Manipulation of light quality is an effective tool to regulate photosynthetic capacity and fruit antioxidant properties of *Solanum lycopersicum* L. cv. 'Microtom' in a controlled environment

Ermenegilda Vitale [1], **Violeta Velikova** [2], **Tsonko Tsonev** [3], **Giulia Costanzo** [1], **Roberta Paradiso** [4], **Carmen Arena** [Corresp. 1, 5]

[1] Department of Biology, University of Naples Federico II, Naples, Italy

[2] Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria

[3] Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

[4] Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy

[5] BAT Center- Center for Studies on Bioinspired Agro-Environmental Technology, Portici, Italy

Corresponding Author: Carmen Arena
Email address: c.arena@unina.it

Light quality plays an essential role in setting plant structural and functional traits, including antioxidant compounds. This paper aimed to assess how manipulating the light spectrum during growth may regulate the photosynthetic activity and fruit bioactive compound synthesis in *Solanum lycopersicum* L. cv. 'Microtom' to improve plant physiological performance and fruit nutritional value.Plants were cultivated under three light quality regimes: Red-Green-Blue LEDs (RGB), Red-Blue LEDs (RB) and white fluorescent lamps (FL), from sowing to fruit ripening. Leaf functional traits, photosynthetic efficiency, Rubisco and D1 protein expression, and antioxidant production in fruits were analyzed. Compared to FL, RGB and RB regimes reduced height and increased leaf number and specific leaf area, enhancing plant dwarf growth. The RGB regime improved photosynthesis and stomatal conductance despite lower biomass, favoring Rubisco synthesis and carboxylation rate than RB and FL regimes. The RB light produced plants with fewer flowers and fruits with a lower ascorbic acid amount but the highest polyphenol content, antioxidant capacity and SOD and CAT activities. Our data indicate that the high percentage of the green wavelength in the RGB regime promoted photosynthesis and reduced plant reproductive capacity compared to FL and RB. Conversely, the RB regime was the best in favoring the production of health-promoting compounds in tomato berries.

Manipulation of light quality is an effective tool to regulate photosynthetic capacity and fruit antioxidant properties of *Solanum lycopersicum* L. cv. 'Microtom' in a controlled environment.

Ermenegilda Vitale[1], VioletaVelikova[2], TsonkoTsonev[3], Giulia Costanzo[1], Roberta Paradiso[4], Carmen Arena [1,5]*

[1] Department of Biology, University of Naples Federico II, Via Cinthia, 80126 Naples, Italy
[2] Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. bl. 21, 1113 Sofia, Bulgaria
[3] Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 21, Sofia 1113, Bulgaria
[4] Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici, Italy.
[5] BAT Center- Center for Studies on Bioinspired Agro-Environmental Technology,80055 Portici, Italy.
*Corresponding author: c.arena@unina.it

Abstract

Light quality plays an essential role in setting plant structural and functional traits, including antioxidant compounds. This paper aimed to assess how manipulating the light spectrum during growth may regulate the photosynthetic activity and fruit bioactive compound synthesis in *Solanum lycopersicum* L. cv. 'Microtom' to improve plant physiological performance and fruit nutritional value.

Plants were cultivated under three light quality regimes: Red-Green-Blue LEDs (RGB), Red-Blue LEDs (RB) and white fluorescent lamps (FL), from sowing to fruit ripening. Leaf functional traits, photosynthetic efficiency, Rubisco and D1 protein expression, and antioxidant production in fruits were analyzed. Compared to FL, RGB and RB regimes reduced height and increased leaf number and specific leaf area, enhancing plant dwarf growth. The RGB regime improved photosynthesis and stomatal conductance despite lower biomass, favoring Rubisco synthesis and carboxylation rate than RB and FL regimes. The RB light produced plants with fewer flowers and fruits with a lower ascorbic acid amount but the highest polyphenol content, antioxidant capacity and SOD and CAT activities. Our data indicate that the high percentage of the green wavelength in the RGB regime promoted photosynthesis and reduced plant reproductive capacity compared to FL and RB. Conversely, the RB regime was the best in favoring the production of health-promoting compounds in tomato berries.

Key words: D1 protein, gas exchanges, leaf functional traits, light quality, photochemistry, Rubisco.

