Scientia Horticulturae 315 (2023) 111970

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Modulation of VOC fingerprint and alteration of physiological responses after supplemental LED light in green- and red-leafed sweet basil (*Ocimum basilicum* L.)

Giulia Lauria [a], Ermes Lo Piccolo [a,b], Anna Davini [a,c], Monica Ruffini Castiglione [c], Ylenia Pieracci [d], Guido Flamini [d], Stefan Martens [e], Andrea Angeli [e], Costanza Ceccanti [a], Lucia Guidi [a], Elisa Pellegrini [a], Luca Incrocci [a], Marco Landi [a,*]

[a] Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto, 80, Pisa 56124, Italy
[b] Department of Agriculture, Food, Environment and Forestry, University of Florence, Florence 50144, Italy
[c] Department of Biology, University of Pisa, Pisa 56126, Italy
[d] Department of Pharmacy, University of Pisa, Pisa 56126, Italy
[e] Fondazione Edmund Mach, Centro Ricerca e Innovazione, San Michele all'Adige, Trento 38098, Italy

ARTICLE INFO

Keywords:
Anthocyanin
Chlorophyll fluorescence
Gas exchange
Metabolomics
Polyphenol
Stomatal density

ABSTRACT

Narrowband green (G), blue (B), red (R), and polychromatic (W – R:G:B, 1:1:1) lights were supplemented (250 μmol photon m^{-2} s^{-1}; 5 h d^{-1}; 21 d) to ambient light with Light Emitting Diodes (LED) in green- ('Tigullio'; TI) and red-leafed ('Red Rubin'; RR) sweet basil (*Ocimum basilicum* L.) plants. The aim of this work was to evaluate LED-triggered variations in biometric and morpho-anatomical traits, photosynthesis and secondary metabolite production to 'photomodulate' plant performance *via* monochromatic LED light supplementation.

In both the cultivars, light supplementation did not alter plant biomass production compared to controls and at the amplitude applied therein any LED supplementation resulted in oxidative stress, as evidenced by slight changes in chlorophyll fluorescence parameters and H_2O_2 staining in leaves. Conversely, monochromatic LEDs selectively modulate metabolomics profile in both green- and red-leafed basil; e.g., B lights increased polyphenol content in TI, whilst G light resulted effective in reducing cyanidin-3-coumaroil-glucoside one of the prevalent acylated anthocyanin of RR. Volatilome profile unveiled profound waveband-dependent alterations though consistently eugenol, characterised by a clove flavor, and also considered one of the major toxic compounds in sweet basil, decreased with all the LED treatments, making green-leafed basil a more pleasant and safer product.

Our dataset offers the clear evidence that monochromatic LED light supplementation represents a valid tool to modulate the accumulation of targeted secondary metabolites *in planta*.

1. Introduction

Light is a pivotal factor controlling plant morpho-anatomical, physiological and biochemical traits (Landi et al., 2020). In particular, light quantity and quality and the photoperiod affect the photosynthetic apparatus (Murchie and Horton, 1997; Walters et al., 2003; Lee et al., 2007). Through the stimulation of photosynthetic pigments and photoreceptors, light quality may promote a *plethora* of responses on plant growth, morphology, physiology, and biochemistry (Folta and Carvalho, 2015; Galvão and Frankhauser, 2015; Paik and Huq, 2019). Through the photosynthetic process, light influences not only CO_2 fixation and primary metabolism, but also the biosynthesis of fundamental compounds involved in plant development and defense (Loi et al., 2021). Some of these molecules, such as carotenoids (Llorente et al., 2017), α-tocopherol, flavonoids, anthocyanins, and other polyphenols (Qian et al., 2016; Jang et al., 2020), play also antioxidant roles in plants (Loi et al., 2021). Furthermore, these compounds play a fundamental role in the prevention of chronic and degenerative diseases, and in helping to preserve human health (Leicach and Chludil, 2014). Thus, the regulation of light spectra is becoming an important aspect of indoor plant cultivation as it allows the control of plant growth, development, nutritional, and nutraceutical quality (Bantis et al., 2018). Moreover, the recent diffusion of Light Emitting Diodes (LEDs) paves the

* Corresponding author.
E-mail address: marco.landi@unipi.it (M. Landi).

https://doi.org/10.1016/j.scienta.2023.111970
Received 25 November 2022; Received in revised form 24 February 2023; Accepted 1 March 2023
Available online 28 March 2023

Abbreviation

Anth	anthocyanins
B	monochromatic blue light supplementation
C_i	intercellular CO_2 concentration
Chl	chlorophyll
DAB	3,3′-diaminobenzidine
ETR	Electron Transport Rate
F_0	minimal fluorescence yield of the dark-adapted state of photosystem II (PSII)
Flav	flavonoids
F_m	maximal fluorescence yield of the dark-adapted state of PSII
F_v/F_m	maximum quantum yield of PSII
G	monochromatic green light supplementation
g_s	stomatal conductance
LA	single leaf area
LED	Light Emitting Diodes
NBI®	Nitrogen Balance Index
NS	control
P_N/C_i	apparent carboxylation efficiency
P_N	net photosynthetic rate
PSII	photosystem II
R	monochromatic red light supplementation
ROS	Reactive Oxygen Species
SD	standard deviation
VOC	Volatile Organic Compound
W	polychromatic (R:G:B; 1:1:1) light supplementation
WUE_i	intrinsic water use efficiency (P_N/g_s)
Φ_{NO}	quantum yield of non-regulated non-photochemical energy loss in PSII
Φ_{NPQ}	quantum yield of regulated non-photochemical energy loss in PSII
Φ_{PSII}	operational quantum yield of PSII photochemistry

way for increasing the knowledge about plant photobiology (Paradiso and Proietti, 2022). In addition, to practical advantages, such as high energy efficiency and lower thermal radiation, LED lamps offer the possibility to finely modulate light intensity and spectrum (Cocetta et al., 2017; Paradiso and Proietti, 2022).

To date, most research deals with exclusively monochromatic environments, which are effective in short-term treatments (e.g., for microgreens), but exert deleterious effect in long-term applications (Landi et al., 2020; Zhang et al., 2020). Conversely, the present study focuses on ambient light enrichment, which is a more valuable practice for long-term cultivation. So far, the growing body of literature about LED light supplementation has mainly focused on the application of supplemental red, blue, or of different combination of the two wavebands. Many authors have reported that blue light enrichment is more effective for the stimulation of secondary metabolites compared to red, green or yellow lights (Kim et al., 2014; Samuolienė et al., 2017; Taulavuori et al., 2018; Zhang et al., 2019). Instead, green wavebands have been barely investigated because are considered less effective in photosynthesis, but recent and a growing body of research has proved green light effects on plant physiology as well as on plant secondary metabolism (Samuolienė et al., 2013; Bian et al., 2019; Dou et al., 2019). Therefore, further research should be carried out to deeply understand the impact of this wavelength on plant metabolism *sensu lato*.

Sweet basil (*Ocimum basilicum* L.) is a highly valued, culinary and medicinal herb, widely cultivated in the Mediterranean countries, belonging to the Lamiaceae family. It is well known as a rich source of a wide variety of compounds, such as polyphenols and terpenoids (Filip, 2017; Chutimanukul et al., 2022), and it is famous for its characteristic scent given by carbon-based volatile compounds. These molecules, due to their high vapor pressure at room temperature, are called volatile organic compounds (VOCs) and are responsible for the basil complex aroma profile (Carvalho et al., 2016; Hammock et al., 2021). The wide variety of compounds synthesized by sweet basil confers this herb high potential health benefits for consumers (Litvin et al., 2020; Hammock et al., 2021). Basil essential oil has shown good antioxidant properties and antimicrobial activity against pathogenic bacteria and fungi (Lee et al., 2005; Hussain et al., 2008).

Experiments conducted in controlled environments reported that monochromatic red and blue LED light can affect yields, photosynthesis, and metabolic profile in basil (Carvalho et al., 2016; Bantis et al., 2016; Hosseini et al., 2019) and that different red:blue ratios can modulate basil biomass accumulation, photosynthetic responses and antioxidant capacities of the leaf extract (Pennisi et al., 2019; Lin et al., 2021; Chutimanukul et al., 2022). Moreover, some authors have evidenced light influence in the variation of plant VOC profile (Owen et al., 2002; Loreto et al., 2006). In particular, volatiles compounds were reported to be affected by both light intensity (Chang et al., 2008; Walters et al., 2021) and profile (Pennisi et al., 2019; Hammock et al., 2021) in sweet basil.

This work aims to describe the physiological and biochemical effects of narrowband LED light supplementation to ambient light on two different cultivars of sweet basil: one is a widely consumed green-leafed cultivar ('Tigullio'), while the other is characterised by red leaves ('Red Rubin') with a high anthocyanin content in the leaf epidermis (Landi et al., 2014). According to other studies, these cultivars are characterised by a different essential oil and VOC profiles (Murárikova et al., 2017; Fernandes et al., 2019). Red (R), blue (B), green (G), and polychromatic (W – R:G:B, 1:1:1) lights were applied. Since most research was conducted in growth chambers, this study was performed in a greenhouse to better simulate the common nursery cultivation and investigate the possibility of influencing and improving the basil aroma profile and the biosynthesis of secondary metabolites with monochromatic LED light supplementation in this highly-valued culinary herb.

2. Materials and methods

2.1. Plant material and growth conditions

Experiments were carried out during the autumn 2021 in a greenhouse at the Department of Agriculture, Food and Environment, University of Pisa (43.704700°N, 10.427269°E). Seeds of green- and red-leafed sweet basil (*O. basilicum* L.) cv. 'Tigullio' (TI) and 'Red Rubin' (RR), (purchased from Franchi Sementi S.P.A., MI, Italy), were sown in rockwool plug trays (Grodan® Plantop Plug). After one month, plantlets were transplanted into 1-L pots filled with a sandy soil-peat mixture (60:40, v:v), and daily irrigated with the following nutrient solution: 10.00 mM $N-NO_3^-$, 0.51 mM $N-NH_4^+$, 1.50 mM P, 9.00 mM K^+, 4.50 mM Ca^{2+}, 2.00 mM Mg^{2+}, 0.78 mM Na^+, 5.37 mM $S-SO_4^{2-}$, 0.68 mM Cl^-, 40.00 μM Fe^{2+}, 40.00 μM BO_3^-, 3.00 μM Cu^{2+}, 10.00 μM Zn^{2+}, 10.00 μM Mn^{2+}, and 1.00 mM Mo^{3+}. Electrical conductivity (EC) was 2.40 mS cm^{-1} and pH was adjusted to 5.6 with diluted sulphuric acid. The average air temperature was 21 °C and the minimum and maximum temperatures reached during the whole period of the experiment were 14 and 33 °C, respectively. Four days after transplanting, eight plants per cultivar were subjected to different supplemental LED light illumination, supplied with lamps (1200 × 68 × 36 mm) purchased from Ambra Elettronica s.r.l. (Bolzano Vicentino, VI, Italy). Treatments applied were G, B, and R (peak 530, 450 and 660 nm), and polychromatic (W – R:G:B, 1:1:1) light supplementation (for light spectra details see Lauria et al.

2021), which radiated additional 250 μmol photon m^{-2} s^{-1} to ambient light, for five hours per day (from 11:00 a.m. to 4:00 p.m., GMT), while the control plants (non-supplemented; NS) received only ambient light and had a panel of the size of a lamp upon them to simulate the lamp shading at midday. Non-destructive analyses were conducted on fully expanded leaves placed at the second to last node after 21 days of treatment. Biometric measurements and leaves samplings took place after 28 days, while anatomical and histochemical analyses were performed after 49 days from the beginning of LED light supplementation. Leaf samples were cryogenised in liquid nitrogen and stored at −80 °C until metabolomics analyses were performed.

2.2. Biometric features

Plant height, number of leaves and mean internode length were measured (n = 3). Fresh weight (n = 3) was obtained by weighing the whole aerial part of the plant. The dry matter (n = 3) was achieved by drying the aerial part at 105 °C in a thermostatic oven (Memmert GmbH + Co. KG Universal Oven UN30, Schwabach, Germany) until a constant weight. Single leaf area (LA; n = 5) was measured using ImageJ software (version 1.52t).

2.3. Anatomical and histochemical analysis

Stomatal count per unit area of leaf surface was performed (n = 30) on impressions of leaf epidermis. Clear nail varnish was applied on the abaxial leaf surface of developed leaves from 3 sample plants for each treatment, and then was allowed to dry. Subsequently, an adhesive tape was used to remove the nail varnish and placed on a glass slide to examine the obtained epidermal leaf impression at × 250 magnification using a Leitz Diaplan optical microscope (Wild Leitz, Wetzlar, Germany). For each impression, at least 10 random microscope fields were analysed and captured by a Leica DCF420 microscope camera with 5-megapixel CCD (Leica, Wetzlar, Germany), with a visual field of 153 mm^2 (340 μm × 450 μm, measured with a slide equipped with a micrometric scale). Stomatal density has been reported as the number of stomata per mm^2 (Bogani et al., 2015). Foliar thickness (n = 10) was measured through foliar sections obtained manually from 5 sample plants for each treatment, mounted with water on slides and examined using a Leitz Diaplan optical microscope (as previously mentioned). Acquisitions were performed with a Leica DCF420 microscope camera (as previously reported) and thickness was measured with ImageJ software. *In situ* qualitative assay of H_2O_2 (n = 3) was performed according to Daudi and O'Brien (2012) on 1 cm-diameter leaf discs of obtained with a hole punch. Samples were placed in 1 mg mL^{-1} 3, 3′-diaminobenzidine (DAB)-HCl followed by vacuum infiltration for 20 min. After that, the discs were incubated overnight at room temperature in the dark. The next day, in order to remove chlorophylls, discs were treated with 96% ethanol in a water bath at 65 °C for 3 h, and ethanol was replaced when needed. Then, samples were washed in 30% ethanol and mounted on slides with 30% glycerol (Menicagli et al., 2022). DAB was oxidized by H_2O_2 in the presence of heme-containing proteins and generated a dark brown precipitate. The presence of H_2O_2 was observed with a WILD Heerbrugg M420 (Leica, Wetzlar, Germany) stereoscopic microscope equipped with a Canon PowerShot S45 (Canon Europa N.V., Amstelveen, The Netherlands) camera. Staining method for this oxidative stress marker were combined with image processing to estimate DAB staining intensity on digitalized leaf image by ImageJ software as arbitrary unit.

2.4. Gas exchange and chlorophyll (Chl) a fluorescence analysis

Gas exchange measurements (n = 6) were performed using a portable infrared gas analyzer LI-6400 system (Li-Cor, Lincoln, NE, USA) on randomly selected fully expanded leaves from 11:00 a.m. to 1:00 p.m. at ambient light intensity (800 μmol photons m^{-2} s^{-1}). Using the CO_2 mixer, the CO_2 concentration inside the leaf chamber was set at 400 μmol mol^{-1}, and the flow rate was 500 μmol s^{-1}. Once the steady state was reached, the following parameters were recorded: net photosynthetic rate (P_N), stomatal conductance to water vapor (g_s), and intercellular CO_2 concentration (C_i). Apparent carboxylation efficiency (P_N/C_i) and intrinsic water use efficiency ($WUE_i = P_N/g_s$) were also calculated. Chl *a* fluorescence parameters were measured from 11:00 a.m. to 2:00 p.m. with a PAM-2000 fluorometer (Walz, Effeltrich, Germany) in leaves consistent with those where gas exchange analysis was performed. Leaves (n = 4) were dark-adapted for 30 min, and the following parameters were recorded: maximum quantum yield of PSII photochemistry (F_v/F_m), the effective quantum yield of PSII photochemistry (Φ_{PSII}), quantum yield of non-regulated non-photochemical energy loss in PSII (Φ_{NO}), the quantum yield of non-photochemical quenching (Φ_{NPQ}), and electron transport rate of PSII (ETR) (Lo Piccolo et al., 2020).

