horticulturae

MDPI

Article

Modulated Light Elicitation and Associated Physiological and Molecular Processes in Phenolic Compounds Production in *Ocimum basilicum* L. Microgreens

Gabriel-Ciprian Teliban [1], Naomi-Eunicia Pavăl [2,*], Gabriela Mihalache [1], Marian Burducea [1], Vasile Stoleru [1,*] and Andrei Lobiuc [2]

[1] Department of Horticulture Technologies, "Ion Ionescu de la Brad" Iasi University of Life Sciences, 700490 Iasi, Romania; gabriel.teliban@iuls.ro (G.-C.T.); gabriela.mihalache@uaic.ro (G.M.); marian.burducea@uaic.ro (M.B.)

[2] Department of Biological and Morphofunctional Sciences, Faculty of Medicine and Biological Sciences, Ştefan cel Mare University, 720229 Suceava, Romania; andrei.lobiuc@usm.ro

* Correspondence: naomi.paval@usm.ro (N.-E.P.); vasile.stoleru@iuls.ro (V.S.)

Abstract: Microgreens represent a valuable source of health-promoting compounds and also a research avenue, since such organisms have a very high plasticity related to environmental cues, allowing biotechnological development with low costs. *Ocimum basilicum* L. species naturally synthesize valuable, phenolic compounds, among which rosmarinic acid is most prominent. Within the current research, basil plantlets were grown for 10 days under either full spectrum light (white light) or modulated blue/red/far-red/UV spectrum elicitation with an additional factorization, by applying fertilization. Biomass accumulation reached up to 0.8 g/20 plantlets, while chlorophyll fluorescence was in the 0.75–0.78 range and remained uniform across treatments, indicating that no significant stress was exerted under modified light treatment. However, total phenolic contents and, in particular, rosmarinic acid contents, were markedly enhanced (up to 7.5 mg/g in the red cultivar) under modulated light treatment and fertilization, compared to full spectrum light. Moreover, in the red cultivar, gene expression was enhanced, 1.3–6.3 fold for genes coding for enzymes involved in phenylpropanoid synthesis pathways, such as phenylalanine ammonia lyase (PAL), tyrosine aminotransferase (TAT), Catechol-O-methyltransferase (COMT) and rosmarinic acid synthetase (RAS). Overall, light modulation coupled with fertilization led to the production of basil microgreens with up to 10% more total phenolics and up to 25% more rosmarinic acid. The results show that, using relatively simple growth equipment and setup, synthesis of health related, valuable compounds can be modulated in microgreens and, hence, serves as an avenue for businesses to develop cost effective biotechnological processes.

Keywords: polyphenols; LED treatment; fertilization; fluorescence; biological activity

Academic Editor: Viktorija Vaštakaitė-Kairienė

Received: 2 December 2024
Revised: 23 December 2024
Accepted: 3 January 2025
Published: 8 January 2025

Citation: Teliban, G.-C.; Pavăl, N.-E.; Mihalache, G.; Burducea, M.; Stoleru, V.; Lobiuc, A. Modulated Light Elicitation and Associated Physiological and Molecular Processes in Phenolic Compounds Production in *Ocimum basilicum* L. Microgreens. *Horticulturae* **2025**, *11*, 56. https://doi.org/10.3390/horticulturae11010056

1. Introduction

1.1. Microgreens as Novel Foods

Microgreens, defined as young plants, usually grown until past the cotyledonary leaves and into the phase of true leaves, exhibit intense synthesis of metabolites related to growth but also to protection from environmental factors. Dietary phenolic in microgreens stand out, as they share a set of very desirable traits related to healthy nutrition: young plants have a high moisture content and, hence, palatability; are easy to grow and have quick harvest turnaround times; are rich in enzymes, chlorophyll pigments, etc. [1]. In the

same time, microgreens are susceptible to influence of many environmental cues that offer the opportunity of modulating specific pathways, and, hence, increase bioactive production [2]. Such experimental inductions of synthesis of specific compounds are known under the concept of elicitation.

1.2. Phenolic Contents in Basil Microgreens

The abundance of phenolic is high in basil microgreens, with the most representative compounds being chicory acid, rosmarinic acid, and caffeic acid, with values up to 4.99 mg/g fresh matter. Regarding the synthesis of these kinds of phenolic acids, it was already proven that they can be increased by modulating the spectrum of light delivered to microgreens, including in basil ones [3], and total phenolic and anthocyanin compounds under different blue/red proportions [4]. Other reports established that certain wavelengths increase nutraceutical and mineral contents [5] or specific bioactive such as rosmarinic acid [6] in various basil cultivars. While the undoubtable effect of light on the synthesis of specific compounds is agreed upon, some works, such as [7,8], report the effect (usually beneficial) of the applied light treatments on the physiology of plants [9], as such effects are fundamental to designing proper technological setups for mass cultivation. Much less frequent are the reports on the effects such treatments have on molecular mechanisms, such as gene expression, in order to truly characterize the process. While red, blue, and UV appear to be main wavelengths affecting phenolic synthesis in microgreens [10], this aspect is addressed in papers focused on *Lamiaceae* species such as *Mentha piperita* [11] or *Salvia verticillata* [12], but not in *Ocimum*, as far as a reasonable literature screening goes.

1.3. Benefits of Phenolic Compounds

Phenolic compounds are secondary metabolites found in high concentration in medicinal and aromatic plants, as well as in micro plantlets that are used in various cuisines. Epidemiologically, a diet rich in polyphenols protects against diseases such as cancer, diabetes, osteoporosis, cardiovascular, and neurological diseases [13], underpinned by mechanisms such as inflammation, oxidative stress, and cell ageing [14]. For instance, inflammation caused by pathogens or toxic compounds [15], such as free radicals, may exceed the antioxidant defense and lead to cell ageing, progressive loss of tissue and organ function [16], associated with the progression of diseases such as diabetes [17], Alzheimer's, and Parkinson's diseases [18,19]. In this sense, dietary bioactive molecules can positively influence tissue metabolism and alleviate oxidative and inflammatory effects at the cellular level [20], while inadequate nutrient consumption may lead to imbalance between antioxidant defense and pro-oxidant load that induces oxidative stress [21]. Relevant examples are polyphenols, which are good electron or hydrogen atom donors that may neutralize free radicals and reactive oxygen species (ROS). Moreover, polyphenols act at different cellular sites, leading to antioxidant, antimicrobial, anti-inflammatory, or other biological functions through several mechanisms, such as regulating the expression of some antioxidant enzymes, such as superoxid dismutase (SOD), glutathione S-transferase (GST) and glutathion peroxidase (GSH-Px) [22,23]. Also, phenolic compounds exert anti-inflammatory through effects on gene expression, such as cyclooxygenase (COX-2), lypooxygenase (LOX), inducible nitric oxide synthase (iNOS) [24], nuclear factor-kB (NF-kB), nuclear factor-erythroid factor 2-related factor 2 (Nrf-2) [13] and activate enzymes such as phase-II antioxidant detoxifying enzymes, mitogen-activated protein kinase (MAPK), protein kinase-C. Furthermore, polyphenols act on different sites of bacterial cells, altering the structure or metabolic pathways, or may inhibit the gene expression related to virulence factors produced by bacterial pathogens, also exhibiting antibacterial properties [25].