Abbreviations: AsA, ascorbic acid; B, blue; CAT, catalase; cv, cultivar; ETR, electron transport rate; FRAP, ferric reducing antioxidant power; G, green; LA, leaf area; LDMC, leaf dry matter content; LEDs, light emitting diodes; FL, white fluorescent light; F_v/F_m, maximum PSII photochemical efficiency; V_{cmax}, maximum rate of Rubisco carboxylation; g_m, mesophyll conductance to CO_2 diffusion; A_N, net CO_2 assimilation; NPQ, non-photochemical quenching; PPFD, photosynthetic photon flux density; ΦPSII, quantum yield of PSII electron transport; R, red; RWC, relative water content; SLA, specific leaf area; g_s, stomatal conductance; SOD, superoxide dismutase.

Introduction

The demand for healthy fresh food has increased according to the global population rise in the last decades. However, satisfying this need has led to the intensification of non- sustainable agriculture practice and the overuse of broad cultivation areas, with consequent overexploitation of natural resources (*FAO, 2017*). Furthermore, the open field cultures are increasingly threatened by the risks and uncertainties associated with biotic and abiotic stresses, such as pest attacks, drought, and frost, exacerbated by the ongoing climate change (*Pandey et al., 2017*), compelling need for new cultivation approaches (*Dutta-Gupta, 2017*; *FAO, 2019)*. The Controlled Environment Agriculture (*CEA*) has emerged as a feasible alternative, as it optimizes the plant growth environment by minimizing the interactions with the external factors (*Dutta-Gupta, 2017*; *Amitrano et al., 2018*; *Pennisi et al., 2019*). The manipulation of light quality in CEA through light-emitting diodes (LEDs) technology modifies plant morphological, anatomical, and physiological traits (*Arena et al., 2016*; *Yang et al., 2017*), allowing to select the more appropriate light regime to improve crop productivity and food quality for a specific crop. This approach is worthy of attention to reduce the overuse of resources needed for massive crop production and plant cultivation in extreme environments such as hot and cold deserts or extraterrestrial platforms. In the view of space colonization, in the last decade, the National Aeronautics and Space Administration (*NASA*) has strongly encouraged the development of CEA and LED-based plant growth systems on the International Space Station (*ISS*) to support the realization of future colonies on the Moon and Mars (*Massa et al., 2006*; *Wheeler & Morrow, 2010*; *Gomez & Izzo, 2018*).

Changes in growth and photosynthesis induced by different light wavelengths are strictly linked to species, but some evidence iswidely recognized. Generally, red and blue wavelengths are most efficiently utilized for photosynthesis and influence the synthesis of PSII D1 protein and Rubisco (*Kato et al., 2015*; *Izzo et al., 2020*; *Vitale et al., 2020*). More specifically, red light influences the photosynthetic apparatus development, biomass accumulation, and stem elongation (*Urbonavičiūte et al., 2007*; *Wang et al., 2009*), and the level of soluble sugars (*Cui et al., 2009*) and fruits antioxidant compounds, like carotenoids and phenols (*Panjai et al., 2017*). Blue light

is mainly involved in vegetative growth regulation, early photomorphogenesis, and stomata control (*Chen et al., 2014*; *Singh et al., 2015*; *Izzo et al., 2020*). A high proportion of blue wavelengths within the light spectrum, being more energetic, may cause light avoidance phenomena in chloroplasts, reducing photosynthesis (*Loreto et al., 2009*; *Pallozzi et al., 2013*) and increasing the antioxidant production (i.e., lettuce, spinach) (*Lester, 2006*; *Ohashi-Kaneko et al., 2007*; *Hasan et al., 2017*) and protein biosynthesis (*Li & Pan, 1994*; *Hasan et al., 2017*) in some leafy vegetables. Finally, green light also plays a fundamental role in plant growth and development, involving seed germination and plant flowering (*Wang & Folta, 2013)*, and modulation of fruits and sprouts (*Samuolienė et al., 2011; Bantis et al., 2016*). In addition, the green wavelengths, penetrating deeply in the leaf mesophyll and lower canopy layers, promote photosynthesis and carbon gain in the deepest chloroplasts and inner canopy (*Terashima et al., 2009*; *Smith et al., 2017*). Besides the traditionally used red and blue lights, green and orange enhance photosynthesis and translocation of assimilates by affecting source/sink relationships among plants. Green and orange bands improve the water use efficiency and promote plant growth through the accumulation of photoassimilates in leaves. This encourages to include the green light, besides red and blue, to project lighting systems in a growth-controlled environment (*Lanoue et al*., 2018). Moreover, the green light strongly influences plant growth by acting on cryptochrome. Indeed, the green wavelengths may reverse cryptochrome blue-light mediated signals, such as dry biomass accumulation, stem growth inhibition, and anthocyanin production (*Bouly et al., 2007*; *Zhang & Folta, 2012*; *Kusuma, et al., 2021*). Based on this evidence, the modulation of light spectral composition may be a practical approach for sustainable agriculture to obtain crops with specific characteristics in CEA and indoor cultivation.

