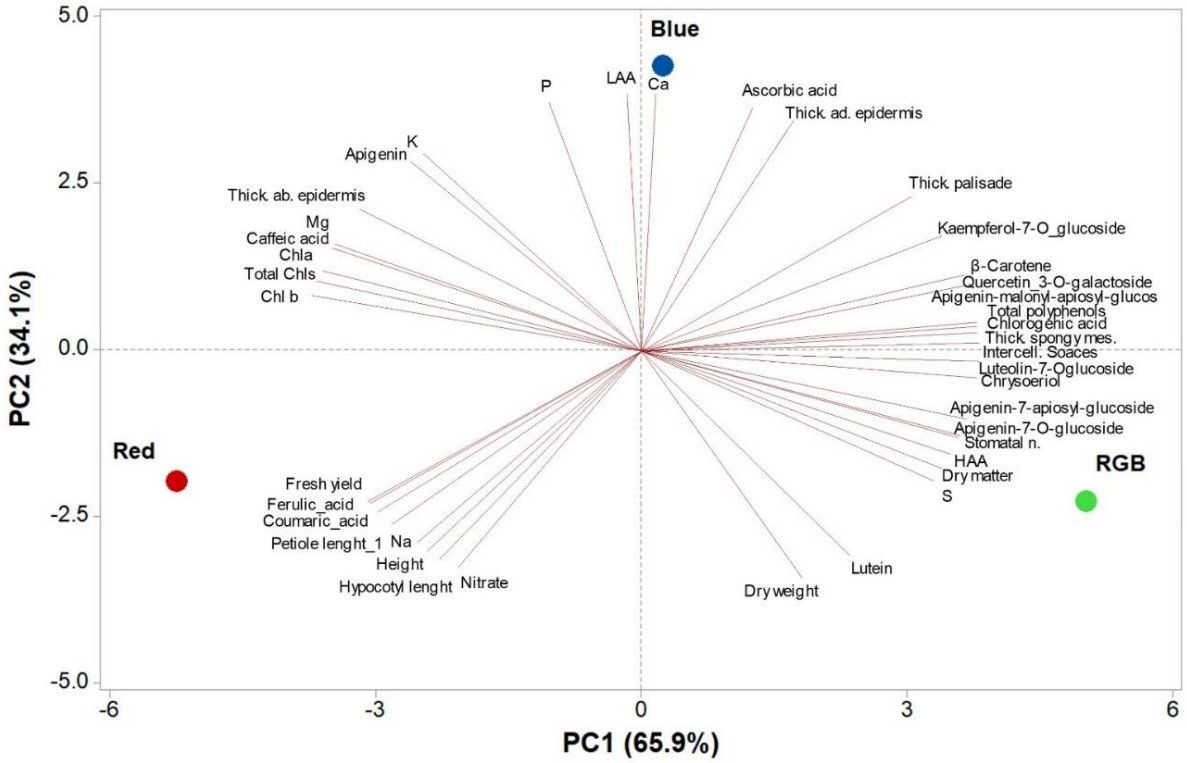

Figure 2. Principal component loading plot and scorses of PCA of morpho-metric analysis, pigments and secondary metabolites of parsley microgreens with reference to the different light treatments (red, blue and RGB: red, green-yellow, blue), LAA = ABTS antioxidant activity.