While the mean dietary phenolic intake ranges from around 255 mg/day in US citizens [26] and up to 1756 mg/day in European citizens [27], major benefits from consumption were described, such as decreased body fat, body mass index (BMI), waist and hip circumference [28], or lower serum pro/anti-inflammatory biomarkers' ratio such as interleukin-10 (IL-10), T helper 1/T helper 2 balance (Th1:Th2), interleukin-1 (IL-1), interleukin-2 (IL-2) and interferon-gamma (IFN-γ) [29]. However, there is a great degree of variability in phenolic consumption, depending on the source, refs. [26,30], or age [31]. However, phenolic substances are truly relevant for human health only by repeated intake, as most are degraded after 1–2 h, with phenolic acids having a longer retention time [32], and are also influenced by conditioning or cooking [33]. Thus, it appears logical that constant consumption of dietary phenolic may be a real solution to the occurrence of some chronic diseases, such as malnutrition, cardiovascular diseases, obesity, diabetes, cancer, and neurodegenerative disorders [34].

1.4. Scope and Aims

Building on previous results, the present paper, based on selected blue/red/UV spectrum, further explores the effects and mechanisms involved in primary and secondary plant metabolism modulation by light. The main aims of the paper are as follows: with the elicited basil microgreens, we aim (1) to quantify physiological processes pertaining to photosynthetic apparatus in order to assess stress levels, (2) to quantify key metabolites related to phenolic synthesis, (3) to quantify specific genes' expression within the phenolic synthesis pathways, and (4) to integrate such data in order to describe the potential benefits of value-added microgreens based on a specific technological cultivation setup.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Two basil cultivars were used for microgreens production, "Sweet Genovese", a green, acyanic cultivar, and "Red Rubin", a red pigmented, cyanic cultivar, the seeds being provided by VS (author) from the research and educational seed stock of the Life Sciences University in Iasi. The seeds originated from the Vegetable Research and Development Station, Buzău, Romania. The experimental design (Figure 1) was a randomized block one, where for each treatment, approximately 150 seeds were sowed in plastic boxes (5 boxes per treatment, 3 used for biochemical analyses and 2 for phenotypic measurements), using a mixture of general-purpose soil and peat moss 2:1. The boxes, made of High-Density Polyethylene (HDPE), sized 10 × 10 × 12 cm (L × l × h), were irrigated daily for 1 min using automated drip systems, with either tap water or fertilizer. The fertilizer was prepared according to the recipe presented in Khater et al. [35] and had the final concentrations: N:P:K = 210:31:234 ppm. The minerals were introduced as the following salts: NH_4NO_3, P_2O_5 and $C_2K_2O_4xH_2O$ (Carl Roth, GmbH, Karlsruhe, Germany). Each light treatment was provided by a Phytofy RL LED unit (OSRAM, Golden Dragon, Munich, Germany), from a distance of 30 cm from the top of the boxes. The two light treatments applied were a control variant, using a white LED program (0:0:0:0:0:1, UV/blue/green/red/far-red/white, in µmoles) and a colored program (1:9:0:9:3:0, UV/blue/green/red/far-red/white), respectively. After seeding, the boxes were kept in the dark for 3 days and afterwards, total PPFD (Photonic Flux Density) for the two treatments were 160 and, respectively, 161 µmol/m^2/s. The emission spectra of LED lights (according to OSRAM software version 1.0.22) used are given in Figure 2. The plants were collected for biochemical and gene expression analyses 10 days after germination.

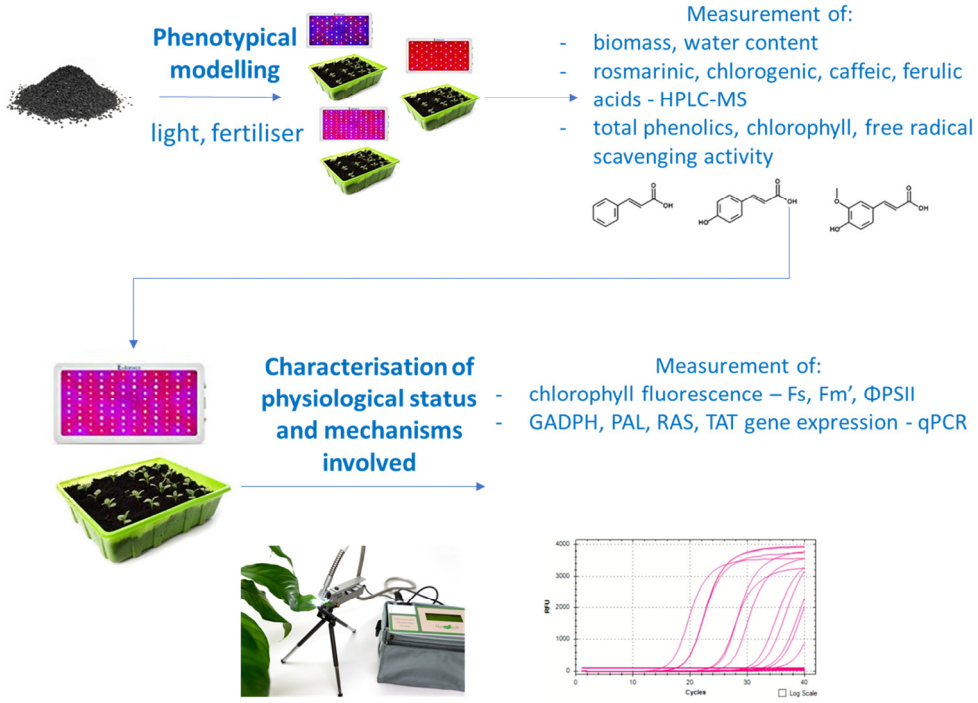

Figure 1. Experimental setup for assessing influence of the synthesis of phenolic compounds in *Ocimum basilicum* L. microgreens.

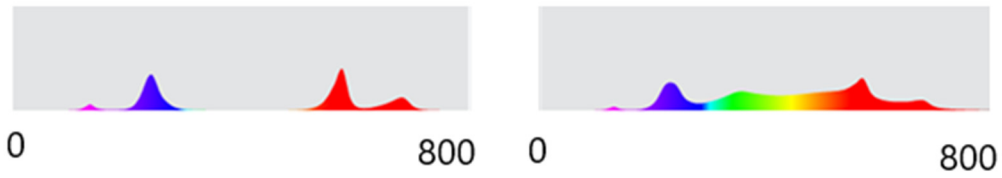

Figure 2. LED emission spectra (**Left**) BRUV—blue/red/UV light treatment treatment; (**Right**) white treatment (wavelengths given in nm).