The manipulation of the light spectrum to modulate photosynthesis and bioactive compound production still represents an open study field because light treatments promoting plant growth could be inappropriate for enhancing nutraceutical quality.

This study aimed to evaluate the effects of three different light quality regimes, white fluorescent (FL), red-green-blue (RGB), and red-blue (RB) LEDslight on growth, photosynthetic performance, and fruit antioxidant properties of *Solanum lycopersicum* L. cv. 'Microtom' plants. Specific attention was devoted to the photosynthetic regulation in response to the different light quality treatments to assess the mechanisms allowing plants to improve productivity. To this purpose, gas exchanges, chlorophyll fluorescence measurements, chlorophyll and carotenoid content, and the expression of PSII D1 protein and Rubisco have been assessed.

The cultivar 'Microtom' was chosen in our experiment for a series of characteristics, such as short life cycle, compact size, fast growth, which makes it ideal for cultivation in small volumes at high plants density, compared to other tomato landraces (*Scott & Harbaugh, 1989*; *Okazaki & Ezura, 2009*; *Saito et al., 2011*; *Shikata et al., 2016*; *Samuolienė et al., 2021*).

The best light quality regime may be utilized to obtain cropswith enhanced productivity and high content of antioxidants, in specific indoor cultivation environments such as Space greenhouses or planetary platforms for providing fresh food to the crew (*Colla et al., 2007*; *Saito et al., 2011*; *De Micco et al., 2014*; *Arena et al., 2019*).

Materials & Methods

Plant material and growth conditions

Seeds of *Solanum lycopersicum* L. cv. 'Microtom', provided by Holland Online Vof (Amsterdam, The Netherlands), were sown in 3.0 L pots filled with peat soil and placed at 10-15 cm from each other (*Scott & Harbaugh, 1989*). Plants were cultivated in a climatized chamber under three different light regimes (five plants per treatment): white fluorescent light (FL) obtained by using fluorescent tubes (Lumilux L360W/640 and L360W/830, Osram, Germany); red-green-blue (RGB) and red-blue (RB) supplied by light-emitting diodes (LEDs) (LedMarket Ltd., Plovdiv, Bulgaria) with the following emission peaks: 630 nm red, 510 nm green, 440 nm blue. The used LEDs have some proportion of the adjacent to red, green and blue colors of the visible spectrum (*Fig. 1*) but for convenience we conditionallyaccept the designations RGB and RB meaning the peak wavelengths. An SR-3000A spectroradiometer was used to measure the spectral composition of the three light regimes (*Fig. 1*) with 10 nm resolution (Macam Photometrics Ltd., Livingston, Scotland, U.K.). Plant growth was followed from sowing to fruit ripening up to 100 DAS (days after sowing) under the following environmental conditions: photosynthetic photon flux density (PPFD) 300 ± 5μmol photons m^{-2} s^{-1} for each light treatment, day/night air temperature 24/18 °C, relative air humidity 60-70%, photoperiod of 12 h. Plants were irrigated to pot capacity with tap water at a two-day interval to reintegrate the water loss for evapotranspiration. Every two weeks, plants were fertilized with Hoagland's solution.

Measurements of plant growth and leaf functional traits

Plant growth measurements were carried out at 100 DAS. We considered: plant height (cm, considering the main stem), leaf number, fruit number, fruit weight (g FW per plant), epigeal plant biomass (EB, g FW per plant) as well as the ratios leaf biomass/epigeal biomass (LB/EB) and fruit biomass/epigeal biomass (FB/EB), where the epigeal biomass corresponds to the whole above-ground biomass. The flower number was monitored starting from 40 up to 70 DAS until the first fruits' appearance, considering for each plant the sum of flowers measured within the range 40-70 DAS.