2.5. Chl content, flavones and anthocyanin indexes and nitrogen balance index (NBI)® analysis

Measurements (n = 28) were performed using a DUALEX® Scientific analyser (Force-A, Orsay, France). Chl were expressed as μg cm^{-2}. Flavones (Flav) and anthocyanins (Anth) were expressed as indexes, while NBI® was a good estimator of leaf nitrogen content and was extrapolated from Chl and Flav indexes (as indicated by the instrument manual).

2.6. Polyphenol extraction and analysis

One hundred milligrams of freeze-dried leaf samples were accurately weighed into a 15-mL plastic tube and extracted with 8 mL of 80% (v/v) aqueous methanol solution in 3 biological replicates. Samples were rotated with a vertical multi-function rotator for 20 min and sonicated for 30 min. After 48 h in the dark at 4 °C, samples were centrifuged at 1000 × g and 4 °C for 10 min. The resulting supernatants were collected and filtered through a 0.22 μm polyvinylidene difluoride filter. The targeted analysis of polyphenol compounds was performed using the LC-MS/MS method, coupled with multiple reaction monitoring (MRM) quantification according to Vrhovsek et al. (2012). The same samples were analyzed for anthocyanins as described by Arapitsas et al. (2012) on a RP Acquity UPLC® BEH C18 column (130 Å, 2.1 × 150 mm, 1.7 μm, waters), protected with an Acquity UPLC® BEH C18 pre-column (130 Å, 2.1 × 5 mm, 1.7 μm, waters).

2.7. Solid phase microextraction and analysis

For headspace-solid phase microextraction (HS-SPME), a Supelco SPME devices coated with polydimethylsiloxane (100 μm; Sigma-Aldrich) was used. SPME sampling was performed (n = 3) using the same fiber, preconditioned according to the manufacturer instructions. Fully expanded leaves were collected all around the upper nodes of plants, immediately inserted into a 100 mL glass conical flask, capped with aluminum foil, and then allowed to equilibrate for 30 min. After the equilibration time, the fiber was exposed to the headspace for 25 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the gas chromatography-mass spectrometry (GC–MS) system. GC–MS analyses were performed with an Agilent 7890B gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an Agilent HP-5MS capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and an Agilent 5977B single quadrupole mass detector. Injector and transfer line temperatures were 250 and 240 °C, respectively. The oven temperature was programmed from 60 to 240 °C at 3 °C min^{-1} ramp, with helium as the carrier gas (flow rate 1 mL min^{-1}) using the splitless injection mode. The acquisition parameters were: full scan; scan range: 30–300 m/z; scan time: 1.0 s. The constituents were identified by comparing the retention times with those of the standard samples and

their linear retention indices relative to a series of *n*-hydrocarbons; experimental mass spectra were matched against a commercial (NIST 2014 and ADAMS) and homemade library of mass spectra built up from pure substances and components of known essential oils and the literature data (Adams, 1995). Quantitative comparisons of relative peaks areas (%) were performed between the same chemicals in the different samples.

2.8. Statistical analysis

All data, except for H_2O_2 staining, were subjected to *Bartlett's* test to evaluate their homoscedasticity, while *Shapiro-Wilk* test was used to evaluate the normal distribution of data. Data were subjected to one-way analysis of variance (*ANOVA*) considering light treatment as the only source of variability. Significant differences between light treatments were determined by Fisher's least significant difference (LSD) *post-hoc* test ($p \leq 0.05$). Statistical analyses were performed using GraphPad (GraphPad, La Jolla, CA, USA). The complete compositions of the HSs of TI and RR samples were subjected to multivariate statistical analysis with Principal Component Analysis (PCA) and the Hierarchical Cluster Analysis (HCA) using JMP Pro 16 (SAS Institute Inc., Cary, NC, USA). The covariance data matrices were a 48 × 5 (48 compounds × 5 samples = 240 data) and a 46 × 5 (46 compounds × 5 samples = 230 data) matrices for TI and RR, respectively. The principal component analysis (PCA) was performed selecting the two highest principal components (PCs) obtained by the linear regressions operated on mean-centered, unscaled data; the chosen PC1 and PC2 for TI covered 76.80 and 21.50% of the variance, respectively, for a total explained variance of 98.30%, while for RR 84.50% and 12.00%, respectively, for a total variance of 96.50%. The hierarchical cluster analysis (HCA) was performed using the Ward's method, with squared Euclidean distances as a measure of similarity.

3. Results

3.1. Biometric features

Light supplementation did not influence the main biometric characteristics of both the basil cultivars compared to NS plants (Table 1). However, LA was affected by light supplementation. In TI, LA decreased under G and B light supplementation compared to NS (−15.5 and −21.9%, respectively), while in RR all the light treatments promoted an increment in LA (up to +61.6% with B light).

Table 1
Total biomass, number of leaves, mean internode length, plant height, plant dry matter and single leaf area (LA) measured in plants of *Ocimum basilicum* L. cv. 'Tigullio' and 'Red Rubin'. Treatments were non supplemented (NS), polychromatic light (W – R:G:B; 1:1:1), narrowband green (G), blue (B) and red (R) light supplementation. Means (n = 3 ± SD except for LA in which n = 5 ± SD) with different letters are significantly different after one-way ANOVA followed by Fisher's LSD *post-hoc* test ($P \leq 0.05$) considering light treatment as the only source of intra-cultivar variability. Significant differences among treatments are given according to P level (* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$; ns = not significant).

	Total fresh biomass [g]	Number of leaves	Mean internode length [cm]	Height [cm]	Dry matter [%]	LA [cm^2]
cv. 'Tigullio'						
NS	32.44 ± 0.92	50.7 ± 3.1	7.23 ± 0.24	37.40 ± 0.72	7.35 ± 0.12^{ab}	70.49 ± 6.02^{a}
W	39.68 ± 2.49	62.0 ± 7.2	7.47 ± 0.72	41.30 ± 1.04	7.49 ±0.13^{ab}	73.67 ± 5.58^{a}
G	36.36 ± 3.53	56.3 ± 3.5	7.33 ± 0.45	40.53 ± 3.07	7.67 ± 0.07^{a}	59.54 ± 5.02^{b}
B	31.56 ± 6.12	51.3 ± 2.3	7.07 ± 0.66	38.30 ± 3.70	7.64 ± 0.34^{a}	55.08 ± 1.62^{b}
R	36.15 ± 2.86	58.0 ± 9.2	7.63 ± 0.43	41.33 ± 3.26	7.16 ± 0.13^{b}	74.49 ± 6.49^{a}
P	ns	ns	ns	ns	*	***
cv. 'Red Rubin'						
NS	14.435 ± 2.38	54.0 ± 6.9	4.53 ± 0.16	29.47 ± 1.00	6.90 ± 0.10	20.96 ± 2.08^{c}
W	16.66 ± 1.26	68.7 ± 4.2	4.70 ± 0.10	31.20 ± 1.05	7.05 ± 0.08	29.19 ± 6.77^{ab}
G	16.71 ± 4.64	60.7 ± 10.3	4.83 ± 0.06	32.47 ± 1.40	6.96 ± 0.11	29.01 ± 1.94^{ab}
B	16.83 ± 1.31	64.0 ± 7.2	4.57 ± 0.25	30.43 ± 1.75	6.92 ± 0.34	33.87 ± 6.37^{a}
R	15.91 ± 2.76	69.3 ± 8.3	4.87 ± 0.29	32.77 ± 2.77	7.07 ± 0.05	27.85 ± 2.71^{b}
P	ns	ns	ns	ns	ns	**

3.2. Anatomical and histochemical analysis

In TI, W, G and B light treatments resulted in a significant increase in stomatal number compared to NS and R treatment (Fig. 1A). On the contrary, in RR cultivar (Fig. 1B), W light did not to alter the stomatal number; with G and B light supplementation red-leafed plants had a greater stomata number (+36.6 and +28.7%, respectively), while R light resulted in a reduced number of stomata (Fig. 1B) as compared with NS. All the light treatments enhanced leaf thickness compared to NS plants, both in TI and RR (Fig. 1C and D) and the highest results were obtained with B light (+31.6% and +60.3% in TI and RR, respectively). In both TI and RR plants. DAB responsiveness, indicative of H_2O_2 formation, was similar for both intensity and distribution in leaves under different treatments (Fig. 2A andB).

3.3. Gas exchange analysis

P_N in TI was not significantly influenced by light supplementation except under R treatment, which significantly decreased the net photosynthetic rate (−18.1%) compared to NS individuals (Fig. 3A). In RR, P_N was significantly influenced only by G supplementation (−9.0%) compared to the other treatments (Fig. 3B). Stomatal conductance was greater with W and B supplementation (+18.8 and +18.3%, respectively) and significantly decreased under G treatment (−21.6%) in green-leafed basil compared to NS plants. At the same time, R light did not influence g_s values (Fig. 3C). Similarly, in RR, g_s increased with B supplementation (+23.5%) and decreased with G treatment (−27.8%) compared to NS (Fig. 3D). Intercellular CO_2 concentration increased in TI with W, B and R lights and decreased with G light supplementation compared to control (Fig. 3E), while in RR C_i increased with B light and decreased under G treatment (Fig. 3F). In green-leafed basil, R light significantly decreased P_N/C_i compared to control (−33.3%), while no other treatment influenced this parameter (Fig. 3G). In RR P_N/C_i did not show significant differences compared to NS (Fig. 3H). In TI, WUE decreased under W, B and R light (up to −22.4% in R treatment), while significantly increased under G treatment (Fig. 3I). Similarly, W and B lights decreased as well as G light increased WUE (+26.9%) in RR (Fig. 3L). R light supplementation in RR showed no variation in any gas exchange parameters compared to control.

3.4. Chl a fluorescence analysis

Fig. 4 shows the kinetics Chl *a* fluorescence results for TI and RR (left and right column, respectively). In TI plants, no variations in F_v/F_m values were observed compared to NS individuals (F_v/F_m = 0.79; Fig. 4A), even if the highest value was reached under B light supplementation (F_v/F_m = 0.83). F_0 decreased significantly only in B-supplemented plants and F_m showed no significant differences among the

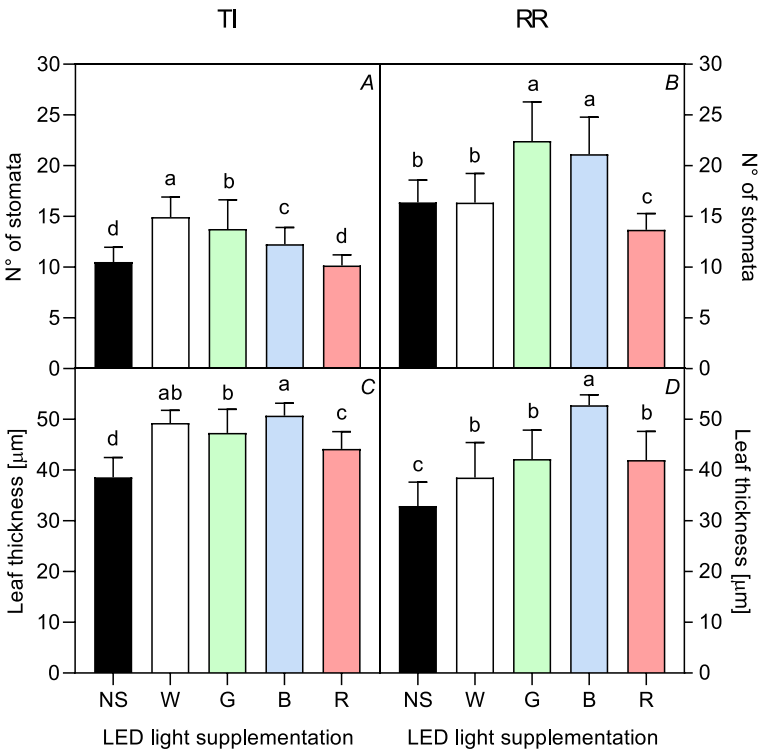

Fig. 1. Stomatal density (A, B) and leaf thickness (C, D) of leaves of *Ocimum basilicum* L. cv. 'Tigullio' (TI; left column) and cv. 'Red Rubin' (RR; right column) leaves. Treatments were non supplemented (NS), polychromatic light (W – R:G:B; 1:1:1), narrowband green (G), blue (B) and red (R) lights supplementation. Means (n = 30 ± SD for stomatal number and n = 10 ± SD for leaf thickness) with different letters are significantly different after one-way ANOVA followed by Fisher's LSD *post-hoc* test ($P \leq 0.05$) considering light treatment as the only source of intra-cultivar variability.

treatments (data not shown). In green-leafed basil plants, ETR was enhanced only with B light supplementation (+24%) compared to NS (Fig. 4C). Values of Φ_{PSII} significantly decreased under G light, while B treatment increased this parameter and consequently a decrease in Φ_{NPQ} values was found (Fig. 4E and G). Conversely, RR cultivar appeared less responsive to different light supplementation at the photochemical level. In these plants, only a decrease in Φ_{PSII} values were measured after B and R light supplementation (Fig. 4F) as compared with the NS individuals, whilst no variation occurred in Φ_{NO}, Φ_{NPQ}, ETR nor F_v/F_m values (Fig. 4B,D,H and L).

3.5. Chlorophyll concentration, flavone, anthocyanin, and nitrogen balance index (NBI)®

TI cultivar did not show any variation in Chl concentration, Flav index and NBI® induced by light supplementation (Table 2). In RR, instead, a significant increase in Chl content was observed in all the supplemented plants (regardless of light color) compared to NS individuals, with the highest amount detected in R-supplemented plants (+20.4%). NBI® paralleled Chl trend and showed the highest value with R light (+24.2%) followed by W treatment (+6.7%); values in G- and B-supplemented plants did not differ from those in NS individuals. Flav index was enhanced by W and B light in red-leafed basil, while only B light promoted the increase in Anth index compared to NS plants (+12.8%).

3.6. Polyphenolic profile

Polyphenol analysis allowed the identification of seven polyphenol derivatives namely caffeic, caftaric, rosmarinic and fertaric acids, quercetin- and kaempferol-3-O-glucoside(glc) and rutin (quercetin-3-O-rutinoside) and three anthocyanin derivatives i.e. cyanidin(Cy)3-(*p*-coumaroyl)-glc (Cy 3-coum-glc), Cy 3-O-arabinoside and Cy 3-(malonyl)-glc (Tables 3 and 4). Flavonoids quercetin 3-glc and kaempferol 3-glc were not detected in TI plants (Table 3). In TI no significant differences among the treatments were detected in rosmarinic acid content, which is the major phenolic acid in basil leaves. The amount of caffeic acid improved with G and B treatments (2.8- and 1.6-fold increase, respectively) compared to NS, whereas caftaric acid content improved under G, W and B lights (up to +40.4% under W treatment). In comparison, rutin content was incremented under W and B lights treatments (1.2- and 1.0-fold increase, respectively) compared to NS (Table 3). No differences emerged in fertaric acid content. Among all these compounds, in RR cultivar, light supplementation affected caffeic and caftaric acids (Table 4). Caffeic acid decreased under G and R treatment (−60.7 and −55.8% respectively) compared to NS, while caftaric acid increased only under B light (+49.5%). Fertaric acid was not detected in RR cultivar. Among the identified anthocyanin derivatives, only Cy 3-coum-Glc was affected by light treatment, showing a decrease in its content under G treatment (−16%).

