



Photosynthetic traits and biochemical responses in strawberry (*Fragaria × ananassa* Duch.) leaves supplemented with LED lights

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Abstract

Selected light wavebands promote plant development and/or the biosynthesis of targeted metabolites. This work offers new insights on the effects of red (R), green (G), blue (B), and white (W – R:G:B; 1:1:1) LED light supplementation on physiochemical traits of strawberry leaves. Gas exchange and chlorophyll fluorescence parameters, photosynthetic pigments, and superoxide anion (O_2^-) content were analysed in plants grown for 1 (T1) and 17 (T17) d with light supplementations. At T1, light supplementations resulted in the enhancement of the de-epoxidation state of xanthophylls and nonphotochemical quenching, but no changes were observed in maximal photosynthetic rate ($P_{N\max}$), irrespective of light spectra. At T17, xanthophyll contents remained higher only in R-supplemented plants. Overall, W light resulted in higher photosynthesis, whilst R and B light depressed $P_{N\max}$ values and promoted O_2^- formation at T17. G light did not induce variations in photosynthetic traits nor induced oxidative stress at both T1 and T17.

Keywords: chlorophyll *a* fluorescence; gas exchange; mesophyll conductance; oxidative stress; superoxide anion; xanthophyll.

Introduction

Artificial light supplementation promotes specific reactions of photosynthesis and/or increases plant performance,

especially in protected and indoor environments, in which plants have to compete for light harvesting (Folta and Childers 2008, Cocetta *et al.* 2017). In recent years, light-emitting diodes (LEDs) have been proficiently used

Highlights

- One-day light supplementation promotes nonphotochemical quenching in strawberry
- W light promotes photosynthesis, while R and B depresses it after 17 d of exposure
- G light does not alter xanthophyll contents and photosynthetic traits

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Abbreviations: B – blue light supplementation; Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DEPS – de-epoxidation state of xanthophyll cycle pigments; ETR – electron transport rate; flav – flavonoids; FM – fresh mass; F_v/F_m – maximum quantum yield of PSII; G – green light supplementation; g_m – mesophyll conductance; g_s – stomatal conductance; J_{1300} – electron transport obtained at saturating light; LED – light emitting diode; NBI – nitrogen balance index; NS – control; P_N – net photosynthetic rate; $P_{N\max}$ – light-saturated net photosynthetic rate; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; R – red light supplementation; R_L – mitochondrial respiration in the light; ROS – reactive oxygen species; SE – standard error; T1/T17 – 1/17 d of light supplementation; TPU – triose phosphate-utilization rate; VAZ – total amount of xanthophyll cycle components; $V_{c\max}$ – maximum rate of carboxylation of Rubisco; W – white (R:G:B; 1:1:1) light supplementation; XTT – 3'-(1-[phenylamino-carbonyl]-3,4-tetrazolium)-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate; α -toc – α -tocopherol; Γ^* – CO_2 compensation point in absence of respiration; Φ_{NPQ} – quantum yield of nonphotochemical quenching; Φ_{PSII} – operational quantum yield of PSII photochemistry.

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as light sources in greenhouse cultivation and they have a great commercial appeal due to their benefits for both research and productive purposes (Bantis *et al.* 2018). LED-based light sources have numerous advantages over traditional light sources: (1) LED allows control over emission spectrum (Bourget 2008, Morrow 2008), (2) they are more energy-efficient (Singh *et al.* 2015); (3) radiate very little heat so can be placed close to the plants thereby increasing the irradiances over the leaf lamina; (4) their compactness reduces shadowing problems (Cocetta *et al.* 2017). LEDs are often used to provide supplemental narrow-band light, which may exert specific and targeted effects on morphoanatomical traits, photosynthesis, as well as secondary metabolism, in particular with 'color-specific' effects (Landi *et al.* 2020), and also activate plant defence against pathogens (Ballaré 2014). Selected LED lights are used in a controlled environment, mainly in growth chambers, whereas only a little research has been conducted on the use of specific LED wavebands in greenhouse-enrichment experiments (Bantis *et al.* 2018).

Blue LED light was reported to increase net photosynthetic rate (P_N) and stomatal conductance (g_s) under high-light conditions (Li *et al.* 2017) as well as decrease electron transport rate (ETR) and PSII quantum yield (Φ_{PSII}) values associated with an increase in heat dissipation from PSII (NPQ and Φ_{NPQ}) (Ouzounis *et al.* 2015). Conversely, red light supplementation was more effective in increasing P_N especially under low-light conditions (Li *et al.* 2017). Green light is usually neglected due to the misconception that plants do not absorb the green region of the spectrum and only reflect those wavelengths (Smith *et al.* 2017). Differently, some studies underlined that green light can be absorbed by chlorophyll (Chl) *b* in the deeper canopy layers (Sun *et al.* 1998) and by carotenoids (Govindjee 1982a,b); furthermore, green light can act as a shade signal (Smith *et al.* 2017) and increase water-use efficiency within the canopy layers (Frechilla *et al.* 2000, Talbott *et al.* 2002, 2006). Green light also regulates some essential processes of the plant, from seed germination to flowering (Golovatskaya and Karnachuk 2015). Given these assumptions, Smith *et al.* (2017) suggested the use of green LED light supplementation for greenhouse production.

The use of selected LED colours exerts a plethora of reactions, in most cases species-specific and waveband-dependent on the secondary metabolism of plants (Landi *et al.* 2020). Overall, the supplementation of LED lights (independently to the emission spectra) generally promotes the increase of Chl content in some leafy greens (Matysiak and Kowalski 2019), whereas, in terms of flavonoid metabolism, blue LED light usually stimulates the activation of anthocyanin pattern (Mizuno *et al.* 2011, Olle and Virsilè 2013). In controlled environments, red light also stimulated the biosynthesis of other polyphenols (Li and Kubota 2009) as well as glucosinolates (Lefsrud *et al.* 2008). In preharvest, the application of red LED increased the antioxidant capacity in lettuce leaves (Zukauskas *et al.* 2011) and reduced the nitrate content in lettuce and onion (Samuolienė *et al.* 2009). Moreover, red light increased shoot fresh biomass in *Eruca sativa* L. and stimulated

leaf elongation of *Valerianella locusta* and garden rocket plants (Matysiak and Kowalski 2019).

In other cases, blue LED light promotes the accumulation of other secondary metabolites, including flavonols (Matysiak and Kowalski 2019), phenolic acids, flavonoids *sensu lato*, and pigments in red leaf lettuce (Ouzounis *et al.* 2015), chlorophylls in cabbage seedlings (Mizuno *et al.* 2011), and anthocyanins and carotenoids in baby leaf lettuce (Li and Kubota 2009). Blue LED light also promoted petiole elongation in cabbage seedlings (Mizuno *et al.* 2011).

Because of the modulatory effect on secondary metabolism, specific narrow-band light supplementation may also promote fruit quality (Landi *et al.* 2020) as well as plant defence reactions. For example, red LEDs significantly reduced the incidence of powdery mildew (*Sphaerotheca fuliginea*) in cucumber plants (Wang *et al.* 2010) and both blue (Kim *et al.* 2013) and red (Xu *et al.* 2017) LED light inhibited the development of grey mould disease (*Botrytis cinerea*) in tomato plants due to the stimulation of plant-defensive compounds.

As the topic is a warrant further investigation, for the present experiment, we selected strawberry (*Fragaria* × *ananassa*) which is a perennial herbaceous species belonging to Rosaceae family, widely cultivated for its fruits, and economically relevant for indoor cultivation. For its small size and short life cycle, it can be considered a good model plant. Despite several studies that have been carried out on this species, scarce information is available on the possible effect of light supplementation in greenhouse-grown plants. Previous experiments conducted with the strawberry highlighted that supplementation of plants with composite LED lighting systems enhanced photosynthetic traits (Hidaka *et al.* 2013). Red-LED supplementation resulted effective in the promotion of the early flowering and fruiting stage (Jamal Uddin *et al.* 2018). Blue light resulted effective in increasing leaflets length (Choi *et al.* 2015), plant height, leaf number and area (Jamal Uddin *et al.* 2018), and strawberry fruit production (Choi *et al.* 2015). To date, no data are available for the green light.