The determination of leaf functional traits (leaf area, LA; specific leaf area, SLA; leaf dry matter content, LDMC; relative water content, RWC), were assessed at 50 DAS on fully expanded leaves, according to methods reported in *Cornelissen et al. (2003)*. LA (cm^2) was measured by acquiring digital images and using ImageJ 1.45 program (Image Analysis Software, NIH, USA). SLA was determined as the ratio between leaf area and dry leaf mass and expressed in cm^2 g^{-1}. LDMC was calculated as dry leaf mass to saturated fresh mass and reported in g g^{-1}. RWC was expressed as a percentage of the ratio (fresh leaf mass – dry leaf mass)/ (saturated leaf fresh mass – dry leaf mass). The saturated fresh mass was obtained by submerging the petiole of leaf blades in distilled water for 48 h in the dark at 15°C, whereas the dry mass was determined after oven-drying leaves at 75°C for 48 h.

Measurements of plant growth and leaf functional traits were determined on five plants for each light regime, collecting five leaves (one leaf per plant).

Gas exchange and chlorophyll *a* fluorescence measurements

Gas exchange and chlorophyll*a*fluorescence measurements were carried out at 50 DAS on five plants per light regime. We selected one fully expanded leaf foreach plant to obtain five replicates per light treatment. The net CO_2 assimilation (A_N) and the stomatal conductance (g_s) were measured using a portable leaf gas exchange system (LCpro+, ADC BioScientific, UK). Thecentral leaflet of each compound leaf (5th from the stem base) was clamped into the gas exchange system cuvette (6.25 cm^2) for measurements at 1000 µmol photons $m^{-2}s^{-1}$ PPFD, 25±2 °C leaf temperature and 50-60% relative humidity. The gas exchange measurements were conducted under red+10% blue light by means of light source of the gas exchange system (LCpro+, ADC BioScientific, UK). The photosynthesis and the stomatal conductance were calculated as indicated in *von Caemmerer& Farquhar (1981)*. The mesophyll conductance to CO_2 diffusion (g_m) was determined using the variable J method (*Loreto et al., 1992*), whereas the maximum rate of Rubisco carboxylation (V_{cmax}) was estimated as proposed by *Farquhar, von Caemmerer and Berry (1980).*

After gas exchange measurements, on the same leaves, chlorophyll *a* fluorescence was assessed by a fluorescence Monitoring System (FMS, Hansatech Instruments, King'Lynn, UK). The background fluorescence signal, F_o, was induced on 20 min dark-adapted leaves, by an inner light of about 2–3 µmol photons m^{-2} s^{-1}, at a frequency of 0.5 kHz. Previous experiments demonstrated that 20 minutes are sufficient to obtain complete re-oxidation of PSII reaction centers (*Shahzad et al., 2020*). The maximum fluorescence level (F_m) in the dark-adapted state was determined with a 1s saturating light pulse of about 6000 µmol photons $m^{-2}s^{-1}$. The maximum PSII photochemical efficiency (F_v/F_m) was calculated as (F_m-F_0)/F_m. Under illumination at plant growth irradiance (PPFD of 300 µmol photons m^{-2} s^{-1}), the steady-state fluorescence (F_s) was measured, and maximum fluorescence (F_m') in the light-adapted state was determined by applying a saturating pulse of 0.8 s with over 6000 µmol photons $m^{-2}s^{-1}$. The quantum yield of PSII electron transport (Φ_{PSII}) was calculated as (F_m'-F_s)/F_m' according to *Genty et al. (1989)*, while the non-photochemical quenching (NPQ) was expressed as (F_m-F_m')/F_m' as reported in *Bilger & Björkman (1990).*

Photosynthetic proteins D1 and Rubisco and pigments content

After chlorophyll fluorescence and gas exchange measurements, the same leaves were collected to perform the protein extraction following the procedure of *Wang et al. (2006*) modified by *Arena et al. (2019)*. Protein extracts were quantified with the *Bradford assay (1976)* and subjected to an SDS-PAGE (12%). The Western Blot analysis started treating the leaf samples with a blocking solution (100mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Tween20, 10% Milk). In order to reveal the selected proteins, samples were then incubated with the respective primary and secondary antibodies (Agrisera, Vännäs, Sweeden): anti-PsbA (chicken, 1: 5000 v/v) for D1

protein, anti-RbcL (rabbit, 1:10000 v/v) for Rubisco, anti-ACT (rabbit, 1:5000 v/v) for Actin. Immuno-revelation was carried out using the kit for chemiluminescence (ECL Western Blotting Analysis System, Ge Healthcare) by the Chemidoc system (Bio-Rad Laboratories). The software Quantity One (Bio-Rad) was used for the densitometric analysis to obtain quantitative information associated with the individual protein bands. The protein actin was used as loading control. The value of each band was normalized to the corresponding actin band. For all treatments, the density value was expressed in arbitrary units and represented as a bar diagram corresponding to the pixel volume of the protein band.