3.7. VOC profile

The complete composition of TI and RR plants HS-SPME are reported in Tables 5 and 6 (and Supplementary material, Figs. S1 and S2, respectively), respectively. TI samples were all characterized by sesquiterpene hydrocarbons (SHs) as major chemical class, mainly represented by *trans*-α-bergamotene (Table 5). The samples radiated with G and R lights had the highest percentages of SHs, as they accounted for 61.1 and 57.1% of the whole compositions, respectively, while the W-radiated one the lowest (46.8%). However, good amounts of these compounds were also found in the control plants NS (54.5%) and in those radiated with B light (54.0%). Oxygenated monoterpenes (OMs) was the second most represented chemical class of compounds in all the TI samples, despite they reached the highest content in the plants radiated with W (38.5%) and B (33.5%) lights (Table 5). Among this class, linalool was the prevalent component, even though good amounts of 1,8-cineole were also detected. The hydrocarbon form of monoterpenes (MHs) was also found in appreciable percentages, as it was comprised between 5.7 and 9.3% in B and W, respectively (Table 5). Finally, phenylpropanoids (PPs) represented an important chemical class, and the only detected compounds within this group were eugenol and methyl eugenol (Table 5). From a quantitative point of view, these secondary metabolites showed a relevant variability among the TI

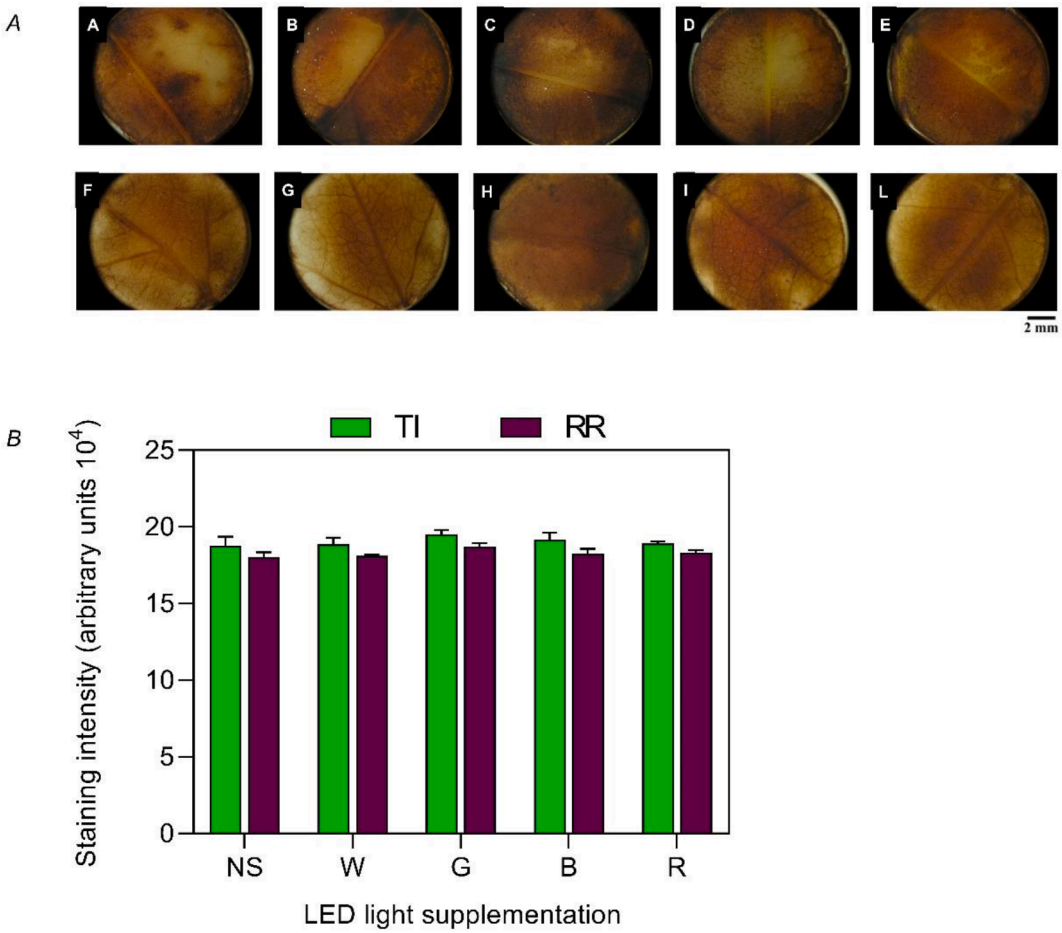

Fig. 2. Representative leaf discs of *Ocimum basilicum* L. cv. 'Tigullio' (A-E) and cv. 'Red Rubin' (F-L) after the qualitative assay of H_2O_2. Treatments were non supplemented (NS), polychromatic light (W – R:G:B; 1:1:1), narrowband green (G), blue (B) and red (R) lights supplementation. Dark brown areas represent H_2O_2 leaf oxidation. B. Quantification of histochemical detection of H_2O_2. The graphs represent means ± SD of three independent experiments. Data, analysed by one-way ANOVA followed by Tukey *post-hoc* test for multiple comparison considering light treatment as the only source of intra-cultivar variability, were statistically not different.

samples, as they accounted for 19.0% in NS, while LED light supplementations determined their significant reduction, mostly at the expense of eugenol. Considering the changes induced by LED light supplementations even for the other chemical classes, MHs did not significant change under any type of LED light supplementation. Oxygenated monoterpenes, instead, greatly increased with W and B light, conversely to sesquiterpene hydrocarbons, which, instead, increased with G and R lights and decreased with W one. In fact, analysing the most representative compounds, 1,8-cineole was enhanced under W light, linalool was spurred under W and B lights and myrcene amount increased under W lights; *trans*-α-bergamotene, instead, increased under G light. Concerning phenylpropanoids, eugenol was negatively influenced by LED light supplementation.

Plants of RR were characterized by phenylpropanoids as main chemical class in all the samples, with the only exception of W one, in which SHs prevailed (Table 6). PPs were comprised between 26.1 and 49.9% in W and R, respectively, while methyl eugenol between 24.8 and 47.9%. The latter compound in TI was detected in very low percentages in only two samples (see Table 5). Within this class, eugenol was also found in all the RR samples, in amounts ranging from 0.4 to 2.6%, while methyl chavicol was detected only in NS in negligible percentages (0.1%) (Table 6). SHs, as well, were well represented: the lowest percentage was found in G, with 27.2%, while the highest in W (46.0%). The main component belonging to this class were β-elemene, β-caryophyllene, and *(E)*-β-farnesene (Table 6). Finally, OMs were characterized in appreciable relative amounts in all the RR samples (18.8 - 30.1%) and, similarly to TI, linalool and 1,8-cineole were the most represented component belonging to this class. Monoterpene hydrocarbons and non-terpene derivatives, both found in low amounts, were the only class influenced by LED light supplementations, as opposed to all the other classes, which instead were not significantly affected. Focusing on the most important volatiles (Table 6), 1,8-cineole, linalool, (E)-*β*-farnesene and myrcene, as well as eugenol and methyl eugenol, did not significantly change among the treatments, while *trans*-*α*-bergamotene enhanced under W light.

The complete compositions of the HSs of both TI and RR plants were subjected to multivariate analysis in order to evaluate the distribution of the samples based on their similarities and dissimilarities. The score plot of the Principal Component Analysis (Fig. 5A) performed on TI samples revealed that B and W, positioned in the bottom left quadrants (PC1 and PC2 < 0), were completely separated from NS, whose positioning in the bottom right quadrant (PC1 > 0; PC2 < 0) was attributable to the eugenol vector (Fig. 5B). Conversely, G and R, despite they were projected in the upper right quadrant (PC1 and PC2 > 0), partially overlapped NS. The same partitioning was also evidenced by the dendrogram of the HCA (Fig. 6A), which showed two main clusters: the red group comprising samples B and W, and a second cluster made up by two subgroups, more similar each other: the green one comprising G and R, and the blue one constituted by NS.

Both HCA and PCA performed on the HSs compositions of RR plants,

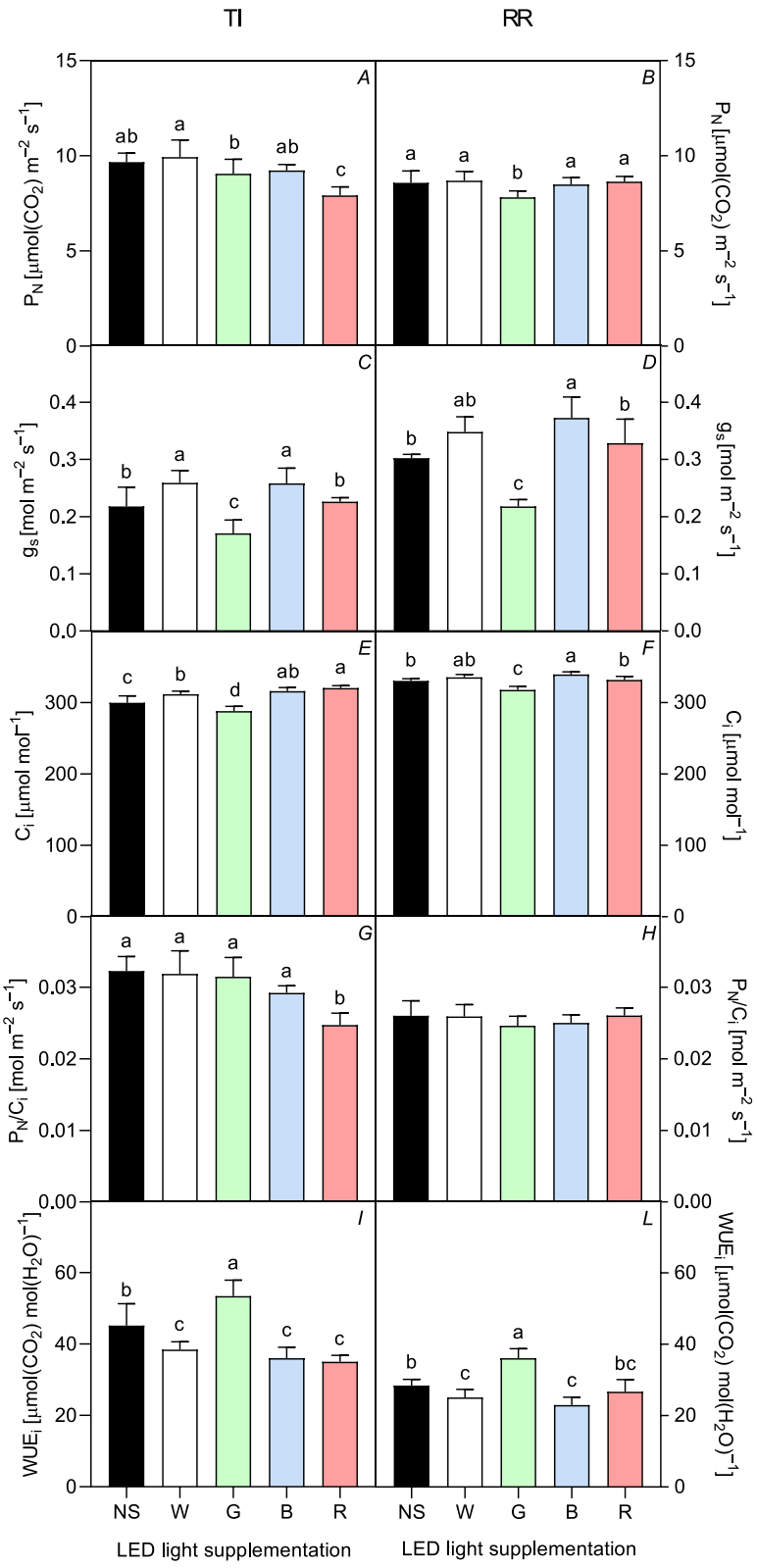

Fig. 3. Net photosynthetic rate (P_N; A, B), stomatal conductance (g_s; C, D), intercellular CO_2 concentration (C_i; E, F), carboxylation efficiency (P_N/Ci; G, H), intrinsic water-use efficiency (WUEi; I, L) of *Ocimum basilicum* L. cv. 'Tigullio' (TI; left column) and cv. 'Red Rubin' (RR; right column). Treatments were non supplemented (NS), polychromatic light (W – R:G:B; 1:1:1), narrowband green (G), blue (B) and red (R) lights supplementation. Means (n = 6 ± SD) with different letters are significantly different after one-way ANOVA followed by Fisher's LSD *post-hoc* test (P≤0.05) considering light treatment as the only source of intra-cultivar variability.

instead, showed that W light strongly influenced the chemical composition. In fact, in the score plot of the PCA (Fig. 5C) W was plotted in the bottom left quadrant, clearly far from NS, which instead, was located in the bottom right one together with B and R. Moreover, G showed a certain similarity to NS, despite it was located in a different quadrant. This similarity, moreover, was confirmed by the dendrogram of the HCA as well (Fig. 6B), which grouped G together with NS, B, and R in the red cluster. Even in the dendrogram, W sample constituted a cluster by itself, the green one, evidencing great differences in the chemical composition.

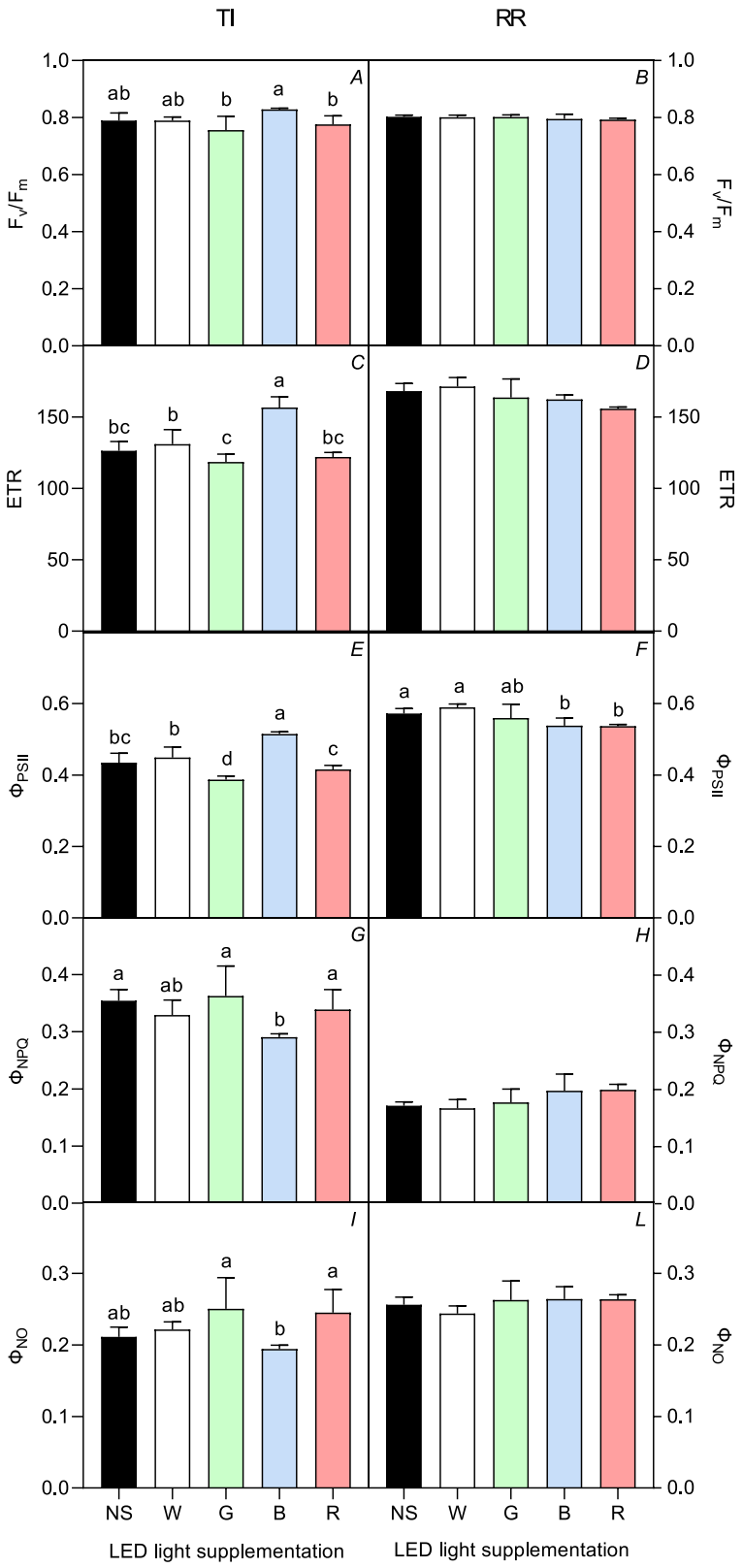

Fig. 4. Maximum quantum yield of primary PSII photochemistry (F_v/F_m; A, B), electron transport rate of PSII (ETR; C; D), effective quantum yield of PSII photochemistry (Φ_{PSII}; E, F), quantum yield of nonphotochemical quenching (Φ_{NPQ}; G, H) and quantum yield of non-regulated non-photochemical energy loss in PSII (Φ_{NO}; I, L) in *Ocimum basilicum* L. cv. 'Tigullio' (TI; left column) and 'Red Rubin' (RR; right column). Treatments were non supplemented (NS), polychromatic light (W – R:G:B; 1:1:1), narrowband green (G), blue (B) and red (R) lights supplementation. Means (n = 4 ± SD) with different letters are significantly different after one-way ANOVA followed by Fisher's LSD *post-hoc* test ($P \leq 0.05$) considering light treatment as the only source of intra-cultivar variability.