The establishment of a positive effect by supplementation of some LED colours (red, green, and blue) on fruit quality and plant resistance against some pathogens (e.g., *B. cinerea*) should be tested. However, it is essential to evaluate the effect of different LED colour supplementation in leaves, this in order to prevent damages to the photosynthetic apparatus as well as avoid improper development of the plants. Indeed, specific red-, green-, blue-light-triggered physiobiochemical changes in leaves could nullify the use of such wavebands to promote fruit quality and pathogen resistance response.

Assuming that little and not consistent information is available about the effect of LED light supplementation in strawberry plants, the present research aimed to investigate the effects of light provided by red (R), green (G), and blue (B) LEDs, and white (W) light supplementation (R:G:B; 1:1:1) on physiological and biochemical features of strawberry leaves. The present work aims to select the best light supplementation to stimulate strawberry

photosynthetic performances. Future research will evaluate the impact of those LED supplementations in fruit quality and disease resistance in indoor-grown strawberry plants.

Materials and methods

Plant material and growth conditions: Twenty-day-old commercial strawberry plants (*Fragaria × ananassa*) were transplanted on 30 June 2020 into a glasshouse located at the Department of Agriculture, Food and Environment (DiSAAA-a), University of Pisa (43.704672°N, 10.427292°E). Each plant was transplanted in a 3-L pot filled with sandy soil–peat mixture (60:40; v:v). Plants were irrigated with deionized water during the first week after transplanting and afterwards with a nutrient solution containing the following nutrient concentrations: 14.0 mM NO_3^- , 1.0 mM NH_4^+ , 1.0 mM P, 6.03 mM K^+ , 3.14 mM Ca^{2+} , 1.7 mM Mg^{2+} , 5.22 mM Na^+ , 2.39 mM SO_4^{2-} , 15.0 μM Fe^{2+} , 20.00 μM BO_3^- , 1.0 μM Cu^{2+} , 5.0 μM Zn^{2+} , 10.0 μM Mn^{2+} , 1.0 μM Mo^{3+} . Electrical conductivity (EC) was 1.9 mS cm^{-1} ; pH values were adjusted to 5.5 with diluted sulfuric acid.

Experiments took place from the end of June to the end of July and climatic parameters were continuously monitored by a weather station placed inside the glasshouse. The minimum and average air temperatures were 24 and 30°C, respectively; the maximum temperature reached up to 32–36°C in sunny hours. In total, 20 plants were used (five replicates per treatment). Control (NS) was exposed to ambient light only, while for other treatments plants were exposed to ambient light with supplemental LED lighting. For LED illumination, four lamps (1,200 × 68 × 36 mm) purchased from *Ambra Elettronica s.r.l.* (Bolzano Vicentino, VI, Italy) were used: red (R; peak 660 nm), blue (B; 450 nm), green (G; 530 nm), and white (W – R:G:B, 1:1:1), radiating 250 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ of light for five hours per day (from 11:00 to 16:00 h). Light spectra of each lamp were reported in Fig. 1. Analysis was conducted on leaves continuously exposed to the treatments from the

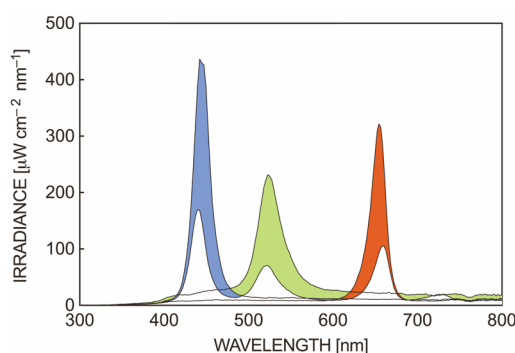


Fig. 1. Spectrum of LED supplemental light sources used in the experiment: blue LED light (peak wavelength at 450 nm); green LED light (peak wavelength at 530 nm); red LED light (peak wavelength at 660 nm); white LED light (peak wavelength at 450, 530, and 660 nm). The spectral distributions were measured with a handheld spectroradiometer (*SpectraPen*, Photon Systems Instruments, Drásov, Czech Republic).

beginning of the trials; flower buds were removed for the maintenance of homogenous conditions. Measurements were taken the day after 24 h of light supplementation (T1) and after 17 d (T17).

Two kinds of analysis were conducted: nondestructive physiological investigations (encompassing gas exchange, Chl *a* fluorescence, and *DUALLEX*[®] measurements) and destructive biochemical analyses (evaluation of chlorophylls, carotenoids, tocopherol content, and oxidative stress markers). For destructive analyses, samples were firstly collected into liquid nitrogen, cryogenically ground, and stored at –80°C.

Gas exchange and Chl *a* fluorescence parameters were measured ($n = 3$) using a portable infrared gas analyser *LI-6400* system (*Li-Cor*, Lincoln, NE, USA) and *PAM-2000* fluorometer (*Walz*, Effeltrich, Germany), respectively.

Gas-exchange measurements were conducted on randomly selected fully expanded leaves from 10:00 to 14:00 h at light of saturating intensity of 1,300 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$. Inside the leaf chamber, the CO_2 concentration was set to 400 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$ by using the CO_2 mixer, the leaf temperature was blocked at ~30°C, relative humidity was maintained at ~50%, and the flow rate was 500 $\mu\text{mol s}^{-1}$. Once the steady state was reached, light-saturated net photosynthetic rate (P_{Nmax}), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were recorded. Immediately after P_N/C_i response curves were conducted on the same leaves. P_N/C_i curves were obtained by adjusting the CO_2 concentration inside the leaf chamber to 400, 200, 150, 50, 400, 600, 800; 1,000; 1,200; 1,500 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$. The maximum rate of carboxylation (V_{cmax}), electron transport obtained at saturating light of 1,300 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ (J_{1300}), and triose phosphate-utilization rate (TPU) were estimated according to Long and Bernacchi (2003), based on the model of Farquhar *et al.* (1980). Mitochondrial respiration in the light (R_L) was considered as half of the mitochondrial respiration (measured during the dark; Gago *et al.* 2013), and the CO_2 -compensation point in absence of respiration (Γ^*) was assumed to be 44.04 at 30°C (Bernacchi *et al.* 2002). Mesophyll conductance (g_m) was estimated using the variable J method (Harley *et al.* 1992), through the combination of gas exchange and Chl *a* fluorescence analysis.

Chl *a* fluorescence parameters were measured from 10:00 to 14:00 h in leaves homogeneous to those used for gas exchange. After 30 min of dark adaptation, operational quantum yield (Φ_{PSII}), nonphotochemical (q_N) and photochemical quenching coefficient (q_P), the maximum quantum yield of PSII (F_v/F_m), and electron transport rate (ETR) of PSII were measured. Analysis was performed on the leaf central section of the three leaflets terminal leaf, avoiding midrib.

Chl, flavonoid indexes, and nitrogen balance index (NBI) analysis: Investigations were performed using a *DUALLEX*[®] (*Force-A*, Orsay, France). NBI was considered a good estimator of leaf nitrogen content. Data

were collected on mature leaves from three samples per treatment. Each sample was analysed thrice on the abaxial and adaxial leaf face.