The photosynthetic pigments content, namely total chlorophylls (*a*+*b*) and carotenoids (x+c), were quantified on leaf samples of known area treated with ice-cold 100% acetone, following the procedure reported by *Lichtenthaler (1987)*. The absorbance was detected at 470, 645 and 662 nm, and pigment content was expressed as $\mu g cm^{-2}$.

Fruit antioxidant characterization

The effect of different light quality regimes on the antioxidant properties of 'Microtom' fruits was evaluated by collecting whole mature berries. Each assay was carried out on five fruits collected from five different plants, considering one berry as one replica. Fresh samples (0.250 g) were grounded in liquid nitrogen and the ascorbic acid (AsA) content, superoxide dismutase (SOD) and catalase (CAT) activities were determined as described in *Arena et al. (2019)*.

The AsA concentration was evaluated with the Ascorbic Acid Assay Kit II (Sigma-Aldrich, St. Louis, MO, USA) based on the ferric reducing/antioxidant and ascorbic acid (FRASC) assay. Antioxidants contained in the sample are involved in reducing Fe^{3+} into Fe^{2+}, resulting in a colored product. After the addition of ascorbate oxidase, any ascorbic acid is oxidized and quantified by measuring the absorbance at 593 nm with a spectrophotometer (UV-VIS Cary 100; Agilent Technologies). The AsA concentration was determined using a standard curve and expressed in mg L^{-1}, as reported in *Costanzo et al. (2020).*

The SOD Assay Kit (Sigma-Aldrich, St. Louis, MO, USA) was used to evaluate the SOD activity by measuring inhibition of the nitro blue tetrazolium (NBT) reduction into blue formazan. The absorbance of the blue color generated during the colourimetric reaction was read at 440 nm with a spectrophotometer (UV-VIS Cary 100; Agilent Technologies). The volume of the sample that caused the 50% inhibition in blue formation was defined as a unit of SOD activity.

The CAT activity was assessed through the Catalase Assay Kit (Sigma-Aldrich, St. Louis, MO, USA). The colourimetric decomposition reaction of H_2O_2 into H_2O and O_2 was spectrophotometrically (UV-VIS Cary 100; Agilent Technologies) followed by monitoring the decreasing absorbance at 520 nm. The amount of enzyme capable of decomposing 1 µmol of H_2O_2 per minute at pH 7.0 and 25°C was considered a CAT activity unit.

The total antioxidant capacity was assessed by the Ferric Reducing Antioxidant Power assay (FRAP) on samples (0.250g) treated with methanol/water solution (60:40, v/v). As reported in *George et al. (2004),* samples were centrifuged at 20 817 g for 15 min at 4°C, mixed with the

FRAP reagents and incubated for 1h in the dark. After the reaction, the absorbance was read at 593 nm. Then, the antioxidant capacity was calculated using a Trolox standard curve and expressed as µmol Trolox equivalents (µmol Trolox eq. g^{-1} FW).
The total polyphenols were quantified on samples (0.02g) extracted with aqueous 80% methanol and subjected to the procedure described in *Costanzo et al. (2020)*. The total polyphenol content was determined with a gallic acid standard curve and expressed as mg gallic acid equivalents (GAE) 100 g^{-1} FW.

Statistical analysis

Results were analyzed using SigmaPlot 12 software (Jandel Scientific, San Rafael, CA, USA). The effect of the different light quality treatments on the investigated parameters was assessed by applying a one-way analysis of variance (ANOVA). The Student-Newman-Keuls test was applied for all pairwise multiple comparison tests with a significance level of $P<0.05$. The Kolmogorov–Smirnov and Shapiro–Wilk tests were performed to check for normality. Data are reported as mean values ± standard error (n=5). All the data obtained for leaves and fruits were represented by two heatmapsto provide an immediate visual summary of information. The heatmaps were generated by means of the program ClustVis (https://biit.cs.ut.ee/clustvis/online, accessed 31 January 2021). The clusters of rows and columns were based on Euclidean distance and average linkage. The numeric differences within each heatmap are indicated by a color scale: red scale from light todark indicated increasing values while blue scale decreasing values.