4. Discussion

In our study, we found that blue light was able to inhibit hypocotyl growth in parsley. Cosgrove and Green [35] proved that in cucumber and sunflower seedlings, blue light inhibited hypocotyl growth by decreasing the yielding properties of the cell walls, thus causing a decrease in turgor and consequently in growth rate. More recently, Ma et al. [36] found that the Arabidopsis blue light photoreceptor cryptochrome 1 (CRY1) when activated was able to physically interact with the phytochrome-interacting factor 4 (PIF4) and inhibit PIF4 transcription activity and repress hypocotyl elongation. However, this effect is totally different from that reported by Kong et al. [37] in which blue light enhanced hypocotyl length, stem extension and petiole length in rocket, cabbage and kale microgreens, while it had no effect on mustard. Cotyledon and petiole lengths were significantly higher under red light. Kozuka et al. [38] demonstrated that petiole and leaf blade undergo the positive growth-promotional effects of the photo-assimilated (e.g., sucrose) whose concentration is highly dependent on the light conditions. However, the enzyme involved in the synthesis of this disaccharide is inhibited by red light and activated by a treatment with combined red/blue light (50/50) [39]. Therefore, most of the responses normally present with red or blue monochromatic lights appear reversed in our study. Probably the presence of a 10% green-yellow component in red and blue LEDs is capable of reversing the mechanisms controlled by these light wavelengths, determining phenotypes different from those normally expected, as also reported by Orlando et al. [20]. Indeed, parsley microgreens treated with red light showed the highest yield, indirectly demonstrating that this plant had the highest photosynthetic light use efficiency and consequently the highest soluble sugars content potentially available for plant growth. RGB light determined the highest leaf length, width, and DM percentage, accordingly with Kim et al. [40] and Orlando et al. [20] who reported that the inclusion of green light in the red/blue light environment promoted dry weight biomass. Green light has the advantage of penetrating into the plant canopy better than red or blue light [41], where the transmitted green light

could be used to boost photosynthesis [42] and enhance plant growth [43]. This positive effect is present where green light percentage remains lower than that of blue light; if not, a green-light-dependent reversal of blue-light-stimulated stomatal opening can occur, that decreases plant growth [40,44].

Growth parameters including upper epidermis thickness and spongy mesophyll thickness were the lowest in parsley microgreens grown under red light, while blue light induced higher upper epidermis thickness as previously found in in vitro-cultured plants of *Alternanthera brasiliana* Kuntze by Macedo et al. [45]. Moreover, the specific spectral band also influenced the differentiation of mesophyll cells. In fact, under red light, the leaves were more compact with less intercellular spaces, while under blue and RGB light they displayed ticker spongy mesophyll with higher percentage of intercellular spaces. These results are in line with those found in sweet pepper (*Capsicum annuum* L.) seedlings under RGB light treatment by Li et al. [46].

The highest nitrate content was present in parsley microgreens under red light, while the lowest was found under blue light, as also found by Fan et al. [47] in pakchoi plants (*Brassica campestris* L. var. Suzhouqing); nonetheless nitrate content under red and RGB light is in harmony with data previously mentioned on parsley microgreens by El-Nakhel et al. [48] and coworkers. In pakchoi plants, the gene expression level and related enzyme activities of genes involved in nitrate reductive assimilation pathway, e.g., nitrate reductase (NR), nitrite reductase (NiR) and glutamine synthetase (GS), were at the highest levels under blue light, thus reducing the nitrate content [47]. On the contrary, Kyriacou et al. [24] reported that nitrate accumulation was higher under monochromatic light (red or blue) because of the dependency of nitrite reductase activity on reduced ferredoxin produced during electron transport to PSI, most efficiently promoted by blue-red light. However, nitrate can be also reduced in roots, using as reducing power that of NADPH produced in the root cytosolic oxidative pentose phosphate pathway OPPP, transferred to ferredoxin by the ferredoxin-NADP-reductase.

P, K, and Ca were significantly higher under blue light. These results were also found in Chinese chive (*Allium tuberosum*) and garlic (*Allium sativum* L.) for P and K [39,49] and Gynostemma (*Gynostemma pentaphyllum*) for Ca [50]. Systematic research on the mechanisms and modes of nutrient absorption and utilization in crop plants under diverse light conditions has recently been focused.