Chl, carotenoids, and α -tocopherol content determination: According to Garcia-Plazaola and Esteban (2016), samples between 0.03–0.05 g of fresh matter (FM) were added to 100% HPLC-grade methanol (1:10, w:v) and stored at 4°C in the dark for 12 h. Later, samples were centrifuged at $12,000 \times g$ for 10 min at 4°C, and supernatants were filtered with 0.22 μm Minisart® SRT 15 filters. For pigments determination, an UltraHPLC Dionex UltiMate 3000 system was used, equipped with an UltiMate autosampler, a Dionex TCC-100 column oven, and a Dionex UVD 170 U UV-Vis detector (Thermo Scientific, Waltham, MA, USA). Each running last for 30 min with a 1 mL min⁻¹ flow. Acetonitrile/methanol (75:25, v:v) was used at 100% for the first 12 min followed by 1.5-min linear gradient to 100% solved B methanol/ethyl acetate (68:32, v:v), 15 min with 100% solvent B, followed by 2-min linear gradient to 100% solvent A to elute respectively xanthophylls on one hand and chlorophylls and β -carotene on the other. A Dionex™ C18 Acclaim 120, Thermo Scientific™ column (5 μm particle size, 4.6 mm internal diameter \times 150 mm length) was used, by setting the column oven at 30°C. Two specific wavelengths were controlled with a UV-Vis detector: 295 nm for α -tocopherol determination and 445 nm for photosynthetic pigments. Results were compared with standards obtained as 1 mg mL⁻¹ solution in pure HPLC methanol and then filtrated with 0.22 μm filters, while calibration solutions were obtained through dilution. Chl, carotenoids, and α -tocopherol quantitative determination data were processed with Chromeleon Chromatography Management System software, version 7.2.10-2019 (Thermo Scientific). Chl *a/b* ratio, total Chl content [Chl (*a* + *b*)], total amount of xanthophyll cycle components (VAZ: violaxanthin + antheraxanthin + zeaxanthin), the de-epoxidation state of xanthophyll cycle pigments (DEPS) calculated as (antheraxanthin + zeaxanthin)/(violaxanthin + antheraxanthin + zeaxanthin), and Car/Chl ratio calculated as (β -carotene + lutein + violaxanthin + antheraxanthin + zeaxanthin)/(Chl *a* + *b*) were also determined.

Superoxide anion determination: The $\cdot\text{O}_2^-$ content was determined using the tetrazolium salt dye, 3'-(1-[phenyl-amino-carbonyl]-3,4-tetra-zolium)-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate (XTT) which can be reduced by $\cdot\text{O}_2^-$ to a soluble formazan that can be readily quantified in solution (Sutherland and Learmonth 1997). Samples [0.10 g(FM)] were extracted with 1 mL of 50 mM (pH 7.8) K₃PO₄ buffer solution. After centrifugation ($10,000 \times g$ for 15 min at 4°C) supernatants were selected. Solution obtained as 287 μL buffer + 3 μL XTT + 10 μL extract, filled each well. XTT reduction was evaluated every 30 min for 180 min total, measuring absorbance at 470 nm. Data obtained at 60 min were selected. $\cdot\text{O}_2^-$ concentration was determined using molar extinction coefficient (ϵ) 21.6 mM⁻¹ cm⁻¹ (Able *et al.* 1998) and expressed as nmol g⁻¹(FM).

Statistical analysis: Each data represents the mean value of three independent replicates (\pm SE), except for *Dualix*® data whose mean is derived from nine replicates. Homoscedasticity of data was evaluated by *Bartlett's* test. All 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 *LSD post-hoc* test ($P \leq 0.05$).

Results

Gas-exchange analysis: At T1, no significant differences between treatments were found for $P_{N_{\text{max}}}$, whereas at T17, W-supplemented plants showed the highest values of $P_{N_{\text{max}}}$ [$17.15 \pm 0.25 \mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$], followed by control (NS) and G. At the same time, in the B- and R-supplemented leaves the lowest values of $P_{N_{\text{max}}}$ were recorded [12.37 ± 0.57 and $12.53 \pm 0.95 \mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$, respectively; Fig. 2A]. At T1, values of g_s were similar in leaves of NS plants and those subjected to R and G treatments; plants supplemented with W light showed higher values than that of the aforementioned treatments, whereas g_s was significantly depressed by B light (Fig. 2B). At T17, no significant differences were observed between B, R, G vs. NS and only W light induced an increase of stomatal conductance compared to NS, B, and G (+15.2, +22.7, and +24.2%, respectively; Fig. 2B). At T1, plants grown with W light supplementation had higher values of C_i for NS, B, and G treatments, whereas only B-enriched plants showed the lowest values among all the treatments [$223.00 \pm 1.00 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$; Fig. 2C]. At T17, B- and R-supplemented plants resulted in the highest values of C_i values for NS, W, and G light (Fig. 2C). At T1, B resulted in increased g_m levels (+21.1% compared with NS), whereas no significant differences were recorded between the other light treatments. At T17, NS plants and those enriched with G light shared similar values of g_m , whilst B and R light induced a decline of this parameter. W resulted in higher values of g_m compared to other light treatments (Fig. 2D). V_{cmax} increased under W and B at T1 compared to NS (+16.9 and +30.1%, respectively), reaching the highest values under B treatment [$100.60 \pm 0.35 \mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$; Fig. 2E]. After 17 d, values of V_{cmax} were similar in NS, W, and G, while B and R resulted in lower carboxylation rates (−25.5 and −34.2% compared with NS, respectively; Fig. 2E). J_{1300} and TPU values had a similar trend: at T1, no significant differences emerged between treatments, whereas at T17, their values decreased in B- and R-supplemented plants (Fig. 2F,G).

Chl *a* fluorescence analysis: No significant variations were observed for Φ_{PSII} , F_v/F_m , q_p (Fig. 3A,C,D), and ETR (data not shown), irrespectively to light treatments and time exposure. At T1, W, B, and G induced an increment of q_N compared with NS (+60.5, +26.7, and +69.6%, respectively) but after 17 d, W- and B-supplemented plants accounted for similar q_N values as NS, whereas R and G light induced a reduction of this parameter (Fig. 3B). Moreover, values of F_v/F_m ranged from 0.78 to 0.80, irrespectively to treatments and time of exposure.