2.2. Analyses

Chlorophyll pigments were analyzed non-destructively, using a MC-100 Chlorophyll Concentration Meter (Apogee Instruments), by measuring 24 leaves/treatment/basil cultivar. Chlorophyll fluorescence-related parameters—Fs—steady state fluorescence, Fm'—maximal light-adapted fluorescence and ΦPSII—quantum efficiency of the photosystem II, were measured using an FMS2 fluorimeter (HansaTech, Norfolk, UK) for 12 cotyledons/treatment, during the light treatment period. Chlorophyll related analyses were performed at the end of the experiment before harvest.

The total phenolic content and antioxidant activity were determined in microtiter plates according to the methods described by Herald [36]. Briefly, total phenolic contents were assayed using Folin–Ciocalteu reagent, expressing results as gallic acid equivalents (GAE)/mg, while antioxidant activity was measured as % inhibition of DPPH free radical in ethanolic extracts. The reads were performed with BioTek Epoch 2 microplate spectrophotometer (Agilent, Santa Clara, CA, USA). The extracts were prepared from the dry plant and 70% (*w*/*w*) ethanol in a ratio of 1:9, by maceration at 50 °C for 60 min. Extracts were prepared in triplicate for each experimental variant. For high-performance liquid chromatography (HPLC) determination, the extracts were filtered through a polyethersulfone (PES) membrane with 0.22 µm diameter pores.

The identification and quantification of the phenolic compounds from samples were performed on a Waters 2695e Alliance HPLC system coupled with a 2998 PDA Detector. The resulting chromatograms were processed using Empower software. Separation was

achieved on a Waters XBridge column C18 column (50 × 4.6 mm, 3.5 µm), maintained at 30 °C. The mobile phase A consisted in a solution of 0.1% trifluoroacetic acid (TFA) in water, while for mobile phase B a solution of 0.1% TFA in acetonitrile was used. The gradient program was as follows: 0–4 min 100% (A), 5–20 min 98% (A), 27–30 min 96% (A), 32–35 min 90% (A), 40–45 min 82% (A), 50–53 min 0% (A), 55–60 min 100% (A). The flow rate was set up at 0.7 mL/min and the injection volume was 20 µL. HPLC/DAD analyses were performed monitoring the 280 nm wavelength. The identification of phenolic compounds was realized by comparing retention time with the available standards. The phenolic compounds quantification was performed using the standard curves of external standards, obtained by plotting HPLC peak areas against the concentrations (µg/mL) ($r^2 > 0.99$).

Gene expression analysis was carried out using qRT-PCR commercial assays on a Applied Biosystems QuantStudio5 real time PCR equipment. Total RNA extraction was performed using RNeasy Plant Mini Kit (Qiagen GmbH, QIAGEN Str. 1, D-40724 Hilden, Germany), from liquid nitrogen frozen cotyledons. RNA extracts were assessed for nucleic acid purity and amount using Qubit fluorometer, then samples were prepared according to manufacturer specifications for amplification and detection, using GoTaq® 1-Step RT-qPCR System (Promega Corp., Madison, WI, USA). ΔΔCt calculations were performed relative to GADPH reference and expressed logarithmically.

2.3. Statistical Tests

For assessing the inter-treatment differences of the analyzed variables (biomass, water content, fluorescence related, phenolic contents related), two-way analysis of variance (cultivar x fertilizer) was performed, followed by post-hoc Tukey testing, for $p < 0.05$. The statistical software used was OriginLab Pro 2024 10.1.0.170 (OriginLab Corporation, Northampton, MA, USA).

3. Results

Plants exposed to light regimes exhibited various grades of effects in phenotypical traits and in biochemical and physiological processes. Regarding biomass accumulation, plants under either colored or light treatment recorded little differences, with respect to the fresh mass/20 plantlets and also to water content (Figure 3). The green cultivar consistently recorded higher biomass accumulation not from water, but rather from organic matter (Figure 2), compared to the red cultivar. The different light spectra induced some significant variation between the water and fertilizer-treated plantlets in the green cultivar.

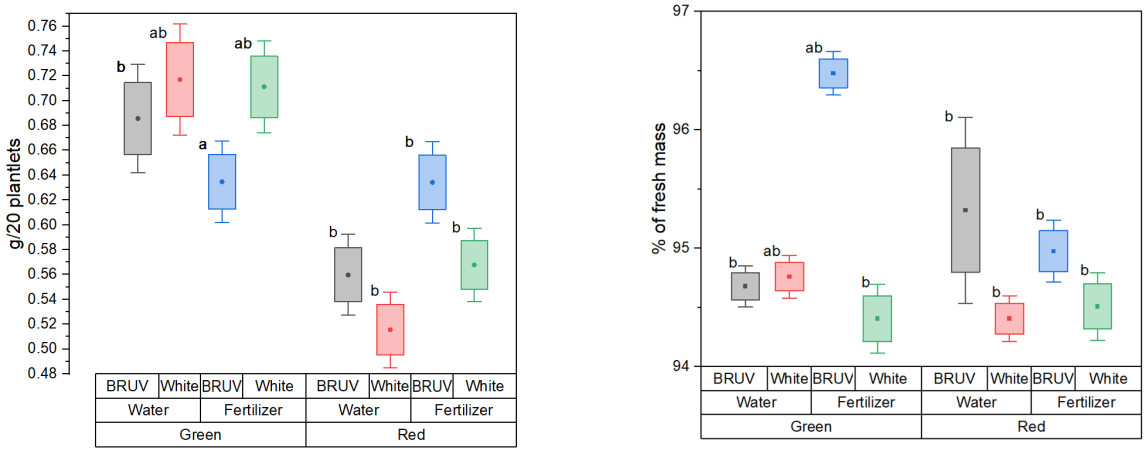

Figure 3. Biomass accumulation (**Left**) and water contents (% of fresh mass) (**Right**) in *Ocimum basilicum* L. (BRUV—blue/red/UV light treatment, white—full spectrum light treatment; different letters indicate statistical significance for $p < 0.05$).

With respect to physiological processes such as the efficiency of the second photosystem, the applied factors (light and fertilization) did not exert any influence. Overall, ΦPSII values ranged between 0.75 and 0.79 in the red cultivar and between 0.76 and 0.78 in the green cultivar (Table 1). The former recorded lower values of both light-adapted steady state fluorescence (Fs) and maximal fluorescence (Fm'), which is attributable to the intrinsically lower chlorophyll contents in this cultivar. The usual fluorescence values range in the 0.75–0.84 domain; however, with shifts from these values depending on leaf properties.

Table 1. Chlorophyll contents and chlorophyll fluorescence markers in *Ocimum basilicum* L. (BRUV—blue/red/UV, white—full spectrum light treatments, different letters—statistical significance for $p < 0.05$, n = 24; values expressed as means ± standard error).