4. Discussion

It has been well established that light quality affects vital processes of the plant influencing its proper physiological development. Photons perception/absorption promotes plant photomorphogenesis and growth through the stimulation of photoreceptors and the associated signaling pathways, inducing changes in gene expression and resulting in the variation of secondary metabolite biosynthesis (Folta and Carvalho, 2015; Thoma et al., 2020). Considering this, typically indoor-cultivated crops can benefit from an accurate understanding of the waveband-dependent responses as both plant growth and bioactive compound biosynthesis can be controlled by manipulating the light

Table 2
Chlorophyll (Chl) content, Nitrogen Balance Index (NBI®), flavone (Flav) and anthocyanin (Anth) indexes measured in plants of *Ocimum basilicum* L. cv. 'Tigullio' and 'Red Rubin'. Treatments were non supplemented (NS), polychromatic light (W – R:G:B; 1:1:1), narrowband green (G), blue (B) and red (R) light supplementation. Means (n = 28 ± SD) with different letters are significantly different after one-way ANOVA followed by Fisher's LSD *post-hoc* test (P≤0.05) considering light treatment as the only source of intra-cultivar variability. Significant differences among treatments are given according to P level (** = P<0.01; *** = P<0.001; ns = not significant).

	Chl [μg cm^{-2}]	NBI®	Flav	Anth
cv. 'Tigullio'				
NS	20.95 ± 3.08	23.57 ± 4.44	0.91 ± 0.13	–
W	21.60 ± 3.38	21.95 ± 4.29	1.00 ± 0.15	–
G	21.93 ± 2.85	25.20 ± 4.32	0.89 ± 0.16	–
B	20.90 ± 2.98	23.95 ± 7.11	0.92 ± 0.19	–
R	21.54 ± 2.91	24.99 ± 5.27	0.89 ± 0.17	–
P	ns	ns	ns	–
		cv. 'Red Rubin'		
NS	30.40 ± 3.87^{c}	51.32 ± 6.90^{c}	0.59 ± 0.03^{b}	0.39 ± 0.04^{b}
W	33.57 ± 3.35^{b}	54.78 ± 6.89^{b}	0.62 ± 0.05^{a}	0.43 ± 0.05^{ab}
G	32.70 ± 3.15^{b}	54.48 ± 5.83^{bc}	0.60 ± 0.05^{ab}	0.41 ± 0.04^{b}
B	32.99 ± 3.65^{b}	53.42 ± 6.74^{bc}	0.62 ± 0.05^{a}	0.44 ± 0.04^{a}
R	36.63 ± 2.61^{a}	63.74 ± 5.57^{a}	0.58 ± 0.04^{b}	0.39 ± 0.03^{b}
P	***	***	**	***

Table 3
Concentration (μg g^{-1} of DW) of polyphenol compounds measured in leaves of *Ocimum basilicum* L. cv. 'Tigullio'. Treatments were non supplemented (NS), polychromatic light (W – R:G:B; 1:1:1), narrowband green (G), blue (B) and red (R) lights supplementation. Means (n = 4 ± SD) with different letters are significantly different after one-way ANOVA followed by Fisher's LSD *post-hoc* test (P≤0.05) considering light treatment as the only source of variability. Significant differences among treatments are given according to P level (** = P<0.01; *** = P<0.001; ns = not significant). Hyphens represented non detected compounds.

	NS	W	G	B	R	P
Polyphenol						
Caffeic acid	80.0 ± 23.6^{c}	132.0 ± 90.2^{bc}	300.0 ± 127.6^{a}	210.0 ± 49.5^{ab}	120.0 ± 55.8^{bc}	**
Caftaric acid	416.0 ± 45.7^{c}	584.0 ± 55.8^{a}	526.0 ± 18.9^{ab}	486.0 ± 33.5^{b}	400.0 ± 28.5^{c}	***
Rosmarinic acid	9572.0 ± 364.4	8718.0 ± 1203.4	10,326.0 ± 779.7	8580.0 ± 1390.7	8170.0 ± 998.6	ns
Quercetin-3-Glc	–	–	–	–	–	–
Fertaric acid	38.0 ± 7.7	46.0 ± 7.7	46.0 ± 4.0	36.0 ± 4.6	42.0 ± 4.0	ns
Rutin	12.0 ± 4.6^{b}	26.0 ± 4.0^{a}	16.0 ± 0.0^{b}	24.0 ± 6.5^{a}	16.0 ± 0.0^{b}	***

spectral composition (Folta and Carvalho, 2015; Landi et al., 2020).

Since the effects of different wavebands on plants are not always consistent among species or genotypes (Landi et al., 2020), plant reaction to light supplementation cannot be considered as exhaustively elucidated. In addition, most studies focused on plant reaction to monochromatic environment, which cannot necessary be consistent with the effect induced by monochromatic enrichment of ambient light. In the present study, therefore, light-waveband dependent morpho-metric, physiological and biochemical responses of the two basil cultivars differing in leaf pigmentation are depicted.

Generally, monochromatic LED light supplementation can variably affect plant growth in different species (Olle and Viršile, 2013), as well as in sweet basil grown under sub-optimal light conditions (Matysiak and Kowalski, 2019). Despite that, in the present experiment no significant differences were observed among light treatments in both the cultivars compared to NS on biometrical features, except for LA and leaf thickness. Supporting our results for TI plants, Lin et al. (2021) reported that major blue and green percentages decreased LA in green basil (cv. '*Caesar*') compared to illumination with higher red percentages. Loughrin and Kasperbauer (2001) obtained similar results in basil cultivated under coloured mulches. According to the literature, red light increased LA or leaf length in red-leafed basil both under a monochromatic environment and greenhouse supplementation (Matysiak and Kowalski, 2019; Lin et al., 2021). In the present experiment, although R light enhanced LA in RR, the highest value was obtained when plants were supplemented with B light. Instead, leaf thickening occurred regardless of the treatment in both TI and RR. This could be due to an increased light condition compared to NS rather than a variation of light quality as, in fact, the increase in light quantity generally increase the leaf thickness (Nobel et al., 1975), probably through an increase in mesophyll cell size and the amount of mesophyll tissue (Chabot and Chabot, 1977). Accordingly, sweet basil plants (irrespectively to the leaf color) showed the highest leaf thickness when supplemented with B light, as also reported for two other species, namely *Ficus benjamina* and *Pelargonium zonale* (Fukuda et al., 2008; Zheng and Van Labeke, 2017).

At physiological level, the significant reduction in P_N values, observed only in TI plants that grew under the R light supplementation, was due to biochemical limitations, as suggested by the reduction in the apparent carboxylation efficiency (P_N/C_i). Our data conformed to the study conducted on *Cucumis sativus* by Wang et al. (2009), who found that plants grown with red light showed a decline of CO_2 carboxylation in leaves, resulting in lower P_N values compared to blue and white light treatments. Indeed, it was observed that plants grown under a pure red-light environment often show severe impairments in the CO_2 assimilation components, such as low Rubisco content (Muneer et al., 2014), and reduced maximum carboxylation efficiency (Trouwborst et al., 2016): the so-called 'red light syndrome' (Trouwborst et al., 2016; Landi et al., 2020). This is due to a lack of the synergistic effect of phytochromes, cryptochromes, and phototropins, e.g. high stimulation of phytochrome due to inadequate far-red light (Landi et al., 2020). Differently, in RR cultivar, only G light supplementation significantly reduced P_N values, mainly due to stomatal limitations. To the best of our knowledge, literature about the effect of green light supplementation on photosynthesis in anthocyanin-rich species is very scarce but, similarly to our results, Dou et al. (2019) observed a significant decrease in P_N, associated with a reduction in g_s, in red-leafed basil plants grown under a high proportion (45%) of green light. However, it is worth noting that the stomatal closure response induced by G light was observed in both TI and RR plants in our experiment. Frechilla et al. (2000) and Talbott et al. (2006) reported that the stomatal opening is regulated predominantly by a photosynthesis-independent mechanism based on the green/blue light antagonism, in which green light is able to reverse the blue light effects on the stomatal opening response (Trojak et al., 2022). Furthermore, it was observed in lettuce (Muneer et al., 2014) and cherry tomato (XiaoYing et al., 2011), that green light also reduced the stomatal density. Conversely, our dataset is supportive for a positive effect exerted by G light in terms of stomatal number, as also reported in tomato and basil (Lim and Kim, 2021; Trojak et al., 2022), when green waveband was added as 10 or 40% of the total spectrum respectively. Contrasting reports about the stomatal density responses under green light supplementation may be due to the different growing conditions (e.g., in our experiment, plants were exposed to the complete light spectrum, supplied with different wavebands). The stomata are crucial passages for gas exchanges between the leaf and the external environment. The increase in g_s has often been associated with a decrease in water use efficiency, with a consequent increase in water waste (Clavijo-Herrera et al., 2018). The lower g_s values observed under G light supplementation were associated with increased values in the water use efficiency (WUE_{int}) in both cultivars. Reduction in g_s and increasing values in WUE_{int} in green-supplemented plants can be useful to plants to tolerate drought stress periods (Bian et al., 2019), utilizing water more efficiently per CO_2 assimilated than non-supplemented plants (Trojak

Table 4
Concentration (μg g^{-1} of DW) of polyphenol compounds and anthocyanins measured in leaves of *Ocimum basilicum* L. cv. 'Red Rubin'. Treatments were non supplemented (NS), polychromatic light (W – R:G:B; 1:1:1), narrowband green (G), blue (B) and red (R) lights supplementation. Means (n = 3 ± SD) with different letters are significantly different after one-way ANOVA followed by Fisher's LSD *post-hoc* test (P≤0.05) considering light treatment as the only source of variability. Significant differences among treatments are given according to P level (* = P<0.05; ns = not significant). Hyphens represented non detected compounds.

	NS	W	G	B	R	P
Polyphenol						
Caffeic acid	162.7 ± 92.0^{a}	191.0 ± 43.5^{a}	64.0 ± 21.2^{b}	178.7 ± 41.1^{a}	71.5 ± 22.0^{b}	*
Caftaric acid	106.7 ± 9.2^{b}	106.7 ± 12.2^{b}	133.3 ± 32.3^{ab}	160.0 ± 32.0^{a}	95.0 ± 8.2^{b}	*
Rosmarinic acid	12,472.0 ± 1203.0	11,781.3 ± 1749.1	13,664.0 ± 1121.5	12,269.3 ± 1057.7	12,284.7 ± 287.0	ns
Quercetin-3-Glc	34.7 ± 12.2	45.3 ± 4.6	29.3 ± 12.2	48.0 ± 16.0	30.6 ± 2.4	ns
Fertaric acid	–	–	–	–	–	–
Kaempferol-3-Glc	13.3 ± 4.6	21.3 ± 4.6	21.3 ± 9.2	18.7 ± 4.6	14.2 ± 5.5	ns
Rutin	18.7 ± 4.6	32.0 ± 8.0	26.7 ± 4.6	32.0 ± 8.0	19.5 ± 4.1	ns
Antocyanin						
Cy-3-coum-Glc	66.7 ± 4.6^{ab}	72.0 ± 8.0^{a}	56.0 ± 0.0^{c}	58.7 ± 4.6^{bc}	64.0 ± 0.0^{abc}	*
Cy 3-arab	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	5.3 ± 4.6	8.0 ± 0.0	ns
Cy 3-malonyl-Glc	13.3 ± 4.6	16.0 ± 0.0	13.3 ± 4.6	13.3 ± 4.6	16.0 ± 0.0	ns

et al., 2022). Moreover, this feature can potentially reduce the water losses of irrigated crops, an aspect of primary importance in the climate change era (Gago et al., 2014).

Regarding the effects of the other light supplementations on stomatal conductance, in both cultivars the observed increase in g_s values in plants subjected to B treatment, is a well-known physiological phenomenon (Wang et al., 2009; Clavijo-Herrera et al., 2018). Blue light is reported to be the driving waveband that affects guard cells regulating stomatal aperture (Assmann, 1988). The higher g_s values found in W compared to NS treatment in TI were likely due to the B wavebands contribution in the W light. Furthermore, increased g_s values were also often correlated with increased stomatal density (Hogewoning et al., 2010; XiaoYing et al., 2011), and, in our experiment, B treatment increased stomatal number in both the cultivars. However, the increase in g_s values without a parallel increase in P_N reduced the WUE_{int}. This reduction, exhibited by both the cultivars, should be considered a negative effect of light supplementation, since W and B lights induced a higher water consumption than NS plants. Further investigation should examine the cost-benefit analysis of light supplementation treatment, and WUE_{int} reduction should be considered.

Chlorophyll *a* fluorescence, coupled with other technique as gas exchange analysis, was a powerful tool to study photosynthesis and monitoring plant stresses (Kalaji et al., 2017). In the case of different stresses not all the amount of absorbed light can be efficiently converted into chemical energy inducing a reduction of F_v/F_m, the maximum quantum yield of photosystem II, electron transport rate and photochemical quenching. On the other hand, non-photochemical quenching values generally increase as a symptom of photoinhibition of PSII (Kalaji et al., 2012). In this experiment Chl *a* fluorescence was performed in the attempt to evaluate if different monochromatic light supplementation could represent a positive input for cultivation or instead had harmful effects (eustress *vs* distress). Several authors reported that blue light was essential for the proper function of PSII than other wavebands, also acting as a booster for the repairing rate of the photosystem (Thum et al., 2001; Hogewoning et al., 2010; Wang et al., 2015). Moreover, Gao et al. (2022) found that blue light supplementation promoted photosynthesis through the up-regulation of related genes involved in the photo-transduction and the photosynthesis-antenna protein pathways. In our experiment, B light supplementation in TI plants resulted in decreased F_0, enhanced Φ_{PSII} and decreased Φ_{NPQ} values compared to NS counterparts, suggesting a higher level of open-state PSII reaction centres compared to controls. Furthermore, according to our results, Gao et al. (2022) reported that an increased proportion of blue light can increase ETR values in green onion, and in this work, B light resulted as the only light treatment able to increase ETR compared to NS. For red-leafed species is reported that anthocyanins, absorbing photons in the blue region of the spectrum, decrease blue light reaching the photosystems, limiting the efficiency of the utilization of the radiant energy as well as inducing alteration at the photosystem level (Horton, 2012; Landi et al., 2021). In this experiment, RR cultivar was barely affected by LED light supplementation at the photochemical level. Only a significant reduction in Φ_{PSII} was recorded with B and R light supplementation and that could match with a slight, not significant increase in Φ_{NPQ}. Conversely, in anthocyanin-less species, red and blue light could enhance energy dissipation in PSII to prevent light-induced damage as reported in basil, calendula and tomato (Aliniaeifard et al., 2018; Yang et al., 2018; Hosseini et al., 2019). Despite these outcomes, however, the not significant variation in F_v/F_m among the treatments, nor in changes the quantum yield of non-regulated energy dissipation in PSII, Φ_{NO}, suggested that no damages at the PSII level occurred after any light supplementations in green- and red-leafed sweet basil.