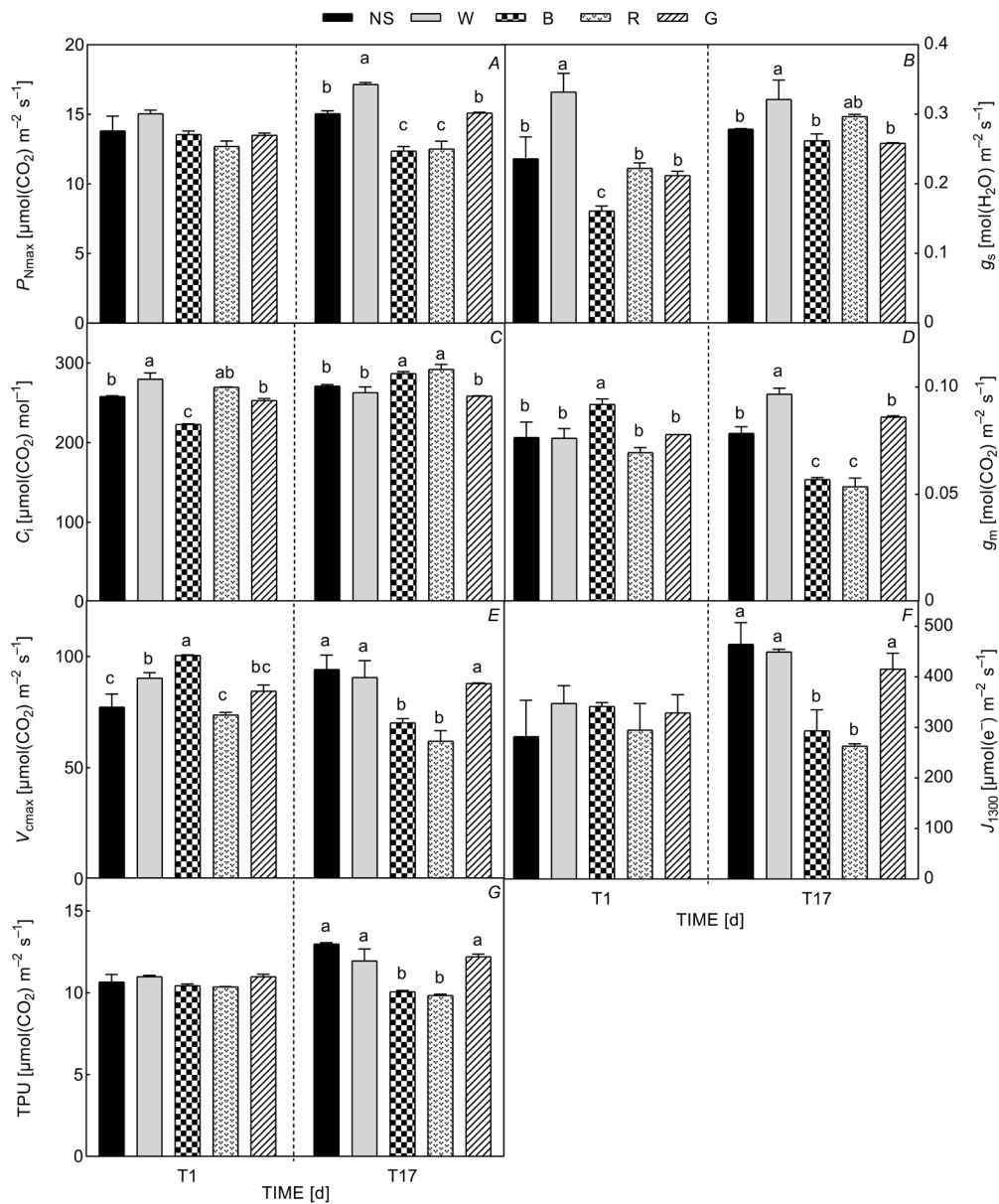


Fig. 2. Light-saturated net photosynthetic rate (P_{Nmax}) (A), stomatal conductance (g_s) (B), intercellular CO_2 concentration (C_i) (C), mesophyll conductance (g_m) (D), maximum rate of carboxylation of Rubisco (V_{cmax}) (E), electron transport obtained at saturating light of $1,300 \mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$ (J_{1300}) (F), and triose phosphate utilization rate (TPU) (G) detected in strawberry leaves. Values were recorded after 1 and 17 d of light supplementation (T1 and T17). Treatments: nonsupplemented control (NS), white light (W – R:G:B, 1:1:1), blue (B), red (R), and green (G) light supplementation. Means (\pm SE, $n = 3$) with different letters are significantly different after one-way ANOVA followed by Tukey's multiple range post-hoc test ($P \leq 0.05$). The absence of letters indicates no significance of the F ratio.

Chl, flavonoid index, and NBI analysis: At T1, no significant differences in NBI were observed at the adaxial leaf surface, while at the abaxial side, only R light induced a decline of this parameter (Table 1). At T17, W and G induced an increment of NBI with G-supplemented plants which accounted for the highest values at the adaxial leaf side. Conversely, B depressed the NBI while R light did not induce significant differences as compared to NS plants. At the abaxial surface, NBI values were higher than

those of NS, in order of G, W, and R; B did not affect this parameter.

For the Chl index, at T1, the only detected difference was related to R light which induced a significant reduction of this index at both leaf surfaces. Conversely, at T17, W induced a Chl boost at each leaf face, whilst G only promoted the Chl index increment at the abaxial side; B- and R-supplemented and NS plants shared similar Chl index values.

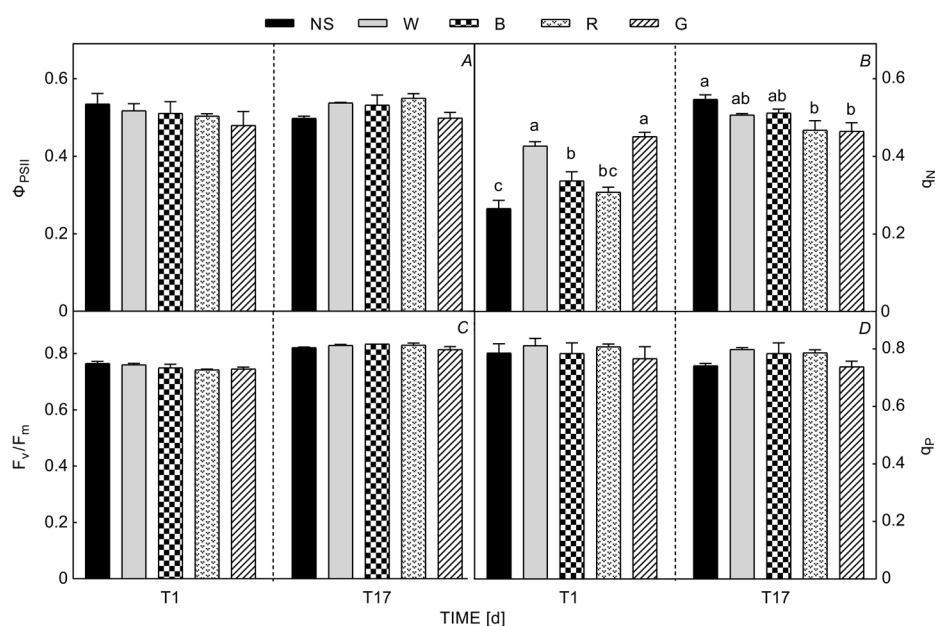


Fig. 3. Values of operational quantum yield (Φ_{PSII}) (A), nonphotochemical quenching coefficient (q_N) (B), maximum quantum yield (F_v/F_m) (C), and photochemical quenching coefficient (q_p) (D) of PSII detected in strawberry leaves. Values were recorded after 1 and 17 d of light supplementation (T1 and T17). Treatments: nonsupplemented control (NS), white light (W – R:G:B, 1:1:1), blue (B), red (R), and green (G) light supplementation. Means (\pm SE, $n = 3$) with *different letters* are significantly different after one-way *ANOVA* followed by *Tukey's* multiple range post-hoc test ($P \leq 0.05$). The absence of letters indicates no significance of the F ratio.

Table 1. Nitrogen balance index (NBI), chlorophyll (Chl) and flavonoid (Flav) index related to adaxial and abaxial strawberry leaf surface. Values were recorded after 1 and 17 d of light supplementation (T1 and T17). Treatments: nonsupplemented control (NS), white light (W – R:G:B, 1:1:1), blue (B), red (R), and green (G) light supplementation. In each column, means (\pm SE, $n = 9$) with *different letters* are significantly different after one-way *ANOVA* followed by *Tukey's* multiple range post-hoc test ($P \leq 0.05$). The absence of the letters indicates no significance of the F ratio.