Cultivar	Fertilizer	Treatment	Fs	Fm'	ΦPSII	Chlorophyll (AU)
Green	Water	BRUV	474.63 ^{a} ± 17.32	2106.89 ^{ab} ± 72.83	0.78 ^{ab} ± 0.01	6.33 ^{a} ± 0.28
		White	539.99 ^{a} ± 21.1	2245.66 ^{a} ± 80.99	0.77 ^{b} ± 0.01	5.16 ^{a} ± 1.91
	Fertilizer	BRUV	561.67 ^{a} ± 89.45	2288.25 ^{ab} ± 343.99	0.76 ^{ab} ± 0.02	-
		White	568.34 ^{a} ± 25.77	2378.59 ^{a} ± 99.4	0.77 ^{ab} ± 0.01	-
Red	Water	BRUV	287.67 ^{b} ± 16.19	1288.27 ^{c} ± 64.63	0.79 ^{a} ± 0.01	5.97 ^{a} ± 0.37
		White	321.5 ^{b} ± 16.79	1388.1 ^{c} ± 54.73	0.78 ^{ab} ± 0.01	3.22 ^{b} ± 0.37
	Fertilizer	BRUV	415.59 ^{ab} ± 20.99	1652.67 ^{bc} ± 90.88	0.75 ^{b} ± 0.01	-
		White	505.34 ^{a} ± 27.53	2083 ^{a} ± 133.11	0.76 ^{ab} ± 0.01	-

As the efficiency of the second photo system was comparable among treatments, no indication of stress development in plants could be observed. In a similar pattern, chlorophyll contents were higher in the green cultivar and the lowest value of chlorophyll contents were recorded in the red cultivar under fertilization.

Total phenolic contents recorded marked differences among treatments, with the lowest values, 3.7–3.8 mg/g of gallic acid equivalents, being recorded in the unfertilized green cultivar plantlets. In the meantime, the highest values were observed in plantlets under modified spectrum illumination in the red basil cultivar, 8.1–14.0 mg/g (unfertilized, fertilized, respectively) (Table 2). Main individual phenolic compounds were vanillic, caffeic, and coumaric acids. Among quantified specific phenolic acids, rosmarinic acid (RA) recorded values of approximately 500 µg/g in the green cultivar. In the meantime, RA was the most abundant phenolic acid with values up to 4.5–8 mg/g under modulated light treatment in the red basil cultivar. Overall, the red cultivar had the highest phenolic acid contents with significant increases under blue, red, and UV illumination (Figure 4).

Table 2. Total phenolic contents and antioxidant activity of *Ocimum basilicum* L. extracts (BRUV—blue/red/UV light treatment, white—full spectrum light treatment, different letters indicate statistical significance for $p < 0.05$; n = 3; values are expressed as means ± standard error).

Cultivar	Fertilizer	Treatment	Total Phenolic Content (µg GAE/g Dry Plant Mass)	Antioxidant Activity (% Inhibition)
Green	Water	BRUV	3739.1 ^{b} ± 202.11	88.08 ± 1.71 ^{a}
		White	3846.48 ^{b} ± 200.94	88.19 ± 4.83 ^{a}
	Fertilizer	BRUV	5739.56 ^{b} ± 313.12	85.28 ± 1.54 ^{a}
		White	4858.96 ^{b} ± 264.71	81.4 ± 3.85 ^{a}
Red	Water	BRUV	8194.1 ^{ab} ± 476.49	86.96 ± 3.97 ^{a}
		White	7219.1 ^{ab} ± 425.58	92.73 ± 1.8 ^{a}
	Fertilizer	BRUV	14,063.79 ^{a} ± 755.69	87.84 ± 1.26 ^{a}
		White	13,145.41 ^{a} ± 768.87	92.33 ± 1.46 ^{a}

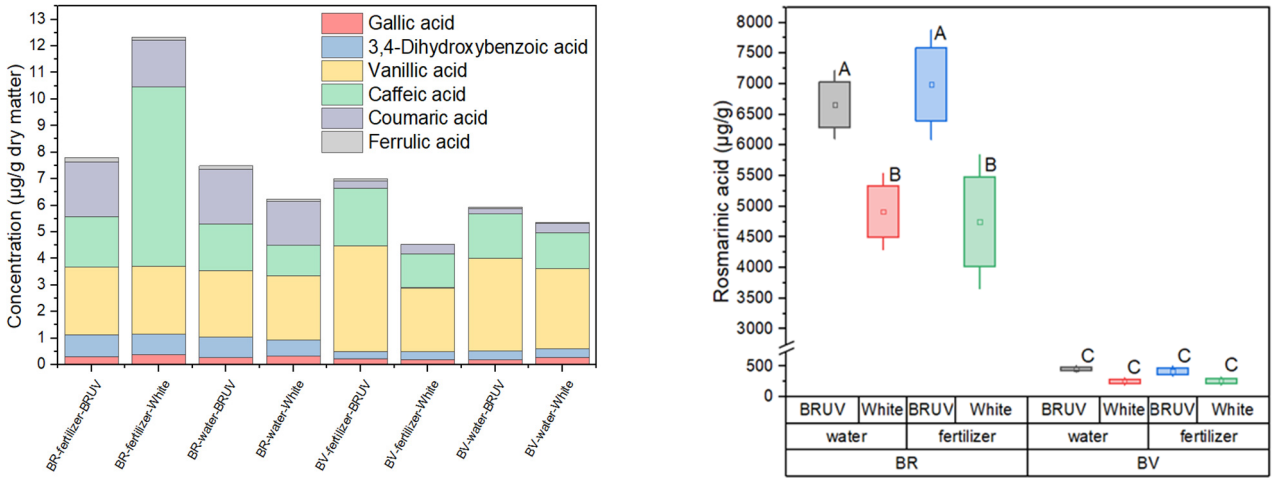

Figure 4. Concentrations of selected phenolic acids (**Left**) and rosmarinic acid (**Right**) in *Ocimum basilicum* L. leaves extracts, analyzed by HPLC, in two Ocimum cultivars under different light treatments (BRUV—blue/red/UV light treatment, white—full spectrum light treatment; BR—Red basil cultivar, BV—Green basil cultivar, different letters indicate statistical significance for $p < 0.05$).

Gene expression of selected phenolic pathways was reduced in plantlets under blue red and UV treatment, with similar values under water or fertilization (Figure 5).

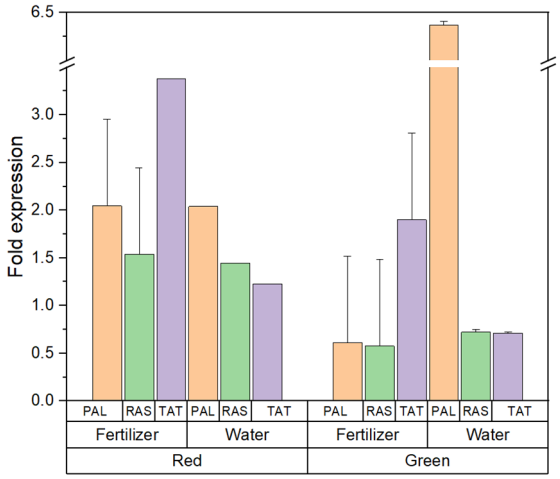

Figure 5. Specific gene expression changes in *Ocimum basilicum* L. plantlets, red cultivar (**left**) and green cultivar (**right**), under different light treatments.