Results

Biometric measurements and leaf functional traits

The morphological parameters and leaf functional traits under the different light quality regimes were reported in Table 1. RGB and RB treatments reduced ($P<0.001$) plant height (Table 1, *Fig.2*) and increased ($P=0.002$, $P<0.001$) leaf number compared to FL light treatment. On the other hand, plants grown under the RGB regime developed the lowest number of flowers ($P=0.006$, $P=0.011$) and fruits ($P=0.001$, $P=0.026$) as well as a reduced ($P<0.001$, $P=0.007$) fruit total biomass than FL and RB plants. The growth under the three light regimes also induced a different partitioning of fresh biomass. More specifically, plants cultivated under RGB and RB light regimes invested more biomass into leaves ($P<0.001$) and stem ($P<0.001$, $P=0.015$)(high ratio LB/EBand SB/EB) compared to FL plants. Conversely, FL and RB plants showed higher ($P<0.001$) partitioning of biomass in fruits (high ratio FB/EB).
Under RGB treatment, LA significantly decreased ($P<0.001$) compared to FL and RB light regimes. An opposite behavior was observed for SLA and LDMC: FL plants showed a lower ($P<0.001$) SLA and a higher ($P<0.001$) LDMC compared to those grown under RGB and RB which exhibited comparable values. Lastly, RWC was not affected by different light quality treatments.

Gas exchange and chlorophyll fluorescence emission measurements

RGB light regime determined a significant increase ($P<0.001$) of A_N and g_m compared to FL and RB treatments *(Fig. 3A, C)*. Conversely, different light quality regimes did not affect g_s (*Fig. 3B*). Consistent with A_N, V_{cmax} was higher ($P<0.001$) in RGB than FL and RB plants. The lowest value of V_{cmax} was measured in RB plants (*Fig. 3D*).
The values of ΦPSII and F_v/F_m were lower in RB compared to RGB ($P<0.001$, $P<0.001$) and FL plants ($P<0.001$, $P=0.001$)(*Fig. 4A, C*). Consistently, RB plants also showed a higher ($P<0.001$, $P=0.005$) NPQ compared to RGB and FL plants (*Fig. 4B*). In particular, plants grown under the RGB regime exhibited the lowest ($P<0.004$) NPQ.

Photosynthetic proteins and leaf pigments content

The plants cultivated under RB light significantly reduced ($P=0.034$, $P=0.031$) the content of D1 protein and Rubisco ($P<0.001$, $P=0.011$) compared to FL and RGB light regimes. No difference in D1 protein amount was found between FL and RGB plants. On the contrary, plants grown under RGB light showed the highest ($P<0.001$) Rubisco amount among light treatments (*Fig. 5*). Compared to FL, plants grown under the RB regime significantly decreased the total chlorophyll and carotenoid content ($P<0.001$, $P=0.027$), while plants developed under the RGB regime only showed a lower chlorophyll concentration ($P=0.022$)(*Fig. 6A, B*). An opposite trend was observed for Chl*a/b* ratio, which resulted higher ($P=0.02$) in RGB and even more in RB ($P<0.001$) compared to FL plants (*Fig. 6C*).

Determination of antioxidants in fruits

The plant cultivation under RB light regime strongly affected the antioxidant properties of fruits. SOD and CAT activities, as well as the antioxidant capacity significantly increased ($P<0.001$, $P<0.001$, $P<0.001$ respectively) in RB compared to FL and RGB fruits (*Fig. 7A, B, C*). SOD and CAT activities did not differ between FL and RGB fruits, conversely to the antioxidant capacity, which was higher ($P<0.001$) in RGB than FL fruits. Furthermore, the total polyphenol content also increased ($P<0.001$) in RB compared to FL and RGB fruits, reaching a concentration about 9 times higher than that found under the other two light regimes (*Fig. 7E*). On the other hand, the RB light regime did not promote the AsA content, which decreased ($P<0.001$) in RB compared to FL and RGB fruits (*Fig. 7D*).