Leaf surface	Treatment	T1			T17		
		NBI	Chl	Flav	NBI	Chl	Flav
Adaxial	NS	21.0 \pm 3.3	34.0 \pm 2.7 ^a	1.64 \pm 0.17 ^{ab}	20.4 \pm 2.2 ^c	32.5 \pm 4.3 ^b	1.59 \pm 0.10 ^{bc}
	W	21.0 \pm 3.6	35.8 \pm 2.6 ^a	1.75 \pm 0.24 ^a	22.9 \pm 2.6 ^b	38.3 \pm 3.2 ^a	1.68 \pm 0.08 ^b
	B	22.1 \pm 4.1	35.2 \pm 2.2 ^a	1.64 \pm 0.26 ^{ab}	16.6 \pm 3.1 ^d	31.7 \pm 6.0 ^b	1.92 \pm 0.15 ^a
	R	18.6 \pm 1.9	29.6 \pm 2.9 ^b	1.59 \pm 0.03 ^{ab}	21.7 \pm 1.6 ^{bc}	34.0 \pm 1.7 ^b	1.46 \pm 0.38 ^c
	G	23.5 \pm 3.5	34.0 \pm 2.8 ^a	1.46 \pm 0.14 ^b	25.0 \pm 1.0 ^a	37.6 \pm 2.7 ^{ab}	1.50 \pm 0.09 ^{bc}
Abaxial	NS	31.5 \pm 5.1 ^a	33.8 \pm 2.2 ^a	1.09 \pm 0.15	26.0 \pm 3.8 ^c	32.0 \pm 4.3 ^b	1.23 \pm 0.05 ^{ab}
	W	31.0 \pm 4.3 ^a	35.5 \pm 3.2 ^a	1.16 \pm 0.08	31.8 \pm 2.5 ^{ab}	37.7 \pm 2.7 ^a	1.19 \pm 0.04 ^b
	B	31.7 \pm 3.1 ^a	34.7 \pm 1.7 ^a	1.10 \pm 0.08	25.9 \pm 4.8 ^c	32.2 \pm 5.9 ^b	1.25 \pm 0.10 ^a
	R	25.9 \pm 3.2 ^b	28.8 \pm 2.6 ^b	1.12 \pm 0.09	29.3 \pm 2.3 ^b	34.7 \pm 1.9 ^{ab}	1.19 \pm 0.07 ^b
	G	31.0 \pm 4.4 ^a	34.2 \pm 2.6 ^a	1.11 \pm 0.10	33.1 \pm 1.8 ^a	37.3 \pm 2.6 ^a	1.11 \pm 0.03 ^c

Flavonoid index values did not differ from the control at the early stage of light treatments, irrespective of the leaf surface, but at T17, the highest value was recorded at the adaxial surface in B-treated plants, while G promoted a reduction of this index at the abaxial leaf side.

Chl, carotenoid, and tocopherol content determination: At T1, R induced a significant increment of total Chl content (+25.9% compared with NS) due to a concomitant

rise of Chl *a* and Chl *b* contents (Table 2). After 17 d of light supplementation, no differences emerged in Chl *a* content between treatments and control. At this sampling time, R and B induced a reduction in Chl *b* content (–27.1% in both treatments), and Chl (*a* + *b*) was reduced under R light (–28.1% compared with NS; Table 2). Concerning the Chl *a/b* ratio, both R and G did not induce significant differences as compared with NS, while plants supplemented with W light showed the highest value

(1.99 ± 0.32), while plants under B were the lowest one (1.21 ± 0.13). At T17, there were no statistical differences between treatments and NS plants, except for B-supplemented plants in which the highest Chl *a/b* ratio was recorded (2.24 ± 0.29).

About xanthophylls (Table 3), G light significantly enhanced violaxanthin content. Antheraxanthin and zeaxanthin contents increased in all the treatments, with a maximum content measured under G light for zeaxanthin, but after 17 d, no significant differences emerged between treatments and control plants in antheraxanthin content, while R resulted as the only light source able to promote a higher zeaxanthin content. At T1, VAZ improvement was significant under G light, while at T17 both G and R treatments enhanced the total amount of xanthophyll cycle components. DEPS index increased in all the supplemented plants at T1 (reaching the highest values under W and B), but at T17, no statistical differences were recorded between LED lights vs. NS.

Lutein contents increased at T1 with B, R, and G supplementation compared with NS (+20.5, +35.1, and +35.1%, respectively), but at T17, only B and G treatments were truly effective for enhancing its content (+28.2 and +20.6%, respectively; Table 4). W light enhanced β -carotene content at T1 (+32.1%), but after 17 d, all the light treatments stimulated the content of this carotenoid, except for R-supplemented plants whose values remained similar to those of NS ones (Table 4). No trace of α -carotene was detected irrespective of light treatment and time of exposure (data not shown). At T1, Car/Chl ratio was enhanced by B treatment (+37.4% compared with NS), whereas at T17, R light supplementation only induced significant differences compared with NS and W (+68.8 and +49.9%, respectively). The content of α -tocopherol increased in R-supplemented plants at T1 (+34.9% compared with NS), while the highest value was observed in plants grown with B-enriched light, which at T1 showed, conversely, the lowest accumulation (Table 4). Notably, at T17, R-supplemented plants showed the lowest values of α -tocopherol.

Superoxide anion determination: An increase in $\cdot\text{O}_2^-$ was initially reported under W and R light (+37.1 and +28.9% compared with NS), while the contents measured in plants

subjected to B and G light enrichment did not differ from those found in NS counterparts. After 17 d, W and B treatments resulted in the highest $\cdot\text{O}_2^-$ production, followed by R light. G treatment did not enhance superoxide anion content nor at T1 or T17 compared with NS (Fig. 4).

Discussion

Light enrichment *via* specific wavebands is nowadays feasible by LEDs, which are inexpensive and eco-friendly lighting devices and therefore can be considered as a valid tool to stimulate the photosynthetic process in indoor cultivation, especially in light-limiting periods (Bantis *et al.* 2018, Matysiak and Kowalski 2019). Different light spectra can selectively stimulate leaf photoreceptors, which in turn induce distinct signalling transduction pathways and gene regulation affecting several physiological and biochemical processes that drive leaf photosynthesis (Wang *et al.* 2009, Landi *et al.* 2020). Therefore, a deep knowledge of the waveband-dependent effects of light enrichment by LED in specific crop species (*e.g.*, strawberry – Choi *et al.* 2015) is essential for efficient management of their indoor cultivation.

To date, most studies agree that there are three main players (among physiological and biochemical processes) that drive photosynthesis in plants: (1) the CO_2 diffusion through stomata (g_s), (2) the CO_2 diffusion through the mesophyll (g_m), and (3) the Rubisco carboxylation activity (Galmés *et al.* 2017, Gago *et al.* 2020). In the present study, the decreased g_s found at T1 under supplemental B light did not affect $P_{N_{\max}}$. The preservation of $P_{N_{\max}}$ (at values similar to those found in NS) was due to the concomitant increase of the efficiency in the diffusion of CO_2 through the mesophyll (g_m) and in the maximum carboxylation rate by Rubisco (V_{cmax}), which resulted in reduced C_i . Changes in these photosynthetic traits highlight that fine coordination between CO_2 diffusion and fixation is critical for achieving the maximum photosynthetic capacity (Galmés *et al.* 2017, Hu *et al.* 2019). However, opposite results concerning the effects of blue light on g_m were detected in *Nicotiana tabacum*, *Platanus orientalis*, and *Populus trichocarpa* (Loreto *et al.* 2009, Momayyezi and Guy 2017), where a reduction in mesophyll conductance was measured immediately

Table 2. Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll [Chl (*a* + *b*)] and chlorophyll *a*-to-chlorophyll *b* ratio (Chl *a/b*) values detected in strawberry leaves. Values were recorded after 1 and 17 d of light supplementation (T1 and T17). Treatments: nonsupplemented control (NS), white light (W – R:G:B, 1:1:1), blue (B), red (R), and green (G) light supplementation. Means (\pm SE, $n = 3$) with different letters are significantly different after one-way ANOVA followed by Tukey's multiple range post-hoc test ($P \leq 0.05$). FM – fresh mass.