Both red lights, by activating phytochromes, and blue lights, activating cryptochromes (CRY1 and CRY2), can induce the accumulation of common downstream signalling components such as constitutive photomorphogenic 1 (COP1), phytochrome interacting factors (PIFs) and long hypocotyl 5 (HY5) [51–53]. PIF4/5 and HY5 transcription factors, in particular, may be involved in the nutrient use-related genes expression or in the regulation of root morphogenesis, thus modifying the architecture and distribution of roots in the soil, enhancing the probability of nutrients uptake and modulating its utilization and use efficiency [54].

Blue light treatment induced in parsley microgreens the highest ascorbic acid content and antioxidant activity (ABTS). Accordingly, Zhang et al. [55] found that blue LED light irradiation upregulated ascorbic acid metabolism (in particular, ascorbic acid biosynthetic and regeneration genes and glutathione-producing genes) in citrus; even if Zha et al. [56] found that the accumulation of ascorbic acid in lettuce promoted by blue light was more due to the upregulation of ascorbic acid regeneration activity than that of the biosynthetic one. Additionally, Kang et al. [57] found that blue light irradiation could increase ascorbic acid content and reduce reactive oxygen species accumulation in Chinese cabbage seedlings.

Chlorophylls were increased under both red and blue LED lights, probably indicating a need to increase the chlorophyll absorbing efficiency when the light spectrum was restricted to almost monochromatic light. In artichoke seedlings, comparable to parsley microgreens for vegetative growth, it was found that chlorophyll content did not vary significantly under natural, red and blue light, while only after long exposure did red light induce a chlorophyll increase [58].

Lutein content strongly increased under RGB LED treatment, as also found in tomatoes grown under LED lighting with an emission spectrum that partially matched the range of 400 to 700 nm [59]. RGB LED light also significantly increased the total polyphenols content and most of the single detected polyphenols, as also found by Gam et al. [60] in *Anoectochilus roxburghii*, an Orchidaceae edible plant used as medicinal plant in Asia. The polyphenols that were mainly increased by the RGB treatments compared to red and blue ones included apigenin-7-O-glucoside, apigenin-malonyl-apiosyl-glucoside, chrysoeriol, all of which are compounds with strong antioxidant properties. In a study in which the antioxidant and anti-inflammatory activities of apigenin-7-O-glucoside, one of the major ethanol extract components from chamomile, were analyzed and compared with those of Trolox, Wang et al. [61] showed that this polyphenol could prevent free radical-induced oxidative damage of DNA, proteins and erythrocytes. Moreover, apigenin-7-O-glucoside, similarly to Trolox, was able to inhibit H_2O_2-induced ROS production in RAW264.7 murine tumors cell lines (RAW309 and WR19M), whereas it was more efficient than Trolox in detoxifying ROS-induced damage in erythrocytes and preventing inflammasome assembly, which is a signal for NLRP3 inflammasome activation in RAW246.7 cells [61]. Apigenin-malonyl-apiosyl-glucoside has been reported as a major flavone derivative from pepper leaves [62]. Chrysoeriol is synthesized from apigenin via luteolin, and, similarly to apigenin derivatives, has antimicrobial and anti-oxidant potential [62]. Coumaric acid in particular, as well as ferulic acid, even if to a lower extent, were increased by red light treatment, as also found in wheat sprouts by Cuong et al. [63], which suggested a red-light dependent upregulation of the expression of genes involved in phenylpropanoid biosynthesis.

5. Conclusions

LEDs with adjustable spectral bandwidths used in controlled environment are the adequate tool to manage and project the final microgreens produce characterized by precise morphological and qualitative traits. Full spectral bandwidths (RGB) permitted to obtain parsley microgreens with higher dry matter, lutein and polyphenols. While blue treatment helped producing compact and shorter parsley microgreens rich in total ascorbic acid, P, Ca and β-carotene, whereas if higher microgreens yield and hypocotyl length are the aim, then red light treatment should be opted for parsley. Although genetic factors always determine most of the morpho-metric and qualitative attributes of microgreens characteristics, preharvest factors modulation such as light quality applied in growing modules definitely leaves a margin for targeted manipulation that leads to a qualitative and yield shift, based on morphological and anatomical adaptations.