Heatmap analyses

An overview of the morphological, photosynthetic and functional traits of 'Microtom' plants in response to FL, RGB and RB light regimes is displayed in *Fig. 8A*.
The heatmap separated FL and RB from RGB plants, evidencing that an elevated amount of green wavelength in the light spectrum effectively promotes gas exchanges and carbon fixation, inducing higher values of A_N, g_s, g_m, V_{cmax}, Rubisco content, and leaf biomass partitioning. Conversely, the FL regime grouped plants with more flowers and fruit biomass, higher photochemistry, photosynthetic pigment content and D1 protein amount. Finally, RB light regime clustered plants with high SLA, leaf number and chlorophyll a/b ratio.

Fig. 8B summarizes the fruit traits, including the antioxidant properties. RB was separated from RGB and FL fruits. In particular, FL light regime induced higher fruit production and fruit weight. Conversely, the RB light regime clustered fruits with a higher antioxidant charge due to higher values of CAT and SOD activities, polyphenols and total antioxidant capacity.

Discussion

Our study showed that different light quality regimes (Figure 1) strongly affect the photosynthetic and morphological traits in 'Microtom' plants and the antioxidant capacity of fruits, confirming that the modulation of the light spectrum is a valuable tool for controlling and selecting specific characters in this cultivar, especially in indoor environments.

Effect of different light quality regimes on photosynthetic and morphological traits of 'Microtom' plants

Regarding plant morphology (Table 1, Figure 2), as previously reported by other authors, the growth under RB and RGB light quality regimes significantly reduced the stem elongation compared to the FL regime (*Xiaoying et al., 2012*; *Arena et al., 2016*; *Dieleman et al., 2019*; *Izzo et al., 2020*). A compact size characterizes the 'Microtom' cultivar, and the induction of further plant compactness may favour tomato growth in a high plant density condition or restricted volumes. Compared to FL, the higher intensityof blue wavelengths composing RB and RGB treatments may be responsible for the more compact size observed in these plants because blue wavelengths by inhibiting cell division and expansion act directly on plant morphogenesis, especially in the early stage of development (*Dougher & Bugbee, 2004*; *Nanya et al., 2012; Izzo et al., 2020*; *Vitale et al., 2021*).

The higher fruit number (Table 1) observed in FL than RB and RGB plants may depend on the far-red portion of the spectrum (2.9 %) in this regime. It is noteworthy that plant morphogenesis is also controlled by phytochrome, regulated by the red/far-red ratio (*Casal & Casal, 2000*). According to other authors, the red/far-red ratio in FL regimes may have promoted stem extension, epigeal biomass, fruit yield and dry mass partitioning to fruits by increasing fruit sink strength in tomato plants (*Ji et al., 2020*; *Kalaitzoglou et al., 2020*).

The growth under RGB and RB regimes has induced biomass partitioning more in leaves than in fruits than in FL plants (Table 1). In the case of RGB plants, the investment toward photosynthetic structures led to a better photosynthetic performance than other light regimes. Likely, the higher intensity of green wavelengths of RGB compared to RB regime may have favored photosynthesis. Indeed, the green component of the light spectrum, penetrating deeper into the leaf and reaching the lower cell layers than red or blue light, may have driven photosynthesis where the other wavelengths were limiting (*Folta, 2005*; *Terashimaet al., 2009; Smith et al., 2017*; *Liu & van Iersel, 2021*), and have favored photo-assimilate translocation in tomato leaves (*Lanoue et al*., 2018). In FL plants, regardless of a high component of green, photosynthesis is lower than RGB, probably due to the lower mesophyll conductance.