Treatment	Chl <i>a</i> [$\mu\text{mol g}^{-1}(\text{FM})$]		Chl <i>b</i> [$\mu\text{mol g}^{-1}(\text{FM})$]		Chl (<i>a</i> + <i>b</i>) [$\mu\text{mol g}^{-1}(\text{FM})$]		Chl <i>a/b</i>	
	T1	T17	T1	T17	T1	T17	T1	T17
NS	1.00 ± 0.15^b	0.79 ± 0.02^{ab}	0.65 ± 0.09^b	0.59 ± 0.02^a	1.64 ± 0.24^b	1.39 ± 0.00^a	1.55 ± 0.04^b	1.33 ± 0.09^b
W	1.22 ± 0.16^{ab}	0.81 ± 0.08^{ab}	0.62 ± 0.04^b	0.59 ± 0.02^a	1.84 ± 0.14^{ab}	1.40 ± 0.09^a	1.99 ± 0.32^a	1.37 ± 0.12^b
B	0.77 ± 0.04^b	0.94 ± 0.02^a	0.64 ± 0.08^b	0.43 ± 0.06^b	1.41 ± 0.09^b	1.37 ± 0.08^a	1.21 ± 0.13^c	2.24 ± 0.29^a
R	1.27 ± 0.16^a	0.57 ± 0.13^b	0.80 ± 0.03^a	0.43 ± 0.08^b	2.07 ± 0.19^a	1.00 ± 0.20^b	1.60 ± 0.17^b	1.31 ± 0.11^b
G	1.05 ± 0.11^{ab}	0.66 ± 0.18^b	0.64 ± 0.06^b	0.64 ± 0.03^a	1.69 ± 0.14^b	1.29 ± 0.19^a	1.63 ± 0.16^b	1.04 ± 0.30^b

Table 3. Violaxanthin, antheraxanthin, VAZ (violaxanthin + zeaxanthin), and DEPS (zeaxanthin + antheraxanthin)/(violaxanthin + antheraxanthin + zeaxanthin) values detected in strawberry leaves. Values were recorded after 1 and 17 d of light supplementation (T1 and T17). Treatments: nonsupplemented control (NS), white light (W – R:G:B, 1:1:1), blue (B), red (R), and green (G) light supplementation. Means (\pm SE, $n = 3$) with *different letters* are significantly different after one-way ANOVA followed by Tukey's multiple range post-hoc test ($P \leq 0.05$). FM – fresh mass.

Treatment	Violaxanthin [$\mu\text{mol g}^{-1}(\text{FM})$]		Antheraxanthin [$\mu\text{mol g}^{-1}(\text{FM})$]		Zeaxanthin [$\mu\text{mol g}^{-1}(\text{FM})$]		VAZ [$\mu\text{mol g}^{-1}(\text{FM})$]		DEPS	
	T1	T17	T1	T17	T1	T17	T1	T17	T1	T17
NS	0.67 \pm 0.08 ^b	0.51 \pm 0.04 ^b	0.00 \pm 0.00 ^b	0.01 \pm 0.00	0.02 \pm 0.01 ^c	0.05 \pm 0.00 ^b	0.69 \pm 0.08 ^b	0.57 \pm 0.03 ^b	0.03 \pm 0.01 ^c	0.09 \pm 0.01
W	0.60 \pm 0.15 ^b	0.42 \pm 0.04 ^b	0.00 \pm 0.00 ^a	0.00 \pm 0.00	0.05 \pm 0.00 ^b	0.04 \pm 0.00 ^b	0.65 \pm 0.16 ^b	0.47 \pm 0.04 ^b	0.08 \pm 0.01 ^a	0.10 \pm 0.01
B	0.76 \pm 0.09 ^b	0.50 \pm 0.14 ^b	0.01 \pm 0.00 ^a	0.01 \pm 0.00	0.06 \pm 0.00 ^{ab}	0.04 \pm 0.00 ^b	0.82 \pm 0.09 ^b	0.55 \pm 0.14 ^b	0.08 \pm 0.01 ^a	0.09 \pm 0.02
R	0.70 \pm 0.19 ^b	0.66 \pm 0.10 ^{ab}	0.01 \pm 0.00 ^a	0.01 \pm 0.00	0.05 \pm 0.01 ^b	0.06 \pm 0.00 ^a	0.76 \pm 0.20 ^b	0.72 \pm 0.10 ^a	0.08 \pm 0.01 ^{ab}	0.09 \pm 0.01
G	1.04 \pm 0.17 ^a	0.75 \pm 0.01 ^a	0.00 \pm 0.00 ^a	0.01 \pm 0.00	0.06 \pm 0.00 ^a	0.05 \pm 0.01 ^{ab}	1.11 \pm 0.17 ^a	0.80 \pm 0.00 ^a	0.06 \pm 0.01 ^b	0.07 \pm 0.01

after the exposition to blue light. It is possible to speculate that the different experimental setup was responsible for this apparent contradiction (*e.g.*, light quality and intensity, environmental conditions, and plant species). To elucidate this pattern, further studies should be conducted to understand the positive transient effect (at 17 d, B plants showed a strong decrease in g_m values indeed) on g_m of supplemental blue light on leaves.

At T17, both g_s and g_m were higher in W-supplemented plants. Given that other photosynthetic parameters, such as V_{cmax} , J_{1300} , and TPU, did not change in W-supplemented plants, it is conceivable that CO_2 diffusive limitations were the main photosynthesis limitations under these experimental conditions. The decrease in P_{Nmax} observed in B and R treatments was attributable to the concomitant limitation occurring at physiological (g_m) and biochemical processes (V_{cmax} , J_{1300} , and TPU). At T17, B treatment induced a significant reduction of g_m , and this response could be partially due to the chloroplast movements, whilst the reduction in V_{cmax} values can be related to the depression in carbonic anhydrase activity by B light (Loreto *et al.* 2009, Momayyezi and Guy 2017). The reduction in photosynthetic performance observed under R treatment was likely related to the reduction in Rubisco content (Muneer *et al.* 2014), and in particular the small subunit (Su *et al.* 2014), or to a lower leaf nitrogen content, responsible, in turn, for a lower chlorophyll content (Kim *et al.* 2004), as also observed in our experiments (Tables 1, 2). Notably, G light did not exert any effect on gas-exchange parameters.

Red, green, blue LED light did not induce any remarkable effect on PSII photochemical phase and, at both T1 and T17, plants belonging to all the treatments showed similar values in maximum quantum yield of PSII (F_v/F_m), which is supportive for no damages to PSII. Irrespectively to the time of exposure, in our experiment, any treatment impacted the effective efficiency of PSII (Φ_{PSII}) and the values of q_p as well, whereas Wang *et al.* (2009) reported that Φ_{PSII} significantly decreased in cucumber plants subjected to different LED lights (in the order: blue, purple, green, yellow, and red) as a consequence of q_p reduction. In contrast to our findings, where R and G reduced the values of q_N at T17, red and green light enhanced nonphotochemical quenching in rice and cucumber plants (Chen *et al.* 2014, Wang *et al.* 2009, respectively). The discrepancy between our results and those available in the literature from plants grown under monochromatic environments underlines the need to add knowledge on the effect of ambient light enrichment by specific wavebands on the photosynthetic process. This is evidence that the photosynthetic behaviour in waveband-specific supplemented plants cannot be simply predicted from those results obtained in plants growing in monochromatic environments.

Under optimal growing conditions, plants usually favour their primary metabolism whereas in case of stress, *e.g.*, nitrogen deficiency, plant metabolism turns towards an increased biosynthesis of secondary metabolites, *e.g.*, polyphenols (Demotes-Mainard *et al.* 2008). In literature, few experiments tested the effect of single-colour LED

Table 4. Lutein, β -carotene, carotenoid-to-chlorophyll ratio (Car/Chl), and α -tocopherol levels detected in strawberry leaves. Values were recorded after 1 and 17 d of light supplementation (T1 and T17). Treatments: nonsupplemented control (NS), white light (W – R:G:B, 1:1:1), blue (B), red (R), and green (G) light supplementation. Means (\pm SE, $n = 3$) with different letters are significantly different after one-way ANOVA followed by Tukey's multiple range post-hoc test ($P \leq 0.05$).