Author Contributions: Conceptualization, C.E.-N.; methodology, C.E.-N., M.G. and A.P.; software, C.E.-N. and A.P.; validation, C.E.-N.; formal analysis, C.E.-N. and V.D.M.; investigation, all authors; resources, M.C.K.; data curation, C.E.-N. and G.G.; writing—original draft preparation, P.C. and C.E.-N.; writing—review and editing, P.C., C.E.-N., V.D.M., M.G., A.P., S.D.P., G.G., A.R., G.A.S., M.C.K. and Y.R.; visualization, P.C. and M.C.K.; supervision, Y.R. and P.C.; project administration, M.C.K. and Y.R.; funding acquisition, M.C.K. and Y.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data is contained within the article.

Acknowledgments: The authors are grateful to Anna Saviano and Danilo Slanzi for their assistance during the experiment and in the laboratory analysis. The authors would like to thank Luigi Formisano for the manuscript formatting.

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

References

1. El-Nakhel, C.; Pannico, A.; Graziani, G.; Kyriacou, M.C.; Giordano, M.; Ritieni, A.; De Pascale, S.; Rouphael, Y. Variation in macronutrient content, phytochemical constitution and in vitro antioxidant capacity of green and red butterhead lettuce dictated by different developmental stages of harvest maturity. *Antioxidants* **2020**, *9*, 300. [CrossRef] [PubMed]
2. Nasri, H.; Baradaran, A.; Shirzad, H.; Rafieian-Kopaei, M. New concepts in nutraceuticals as alternative for pharmaceuticals. *Int. J. Prev. Med.* **2014**, *5*, 1487. [PubMed]
3. World Health Organization. *The First Action Plan for Food and Nutrition Policy: WHO European Region 2000–2005*; WHO Regional Office for Europe: Copenhagen, Denmark, 2001.
4. CREA. Linee Guida per una Sana Alimentazione. Revisione 2018. Available online: https://www.salute.gov.it/imgs/C_17_pubblicazioni_2915_allegato.pdf (accessed on 22 May 2022).
5. COM. European Commission Communication COM/2020/381, 2020. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions: A Farm to Fork Strategy for a Fair, Healthy and Environmentally-Friendly Food System. Available online: https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:52020DC0381 (accessed on 22 May 2022).
6. Kyriacou, M.C.; Rouphael, Y. Towards a new definition of quality for fresh fruits and vegetables. *Sci. Hortic.* **2018**, *234*, 463–469. [CrossRef]
7. Pinto, E.; Almeida, A.A.; Aguiar, A.A.; Ferreira, I.M. Comparison between the mineral profile and nitrate content of microgreens and mature lettuces. *J. Food Compos. Anal.* **2015**, *37*, 38–43. [CrossRef]
8. Rouphael, Y.; Colla, G.; De Pascale, S. Sprouts, microgreens and edible flowers as novel functional foods. *Agronomy* **2021**, *11*, 2568. [CrossRef]
9. Xiao, Z.; Lester, G.E.; Luo, Y.; Wang, Q. Assessment of vitamin and carotenoid concentrations of emerging food products: Edible microgreens. *J. Agric. Food Chem.* **2012**, *60*, 7644–7651. [CrossRef]
10. Kyriacou, M.C.; De Pascale, S.; Kyratzis, A.; Rouphael, Y. Microgreens as a component of space life support systems: A cornucopia of functional food. *Front. Plant Sci.* **2017**, *8*, 1587. [CrossRef]
11. Kyriacou, M.C.; El-Nakhel, C.; Graziani, G.; Pannico, A.; Soteriou, G.A.; Giordano, M.; Ritieni, A.; De Pascale, S.; Rouphael, Y. Functional quality in novel food sources: Genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species. *Food Chem.* **2019**, *277*, 107–118. [CrossRef]