The fact that light supplementation did not induce any considerable stress condition was confirmed also by H_2O_2 assay. In fact, accumulation of reactive oxygen species (ROS), and among them hydrogen peroxide, is usually a consistent response of plants to different stressful conditions, including light excess/imbalance (Zhu, 2016). It was suggested that in condition of excessive light, accumulation of H_2O_2 in the chloroplast, which occurs primarily in the bundle sheath and then translocated as a systemic signaling, depends mainly on the Mehler reaction and the operation of the water-water cycle (Asada, 1999; Ort and Baker, 2002; Fryer et al., 2003). In the case of a monochromatic environment, red and blue lights are reported to enhance the energy dissipation requirement induced by unbalanced light absorption by both the photosystems, increasing consequently the production of ROS (Landi et al., 2020). Conversely, in the present experiment, as Φ_{NPQ} was not significantly increased by any of the treatments compared to NS in both the cultivars, the lack of increment in H_2O_2 staining is a further evidence light-induced oxidative burst did not take place. This supports the fact that light supplementation, at the doses supplied in the present experiment, does not induce a harmful stress condition in the green- and red-leafed cultivars of sweet basil.

In literature it is reported that red, blue and polychromatic LED light supplementation increased Chl content compared to control plants (Choi et al., 2015; Matysiak and Kowalski, 2019; Lauria et al., 2021). In particular, in red-leafed basil, blue light resulted more effective compared to red light for enhancing Chl content in leaves. In the present experiment, contrastingly to the literature, TI did not show any variation in Chl content under the different treatments compared to NS, while, in RR, the higher content of Chl was recorded under R light. The general significant increment observed in the red-leafed cultivar subjected to light supplementations, compared to NS, suggests that light quantity was responsible for the Chl increment more than light quality.

Monochromatic light environments can selectively stimulate secondary metabolism, especially the biosynthetic pathway of those metabolites involved in leaf photoprotection, such as polyphenols, which

Table 5
In vivo headspace-solid phase microextraction (Relative Abundance -%) measured in leaves of *Ocimum basilicum* L. cv. 'Tigullio'. Treatments were non supplemented (NS), polychromatic light (W – R:G:B; 1:1:1), narrowband green (G), blue (B) and red (R) lights supplementation. Means (n = 3 ± SD) with different letters are significantly different after one-way ANOVA followed by Fisher's LSD *post-hoc* test (P≤0.05) considering light treatment as the only source of variability. Hyphens represented non detected compounds.

Compounds	l.r.i. [1]	Class.	Relative Abundance (%) ± SD				
			NS	W	G	B	R
α-pinene	933	mh	0.4 ± 0.23	0.5 ± 0.01	0.3 ± 0.28	0.3 ± 0.02	0.4 ± 0.19
camphene	948	mh	0.1 ± 0.07	0.1 ± 0.05	–	–	–
sabinene	973	mh	0.5 ± 0.20	0.7 ± 0.02	0.7 ± 0.18	0.4 ± 0.03	0.7 ± 0.4
β-pinene	977	mh	0.8 ± 0.39	1.3 ± 0.02	0.6 ± 0.62	0.7 ± 0.05	0.8 ± 0.29
myrcene	991	mh	0.8 ± 0.35 ^{b}	2.2 ± 0.02 ^{a}	1.1 ± 0.63 ^{b}	1.5 ± 0.14 ^{b}	1.3 ± 0.43 ^{b}
3-octanol	995	nt	–	0.1 ± 0.06	–	–	–
limonene	1029	mh	0.7 ± 0.27	0.9 ± 0.01	0.9 ± 0.16	0.5 ± 0.04	1 ± 0.48
1,8-cineole	1031	om	3.6 ± 1.72 ^{bc}	6.6 ± 0.10 ^{a}	1.6 ± 1.35 ^{d}	4.5 ± 0.38 ^{b}	2.0 ± 0.73 ^{cd}
(Z)-β-ocimene	1036	mh	–	0.1 ± 0.00	–	–	0.1 ± 0.08
(E)-β-ocimene	1047	mh	2.1 ± 0.98	2.9 ± 0.01	2.1 ± 1.45	1.8 ± 0.13	3.0 ± 1.27
γ-terpinene	1058	mh	0.2 ± 0.25	–	–	–	–
cis-sabinene hydrate	1066	om	–	0.1 ± 0.06	–	–	–
terpinolene	1089	mh	0.5 ± 0.23	0.7 ± 0.02	0.7 ± 0.13	0.4 ± 0.02	0.8 ± 0.37
linalool	1101	om	13.8 ± 4.84 ^{c}	31.3 ± 0.02 ^{a}	19.2 ± 1.03 ^{b}	28.6 ± 0.12 ^{a}	21.6 ± 2.43 ^{b}
nonanal	1105	nt	–	–	0.3 ± 0.31	–	–
4-terpineol	1177	om	0.4 ± 0.36	–	–	–	–
α-terpineol	1191	om	0.5 ± 0.05 ^{a}	0.5 ± 0.02 ^{a}	0.5 ± 0.03 ^{a}	0.4 ± 0.02 ^{b}	0.5 ± 0.1 ^{a}
decanal	1205	nt	–	0.4 ± 0.40	0.8 ± 0.62	–	–
bornyl acetate	1286	om	0.9 ± 0.69 ^{a}	–	0.2 ± 0.03 ^{b}	0.1 ± 0.05 ^{b}	0.2 ± 0.03 ^{b}
undecanal	1308	nt	–	–	0.1 ± 0.06	–	–
δ-elemene	1338	sh	0.3 ± 0.04 ^{ab}	0.3 ± 0.03 ^{bc}	0.2 ± 0.07 ^{c}	0.4 ± 0.04 ^{a}	0.4 ± 0.01 ^{a}
α-cubebene	1350	sh	0.2 ± 0.00 ^{e}	0.2 ± 0.01 ^{b}	0.2 ± 0.00 ^{d}	0.2 ± 0.01 ^{a}	0.2 ± 0.00 ^{c}
eugenol	1357	pp	18.8 ± 3.79 ^{a}	3.0 ± 0.01 ^{c}	7.4 ± 3.10 ^{bc}	5.6 ± 0.86 ^{bc}	8.4 ± 3.54 ^{b}
α-copaene	1376	sh	0.4 ± 0.04 ^{d}	0.6 ± 0.02 ^{b}	0.5 ± 0.01 ^{c}	0.6 ± 0.01 ^{a}	0.5 ± 0.04 ^{b}
β-cubebene	1390	sh	0.3 ± 0.02 ^{d}	0.4 ± 0.01 ^{b}	0.4 ± 0.00 ^{c}	0.5 ± 0.00 ^{a}	0.4 ± 0.02 ^{b}
β-elemene	1392	sh	2.3 ± 0.17 ^{a}	2.1 ± 0.02 ^{a}	1.6 ± 0.20 ^{b}	2.2 ± 0.08 ^{a}	2.2 ± 0.21 ^{a}
methyl eugenol	1405	pp	0.2 ± 0.18	–	–	–	0.2 ± 0.20
dodecanal	1409	nt	–	–	0.1 ± 0.06	–	–
cis-α-bergamotene	1416	sh	0.3 ± 0.02 ^{c}	0.3 ± 0.00 ^{bc}	0.3 ± 0.01 ^{c}	0.3 ± 0.01 ^{ab}	0.3 ± 0.03 ^{a}
β-caryophyllene	1419	sh	0.5 ± 0.05 ^{c}	0.5 ± 0.02 ^{c}	0.4 ± 0.03 ^{c}	0.5 ± 0.02 ^{b}	0.6 ± 0.01 ^{a}
β-copaene	1426	sh	0.1 ± 0.05	–	–	0.1 ± 0.06	0.1 ± 0.00
trans-α-bergamotene	1436	sh	29.7 ± 3.12 ^{b}	27.9 ± 0.41 ^{b}	39.0 ± 6.13 ^{a}	30.3 ± 0.23 ^{b}	32.7 ± 3.47 ^{b}
α-guaiene	1439	sh	0.7 ± 0.03 ^{cd}	1.0 ± 0.01 ^{a}	0.7 ± 0.01 ^{d}	0.9 ± 0.01 ^{b}	0.8 ± 0.09 ^{c}
(Z)-β-farnesene	1444	sh	0.3 ± 0.03	0.2 ± 0.02	0.3 ± 0.03	0.3 ± 0.00	0.3 ± 0.01
cis-muurola-3,5-diene	1447	sh	0.3 ± 0.03 ^{b}	0.4 ± 0.02 ^{a}	0.3 ± 0.03 ^{b}	0.4 ± 0.02 ^{a}	0.4 ± 0.01 ^{a}
α-humulene	1453	sh	1.5 ± 0.57	0.9 ± 0.02	1.0 ± 0.01	0.9 ± 0.08	1.3 ± 0.03
(E)-β-farnesene	1458	sh	5.7 ± 0.63 ^{a}	2.8 ± 0.02 ^{d}	5.0 ± 0.11 ^{b}	3.4 ± 0.36 ^{d}	4.0 ± 0.00 ^{c}
cis-muurola-4(14),5-diene	1463	sh	0.6 ± 0.05 ^{b}	0.6 ± 0.03 ^{a}	0.6 ± 0.01 ^{a}	0.7 ± 0.03 ^{a}	0.7 ± 0.02 ^{a}
germacrene D	1481	sh	3.1 ± 0.01 ^{c}	2.6 ± 0.01 ^{d}	2.4 ± 0.34 ^{d}	3.8 ± 0.05 ^{a}	3.5 ± 0.09 ^{b}
α-amorphene	1482	sh	1.4 ± 0.13	–	1.5 ± 0.15	1.3 ± 0.03	1.4 ± 0.06
bicyclogermacrene	1496	sh	1.0 ± 0.11 ^{b}	1.0 ± 0.04 ^{b}	0.8 ± 0.08 ^{c}	1.0 ± 0.08 ^{b}	1.2 ± 0.00 ^{a}
aciphyllene	1499	sh	–	0.1 ± 0.08	–	–	0.1 ± 0.01
α-bulnesene	1505	sh	1.3 ± 0.00 ^{c}	1.4 ± 0.01 ^{b}	1.2 ± 0.10 ^{d}	1.6 ± 0.01 ^{a}	1.4 ± 0.01 ^{c}
β-bisabolene	1509	sh	0.3 ± 0.03 ^{a}	0.2 ± 0.00 ^{bc}	0.3 ± 0.01 ^{ab}	0.2 ± 0.02 ^{c}	0.2 ± 0.01 ^{bc}
trans-γ-cadinene	1514	sh	3.2 ± 0.36 ^{a}	2.5 ± 0.03 ^{b}	3.4 ± 0.03 ^{a}	3.3 ± 0.11 ^{a}	3.2 ± 0.03 ^{a}
β-sesquiphellandrene	1524	sh	1.2 ± 0.07 ^{a}	0.9 ± 0.01 ^{c}	1.2 ± 0.01 ^{b}	1.1 ± 0.02 ^{b}	1.3 ± 0.03 ^{a}
1,10-di-*epi*-cubenol	1615	os	0.2 ± 0.03 ^{b}	0.1 ± 0.08 ^{c}	0.3 ± 0.01 ^{a}	0.2 ± 0.02 ^{b}	0.3 ± 0.05 ^{a}
T-cadinol	1641	os	0.8 ± 0.09 ^{c}	0.9 ± 0.04 ^{c}	1.8 ± 0.22 ^{a}	0.8 ± 0.11 ^{c}	1.4 ± 0.05 ^{b}

Chemical classes	NS	W	G	B	R
Monoterpene hydrocarbons (MHs)	6.2 ± 2.94	9.3 ± 0.09	6.3 ± 3.44	5.7 ± 0.42	8.2 ± 3.50
Oxygenated monoterpenes (OMs)	19.1 ± 6.19 ^{b}	38.5 ± 0.00 ^{a}	21.5 ± 0.33 ^{b}	33.5 ± 0.20 ^{a}	24.2 ± 3.08 ^{b}
Sesquiterpene hydrocarbons SHs)	54.5 ± 5.26 ^{ab}	46.8 ± 0.24 ^{b}	61.1 ± 5.62 ^{a}	54.0 ± 0.38 ^{ab}	57.1 ± 3.43 ^{a}
Oxygenated sesquiterpenes (OSs)	1.0 ± 0.12 ^{c}	0.9 ± 0.04 ^{c}	2.1 ± 0.21 ^{a}	1.0 ± 0.12 ^{c}	1.7 ± 0.10 ^{b}
Phenylpropanoids (PPs)	19.0 ± 3.98 ^{a}	3.0 ± 0.01^{b}	7.4 ± 3.10 ^{b}	5.6 ± 0.86 ^{b}	8.6 ± 3.73 ^{b}
Other non-terpene derivatives (NTs)	–	0.1 ± 0.06	0.4 ± 0.33	–	–
Total identified (%)	99.9 ± 0.01	98.6 ± 0.05	98.9 ± 0.72	99.7 ± 0.01	99.7 ± 0.02

[1] Linear retention index on a HP 5-MS capillary column.

not only abate a proportion of light otherwise striking chloroplast, but also prevent and scavenge the excessive formation of ROS (Landi et al., 2020). B light supplementation enhanced flavonoid biosynthesis in several leafy species, e.g., green basil, lamb's lettuce, garden rocket, strawberry leaves, tatsoi and red pak choi (Li and Kubota, 2009; Choi et al., 2015; Matysiak and Kowalski, 2019; Lauria et al., 2021). Blue and red lights supplementation and high light intensity are reported to spur polyphenol biosynthesis (Landi et al., 2020; Loi et al., 2021) and anthocyanin accumulation was reported to be enhanced with increased blue light proportion in red holy basil and red-leafed lettuce (Li and Kubota, 2009; Chutimanukul et al., 2022). The main polyphenols characterizing green-leafed basil leaves are rosmarinic acid, followed by other phenolic acids such as caffeic acid and its derivative as caftaric acid, and flavonol-glycosides as rutin (Jayasinghe et al., 2003; Nguyen and Niemeyer, 2008; Lee and Scagel, 2009; Hossain et al., 2010; Tenore et al., 2017), while principal anthocyanins synthetized in red leafed basil are coumaroyl derivatives of cyanidin and glycosides of cyanidin and pelargonidin (Tattini et al., 2014). In this work, LED light

Table 6
In vivo headspace-solid phase microextraction (Relative Abundance -%) measured in leaves of *Ocimum basilicum* L. cv. 'Red Rubin'. Treatments were non supplemented (NS), polychromatic light (W – R:G:B; 1:1:1), narrowband green (G), blue (B) and red (R) lights supplementation. Means (n = 3 ± SD) with different letters are significantly different after one-way ANOVA followed by Fisher's LSD *post-hoc* test (P≤0.05) considering light treatment as the only source of variability. Hyphens represented non detected compounds.