Treatment	Lutein [$\mu\text{mol g}^{-1}(\text{FM})$]		β -carotene [$\mu\text{mol g}^{-1}(\text{FM})$]		Car/Chl		α -tocopherol [$\mu\text{mol g}^{-1}(\text{FM})$]	
	T1	T17	T1	T17	T1	T17	T1	T17
NS	6.16 \pm 0.65 ^b	5.82 \pm 0.39 ^b	0.56 \pm 0.06 ^b	0.32 \pm 0.07 ^b	4.55 \pm 0.64 ^b	4.84 \pm 0.24 ^b	7.39 \pm 1.21 ^b	5.79 \pm 0.07 ^b
W	6.43 \pm 0.84 ^b	6.53 \pm 0.88 ^{ab}	0.74 \pm 0.02 ^a	0.56 \pm 0.06 ^a	4.25 \pm 0.49 ^b	5.45 \pm 1.00 ^b	7.29 \pm 0.90 ^{bc}	6.07 \pm 0.62 ^b
B	7.42 \pm 0.63 ^a	7.46 \pm 0.42 ^a	0.53 \pm 0.07 ^b	0.58 \pm 0.11 ^a	6.25 \pm 0.81 ^a	6.28 \pm 0.43 ^{ab}	5.81 \pm 0.32 ^c	7.11 \pm 0.12 ^a
R	8.32 \pm 0.69 ^a	6.76 \pm 0.26 ^{ab}	0.63 \pm 0.09 ^{ab}	0.44 \pm 0.01 ^b	4.74 \pm 0.76 ^b	8.17 \pm 1.98 ^a	9.97 \pm 0.62 ^a	4.22 \pm 0.93 ^c
G	8.32 \pm 0.39 ^a	7.02 \pm 0.63 ^a	0.58 \pm 0.07 ^b	0.60 \pm 0.06 ^a	5.96 \pm 0.70 ^{ab}	6.56 \pm 0.52 ^{ab}	5.86 \pm 0.93 ^{bc}	5.65 \pm 0.06 ^b

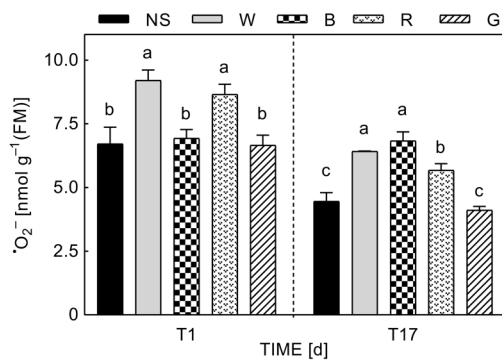


Fig. 4. Superoxide anion (O_2^-) content detected in strawberry leaves. Values were recorded after 1 and 17 d of light supplementation (T1 and T17). Treatments: nonsupplemented control (NS), white light (W – R:G:B, 1:1:1), blue (B), red (R), and green (G) light supplementation. Means (\pm SE, $n = 3$) with different letters are significantly different after one-way ANOVA followed by Tukey's multiple range post-hoc test ($P \leq 0.05$). FM – fresh mass.

light supplementation over ambient light, while most of the studies were conducted in growth chambers with selected wavebands: these differences in experimental conditions make the comparison with literature, again, very difficult. Anyway, it is reported that polychromatic light provided in growth chambers enhanced NBI in tatsoi (Viršilė *et al.* 2019), while red and far-red lights depressed NBI values in hazelnut plantlets (Silvestri *et al.* 2019). To date, no information is available for green and blue light supplementation. From our results, we can assume that W treatment stimulated photosynthesis and primary metabolism as already observed through gas exchange analysis. Previous studies conducted in green- and purple-leafed sweet basil showed that red light supplementation induced a lower chlorophyll accumulation compared to white and blue lights (Matysiak and Kowalski 2019); green light diminished Chl content in *Brassica rapa* var. *chinensis* (Mickens *et al.* 2019), while, according to Choi *et al.* (2015), lower Chl accumulation was observed in strawberry plants under blue light. No significant differences in terms of Chl content emerged in other species when subjected to specific wavebands

(Bagdonavičienė *et al.* 2015, Matysiak and Kowalski 2019), thereby suggesting that supplementation of selective wavebands and Chl content relation was species-specific and also dependent on the experimental conditions. Finally, according to our findings, it was reported that blue LED light supplementation stimulated phenol biosynthesis in various leafy greens (Bantis *et al.* 2016, Matysiak and Kowalski 2019). The present experiment highlights that B treatment enhanced flavonoid contents in the adaxial leaf surface, while a significant decrease was observed under G light on the abaxial side. Generally, the enhancement in leaf flavonoid content is a suitable marker for plant acclimation to unfavourable conditions (Viršilė *et al.* 2018), but phenylpropanoid metabolism boost can also be attributable to selective stimulation of this branch of secondary metabolism by B wavelengths, while G light has often opposite effects (Landi *et al.* 2020).

Our work did not underline significant differences between LED-supplemented and NS plants in terms of Chl content induced by light supplementation, except for a final reduction under R treatment. Choi *et al.* (2015) reported that blue light was less effective for Chl stimulation in strawberry plants, while in other cases blue light, more than red, was able to promote Chl accumulation (Mizuno *et al.* 2011, Li *et al.* 2012). Hernández and Kubota (2014) also found higher Chl concentration in cucumber leaves grown under ambient light and supplemented with blue LEDs. According to our results, Wang *et al.* (2009) reported that the Chl *a/b* ratio was greater under 100% blue light in cucumber plants, while the lowest value was obtained in plants grown under green light. Variation in the Chl *a/b* ratio could be related to the ability to balance light absorption by the two photosystems: an increase in Chl *a/b* was the result of an optimal acclimation to high light (Kitajima and Hogan 2003), as also observed in our experiments under B light. Moreover, Chl *a/b* variation can be considered as a valid marker of N partition in the leaf (Terashima and Hikosaka 1995). In our work, the highest Chl *a/b* ratio was measured in plants subjected to B enrichment which, in turn, had the lowest NBI value. According to this hypothesis, an increase of the Chl *a/b* ratio was observed when N availability decreased, especially under high-light conditions. Indeed, when N supply becomes a limiting factor, N is mainly allocated to protein-based structures of

PSII core at the expenses of N allocation to Rubisco. At the same time, N allocation to LHCII (characterized by greater Chl *b* content), is maintained at a similar level (Hikosaka and Terashima 1995). Consequently, PSII/LHCII and Chl *a/b* ratio most likely increase with the decrease of N availability (Kitajima and Hogan 2003). In addition, Chl *a/b* ratio variation is correlated with PSII light-harvesting antenna size changes (Leong and Anderson 1984) and PSII:PSI content, as Chl *a/b* ratio might be related to the reduction of Φ_{PSII} (Wang *et al.* 2009). There was no evidence in our work to verify this hypothesis.

Besides chlorophylls, carotenoids carry out the fundamental role of plant protection against photooxidative damage and are closely related to photosynthesis as they assist chlorophylls in light absorption (Jahns and Holzwarth 2012, Brazaitytė *et al.* 2015). Carotenoids are pivotal pigments for both their direct scavenger capacity and/or the nonphotochemical quenching activation (Jahns and Holzwarth 2012); light intensity (Thoma *et al.* 2020), as well as different light spectra, can strongly influence the carotenoid metabolism (Landi *et al.* 2020). It has been therefore reported that sunlight exposed leaves accumulated more carotenoids (β -carotene and xanthophylls; Czacuga 1987, Demmig-Adams and Adams 1992, Demmig-Adams *et al.* 1996).