12. Turner, E.R.; Luo, Y.; Buchanan, R.L. Microgreen nutrition, food safety, and shelf life: A review. *J. Food Sci.* **2020**, *85*, 870–882. [CrossRef]
13. Kelly, N.; Vaštakaitė-Kairienė, V.; Runkle, E.S. Indoor lighting effects on plant nutritional compounds. In *Plant Factory Basics, Applications and Advances*; Elsevier: Cambridge, MA, USA, 2022; pp. 329–349.
14. Puccinelli, M.; Maggini, R.; Angelini, L.G.; Santin, M.; Landi, M.; Tavarini, S.; Castagna, A.; Incrocci, L. Can Light Spectrum Composition Increase Growth and Nutritional Quality of *Linum usitatissimum* L. Sprouts and Microgreens? *Horticulturae* **2022**, *8*, 98. [CrossRef]
15. Batista, D.S.; Felipe, S.H.S.; Silva, T.D.; de Castro, K.M.; Mamedes-Rodrigues, T.C.; Miranda, N.A.; Ríos-Ríos, A.M.; Faria, D.V.; Fortini, E.A.; Chagas, K. Light quality in plant tissue culture: Does it matter? *In Vitro Cell. Dev. Biol. Plant* **2018**, *54*, 195–215. [CrossRef]
16. Liu, X.; Li, Y.; Zhong, S. Interplay between light and plant hormones in the control of Arabidopsis seedling chlorophyll biosynthesis. *Front. Plant Sci.* **2017**, *8*, 1433. [CrossRef] [PubMed]
17. Galvão, V.C.; Fankhauser, C. Sensing the light environment in plants: Photoreceptors and early signaling steps. *Curr. Opin. Neurobiol.* **2015**, *34*, 46–53. [CrossRef]
18. Paradiso, R.; Proietti, S. Light-quality manipulation to control plant growth and photomorphogenesis in greenhouse horticulture: The state of the art and the opportunities of modern LED systems. *J. Plant Growth Regul.* **2022**, *41*, 742–780. [CrossRef]
19. Alrifai, O.; Hao, X.; Marcone, M.F.; Tsao, R. Current Review of the Modulatory Effects of LED Lights on Photosynthesis of Secondary Metabolites and Future Perspectives of Microgreen Vegetables. *J. Agric. Food Chem.* **2019**, *67*, 6075–6090. [CrossRef]
20. Orlando, M.; Trivellini, A.; Incrocci, L.; Ferrante, A.; Mensuali, A. The inclusion of green light in a red and blue light background impact the growth and functional quality of vegetable and flower microgreen species. *Horticulturae* **2022**, *8*, 217. [CrossRef]
21. Alrifai, O.; Hao, X.; Liu, R.; Lu, Z.; Marcone, M.F.; Tsao, R. Amber, red and blue LEDs modulate phenolic contents and antioxidant activities in eight Cruciferous microgreens. *J. Food Bioact.* **2020**, *11*. [CrossRef]
22. Brazaitytė, A.; Sakalauskienė, S.; Samuolienė, G.; Jankauskienė, J.; Viršilė, A.; Novičkovas, A.; Sirtautas, R.; Miliauskienė, J.; Vaštakaitė, V.; Dabašinskas, L. The effects of LED illumination spectra and intensity on carotenoid content in Brassicaceae microgreens. *Food Chem.* **2015**, *173*, 600–606. [CrossRef]
23. Craver, J.K.; Gerovac, J.R.; Lopez, R.G.; Kopsell, D.A. Light intensity and light quality from sole-source light-emitting diodes impact phytochemical concentrations within Brassica microgreens. *J. Am. Soc. Hortic. Sci.* **2017**, *142*, 3–12. [CrossRef]
24. Kyriacou, M.C.; El-Nakhel, C.; Pannico, A.; Graziani, G.; Soteriou, G.A.; Giordano, M.; Zarrelli, A.; Ritieni, A.; De Pascale, S.; Rouphael, Y. Genotype-specific modulatory effects of select spectral bandwidths on the nutritive and phytochemical composition of microgreens. *Front. Plant Sci.* **2019**, *10*, 1501. [CrossRef]