Compounds	l.r.i. [1]	Class.	Relative Abundance (%) ± SD				
			NS	W	G	B	R
α-pinene	399	mh	0.2 ± 0.04 ^{b}	0.4 ± 0.12 ^{a}	0.3 ± 0.05 ^{b}	0.2 ± 0.00 ^{b}	0.2 ± 0.02 ^{b}
sabinene	973	mh	0.2 ± 0.04 ^{b}	0.5 ± 0.16 ^{a}	0.3 ± 0.06 ^{b}	0.2 ± 0.04 ^{b}	0.2 ± 0.03 ^{b}
β-pinene	977	mh	0.3 ± 0.06 ^{b}	0.6 ± 0.19 ^{a}	0.5 ± 0.16 ^{ab}	0.3 ± 0.07 ^{b}	0.4 ± 0.04 ^{b}
myrcene	991	mh	0.4 ± 0.07	0.6 ± 0.03	0.7 ± 0.52	0.3 ± 0.07	0.4 ± 0.02
limonene	1029	mh	0.6 ± 0.13 ^{b}	1.3 ± 0.39 ^{a}	0.7 ± 0.19 ^{b}	0.7 ± 0.12 ^{b}	0.7 ± 0.10
1,8-cineole	1031	om	6.4 ± 0.50	1.8 ± 0.28	8.0 ± 5.70	4.7 ± 2.84	6.3 ± 0.06
cis-sabinene hydrate	1066	om	0.1 ± 0.01	0.1 ± 0.00	0.2 ± 0.17	0.1 ± 0.01	0.2 ± 0.03
terpinolene	1089	mh	–	0.9 ± 0.35	–	–	–
fenchone	1089	om	1.4 ± 0.15	–	1.0 ± 0.34	1.4 ± 0.57	1.4 ± 0.14
linalool	1101	om	11.0 ± 2.96	15.5 ± 0.29	18.4 ± 13.93	10.4 ± 3.65	8.2 ± 0.14
fenchol	1114	om	0.3 ± 0.04	–	0.2 ± 0.19	0.1 ± 0.12	0.2 ± 0.01
α-terpineol	1191	om	0.4 ± 0.04	0.6 ± 0.1	0.7 ± 0.19	0.5 ± 0.20	0.4 ± 0.02
methyl chavicol	1201	pp	0.1 ± 0.06	–	–	–	–
decanal	1206	nt	0.2 ± 0.04 ^{b}	0.2 ± 0.05 ^{b}	0.3 ± 0.12 ^{b}	0.2 ± 0.06 ^{b}	0.6 ± 0.23 ^{a}
(endo) fenchyl acetate	1221	om	2.6 ± 0.58	2.5 ± 0.06	1.7 ± 0.52	1.7 ± 0.63	2.2 ± 0.69
2-hydroxy-1,8-cineole	1228	om	–	–	–	0.1 ± 0.07	–
bornyl acetate	1286	om	0.1 ± 0.06	–	–	0.1 ± 0.06	–
δ-elemene	1338	sh	1.1 ± 0.15 ^{a}	0.8 ± 0.04 ^{a}	0.5 ± 0.11 ^{b}	0.8 ± 0.30 ^{a}	0.4 ± 0.10 ^{b}
α-cubebene	1350	sh	0.2 ± 0.02 ^{c}	0.3 ± 0.02 ^{a}	0.2 ± 0.00 ^{d}	0.3 ± 0.01 ^{b}	0.2 ± 0.03 ^{b}
eugenol	1357	pp	0.4 ± 0.21	1.4 ± 0.14	2.6 ± 0.53	1.2 ± 0.85	2.0 ± 1.94
α-copaene	1376	sh	0.5 ± 0.03 ^{c}	0.7 ± 0.04 ^{a}	0.4 ± 0.00 ^{d}	0.6 ± 0.01 ^{b}	0.6 ± 0.05 ^{b}
β-cubebene	1390	sh	0.4 ± 0.01 ^{b}	0.5 ± 0.00 ^{a}	0.3 ± 0.00 ^{c}	0.5 ± 0.01 ^{a}	0.5 ± 0.04 ^{a}
β-elemene	1392	sh	4.7 ± 0.50 ^{ab}	6.0 ± 1.14 ^{a}	2.7 ± 1.20 ^{b}	3.4 ± 1.84 ^{b}	2.7 ± 0.88 ^{b}
methyl eugenol	1405	pp	38.1 ± 0.66	24.8 ± 0.96	35.8 ± 15.83	43 ± 12.82	47.9 ± 8.27
β-caryophyllene	1419	sh	4.8 ± 0.13	5.4 ± 0.61	3.8 ± 0.75	5.3 ± 1.56	3.7 ± 0.15
β-gurjunene	1428	sh	–	–	–	–	0.1 ± 0.07
β-copaene	1428	sh	0.1 ± 0.01	0.1 ± 0.03	–	–	0.1 ± 0.07
trans-α-bergamotene	1436	sh	1.3 ± 0.02 ^{bc}	2.3 ± 0.55 ^{a}	1.3 ± 0.15 ^{bc}	1.4 ± 0.04 ^{b}	0.9 ± 0.28 ^{c}
α-guaiene	1439	sh	1.4 ± 0.10 ^{b}	2.0 ± 0.09 ^{a}	1.0 ± 0.17 ^{c}	1.2 ± 0.33 ^{bc}	1.1 ± 0.18 ^{bc}
aromadendrene	1440	sh	–	0.1 ± 0.07	–	–	–
cis-muurola-3,5-diene	1447	sh	0.3 ± 0.03 ^{b}	0.4 ± 0.04 ^{a}	0.2 ± 0.00 ^{cd}	0.2 ± 0.02 ^{bc}	0.2 ± 0.01 ^{d}
α-humulene	1453	sh	2.1 ± 0.30	2.2 ± 0.21	1.7 ± 0.76	2.0 ± 0.49	1.3 ± 0.16
(E)-β-farnesene	1458	sh	6.0 ± 0.52	9.2 ± 1.5	6.8 ± 1.09	5.4 ± 4.69	7.5 ± 4.48
cis-muurola-4(14),5-diene	1463	sh	0.5 ± 0.01 ^{b}	0.7 ± 0.03 ^{a}	0.4 ± 0.00 ^{c}	0.5 ± 0.05 ^{b}	0.4 ± 0.02 ^{d}
γ-muurolene	1477	sh	–	0.1 ± 0.02	–	–	–
germacrene D	1481	sh	3.8 ± 0.32 ^{ab}	4.7 ± 0.81 ^{a}	2.1 ± 0.33 ^{c}	3.9 ± 1.58 ^{ab}	2.9 ± 0.52 ^{bc}
α-amorphene	1485	sh	–	0.1 ± 0.05	–	–	–
bicyclogermacrene	1496	sh	3.2 ± 0.29 ^{a}	2.6 ± 0.11 ^{a}	1.7 ± 0.34 ^{b}	2.5 ± 0.7 ^{a}	1.3 ± 0.32 ^{b}
aciphyllene	1499	sh	0.3 ± 0.03	0.4 ± 0.07	0.3 ± 0.09	0.3 ± 0.07	0.3 ± 0.01
α-bulnesene	1505	sh	2.3 ± 0.15 ^{b}	3.2 ± 0.30 ^{a}	1.5 ± 0.41 ^{c}	2.0 ± 0.46 ^{bc}	1.6 ± 0.40 ^{bc}
β-bisabolene	1509	sh	–	0.1 ± 0.05	–	–	0.1 ± 0.06
trans-γ-cadinene	1514	sh	2.6 ± 0.19 ^{b}	3.5 ± 0.40 ^{a}	2.0 ± 0.31 ^{cd}	2.5 ± 0.24 ^{bc}	1.9 ± 0.07 ^{d}
δ-cadinene	1524	sh	0.3 ± 0.01 ^{bc}	0.5 ± 0.03 ^{a}	0.3 ± 0.07 ^{bc}	0.3 ± 0.06 ^{b}	0.3 ± 0.03 ^{c}
α-cadinene	1537	sh	–	0.1 ± 0.02	–	0.1 ± 0.05	–
1,10-di-*epi*-cubenol	1615	os	0.2 ± 0.06	0.4 ± 0.07	0.2 ± 0.03	0.2 ± 0.10	0.2 ± 0.07
T-cadinol	1641	os	0.9 ± 0.34	2.0 ± 0.81	1.2 ± 0.29	1.2 ± 0.62	0.8 ± 0.32

Chemical classes	NS	W	G	B	R
Monoterpene hydrocarbons (MHs)	1.7 ± 0.33 ^{b}	4.3 ± 1.17 ^{a}	2.4 ± 0.97 ^{b}	1.7 ± 0.02 ^{b}	1.8 ± 0.21 ^{b}
Oxygenated monoterpenes (OMs)	22.4 ± 1.66	20.5 ± 0.52	30.1 ± 19.99	19.0 ± 0.28	18.8 ± 0.37
Sesquiterpene hydrocarbons SHs)	35.7 ± 2.68	46.0 ± 0.55	27.2 ± 5.76	33.3 ± 12.33	27.7 ± 7.5
Oxygenated sesquiterpenes (OSs)	1.1 ± 0.40	2.3 ± 0.88	1.4 ± 0.32	1.4 ± 0.71	1.0 ± 0.39
Phenylpropanoids (PPs)	38.6 ± 0.39	26.1 ± 0.82	38.3 ± 15.31	44.2 ± 11.97	49.9 ± 6.33
Other non-terpene derivatives (NTs)	0.2 ± 0.04 ^{b}	0.2 ± 0.05 ^{b}	0.3 ± 0.12 ^{b}	0.2 ± 0.06 ^{b}	0.6 ± 0.23 ^{a}
Total identified (%)	99.6 ± 0.06	99.5 ± 0.1	99.8 ± 0.09	99.8 ± 0.13	99.8 ± 0.02

[1] Linear retention index on a HP 5-MS capillary column.

supplementation did not influence TI Flav index, but green-leafed basil metabolome analysis confirmed B light influence on some metabolite biosynthesis such as caffeic and caftaric acids and rutin. Nevertheless, abundance of the main phenolic carboxylic acid in sweet basil, namely rosmarinic acid, was not affected by different spectral composition. Lastly, it is conceivable that the positive results exerted in TI plants by the W light are mainly attributable to the enrichment in blue portion of the spectrum, while green light contribution to enhance caffeic acid content in basil leaves contrasted with literature results (Loi et al., 2021). In RR cultivar, accordingly to literature, B light was the most effective in stimulating Flav and Anth indexes. This is probably due to the phenol link with blue light receptors as these compounds were closely regulated by cryptochromes (Landi et al., 2020; Li and Kubota, 2009). Moreover, as already mentioned, blue light is composed of high energy wavebands which could induce photoinhibition in leaves and thus translate into the necessity of plants to synthetize metabolites with photoprotective prerogatives. Even in RR plants, rosmarinic acid was not affected by light supplementation and only caftaric acid showed the expected increment under B light. Anthocyanin increment with B light could be related to the anthocyanin role of photoprotector against photoinhibition (Steyn et al., 2002; Gould et al., 2018), as in this experiment a decrease in Φ_{PSII} was observed under B light

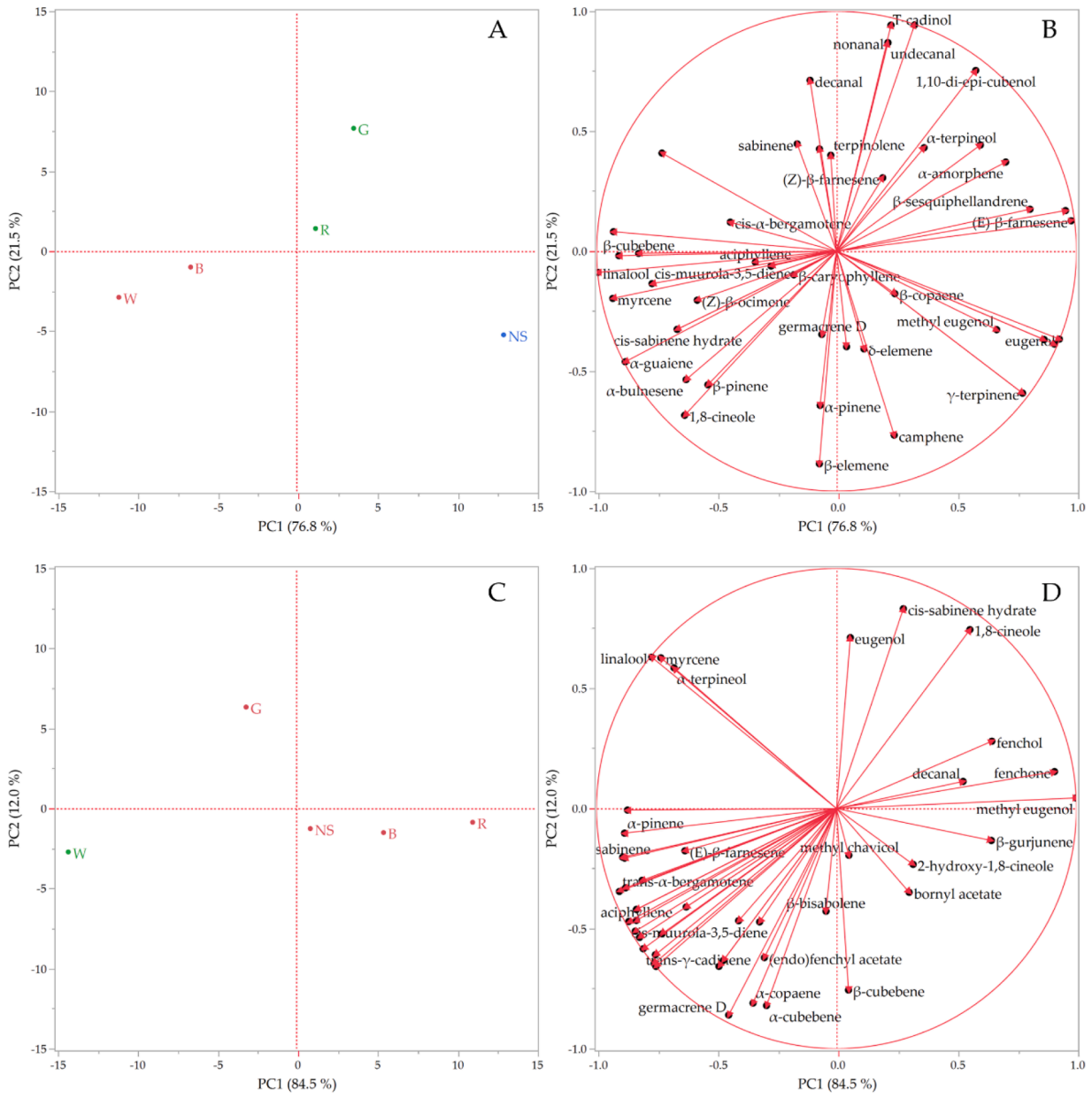

Fig. 5. Score plot (A, C) and loading plot (B, D) of the principal component analysis in leaves of *Ocimum basilicum* L. cv. 'Tigullio' (above) and cv. 'Red Rubin' (below) for the principal component analysis of VOCs. Treatments were non supplemented (NS), polychromatic light (W – R:G:B; 1:1:1), narrowband green (G), blue (B) and red (R) lights supplementation.

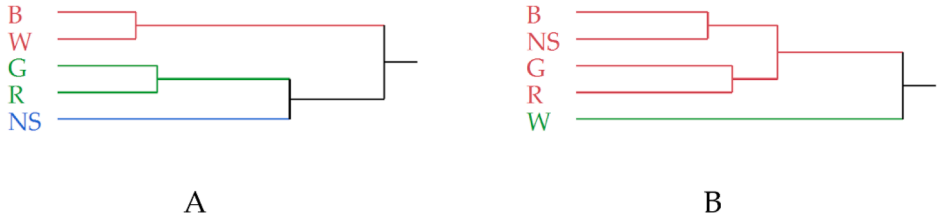

Fig. 6. Dendrogram of the HCA performed on the complete composition of the headspace-solid phase microextraction of the leaves of *Ocimum basilicum* L. cv. 'Tigullio' (A) and 'Red Rubin' (B).

supplementation. From metabolomics analysis emerged that only Cy-3-coum-Glc among the three detected anthocyanin derivatives appeared to be affected by light quality, with a significant reduction after G light supplementation. Though it is well known that anthocyanins absorb mainly in the green region of the spectrum (Gould et al., 2010), a significant reduction of anthocyanins under green light exposure has already been observed (Folta, 2004; Bouly et al., 2007), as green light often reverses blue light effects (Frechilla et al., 2000; Folta and Carvalho, 2015).

Some experiments have already been performed to study the volatile

compounds variation due to different light fluxes (Chang et al., 2008; Walters et al., 2021). However, previous works analysed only green-leafed sweet basil VOC profile, and to the best of our knowledge, the effects of light quality on the aroma profile of RR plants has never been described. Moreover, investigations were performed on the light-dependent responses of VOCs in plants grown in monochromatic environments (or with different proportion of monochromatic LED), so the effects of ambient light enrichment by monochromatic light on volatiles has never been established. Generally, higher irradiances lead to an increase in linalool, 1,8-cineole and eugenol, while methyl eugenol increases in shading conditions (Chang et al., 2008; Walters et al., 2021). In the present experiment, in TI was observed this trend for linalool, a compound characterized by a sweet floral aroma (Hammock et al., 2021) endowed with antioxidants, anti-inflammatory and neuroprotective properties (Park et al., 2016), and for methyl eugenol, considered somewhat toxic and unpleasant (De Vincenzi et al., 2000; Hammock et al., 2021), which was not detected in any of the supplemented treatments (except R one). Eugenol, characterised by clove flavor, and also considered toxic (Nejad et al., 2017), decreased with all the light treatments, making green-leafed basil a safer product. Walters et al. (2021) reported that higher acceptance of basil profile was characterised by higher linalool concentration and lower eugenol content. This suggests that all the LED light supplementation could enhance TI consumer pleasantness compared to NS. Reports about LED light supplementation are controversial. Carvalho et al. (2016) proposed that green light induced the accumulation of monoterpenoids and the decrease of phenylpropanoids, while Hammock et al. (2021) recommended a blue:red ratio of 20:80 and 50:50 for enhancing monoterpenes and diterpenes in basil. Pennisi et al. (2019) reported that higher blue light percentages affected the main basil VOCs, decreasing linalool and increasing α-bergamotene. In RR only W light resulted in a significant alteration of basil VOC profile, and in particular only monoterpene and sesquiterpene hydrocarbons were affected. Although the results were not significantly different, some variations in the percentage of the main compounds were observed, suggesting that a monochromatic light supplementation could influence the RR aroma profile. For example, linalool and 1,8-cineole increased under G light, according to what reported by Carvalho et al. (2016). These compounds are known to have antioxidant, anti-inflammatory and neuroprotective properties (Park et al., 2016; Ryu et al., 2014). Monochromatic light supplementation did not affect strongly the red-leafed basil volatilome profile, but the balanced ratio between R, G and B lights presented in W light contributed to a significant variation in VOC content.