Under light excess conditions, an accumulation of zeaxanthin occurs in concomitance with electron transport saturation and PSII overreduction to avoid undesirable dissipation of excitation energy (*e.g.*, Mehler reaction and ROS production) (Kalitaho *et al.* 2007). In our experiments, a build-up of zeaxanthin at T1 in plants subjected to light enrichment was observed (as compared to NS plants), which is supportive for their need to be photoprotected after a 1-d imposition of supplemented light, *e.g.*, via the activation of the zeaxanthin-dependent quenching, q_z , and feedback de-excitation q_E (Li *et al.* 2002) of nonphotochemical quenching. Indeed, the level of DEPS increased in parallel in plants subjected to light enrichment, irrespectively to the light spectra. However, after 17 d, only zeaxanthin contents remained higher in R-supplemented plants than that in NS ones, which highlights the capability of the strawberry plants grown under W, B, and G lights to acclimate to higher irradiances after 17 d of supplementation. This is in accordance with a higher level of VAZ observed in R-supplemented plants than NS ones, as the unchanged level of DEPS recorded at T17. Notably, a higher level of VAZ measured at both T1 and T17 in G-supplemented plants hints at the plant necessity to increase the content of oxygenated carotenoids once the plants are subjected to those wavebands of the solar spectrum which are normally poorly absorbed by the leaf (Brazaitytė *et al.* 2015).

Lutein is the most abundant xanthophyll in higher plants and supports several pivotal functions in the photosynthetic apparatus, including the structural stabilization of antenna proteins, formation of light-harvesting complexes, quenching of ^3Chl states, and it has been also proposed to be involved in the quenching of ^1Chl (Jahns and Holzwarth 2012). The increase in lutein content measured at T1 in all the plants supplemented with R,

B, and G (but not in W) further confirms the initial perturbation of photosynthetic apparatus by light enrichment, as above reported in terms of zeaxanthin and DEPS. In this case, the lack of enhancement of lutein in W-supplemented plants can support the need for this carotenoid in cases of the unbalanced level of some particular wavebands instead of in polychromatic light stress. Indeed, at T17, the lutein content only remained higher in B and G light, which are the wavebands mostly absorbed by this pigment (Solovchenko 2010).

Among nonoxygenated carotenoids, β -carotene (the main carotenoid *in planta*) also plays imperative functions as it is associated with the core of PSI and PSII, and is present in all organisms performing oxygenic photosynthesis (Qin *et al.* 2015). Our results confirm the accumulation of β -carotene in plants that sensed increasing light irradiances, but differently from lutein, only W (polychromatic light) promoted the increment of this carotenoid at T1. This suggests that lutein (the main xanthophyll) and β -carotene (the main nonoxygenated carotenoid) might play compensatory effects in case of unbalance of the light intensity or light spectra. At T17, the protraction of the light enrichment promoted the accumulation of β -carotene in all treatments except for R, compared to control. Our findings are in agreement with Samuolienė *et al.* (2016) who observed that red light did not promote the accumulation of β -carotene in basil. More solid results are needed to give a consistency of the relationship between light enrichment by selected wavebands and carotenoid metabolism.

Overall, the Car/Chl ratio represents a good marker of vegetation photosynthetic activity, developmental changes, and photosynthetic stress responses (Young 1993, Gitelson 2020), which allows physiological and phenological status characterization (Peñuelas *et al.* 1995, Solovchenko 2010, Zhou *et al.* 2019). In healthy plants, a constant Car/Chl ratio due to conserved stoichiometry of Chl and photosynthetic Car is also detected, while in stressed or senescing leaves, Chl and Car content and proportion vary strongly (Gitelson 2020), with a faster reduction of Chl amount over the Car content (Zhou *et al.* 2019). An increased Car/Chl ratio with increased light intensity revealed the activation of the photoprotection mechanism against excessive light absorption (Lee *et al.* 2007). In our work, R supplementation was able to stimulate the activation of the photoprotective mechanism, even though in literature is reported that 100% blue light induced a higher Car/Chl ratio compared to red and white light in tomato plants (Izzo *et al.* 2020).

It is well known that, when dissipation mechanisms are not sufficient to prevent an excessive light absorption concerning the CO_2 fixation ability, plants may incur ROS formation and oxidative burst with harmful effects at cellular/subcellular level (Foyer 2018). Carotenoids and tocopherols are nonenzymatic antioxidants apt for oxidative stress prevention. Tocopherols are fat-soluble compounds, located in plant chloroplast, where they neutralize peroxy radicals and extinguish $^1\text{O}_2$; α -tocopherol is the most abundant in green-leaved plants (Munné-Bosch and Alegre 2002). Only a few reports are available on the

effect of supplemented light on tocopherol contents. For example, increased α -tocopherol content was registered in basil plants cultivated in growth chambers under red light supplementation (Samuolienė *et al.* 2016). These results are in agreement with our findings, where R-supplemented plants showed the highest content of α -tocopherol at T1, which could be a preferential mechanism adopted to protect themselves against photooxidative damage and oxidative stress. Conversely, as stated above, at T17, R-supplemented plants showed a marked loss of Chl ($a + b$) and a strong enhancement of the Car/Chl ratio as an alternative photoprotective mechanism. Differently, α -tocopherol increased sensibly in B-enriched plants at T17, supporting a role of this molecule as a radical scavenger in a longer-term exposure, when plants are subjected to the highest energetic (shortest waveband) B-light enrichment.

Among all ROS species, $\cdot\text{O}_2^-$ formation is caused by an alteration in photosynthetic reactions due to environmental stresses so that excessive light energy cannot be used for chloroplast ferredoxin NADP^+ reduction (Asada and Takahashi 1987). Thus, superoxide anion molecules can be generated in two different ways: one derived from the plastoquinone pool, carrying one electron from Q_A^- to an oxygen molecule, while the second was produced during Mehler reaction on the PSI acceptor side. Superoxide dismutase (SOD) converted $\cdot\text{O}_2^-$ into H_2O_2 , which in turn was converted in H_2O by ascorbate peroxidase (Caverzan *et al.* 2012). In our experiments, the faster increment of oxidative stress (in terms of $\cdot\text{O}_2^-$ production) occurred for W- and R-supplemented plants as reported at T1, whereas at T17, also B-enriched plants showed a higher content of superoxide anion. Our findings agree with Agarwal *et al.* (2018), in which emerged that blue and red light induced higher oxidative burst. In particular, the highest level of $\cdot\text{O}_2^-$ formation observed in the present experiment, promoted by B light, can be related to the higher energetic level of this waveband (Landi *et al.* 2020). However, even a condition of W supplementation (which is composed of 33% R and 33% B light) induced a higher generation of $\cdot\text{O}_2^-$. Notably, G light nor at T1 or T17 promoted $\cdot\text{O}_2^-$ accumulation. We assume, therefore, that G LED light did not induce oxidative stress enhancement compared to NS plants. The reason behind this effect may be the efficient activation of the xanthophyll cycle and a general carotenoid synthesis stimulation as well as the poor absorbance of these wavebands by the Chl molecules.

In conclusion, W light treatment was able to spur photosynthesis without any stress macroscopic symptoms nor changes in chlorophyll and flavonoid content. G wavelengths did not induce significant alteration in plant photosynthetic processes and level of oxidative stress; thus G LEDs could be tested in further light-enrichment experiments. Instead, R and B lights enhanced the stress levels in strawberry plants and hampered key physiological and biochemical processes that drive leaf photosynthesis which, however, did not result in visible injury. In light of Roberts and Paul (2006) argumentations, that light-mediated ROS production can act as a starter for plant defence responses (including pathogens), further

research will investigate the efficiency of less impacting wavelengths (such as W and G) for testing their efficiency in promoting strawberry quality as much as disease resistance.

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