25. Lobiuc, A.; Vasilache, V.; Pintilie, O.; Stoleru, T.; Burducea, M.; Oroian, M.; Zamfirache, M.-M. Blue and red LED illumination improves growth and bioactive compounds contents in acyanic and cyanic *Ocimum basilicum* L. microgreens. *Molecules* **2017**, *22*, 2111. [CrossRef] [PubMed]
26. Samuolienė, G.; Brazaitytė, A.; Viršilė, A.; Jankauskienė, J.; Sakalauskienė, S.; Duchovskis, P. Red light-dose or wavelength-dependent photoresponse of antioxidants in herb microgreens. *PLoS ONE* **2016**, *11*, e0163405. [CrossRef] [PubMed]
27. Ying, Q.; Jones-Baumgardt, C.; Zheng, Y.; Bozzo, G. The Proportion of blue light from light-emitting diodes alters microgreen phytochemical profiles in a species-specific manner. *HortScience* **2021**, *56*, 13–20. [CrossRef]
28. Pannico, A.; El-Nakhel, C.; Kyriacou, M.C.; Giordano, M.; Stazi, S.R.; De Pascale, S.; Rouphael, Y. Combating micronutrient deficiency and enhancing food functional quality through selenium fortification of select lettuce genotypes grown in a closed soilless system. *Front. Plant Sci.* **2019**, *10*, 1495. [CrossRef]
29. De Micco, V.; De Francesco, S.; Amitrano, C.; Arena, C. Comparative Analysis of the effect of carbon-and titanium-ions irradiation on morpho-anatomical and biochemical traits of Dolichos melanophthalmus DC. Seedlings aimed to space exploration. *Plants* **2021**, *10*, 2272. [CrossRef] [PubMed]
30. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. [CrossRef]
31. Kampfenkel, K.; Vanmontagu, M.; Inzé, D. Extraction and determination of ascorbate and dehydroascorbate from plant tissue. *Anal. Biochem.* **1995**, *225*, 165–167. [CrossRef]
32. Fogliano, V.; Verde, V.; Randazzo, G.; Ritieni, A. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *J. Agric. Food Chem.* **1999**, *47*, 1035–1040. [CrossRef]
33. Pellegrini, N.; Re, R.; Yang, M.; Rice-Evans, C. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2 0 -azinobis(3-ethylenebenzothiazoline-6-sulfonic acid radical cation decolorization assay. *Methods Enzymol.* **1999**, *299*, 379–384. [CrossRef]
34. Ciarmiello, L.F.; Piccirillo, P.; Carillo, P.; De Luca, A.; Woodrow, P. Determination of the genetic relatedness of fig (*Ficus carica* L.) accessions using RAPD fingerprint and their agro-morphological characterization. *S. Afr. J. Bot.* **2015**, *97*, 40–47. [CrossRef]
35. Cosgrove, D.J.; Green, P.B. Rapid suppression of growth by blue light: Biophysical mechanism of action. *Plant Physiol.* **1981**, *68*, 1447–1453. [CrossRef] [PubMed]
36. Ma, D.; Li, X.; Guo, Y.; Chu, J.; Fang, S.; Yan, C.; Noel, J.P.; Liu, H. Cryptochrome 1 interacts with PIF4 to regulate high temperature-mediated hypocotyl elongation in response to blue light. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 224–229. [CrossRef] [PubMed]