5. Conclusions

In conclusion, despite no morphological variation occurred in the two cultivars after light supplementation, monochromatic wavebands stimulate physiological and biochemical changes in green- and red-leafed basil. B light affected LA and thickness in both the cultivars and further investigation should deepen the effect of B light on mesophyll cells and tissue. Moreover, B light also affected stomata, inducing excessive water consumption, which could hamper a higher efficiency of greenhouse cultivation of basil. Further analysis will also be necessary to clarify the R light role in TI photosynthesis and Calvin-Benson-Bassham cycle and G light role in RR cultivar. As no serious stress conditions were imposed by the present doses of monochromatic light supplementation, probably the treatments induce a slight level of stress (i.e., an eustress), which was able to stimulate secondary metabolite biosynthesis. Indeed, the volatilome profile was influenced by the light treatments, and in particular by quantity of light rather than its quality. This suggests a practical application for the greenhouse cultivation of basil, as light supplementation could confer a better pleasantness to TI plants thanks to its influence on volatile compounds as well as a reduced content of eugenol, considered one of the major and most toxic compounds in sweet basil.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Giulia Lauria: Conceptualization, Formal analysis, Writing – original draft. **Ermes Lo Piccolo:** Conceptualization, Formal analysis, Writing – original draft. **Anna Davini:** Formal analysis. **Monica Ruffini Castiglione:** Formal analysis, Writing – review & editing. **Ylenia Pieracci:** Formal analysis. **Guido Flamini:** Formal analysis, Writing – review & editing. **Stefan Martens:** Formal analysis. **Andrea Angeli:** Formal analysis. **Costanza Ceccanti:** Formal analysis, Writing – original draft. **Lucia Guidi:** Writing – review & editing. **Elisa Pellegrini:** Writing – review & editing. **Luca Incrocci:** Writing – review & editing. **Marco Landi:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.111970.

References

Adams, R.P., 1995. Identifcation of Essential Oil Components By Gas Cromatography and Mass Spectroscopy. Allured Publishing Corp, Illinois.

Aliniaeifard, S., Seif, M., Arab, M., Zare Mehrjerdi, M., Li, T., Lastochkina, O., 2018. Growth and photosynthetic performance of *Calendula officinalis* under monochromatic red light. Int. J. Hortic. Sci. Technol. 5, 123–132. https://doi.org/10.22059/IJHST.2018.261042.248.

Arapitsas, P., Perenzoni, D., Nicolini, G., Mattivi, F., 2012. Study of Sangiovese wines pigment profile by UHPLC-MS/MS. J. Agric. Food Chem. 60, 10461–10471. https://doi.org/10.1021/jf302617e.

Asada, K., 1999. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1), 601–639.

Assmann, S.M., 1988. Enhancement of the stomatal response to blue light by red light, reduced intercellular concentrations of CO_2, and low vapor pressure differences. Plant Physiol. 87, 226–231.

Bantis, F., Ouzounis, T., Radoglou, K., 2016. Artificial LED lighting enhances growth characteristics and total phenolic content of *Ocimum basilicum*, but variably affects transplant success. Sci. Hortic. 198, 277–283. https://doi.org/10.1016/j.scienta.2015.11.014.

Bantis, F., Smirnakou, S., Ouzounis, T., Koukounaras, A., Ntagkas, N., Radoglou, K., 2018. Current status and recent achievements in the field of horticulture with the use of light-emitting diodes (LEDs). Sci. Hortic. 235, 437–451. https://doi.org/10.1016/j.scienta.2018.02.058.

Bian, Z., Zhang, X., Wang, Y., Lu, C., 2019. Improving drought tolerance by altering the photosynthetic rate and stomatal aperture *via* green light in tomato (*Solanum lycopersicum* L.) seedlings under drought conditions. Environ. Exp. Bot. 167, 103844 https://doi.org/10.1016/j.envexpbot.2019.103844.

Bogani, P., Calistri, E., Biricolti, S., Ruffini Castiglione, M., Turrini, A., Giovannetti, M., Buiatti, M., 2015. The rat glucocorticoid receptor integration in *Nicotiana langsdorffii* genome affects plant responses to abiotic stresses and to arbuscular mycorrhizal symbiosis. Plant Biotechnol. Rep. 9, 209–220. https://doi.org/10.1007/s11816-015-0358-3.

Bouly, J.P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Van Der Straeten, D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R., Ahmad, M., 2007. Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. J. Biol. Chem. 282 (13), 9383–9391. https://doi.org/10.1074/jbc.M609842200.

Carvalho, S.D., Schwieterman, M.L., Abrahan, C.E., Colquhoun, T.A., Folta, K.M., 2016. Light quality dependent changes in morphology, antioxidant capacity, and volatile

production in sweet basil (*Ocimum basilicum*). Front. Plant Sci. 7, 1328. https://doi.org/10.3389/fpls.2016.01328.
Chabot, B.F., Chabot, J.F., 1977. Effects of light and temperature on leaf anatomy and photosynthesis in *Fragaria vesca*. Oecologia 26 (4), 363377.
Chang, X., Alderson, P.G., Wright, C.J., 2008. Solar irradiance level alters the growth of basil (*Ocimum basilicum* L.) and its content of volatile oils. Environ. Exp. Bot. 63 (1–3), 216–223. https://doi.org/10.1016/j.envexpbot.2007.10.017.
Choi, H.G., Moon, B.Y., Kang, N.J., 2015. Effects of LED light on the production of strawberry during cultivation in a plastic greenhouse and in a growth chamber. Sci. Hortic. 189, 22–31. https://doi.org/10.1016/j.scienta.2015.03.022.
Chutimanukul, P., Wanichananan, P., Janta, S., Toojinda, T., Darwell, C.T., Mosaleeyanon, K., 2022. The influence of different light spectra on physiological responses, antioxidant capacity and chemical compositions in two holy basil cultivars. Sci. Rep. 12 (1), 1–17. https://doi.org/10.1038/s41598-021-04577-x.
Clavijo-Herrera, J., Van Santen, E., Gómez, C., 2018. Growth, water-use efficiency, stomatal conductance, and nitrogen uptake of two lettuce cultivars grown under different percentages of blue and red light. Horticulturae 4 (3), 16. https://doi.org/10.3390/horticulturae4030016.
Cocetta, G., Casciani, D., Bulgari, R., Musante, F., Kołton, A., Rossi, M., Ferrante, A., 2017. Light use efficiency for vegetables production in protected and indoor environments. Eur. Phys. J. Plus 132 (1), 1–15. https://doi.org/10.1140/epjp/i2017-11298-x.
Daudi, A., O'Brien, J.A., 2012. Detection of hydrogen peroxide by DAB staining in *Arabidopsis* leaves. Bio-Protoc. 2 (18), e263. https://doi.org/10.21769/BioProtoc.263.
De Vincenzi, M., Silano, M., Stacchini, P., Scazzocchio, B., 2000. Constituents of aromatic plants: I. Methyleugenol. Fitoterapia 71 (2), 216–221. https://doi.org/10.1016/S0367-326X(99)00150-1.
Dou, H., Niu, G., Gu, M., 2019. Photosynthesis, morphology, yield, and phytochemical accumulation in basil plants influenced by substituting green light for partial red and/or blue light. HortScience 54 (10), 1769–1776. https://doi.org/10.21273/HORTSCI14282-19.
Fernandes, F., Pereira, E., Círić, A., Soković, M., Calhelha, R.C., Barros, L., Ferreira, I.C., 2019. *Ocimum basilicum* var. purpurascens leaves (red rubin basil): a source of bioactive compounds and natural pigments for the food industry. Food Funct. 10 (6), 3161–3171. https://doi.org/10.1039/C9FO00578A.
Filip, S., 2017. Basil (*Ocimum basilicum* L.) a source of valuable phytonutrients. Int. J. Clin. Nutr. Diet 3, 118. https://doi.org/10.15344/2456-8171/2017/118.
Folta, K.M., 2004. Green light stimulates early stem elongation, antagonizing light-mediated growth inhibition. Plant Physiol. 135 (3), 407–1416. https://doi.org/10.1104/pp.104.038893.
Folta, K.M., Carvalho, S.D., 2015. Photoreceptors and control of horticultural plant traits. HortScience 50 (9), 1274–1280. https://doi.org/10.21273/HORTSCI.50.9.1274.
Frechilla, S., Talbott, L.D., Bogomolni, R.A., Zeiger, E., 2000. Reversal of blue light-stimulated stomatal opening by green light. Plant Cell Physiol. 41 (2), 171–176. https://doi.org/10.1093/pcp/41.2.171.
Fryer, M.J., Ball, L., Oxborough, K., Karpinski, S., Mullineaux, P.M., Baker, N.R., 2003. Control of *Ascorbate Peroxidase 2* expression by hydrogen peroxide and leaf water status during excess light stress reveals a functional organisation of *Arabidopsis* leaves. Plant J. 33 (4), 691–705. https://doi.org/10.1046/j.1365-313X.2003.01656.x.
Fukuda, N., Fujita, M., Ohta, Y., Sase, S., Nishimura, S., Ezura, H., 2008. Directional blue light irradiation triggers epidermal cell elongation of abaxial side resulting in inhibition of leaf epinasty in geranium under red light condition. Sci. Hortic. 115 (2), 176–182. https://doi.org/10.1016/j.scienta.2007.08.006.
Gago, J., Douthe, C., Florez-Sarasa, I., Escalona, J.M., Galmes, J., Fernie, A.R., Flexas, J., Medrano, H., 2014. Opportunities for improving leaf water use efficiency under climate change conditions. Plant Sci. 226, 108–119. https://doi.org/10.1016/j.plantsci.2014.04.007.
Galvão, V.C., Fankhauser, C., 2015. Sensing the light environment in plants: photoreceptors and early signaling steps. Curr. Opin. Neurobiol. 34, 46–53. https://doi.org/10.1016/j.conb.2015.01.013.
Gao, S., Wang, K., Li, N., Lv, Y., Cao, B., Chen, Z., Xu, K., 2022. The growth and photosynthetic responses of white LEDs with supplemental blue light in green onion (*Allium fistulosum* L.) unveiled by Illumina and single-molecule real-time (SMRT) RNA-sequencing. Environ. Exp. Bot. 197 https://doi.org/10.1016/j.envexpbot.2022.104835.
Gould, K.S., Dudle, D.A., Neufeld, H.S., 2010. Why some stems are red: cauline anthocyanins shield photosystem II against high light stress. J. Exp. Bot. 61 (10), 2707–2717. https://doi.org/10.1093/jxb/erq106.
Gould, K.S., Jay-Allemand, C., Logan, B.A., Baissac, Y., Bidel, L.P., 2018. When are foliar anthocyanins useful to plants? Re-evaluation of the photoprotection hypothesis using *Arabidopsis thaliana* mutants that differ in anthocyanin accumulation. Environ. Exp. Bot. 154, 11–22. https://doi.org/10.1016/j.envexpbot.2018.02.006.
Hammock, H.A., Kopsell, D.A., Sams, C.E., 2021. Narrowband blue and red LED supplements impact key flavor volatiles in hydroponically grown basil across growing seasons. Front Plant. Sci. 12, 623314 https://doi.org/10.3389/fpls.2021.623314.
Hogewoning, S.W., Trouwborst, G., Maljaars, H., Poorter, H., van Ieperen, W., Harbinson, J., 2010. Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. J. Exp. Bot. 61 (11), 3107–3117. https://doi.org/10.1093/jxb/erq132.
Horton, P., 2012. Optimization of light harvesting and photoprotection: molecular mechanisms and physiological consequences. Philos. Trans. R. Soc. Lond. B, Biol. Sci. 367 (1608), 3455–3465. https://doi.org/10.1098/rstb.2012.0069.
Hossain, M.B., Rai, D.K., Brunton, N.P., Martin-Diana, A.B., Barry-Ryan, C., 2010. Characterization of phenolic composition in Lamiaceae spices by LC-ESI-MS/MS. J. Agric. Food Chem. 58 (19), 10576–10581. https://doi.org/10.1021/jf102042g.
Hosseini, A., Zare Mehrjerdi, M., Aliniaeifard, S., Seif, M., 2019. Photosynthetic and growth responses of green and purple basil plants under different spectral compositions. Physiol. Mol. Biol. Plants 25 (3), 741–752. https://doi.org/10.1007/s12298-019-00647-7.
Hussain, A.I., Anwar, F., Sherazi, S.T.H., Przybylski, R., 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem. 108 (3), 986–995. https://doi.org/10.1016/j.foodchem.2007.12.010.
Jang, E.B., Ho, T.T., Park, S.Y., 2020. Effect of light quality and tissue origin on phenolic compound accumulation and antioxidant activity in *Camellia japonica* calli. *In Vitro* Cell Dev. Biol. Plant 56 (5), 567–577. https://doi.org/10.1007/s11627-020-10121-9.
Jayasinghe, C., Gotoh, N., Aoki, T., Wada, S., 2003. Phenolics composition and antioxidant activity of sweet basil (*Ocimum basilicum* L.). J. Agric. Food Chem. 51 (15), 4442–4449. https://doi.org/10.1021/jf034269o.
Kalaji, H.M., Carpentier, R., Allakhverdiev, S.I., Bosa, K., 2012. Fluorescence parameters as early indicators of light stress in barley. J. Photochem. Photobiol. B 112, 1–6. https://doi.org/10.1016/j.jphotobiol.2012.03.009.
Kalaji, H.M., Schansker, G., Brestic, M., Bussotti, F., Calatayud, A., Ferroni, L., Goltsev, V., Guidi, L., Jajoo, A., Li, P., Losciale, P., Mishra, V.K., Misra, A.N., Nebauer, S.G., Pancaldi, S., Penella, C., Pollastrini, M., Suresh, K., Tambussi, E., Yanniccari, M., Zivcak, M., Cetner, M.D., Samborska, I.A., Stirbet, A., Olsovska, K., Kunderlikova, K., Shelonzek, H., Rusinowski, S., Bąba, W., 2017. Frequently asked questions about chlorophyll fluorescence, the sequel. Photosyn. Res. 132 (1), 13–66. https://doi.org/10.1007/s11120-016-0318-y.
Kim, E.Y., Park, S., Park, B.J., Lee, Y., Oh, M.M., 2014. Growth and antioxidant phenolic compounds in cherry tomato seedlings grown under monochromatic light-emitting diodes. Hortic. Environ. Biotechnol. 55 (6), 506–513. https://doi.org/10.1007/s13580-014-0121-7.
Landi, M., Agati, G., Fini, A., Guidi, L., Sebastiani, F., Tattini, M., 2021. Unveiling the shade nature of cyanic leaves: a view from the "blue absorbing side" of anthocyanins. Plant. Cell Environ. 44 (4), 1119–1129. https://doi.org/10.1111/pce.13818.
Landi, M., Guidi, L., Pardossi, A., Tattini, M., Gould, K.S., 2014. Photoprotection by foliar anthocyanins mitigates effects of boron toxicity in sweet basil (*Ocimum basilicum*). Planta 240 (5), 941–953. https://doi.org/10.1007/s00425-014-2087-1.
Landi, M., Zivcak, M., Sytar, O., Brestic, M., Allakhverdiev, S.I., 2020. Plasticity of photosynthetic processes and the accumulation of secondary metabolites in plants in response to monochromatic light environments: a review. BBA-Bioenergetics 1861 (2), 148131. https://doi.org/10.1016/j.bbabio.2019.148131.
Lauria, G., Lo Piccolo, E., Pellegrini, E., Bellini, E., Giordani, T., Guidi, L., Lorenzini, G., Malorgio, F., Massai, R., Nali, C., Paoli, L., Remorini, D., Sanita' Di Toppi, L., Vernieri, P., Landi, M., 2021. Photosynthetic traits and biochemical responses in strawberry (*Fragaria* × *ananassa* Duch.) leaves supplemented with LED lights. Photosynthetica 59 (4), 557–569. https://doi.org/10.32615/ps.2021.048.
Lee, J., Scagel, C.F., 2009. Chicoric acid found in basil (*Ocimum basilicum* L.) leaves. Food Chem. 115 (2), 650–656. https://doi.org/10.1016/j.foodchem.2008.12.075.
Lee, S.H., Tewari, R.K., Hahn, E.J., Paek, K.Y., 2007. Photon flux density and light quality induce changes in growth, stomatal development, photosynthesis and transpiration of *Withania Somnifera* (L.) Dunal. plantlets. Plant Cell Tiss. Organ. Cult. 90 (2), 141–151. https://doi.org/10.1007/s11240-006-9191-2.
Lee, S.J., Umano, K., Shibamoto, T., Lee, K.G., 2005. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chem. 91 (1), 131–137. https://doi.org/10.1016/j.foodchem.2004.05.056.
Leicach, S.R., Chludil, H.D., 2014. Plant secondary metabolites: structure–activity relationships in human health prevention and treatment of common diseases. Stud. Nat. Prod. Chem. 42, 267–304. https://doi.org/10.1016/B978-0-444-63281-4.00009-4.
Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67 (1), 59–64. https://doi.org/10.1016/j.envexpbot.2009.06.011.
Lim, S., Kim, J., 2021. Light quality affects water use of sweet basil by changing its stomatal development. Agronomy 11 (2), 303. https://doi.org/10.3390/agronomy11020303.
Lin, K.H., Huang, M.Y., Hsu, M.H., 2021. Morphological and physiological response in green and purple basil plants (*Ocimum basilicum*) under different proportions of red, green, and blue LED lightings. Sci. Hortic. 275, 109677 https://doi.org/10.1016/j.scienta.2020.109677.
Litvin, A.G., Currey, C.J., Wilson, L.A., 2020. Effects of supplemental light source on basil, dill, and parsley growth, morphology, aroma, and flavor. J. Am. Soc. Hortic. Sci. 145 (1), 18–29. https://doi.org/10.21273/JASHS04746-19.
Llorente, B., Martinez-Garcia, J.F., Stange, C., Rodriguez-Concepcion, M., 2017. Illuminating colors: regulation of carotenoid biosynthesis and accumulation by light. Curr. Opin. Plant Biol. 37, 49–55. https://doi.org/10.1016/j.pbi.2017.03.011.
Lo Piccolo, E., Landi, M., Giordani, T., Lorenzini, G., Malorgio, F., Massai, R., Nali, C., Pellegrini, E., Rallo, G., Remorini, D., Vernieri, P., Guidi, L., 2020. Can anthocyanin presence ameliorate the photosynthetic performance of *Prunus* saplings subjected to polyethylene glycol-simulated water stress? Photosynthetica 58 (3), 799–807. https://doi.org/10.32615/ps.2020.017.
Loi, M., Villani, A., Paciolla, F., Mulè, G., Paciolla, C., 2021. Challenges and opportunities of light-emitting diode (LED) as key to modulate antioxidant compounds in plants. A review. Antioxidants 10 (1), 42. https://doi.org/10.3390/antiox10010042.