4. Discussion

With an astounding structural diversity, natural phenolic substances are known for their valuable, health-promoting properties and offer the opportunity to be used as part of a regular diet or may serve as starting points for further enhancement of structure and function [25]. Phenolic compounds are a class of secondary metabolites that play pivotal roles in plant physiology and adaptation to the environment. They are involved in a wide range of processes, including defense against pathogens, protection from UV radiation, attraction of pollinators, and modulation of plant–microbe interactions [37].

Our results point to the fact that phenolic acids production was increased, as a result of elicitation under light modulation and due to the fact that the quality of light can be perceived by plants as a cue for the need to protect from excess energy and that phenylpropanoid pathways are involved in such protection [38]. In the arsenal of plant defense mechanisms against harsh light environments and the overproduction of reactive oxygen species (ROS), phenolic compounds stand out for their significant antioxidative capability. While plants

employ various strategies to avert ROS accumulation—including UV-protective epidermal layers, dissipation of surplus light energy as heat, optimizing the architecture of leaves, moving chloroplasts, and transitioning photosystem states—phenolics play a pivotal role in the detoxification process. These compounds are part of the plant's sophisticated antioxidant system that springs into action under stress to neutralize ROS. Alongside enzymatic antioxidants like (SOD), catalase (CAT), and ascorbate peroxidase (APX), phenolics are integral low-molecular-weight antioxidants. They work in concert with other antioxidants such as tocopherols, ascorbate, glutathione, and carotenoids, ensuring the equilibrium between ROS production and scavenging is maintained in non-stressful conditions [39].

The main differences between the light treatments used in our setup was the exclusion of several wavelengths and the addition of ultraviolet wavelengths, by using discrete illumination units, tuned for specific light intervals. It is important to note that the overall photosynthetic active photonic flux density (PPFD) used was not different among treatments and, as such, the observed differences come from eliciting metabolic responses as a result of different energies perceived and used by the photosynthetic apparatus of the plants.

The spectral composition of light plays a significant role in photosynthesis and the overall functioning of photosystems in plants. The diversity of effects observed due to variations in light spectrum is attributed to three main factors: the activation of different photoreceptors, the variable efficiency of different spectral components in driving photosynthesis, and the depth of penetration of these spectral components into the leaf [40]. Different wavelengths of light are known to trigger various photoreceptors in plants, such as phytochromes for red light and cryptochromes for blue light, which subsequently influence plant growth and metabolism. The influence of blue light (maximum 450 nm) and red light (maximum 660 nm) on plant growth and metabolism is recognized, but not fully understood; however, the spectral composition of light modifies the expression of light-dependent genes and impacts the growth, photosynthesis, and physiological responses in plants, as observed in seedlings [41,42].

Blue and red lights are known to have significant effects on PSII and PSI [43]. Blue light, particularly in the range of 400–500 nm, has been shown to enhance the rate of photosynthesis and stomatal opening, leading to increased CO_2 assimilation [44,45]. Also, the use of red and blue LED spectra has been shown to increase the accumulation of polyphenols, flavonoids, and other phytochemicals, although not necessarily enhancing antioxidant activity [46], possibly due to premature plant allocation of metabolites to alternative pathways (such as curcumin synthesis). The mechanisms appears to be related to the stress, induced by high light intensity or specific light spectral compositions, which activate plant response mechanisms that include the production of phenolic compounds through hormonal pathways [47].

Red light, predominantly absorbed by chlorophyll, increases the efficiency of PSII [48], while far-red wavelengths, such as those used in our research, lead to higher yield also by enhancing PSII efficiency through reducing the heat dissipation of PSII, increasing the light energy available for photosynthesis and decreasing NPQ through the faster reoxidation of plastoquinone and reopening of the PSII reaction center [49].

Such effects explain our results, with significantly increased phenolic synthesis under a modulated light spectrum; however, we should note the major differences in the responses of green and reed cultivars. Furthermore, these differences probably stem from two reasons: (a) the presence of anthocyanins in the cells of red cultivar, which additionally absorb photons' energy, and (b) different gene expression levels between cultivars.

The biosynthesis of phenolic compounds in plants is a complex process, involving various enzymes and associated genes such as phenylalanine ammonia lyase (PAL), caffeic

acid O-methyltransferase (COMT), rosmarinic acid synthase (RAS), and tyrosine aminotransferase (TAT). Phenylalanine ammonia lyase is a key enzyme in the phenolic synthesis pathway, catalyzing the deamination of phenylalanine to form cinnamic acid, the entry point for phenolic biosynthesis in plants [50]. Meanwhile, caffeic acid O-methyltransferase is involved in the methylation of hydroxycinnamic acids, converting them into their corresponding methyl esters. This enzyme contributes to lignin biosynthesis and regulates the accumulation of various phenolic compounds [51]. Rosmarinic acid synthase, responsible for the synthesis of rosmarinic acid, catalyzes the condensation of caffeic acid with 3,4-dihydroxyphenyllactic acid. Tyrosine aminotransferase is involved in the conversion of tyrosine to p-coumaric acid, a precursor of various phenolic compounds, and is crucial for the biosynthesis of flavonoids and other phenolics [52,53].

The increased phenolic levels in our red cultivar under modulated light and the increased PAL expression observed may come from the fact that, UV light, particularly UV-B (280–315 nm), has been proven to increase phenolic acids synthesis through the UVR8 photoreceptor, which interacts with the COP1/HYH/HY5 signaling pathway and leads to increases in mRNA levels and activities of phenylalanine ammonia lyase (PAL), cinnamic acid 4-hydroxylase (C4H), 4-coumarate coenzyme A ligase (4CL), p-coumaric acid 3-hdroxylase (C3H), caffeic acid O-methyltransferase (COMT) [54]. UV also increases phenolic contents by increasing PAL activity as a response to induced energy excess in mitochondrial electron transport chain and reactive oxygen species (ROS) generation and by enhancing vitamin C production and, thus, the protection of phenolic substances from degradation [55].

Rosmarinic acid production, in particular, was shown to increase by a few folds in *Lamiaceae* plants by exposure to blue/red/far-red treatments, following increases in PAL, TAT, and hydroxyphenyl pyruvate reductase (HPPR) enzymes, but with minimal effects on chlorophyll contents, as shown in our study [56]. The mechanisms appears to be a modified balance of transcript levels of downstream genes (C4H, chalcone synthase (CHS), chalcone isomerase (CHI), and (RAS) and upstream genes (PAL, TAT, and HPPR) [57].

Several studies have highlighted that the spectral quality of light can significantly influence the production of phenolic compounds. For instance, an experiment with spring barley acclimated to different spectral qualities—white, blue, green, and red—at various irradiances found a complex interaction between photosynthetically active radiation (PAR) irradiance and spectral components in the accumulation of phenolic compounds [58,59]. The impact of light quantity (intensity and photoperiod) and quality (spectral composition) extends to plant growth and physiology, interacting with other environmental parameters and cultivation factors. This complexity influences plant behavior and metabolism, including the synthesis of phenolic compounds, as was seen when comparing the effects of blue, red, and a combination of blue and red lights on metabolism of young wheat plants, which is related to stress responses and secondary metabolite production [39].