37. Kong, Y.; Kamath, D.; Zheng, Y. Blue versus red light can promote elongation growth independent of photoperiod: A study in four Brassica microgreens species. *HortScience* **2019**, *54*, 1955–1961. [CrossRef]
38. Kozuka, T.; Horiguchi, G.; Kim, G.-T.; Ohgishi, M.; Sakai, T.; Tsukaya, H. The different growth responses of the Arabidopsis thaliana leaf blade and the petiole during shade avoidance are regulated by photoreceptors and sugar. *Plant Cell Physiol.* **2005**, *46*, 213–223. [CrossRef] [PubMed]
39. Chen, X.-L.; Wang, L.-C.; Li, Y.-L.; Yang, Q.-C.; Guo, W.-Z. Alternating red and blue irradiation affects carbohydrate accumulation and sucrose metabolism in butterhead lettuce. *Sci. Hortic.* **2022**, *302*, 111177. [CrossRef]
40. Kim, H.-H.; Goins, G.D.; Wheeler, R.M.; Sager, J.C. Stomatal conductance of lettuce grown under or exposed to different light qualities. *Ann. Bot.* **2004**, *94*, 691–697. [CrossRef]
41. Klein, R.M. Effects of green light on biological systems. *Biol. Rev.* **1992**, *67*, 199–284. [CrossRef]
42. Nishio, J. Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. *Plant Cell Environ.* **2000**, *23*, 539–548. [CrossRef]
43. Kim, H.H.; Wheeler, R.M.; Sager, J.C.; Gains, G.; Naikane, J. Evaluation of lettuce growth using supplemental green light with red and blue light-emitting diodes in a controlled environment-A review of research at Kennedy Space Center. In Proceedings of the V International Symposium on Artificial Lighting in Horticulture, Lillehammer, Norway, 21–24 June 2005; Volume 711, pp. 111–120.
44. Johkan, M.; Shoji, K.; Goto, F.; Hahida, S.-N.; Yoshihara, T. Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in Lactuca sativa. *Environ. Exp. Bot.* **2012**, *75*, 128–133. [CrossRef]
45. Macedo, A.F.; Leal-Costa, M.V.; Tavares, E.S.; Lage, C.L.S.; Esquibel, M.A. The effect of light quality on leaf production and development of in vitro-cultured plants of Alternanthera brasiliana Kuntze. *Environ. Exp. Bot.* **2011**, *70*, 43–50. [CrossRef]
46. Li, Y.; Xin, G.; Liu, C.; Shi, Q.; Yang, F.; Wei, M. Effects of red and blue light on leaf anatomy, CO2 assimilation and the photosynthetic electron transport capacity of sweet pepper (*Capsicum annuum* L.) seedlings. *BMC Plant Biol.* **2020**, *20*, 318. [CrossRef] [PubMed]
47. Fan, X.-X.; Xue, F.; Song, B.; Chen, L.-Z.; Xu, G.; Xu, H. Effects of Blue and Red Light On Growth And Nitrate Metabolism In Pakchoi. *Open Chem.* **2019**, *17*, 456–464. [CrossRef]
48. El-Nakhel, C.; Pannico, A.; Graziani, G.; Giordano, M.; Kyriacou, M.C.; Ritieni, A.; De Pascale, S.; Rouphael, Y. Mineral and antioxidant attributes of Petroselinum crispum at different stages of ontogeny: Microgreens vs. baby greens. *Agronomy* **2021**, *11*, 857. [CrossRef]
49. Yang, X.J. Effects of Light Quality on the Physiological Characteristics and Quality in Garlic Seedling. Ph.D. Thesis, Shandong Agricultural University, Tai'an, China, 2011.