Loreto, F., Barta, C., Brilli, F., Nogues, I., 2006. On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. Plant. Cell Environ. 29 (9), 1820–1828. https://doi.org/10.1111/j.1365-3040.2006.01561.x.

Loughrin, J.H., Kasperbauer, M.J., 2001. Light reflected from colored mulches affects aroma and phenol content of sweet basil (*Ocimum basilicum* L.) leaves. J. Agric. Food Chem. 49 (3), 1331–1335. https://doi.org/10.1021/jf0012648.

Matysiak, B., Kowalski, A., 2019. White, blue and red LED lighting on growth, morphology and accumulation of flavonoid compounds in leafy greens. Zemdirbyste 106 (3), 281–286. https://doi.org/10.13080/z-a.2019.106.036.

Menicagli, V., Ruffini Castiglione, M., Balestri, E., Giorgetti, L., Bottega, S., Source, C., Spanò, C., Lardicci, C., 2022. Early evidence of the impacts of microplastic and nanoplastic pollution on the growth and physiology of the seagrass *Cymodocea nodosa*. Sci. Total. Environ. 838, 156514 https://doi.org/10.1016/j.scitotenv.2022.156514.

Muneer, S., Kim, E.J., Park, J.S., Lee, J.H., 2014. Influence of green, red and blue light emitting diodes on multiprotein complex proteins and photosynthetic activity under different light intensities in lettuce leaves (*Lactuca sativa* L.). Int. J. Mol. Sci. 15 (3), 4657–4670. https://doi.org/10.3390/ijms15034657.

Muráriková, A., Ťažký, A., Neugebauerová, J., Planková, A., Jampílek, J., Mučaji, P., Mikuš, P., 2017. Characterization of essential oil composition in different basil species and pot cultures by a GC–MS method. Molecules 22 (7), 1221. https://doi.org/10.3390/molecules22071221.

Murchie, E.H., Horton, P., 1997. Acclimation of photosynthesis to irradiance and spectral quality in British plant species: chlorophyll content, photosynthetic capacity and habitat preference. Plant Cell Environ. 20 (4), 438–448. https://doi.org/10.1046/j.1365-3040.1997.d01-95.x.

Nejad, S.M., Özgüneş, H., Başaran, N., 2017. Pharmacological and toxicological properties of eugenol. Turk. J. Pharm. Sci. 14 (2), 201. https://doi.org/10.4274/tjps.62207.

Nguyen, P.M., Niemeyer, E.D., 2008. Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). J. Agric. Food Chem. 56 (18), 8685–8691. https://doi.org/10.1021/jf801485u.

Nobel, P.S., Zaragoza, L.J., Smith, W.K., 1975. Relation between mesophyll surface area, photosynthetic rate, and illumination level during development for leaves of *Plectranthus parviflorus* Henckel. Plant Physiol. 55 (6), 1067–1070. https://doi.org/10.1104/pp.55.6.1067.

Olle, M., Viršile, A., 2013. The effects of light-emitting diode lighting on greenhouse plant growth and quality. Agric. Food Sci. 22 (2), 223–234. https://doi.org/10.23986/afsci.7897.

Ort, D.R., Baker, N.R., 2002. A photoprotective role for O2 as an alternative electron sink in photosynthesis? Curr. Opin. Plant Biol. 5 (3), 193–198. https://doi.org/10.1016/S1369-5266(02)00259-5.

Owen, S.M., Harley, P., Guenther, A., Hewitt, C.N., 2002. Light dependency of VOC emissions from selected Mediterranean plant species. Atmos. Environ. 36 (19), 3147–3159. https://doi.org/10.1016/S1352-2310(02)00235-2.

Paik, I., Huq, E., 2019. Plant photoreceptors: multi-functional sensory proteins and their signaling networks. Semin. Cell Dev. Biol. 92, 114–121. https://doi.org/10.1016/j.semcdb.2019.03.007.

Paradiso, R., Proietti, S., 2022. 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. 41, 742–780. https://doi.org/10.1007/s00344-021-10337-y.

Park, H., Seol, G.H., Ryu, S., Choi, I.Y., 2016. Neuroprotective effects of (−)-linalool against oxygen-glucose deprivation-induced neuronal injury. Arch. Pharm. Res. 39 (4), 555–564. https://doi.org/10.1007/s12272-016-0714-z.

Pennisi, G., Blasioli, S., Cellini, A., Maia, L., Crepaldi, A., Braschi, I., Spinelli, F., Nicola, S., Fernandez, J.A., Stanghellini, C., Marcelis, L.F.M., Orsini, F., Gianquinto, G., 2019. Unraveling the role of red: blue LED lights on resource use efficiency and nutritional properties of indoor grown sweet basil. Front. Plant Sci. 10, 305. https://doi.org/10.3389/fpls.2019.00305.

Qian, H., Liu, T., Deng, M., Miao, H., Cai, C., Shen, W., Wang, Q., 2016. Effects of light quality on main health-promoting compounds and antioxidant capacity of Chinese kale sprouts. Food Chem. 196, 1232–1238. https://doi.org/10.1016/j.foodchem.2015.10.055.

Ryu, S., Park, H., Seol, G.H., Choi, I.Y., 2014. 1, 8-Cineole ameliorates oxygen-glucose deprivation/reoxygenation-induced ischaemic injury by reducing oxidative stress in rat cortical neuron/glia. J. Pharm. Pharmacol. 66 (12), 1818–1826. https://doi.org/10.1111/jphp.12295.

Samuolienė, G., Brazaitytė, A., Sirtautas, R., Viršilė, A., Sakalauskaitė, J., Sakalauskienė, S., Duchovskis, P., 2013. LED illumination affects bioactive compounds in romaine baby leaf lettuce. J. Sci. Food Agric. 93 (13), 3286–3291. https://doi.org/10.1002/jsfa.6173.

Samuolienė, G., Viršilė, A., Brazaitytė, A., Jankauskienė, J., Sakalauskienė, S., Vaštakaitė, V., Novičkovas, A., Viškelienė, A., Sasnauskas, A., Duchovskis, P., 2017. Blue light dosage affects carotenoids and tocopherols in microgreens. Food Chem. 228, 50–56. https://doi.org/10.1016/j.foodchem.2017.01.144.

Steyn, W.J., Wand, S.J.E., Holcroft, D.M., Jacobs, G.J.N.P., 2002. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol. 155 (3), 349–361. https://doi.org/10.1046/j.1469-8137.2002.00482.x.

Talbott, L.D., Hammad, J.W., Harn, L.C., Nguyen, V.H., Patel, J., Zeiger, E., 2006. Reversal by green light of blue light-stimulated stomatal opening in intact, attached leaves of *Arabidopsis* operates only in the potassium-dependent, morning phase of movement. Plant Cell Physiol. 47, 332–339. https://doi.org/10.1093/pcp/pci249.

Tattini, M., Landi, M., Brunetti, C., Giordano, C., Remorini, D., Gould, K.S., Guidi, L., 2014. Epidermal coumaroyl anthocyanins protect sweet basil against excess light stress: multiple consequences of light attenuation. Physiol. Plantarum 152 (3), 585–598. https://doi.org/10.1111/ppl.12201.

Taulavuori, K., Pyysalo, A., Taulavuori, E., Julkunen-Tiitto, R., 2018. Responses of phenolic acid and flavonoid synthesis to blue and blue-violet light depends on plant species. Environ. Exp. Bot. 150, 183–187. https://doi.org/10.1016/j.envexpbot.2018.03.016.

Tenore, G.C., Campiglia, P., Ciampaglia, R., Izzo, L., Novellino, E., 2017. Antioxidant and antimicrobial properties of traditional green and purple "Napoletano" basil cultivars (*Ocimum basilicum* L.) from Campania region (Italy). Nat. Prod. Res. 31 (17), 2067–2071. https://doi.org/10.1080/14786419.2016.1269103.

Thoma, F., Somborn-Schulz, A., Schlehuber, D., Keuter, V., Deerberg, G., 2020. Effects of light on secondary metabolites in selected leafy greens: a review. Front. Plant Sci. 11, 497. https://doi.org/10.3389/fpls.2020.00497.

Thum, K.E., Kim, M., Christopher, D.A., Mullet, J.E., 2001. Cryptochrome 1, cryptochrome 2, and phytochrome A co-activate the chloroplast psbD blue light–responsive promoter. Plant Cell 13 (12), 2747–2760. https://doi.org/10.1105/tpc.010345.

Trojak, M., Skowron, E., Sobala, T., Kocurek, M., Pałyga, J., 2022. Effects of partial replacement of red by green light in the growth spectrum on photomorphogenesis and photosynthesis in tomato plants. Photosyn. Res. 151 (3), 295–312. https://doi.org/10.1007/s11120-021-00879-3.

Trouwborst, G., Hogewoning, S.W., van Kooten, O., Harbinson, J., van Ieperen, W., 2016. Plasticity of photosynthesis after the 'red light syndrome' in cucumber. Environ. Exp. Bot. 121, 75–82. https://doi.org/10.1016/j.envexpbot.2015.05.002.

Vrhovsek, U., Masuero, D., Gasperotti, M., Franceschi, P., Caputi, L., Viola, R., Mattivi, F., 2012. A versatile targeted metabolomics method for the rapid quantification of multiple classes of phenolics in fruits and beverages. J. Agric. Food Chem. 60 (36), 8831–8840. https://doi.org/10.1021/jf2051569.

Walters, K.J., Lopez, R.G., Behe, B.K., 2021. Leveraging controlled-environment agriculture to increase key basil terpenoid and phenylpropanoid concentrations: the effects of radiation intensity and CO_2 concentration on consumer preference. Front. Plant Sci. 11, 598519 https://doi.org/10.3389/fpls.2.

Walters, R.G., Shephard, F., Rogers, J.J., Rolfe, S.A., Horton, P., 2003. Identification of mutants of *Arabidopsis* defective in acclimation of photosynthesis to the light environment. Plant Physiol. 131 (2), 472–481. https://doi.org/10.1104/pp.015479.

Wang, H., Gu, M., Cui, J., Shi, K., Zhou, Y., Yu, J., 2009. Effects of light quality on CO_2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. J. Photochem. Photobiol. B 96 (1), 30–37. https://doi.org/10.1016/j.jphotobiol.2009.03.010.

Wang, X.Y., Xu, X.M., Cui, J., 2015. The importance of blue light for leaf area expansion, development of photosynthetic apparatus, and chloroplast ultrastructure of *Cucumis sativus* grown under weak light. Photosynthetica 53 (2), 213–222. https://doi.org/10.1007/s11099-015-0083-8.

XiaoYing, L., ShiRong, G., ZhiGang, X., XueLei, J., Tezuka, T., 2011. Regulation of chloroplast ultrastructure, cross-section anatomy of leaves, and morphology of stomata of cherry tomato by different light irradiations of light-emitting diodes. HortScience 46 (2), 217–221. https://doi.org/10.21273/HORTSCI.46.2.217.

Yang, X., Xu, H., Shao, L., Li, T., Wang, Y., Wang, R., 2018. Response of photosynthetic capacity of tomato leaves to different LED light wavelength. Environ. Exp. Bot. 150, 161–171. https://doi.org/10.1016/j.envexpbot.2018.03.013.

Zhang, X., Bian, Z., Li, S., Chen, X., Lu, C., 2019. Comparative analysis of phenolic compound profiles, antioxidant capacities, and expressions of phenolic biosynthesis-related genes in soybean microgreens grown under different light spectra. J. Agric. Food Chem. 67 (49), 13577–13588. https://doi.org/10.1021/acs.jafc.9b05594.

Zhang, X., Bian, Z., Yuan, X., Chen, X., Lu, C., 2020. A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. Trends Food Sci. Technol. 99, 203–216. https://doi.org/10.1016/j.tifs.2020.02.031.

Zheng, L., Van Labeke, M.C., 2017. Long-term effects of red-and blue-light emitting diodes on leaf anatomy and photosynthetic efficiency of three ornamental pot plants. Front. Plant Sci. 8, 917. https://doi.org/10.3389/fpls.2017.00917.

Zhu, J.K., 2016. Abiotic stress signaling and responses in plants. Cell 167 (2), 313–324. https://doi.org/10.1016/j.cell.2016.08.029.