However, the limitation of this study was the relatively low number of plants used, especially considering the variability in the experimental cultivars used. As such, a difference in gene expression was observed in the two cultivars, when TAT, COMT and RAS enzymes were overexpressed in the green one, but not in the red one. Such differences point to the need of more precise standardization of cultivation parameters, such as placement of plants relative to light, periodic rotation between plants, and precise temperature control.

5. Conclusions

The spectral composition of light is a critical factor that influences the functioning of photosystems in plants, affecting a wide range of physiological and developmental processes. Our results showed that a modulation of light quality and quantity, alongside

basic fertilization of *Ocimum basilicum* plants, can lead to significant increases in the production of the valuable phenolic substances, in particular rosmarinic acid, with up to 25%. Moreover, basil microgreens retain their biomass production; thus, the light treatment does not impede economic reasons and no significant stress is recorded in plants. The breadth of the influence of light spectrum variation on plant life underscores the importance of this area of study, particularly in the context of artificial lighting in agriculture and the potential for targeted manipulation of light spectra to enhance plant growth and productivity.

Author Contributions: Conceptualization, A.L. and N.-E.P.; methodology, A.L.; software, G.-C.T.; validation, V.S. and M.B.; formal analysis, A.L. and N.-E.P.; resources, G.M.; data curation, G.M.; writing—original draft preparation, G.-C.T., N.-E.P. and A.L.; writing—review and editing, M.B. and V.S.; supervision, V.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by a grant from the Ministry of Research, Innovation and Digitization, CCCDI-UEFISCDI, project number PN-III-P2-2.1-PED-2021-4380, within PNCDI III.

Data Availability Statement: Data are contained within the article.

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

References

1. Michell, K.A.; Isweiri, H.; Newman, S.E.; Bunning, M.; Bellows, L.L.; Dinges, M.M.; Grabos, L.E.; Rao, S.; Foster, M.T.; Heuberger, A.L.; et al. Microgreens: Consumer Sensory Perception and Acceptance of an Emerging Functional Food Crop. *J. Food Sci.* **2020**, *85*, 926–935. [CrossRef] [PubMed]
2. Galieni, A.; Falcinelli, B.; Stagnari, F.; Datti, A.; Benincasa, P. Sprouts and Microgreens: Trends, Opportunities, and Horizons for Novel Research. *Agronomy* **2020**, *10*, 1424. [CrossRef]
3. Lobiuc, A.; Vasilache, V.; Oroian, M.; Stoleru, T.; Burducea, M.; Pintilie, O.; 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]
4. Hosseini, A.; Zare Mehrjerdi, M.; Aliniaeifard, S. Alteration of Bioactive Compounds in Two Varieties of Basil (*Ocimum basilicum*) Grown Under Different Light Spectra. *J. Essent. Oil Bear. Plants* **2018**, *21*, 913–923. [CrossRef]
5. Viršilė, A.; Laužikė, K.; Sutulienė, R.; Brazaitytė, A.; Kudirka, G.; Samuolienė, G. Distinct Impacts of UV-A Light Wavelengths on Nutraceutical and Mineral Contents in Green and Purple Basil Cultivated in a Controlled Environment. *Horticulturae* **2023**, *9*, 1168. [CrossRef]
6. Shiga, T.; Shoji, K.; Shimada, H.; Hashida, S.; Goto, F.; Yoshihara, T. Effect of Light Quality on Rosmarinic Acid Content and Antioxidant Activity of Sweet Basil, *Ocimum basilicum* L. *Plant Biotechnol.* **2009**, *26*, 255–259. [CrossRef]
7. Vodnik, D.; Vogrin, Ž.; Šircelj, H.; Grohar, M.C.; Medič, A.; Carović-Stanko, K.; Safner, T.; Lazarević, B. Phenotyping of Basil (*Ocimum basilicum* L.) Illuminated with UV-A Light of Different Wavelengths and Intensities. *Sci. Hortic.* **2023**, *309*, 111638. [CrossRef]
8. Chutimanukul, P.; Wanichananan, P.; Janta, S.; Toojinda, T.; Darwell, C.T.; Mosaleeyanon, K. The Influence of Different Light Spectra on Physiological Responses, Antioxidant Capacity and Chemical Compositions in Two Holy Basil Cultivars. *Sci. Rep.* **2022**, *12*, 588. [CrossRef]
9. Branca, F.; Treccarichi, S.; Ruberto, G.; Renda, A.; Argento, S. Comprehensive Morphometric and Biochemical Characterization of Seven Basil (*Ocimum basilicum* L.) Genotypes: Focus on Light Use Efficiency. *Agronomy* **2024**, *14*, 224. [CrossRef]
10. Zhang, S.; Zhang, L.; Zou, H.; Qiu, L.; Zheng, Y.; Yang, D.; Wang, Y. Effects of Light on Secondary Metabolite Biosynthesis in Medicinal Plants. *Front. Plant Sci.* **2021**, *12*, 781236. [CrossRef]
11. Gholamnia, A.; Mosleh Arani, A.; Sodaeizadeh, H.; Tarkesh Esfahani, S.; Ghasemi, S. Expression Profiling of Rosmarinic Acid Biosynthetic Genes and Some Physiological Responses from *Mentha piperita* L. Under Salinity and Heat Stress. *Physiol. Mol. Biol. Plants* **2022**, *28*, 545–557. [CrossRef] [PubMed]
12. Rizi, M.R.; Azizi, A.; Sayyari, M.; Mirzaie-Asl, A.; Conti, L. Increased Phenylpropanoids Production in UV-B Irradiated Salvia Verticillata as a Consequence of Altered Genes Expression in Young Leaves. *Plant Physiol. Biochem.* **2021**, *167*, 174–184. [CrossRef] [PubMed]
13. Rahman, M.d.M.; Rahaman, M.d.S.; Islam, M.d.R.; Rahman, F.; Mithi, F.M.; Alqahtani, T.; Almikhlafi, M.A.; Alghamdi, S.Q.; Alruwaili, A.S.; Hossain, M.d.S.; et al. Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. *Molecules* **2021**, *27*, 233. [CrossRef] [PubMed]