50. Lee, H.-J.; Ha, J.-H.; Kim, S.-G.; Choi, H.-K.; Kim, Z.H.; Han, Y.-J.; Kim, J.-I.; Oh, Y.; Fragoso, V.; Shin, K. Stem-piped light activates phytochrome B to trigger light responses in Arabidopsis thaliana roots. *Sci. Signal.* **2016**, *9*, ra106. [CrossRef] [PubMed]
51. Huang, X.; Ouyang, X.; Deng, X.W. Beyond repression of photomorphogenesis: Role switching of COP/DET/FUS in light signaling. *Curr. Opin. Plant Biol.* **2014**, *21*, 96–103. [CrossRef] [PubMed]
52. Leivar, P.; Monte, E. PIFs: Systems integrators in plant development. *Plant Cell* **2014**, *26*, 56–78. [CrossRef]
53. Qiu, Z.; Wang, H.; Li, D.; Yu, B.; Hui, Q.; Yan, S.; Huang, Z.; Cui, X.; Cao, B. Identification of candidate HY5-dependent and-independent regulators of anthocyanin biosynthesis in tomato. *Plant Cell Physiol.* **2019**, *60*, 643–656. [CrossRef]
54. Xu, J.; Guo, Z.; Jiang, X.; Ahammed, G.J.; Zhou, Y. Light regulation of horticultural crop nutrient uptake and utilization. *Hortic. Plant J.* **2021**, *7*, 367–379. [CrossRef]
55. Zhang, L.; Ma, G.; Yamawaki, K.; Ikoma, Y.; Matsumoto, H.; Yoshioka, T.; Ohta, S.; Kato, M. Regulation of ascorbic acid metabolism by blue LED light irradiation in citrus juice sacs. *Plant Sci.* **2015**, *233*, 134–142. [CrossRef]
56. Zha, L.; Liu, W.; Yang, Q.; Zhang, Y.; Zhou, C.; Shao, M. Regulation of ascorbate accumulation and metabolism in lettuce by the red: Blue ratio of continuous light using LEDs. *Front. Plant Sci.* **2020**, *11*, 704. [CrossRef]
57. Kang, C.H.; Yoon, E.K.; Muthusamy, M.; Kim, J.A.; Jeong, M.-J.; Lee, S.I. Blue LED light irradiation enhances L-ascorbic acid content while reducing reactive oxygen species accumulation in Chinese cabbage seedlings. *Sci. Hortic.* **2020**, *261*, 108924. [CrossRef]
58. Rabara, R.C.; Behrman, G.; Timbol, T.; Rushton, P.J. Effect of spectral quality of monochromatic LED lights on the growth of artichoke seedlings. *Front. Plant Sci.* **2017**, *8*, 190. [CrossRef] [PubMed]
59. Dannehl, D.; Schwend, T.; Veit, D.; Schmidt, U. Increase of yield, lycopene, and lutein content in tomatoes grown under continuous PAR spectrum LED lighting. *Front. Plant Sci.* **2021**, *12*, 299. [CrossRef] [PubMed]
60. Gam, D.T.; Khoi, P.H.; Ngoc, P.B.; Linh, L.K.; Hung, N.K.; Anh, P.T.L.; Thu, N.T.; Hien, N.T.T.; Khanh, T.D.; Ha, C.H. LED lights promote growth and flavonoid accumulation of Anoectochilus roxburghii and are linked to the enhanced expression of several related genes. *Plants* **2020**, *9*, 1344. [CrossRef]
61. Wang, W.; Yue, R.-F.; Jin, Z.; He, L.-M.; Shen, R.; Du, D.; Tang, Y.-Z. Efficiency comparison of apigenin-7-O-glucoside and trolox in antioxidative stress and anti-inflammatory properties. *J. Pharm. Pharmacol.* **2020**, *72*, 1645–1656. [CrossRef]
62. Assefa, S.T.; Yang, E.-Y.; Asamenew, G.; Kim, H.-W.; Cho, M.-C.; Lee, J. Identification of α-Glucosidase Inhibitors from Leaf Extract of Pepper (Capsicum spp.) through Metabolomic Analysis. *Metabolites* **2021**, *11*, 649. [CrossRef]
63. Cuong, D.M.; Ha, T.W.; Park, C.H.; Kim, N.S.; Yeo, H.J.; Chun, S.W.; Kim, C.; Park, S.U. Effects of LED lights on expression of genes involved in phenylpropanoid biosynthesis and accumulation of phenylpropanoids in wheat sprout. *Agronomy* **2019**, *9*, 307. [CrossRef]