14. Sun, W.; Shahrajabian, M.H. Therapeutic Potential of Phenolic Compounds in Medicinal Plants—Natural Health Products for Human Health. *Molecules* **2023**, *28*, 1845. [CrossRef]
15. Ambriz-Perez, D.L.; Leyva-Lopez, N.; Gutierrez-Grijalva, E.P.; Heredia, J.B. Phenolic Compounds: Natural Alternative in Inflammation Treatment. A Review. *Cogent Food Agric.* **2016**, *2*, 1131412. [CrossRef]
16. Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciatore, F.; Bonaduce, D.; et al. Oxidative Stress, Aging, and Diseases. *Clin. Interv. Aging* **2018**, *13*, 757–772. [CrossRef]
17. Yaribeygi, H.; Sathyapalan, T.; Atkin, S.L.; Sahebkar, A. Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. *Oxidative Med. Cell. Longev.* **2020**, *2020*, 8609213. [CrossRef]
18. Blesa, J.; Trigo-Damas, I.; Quiroga-Varela, A.; Jackson-Lewis, V.R. Oxidative Stress and Parkinson's Disease. *Front. Neuroanat.* **2015**, *9*, 91. [CrossRef]
19. Huang, W.-J.; Zhang, X.; Chen, W.-W. Role of Oxidative Stress in Alzheimer's Disease. *Biomed. Rep.* **2016**, *4*, 519–522. [CrossRef]
20. Cianciosi, D.; Forbes-Hernández, T.; Afrin, S.; Gasparrini, M.; Reboredo-Rodriguez, P.; Manna, P.; Zhang, J.; Bravo Lamas, L.; Martínez Flórez, S.; Agudo Toyos, P.; et al. Phenolic Compounds in Honey and Their Associated Health Benefits: A Review. *Molecules* **2018**, *23*, 2322. [CrossRef]
21. Saha, S.K.; Lee, S.B.; Won, J.; Choi, H.Y.; Kim, K.; Yang, G.-M.; Dayem, A.A.; Cho, S. Correlation between Oxidative Stress, Nutrition, and Cancer Initiation. *Int. J. Mol. Sci.* **2017**, *18*, 1544. [CrossRef] [PubMed]
22. Yan, Z.; Zhong, Y.; Duan, Y.; Chen, Q.; Li, F. Antioxidant Mechanism of Tea Polyphenols and Its Impact on Health Benefits. *Anim. Nutr.* **2020**, *6*, 115–123. [CrossRef] [PubMed]
23. Zeb, A. Concept, Mechanism, and Applications of Phenolic Antioxidants in Foods. *J. Food Biochem.* **2020**, *44*, e13394. [CrossRef] [PubMed]
24. Hussain, T.; Tan, B.; Yin, Y.; Blachier, F.; Tossou, M.C.B.; Rahu, N. Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? *Oxidative Med. Cell. Longev.* **2016**, *2016*, 7432797. [CrossRef]
25. Lobiuc, A.; Pavăl, N.-E.; Mangalagiu, I.I.; Gheorghiță, R.; Teliban, G.-C.; Amăriucăi-Mantu, D.; Stoleru, V. Future Antimicrobials: Natural and Functionalized Phenolics. *Molecules* **2023**, *28*, 1114. [CrossRef]
26. Lee, B.H.; Nam, T.G.; Park, N.Y.; Chun, O.K.; Koo, S.I.; Kim, D.-O. Estimated Daily Intake of Phenolics and Antioxidants from Green Tea Consumption in the Korean Diet. *Int. J. Food Sci. Nutr.* **2016**, *67*, 344–352. [CrossRef]
27. Grosso, G.; Stepaniak, U.; Topor-Mądry, R.; Szafraniec, K.; Pająk, A. Estimated Dietary Intake and Major Food Sources of Polyphenols in the Polish Arm of the HAPIEE Study. *Nutrition* **2014**, *30*, 1398–1403. [CrossRef]
28. Kapolou, A.; Karantonis, H.C.; Rigopoulos, N.; Koutelidakis, A.E. Association of Mean Daily Polyphenols Intake with Mediterranean Diet Adherence and Anthropometric Indices in Healthy Greek Adults: A Retrospective Study. *Appl. Sci.* **2021**, *11*, 4664. [CrossRef]
29. Wisnuwardani, R.W.; De Henauw, S.; Ferrari, M.; Forsner, M.; Gottrand, F.; Huybrechts, I.; Kafatos, A.G.; Kersting, M.; Knaze, V.; Manios, Y.; et al. Total Polyphenol Intake Is Inversely Associated with a Pro/Anti-Inflammatory Biomarker Ratio in European Adolescents of the HELENA Study. *J. Nutr.* **2020**, *150*, 1610–1618. [CrossRef]
30. Zamora-Ros, R.; Knaze, V.; Rothwell, J.A.; Hémon, B.; Moskal, A.; Overvad, K.; Tjønneland, A.; Kyrø, C.; Fagherazzi, G.; Boutron-Ruault, M.-C.; et al. Dietary Polyphenol Intake in Europe: The European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *Eur. J. Nutr.* **2016**, *55*, 1359–1375. [CrossRef]
31. Wisnuwardani, R.W.; De Henauw, S.; Androutsos, O.; Forsner, M.; Gottrand, F.; Huybrechts, I.; Knaze, V.; Kersting, M.; Le Donne, C.; Marcos, A.; et al. Estimated Dietary Intake of Polyphenols in European Adolescents: The HELENA Study. *Eur. J. Nutr.* **2019**, *58*, 2345–2363. [CrossRef] [PubMed]
32. Scalbert, A.; Williamson, G. Dietary Intake and Bioavailability of Polyphenols. *J. Nutr.* **2000**, *130*, 2073S–2085S. [CrossRef] [PubMed]
33. Knaze, V.; Rothwell, J.A.; Zamora-Ros, R.; Moskal, A.; Kyrø, C.; Jakszyn, P.; Skeie, G.; Weiderpass, E.; Santucci de Magistris, M.; Agnoli, C.; et al. A New Food-Composition Database for 437 Polyphenols in 19,899 Raw and Prepared Foods Used to Estimate Polyphenol Intakes in Adults from 10 European Countries. *Am. J. Clin. Nutr.* **2018**, *108*, 517–524. [CrossRef] [PubMed]
34. Bhaswant, M.; Shanmugam, D.K.; Miyazawa, T.; Abe, C.; Miyazawa, T. Microgreens—A Comprehensive Review of Bioactive Molecules and Health Benefits. *Molecules* **2023**, *28*, 867. [CrossRef]
35. Khater, E.-S.; Bahnasawy, A.; Abass, W.; Morsy, O.; El-Ghobashy, H.; Shaban, Y.; Egela, M. Production of Basil (*Ocimum basilicum* L.) Under Different Soilless Cultures. *Sci. Rep.* **2021**, *11*, 12754. [CrossRef]
36. Herald, T.J.; Gadgil, P.; Tilley, M. High-throughput Micro Plate Assays for Screening Flavonoid Content and DPPH-scavenging Activity in Sorghum Bran and Flour. *J. Sci. Food Agric.* **2012**, *92*, 2326–2331. [CrossRef]
37. Dixon, R.A.; Paiva, N.L. Stress-Induced Phenylpropanoid Metabolism. *Plant Cell* **1995**, 1085–1097. [CrossRef]
38. Marchica, A.; Cotrozzi, L.; Detti, R.; Lorenzini, G.; Pellegrini, E.; Petersen, M.; Nali, C. The Biosynthesis of Phenolic Compounds Is an Integrated Defence Mechanism to Prevent Ozone Injury in Salvia Officinalis. *Antioxidants* **2020**, *9*, 1274. [CrossRef]

39. Pech, R.; Volná, A.; Hunt, L.; Bartas, M.; Červeň, J.; Pečinka, P.; Špunda, V.; Nezval, J. Regulation of Phenolic Compound Production by Light Varying in Spectral Quality and Total Irradiance. *Int. J. Mol. Sci.* **2022**, *23*, 6533. [CrossRef]
40. Ptushenko, O.S.; Ptushenko, V.V.; Solovchenko, A.E. Spectrum of Light as a Determinant of Plant Functioning: A Historical Perspective. *Life* **2020**, *10*, 25. [CrossRef]
41. Pashkovskiy, P.; Ivanov, Y.; Ivanova, A.; Kreslavski, V.D.; Vereshchagin, M.; Tatarkina, P.; Kuznetsov, V.V.; Allakhverdiev, S.I. Influence of Light of Different Spectral Compositions on Growth Parameters, Photosynthetic Pigment Contents and Gene Expression in Scots Pine Plantlets. *Int. J. Mol. Sci.* **2023**, *24*, 2063. [CrossRef] [PubMed]
42. Tarakanov, I.G.; Tovstyko, D.A.; Lomakin, M.P.; Shmakov, A.S.; Sleptsov, N.N.; Shmarev, A.N.; Litvinskiy, V.A.; Ivlev, A.A. Effects of Light Spectral Quality on Photosynthetic Activity, Biomass Production, and Carbon Isotope Fractionation in Lettuce, *Lactuca sativa* L., Plants. *Plants* **2022**, *11*, 441. [CrossRef] [PubMed]
43. 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]
44. Lazzarin, M.; Meisenburg, M.; Meijer, D.; Van Ieperen, W.; Marcelis, L.F.M.; Kappers, I.F.; Van Der Krol, A.R.; Van Loon, J.J.A.; Dicke, M. LEDs Make It Resilient: Effects on Plant Growth and Defense. *Trends Plant Sci.* **2021**, *26*, 496–508. [CrossRef]
45. Pál, M.; Hamow, K.Á.; Rahman, A.; Majláth, I.; Tajti, J.; Gondor, O.K.; Ahres, M.; Gholizadeh, F.; Szalai, G.; Janda, T. Light Spectral Composition Modifies Polyamine Metabolism in Young Wheat Plants. *Int. J. Mol. Sci.* **2022**, *23*, 8394. [CrossRef]
46. Marchant, M.J.; Molina, P.; Montecinos, M.; Guzmán, L.; Balada, C.; Castro, M. Effects of LED Light Spectra on the Development, Phytochemical Profile, and Antioxidant Activity of Curcuma Longa from Easter Island. *Plants* **2022**, *11*, 2701. [CrossRef]
47. Makowski, W.; Tokarz, B.; Banasiuk, R.; Królicka, A.; Dziurka, M.; Wojciechowska, R.; Tokarz, K.M. Is a Blue–Red Light a Good Elicitor of Phenolic Compounds in the Family Droseraceae? A Comparative Study. *J. Photochem. Photobiol. B Biol.* **2019**, *201*, 111679. [CrossRef]
48. Zhen, S.; Haidekker, M.; Van Iersel, M.W. Far-Red Light Enhances Photochemical Efficiency in a Wavelength-Dependent Manner. *Physiol. Plant.* **2019**, *167*, 21–33. [CrossRef]
49. Tan, T.; Li, S.; Fan, Y.; Wang, Z.; Ali Raza, M.; Shafiq, I.; Wang, B.; Wu, X.; Yong, T.; Wang, X.; et al. Far-Red Light: A Regulator of Plant Morphology and Photosynthetic Capacity. *Crop J.* **2022**, *10*, 300–309. [CrossRef]
50. Koshiba, T.; Saito, E.; Ono, N.; Yamamoto, N.; Sato, M. Purification and Properties of Flavin- and Molybdenum-Containing Aldehyde Oxidase from Coleoptiles of Maize. *Plant Physiol.* **1996**, *110*, 781–789. [CrossRef]
51. Boudet, A.M.; Kajita, S.; Grima-Pettenati, J.; Goffner, D. Lignins and Lignocellulosics: A Better Control of Synthesis for New and Improved Uses. *Trends Plant Sci.* **2003**, *8*, 576–581. [CrossRef] [PubMed]
52. Petersen, M.; Häusler, E.; Karwatzki, B.; Meinhard, J. Proposed Biosynthetic Pathway for Rosmarinic Acid in Cell Cultures of Coleus Blumei Benth. *Planta* **1993**, *189*, 10–14. [CrossRef]
53. Petersen, M.; Abdullah, Y.; Benner, J.; Eberle, D.; Gehlen, K.; Hücherig, S.; Janiak, V.; Kim, K.H.; Sander, M.; Weitzel, C.; et al. Evolution of Rosmarinic Acid Biosynthesis. *Phytochemistry* **2009**, *70*, 1663–1679. [CrossRef]
54. Wang, M.; Leng, C.; Zhu, Y.; Wang, P.; Gu, Z.; Yang, R. UV-B Treatment Enhances Phenolic Acids Accumulation and Antioxidant Capacity of Barley Seedlings. *LWT* **2022**, *153*, 112445. [CrossRef]
55. Rabelo, M.C.; Bang, W.Y.; Nair, V.; Alves, R.E.; Jacobo-Velázquez, D.A.; Sreedharan, S.; De Miranda, M.R.A.; Cisneros-Zevallos, L. UVC Light Modulates Vitamin C and Phenolic Biosynthesis in Acerola Fruit: Role of Increased Mitochondria Activity and ROS Production. *Sci. Rep.* **2020**, *10*, 21972. [CrossRef]
56. Chai, W.Y.; Goh, J.K.; Kalavally, V.; Rahman, S.; Lim, Y.Y.; Choo, W.S. Enhancing Rosmarinic Acid Production and Regulating Enzyme Activity in *Melissa officinalis* L. Using Spectrally Tunable Light-Emitting Diodes. *Ind. Crops Prod.* **2023**, *204*, 117332. [CrossRef]
57. Park, W.T.; Yeo, S.K.; Sathasivam, R.; Park, J.S.; Kim, J.K.; Park, S.U. Influence of Light-Emitting Diodes on Phenylpropanoid Biosynthetic Gene Expression and Phenylpropanoid Accumulation in *Agastache rugosa*. *Appl. Biol. Chem.* **2020**, *63*, 25. [CrossRef]
58. Kivimäenpä, M.; Mofikoya, A.; Abd El-Raheem, A.M.; Riikonen, J.; Julkunen-Tiitto, R.; Holopainen, J.K. Alteration in Light Spectra Causes Opposite Responses in Volatile Phenylpropanoids and Terpenoids Compared with Phenolic Acids in Sweet Basil (*Ocimum basilicum*) Leaves. *J. Agric. Food Chem.* **2022**, *70*, 12287–12296. [CrossRef]
59. Taulavuori, K.; Hyöky, V.; Oksanen, J.; Taulavuori, E.; Julkunen-Tiitto, R. Species-Specific Differences in Synthesis of Flavonoids and Phenolic Acids Under Increasing Periods of Enhanced Blue Light. *Environ. Exp. Bot.* **2016**, *121*, 145–150. [CrossRef]