The different light quality regimes also affected leaf functional traits (Table 1) indicating that leaf structural adjustments are required to allow plant acclimation to the surrounding light environment.
The growth under RGB and RB reduced the LDMC and increased the SLA compared to the FL regime indicating differences in the potential relative growth rate (*Maizane & Shipley,1999*). Both SLA and LDMC are involved in the trade-off between quick biomass production (high SLA, low LDMC species) and efficient conservation of nutrients (low SLA, high LDMC species) (*Poorter & De Jong, 1999*); thus, the higher SLA in RGB and RB compared to FL plants suggests a more efficient growth strategy, under these specific light quality regimes.
Light-induced modifications of leaf structure strongly impact gas exchanges and photosynthetic carbon gain in 'Microtom' plants as determine changes in the resistances along the CO_2 diffusion pathway inside leaves (Figure 3A-D) (*Johkanet al., 2012*; *Arena et al., 2016*; *Vitale et al., 2020*). Despite similar values of SLA and LDMC, RGB and RB plants did not show the same photosynthetic efficiency. The higher A_N in RGB compared to FL and RB plants was not due to the difference in stomatal conductance (g_s) but increased mesophyll conductance (g_m), indicating a reduced limitation to CO_2 diffusion in mesophyll cells.
Thinner leaves, as well as less dense tissues in RGB plants (low LDMC), may reduce the limitations to the CO_2 diffusion in the mesophyll (*Niinemets et al., 2009*; *Tomás et al., 2013*), leading to a higher amount of the CO_2 available at the carboxylation sites, which, in turn, led to the significant increase of the maximum rate of Rubisco carboxylation (V_{cmax}) and net CO_2 assimilation. This hypothesis is consistent with the highest level of Rubisco found in RGB plants (Figure 5B).
The lack of significant differences in the stomatal conductance between RGB and RB plants suggested that A_N decline in RB compared to RGB plants was not due to stomatal limitation but rather to other causes such as a decline of Rubisco activity. Indeed, it is noteworthy that a decreased capacity of ribulose-1,5-bisphosphate (RuBP) carboxylation or regeneration may be associated with lower photosynthetic performance (*Onoda et al., 2005*). Therefore, the low Rubisco expression may likely induce the V_{cmax} and A_N drop observed in RB compared to FL and RGB plants (Figure 3A, D). Consistent with our results, Miao et al. (2016) demonstrated in cucumber plants that the RB treatment (R: B 8:1) determined no change in g_s but decreased V_{cmax} and photosynthesis compared to white fluorescent light.
The addition of green to red and blue wavelengths does not always produce positive effects on Rubisco expression and photosynthesis (*Wang et al., 2009*; *Suet al., 2014*); however, in our case, the more homogeneous light distribution within the leaf mesophyll (i.e., red and blue wavelengths on surface and green wavelength deeper in leaf parenchyma) may have induced stimulation of Rubisco synthesis (*Terashima et al., 2009*). Furthermore, similarlyto *Liu et al. (2019)*, adding green to red and blue wavelengths in our case also promoted D1 protein expression (Figure 5A). The D1 levels were comparable to those found in FL plants, leading to a similar PSII photochemical efficiency (Figure 4C). It is likely to suppose that in the RB regime,

the high proportion of red wavelength did not favor photosynthesis because it negatively affected the Rubisco and D1 protein synthesis.

The growth under RB regime induced different partitioning of absorbed light energy within photosystems, promoting the heat dissipation processes instead of PSII photochemistry (Figure 4A, B). Furthermore, the lowest content of total chlorophylls and carotenoids in RB compared to FL and RGB plants (Figure 6A, B) also indicated a lower capability of light harvesting for these plants (*Chen et al., 2014*).

We cannot exclude that the down-regulation of photosynthetic pigments may represent in RB plants a safety strategy to reduce the light absorption, thus avoiding photodamages to PSII under limited photosynthetic activity. This hypothesis is supported by the increase of the chlorophyll *a/b* ratio that generally occurs in leaves exposed to higher light intensities (*Kitajima & Hogan, 2003; Li et al., 2016*). The increment of the Chl *a/b* ratio clearly indicates an adjustment of the light-harvesting system in RB plants, and more specifically, a reduction of Chl *b* mainly involved in the absorption of high-energy blue wavelengths (*Wang et al., 2009*). The Chl *a/b* ratios observed in this study are peculiar, as they deviate from the usual 3-4. However, many species of plantsshow values lower than the most commonly found. This may be considered a specific response to different light intensities or different light quality spectra, especially after long-term exposure to R, B and RB light (*Kitajima & Hogan, 2003; Li et al., 2016; Zheng & Van Labeke, 2017*).

The maintenance of the PSII activity is strictly related to the pigment concentration and the turnover of the D1 protein encoded by the *psbA* gene. The F_v/F_m decline in RB compared to FL and RGB plants (Figure 4C) may indicate a slowdown of D1 turnover resulting from the imbalance between D1 degradation and replacement (*Miao et al., 2016*). As previously observed by *Bian et al. (2018)* in lettuce plants, in 'Microtom', the continuous RB light growth regime may have induced oxidative stress responsible for the downregulation of *psbA* expression and photosynthetic decline.

Effect of different light quality regimes on antioxidant properties of 'Microtom' fruits.

The growth under different light qualities modified the antioxidant properties of tomato fruits (Figure 7), evidencing that it is possible to obtain fruits richer in bioactive compounds for the human diet by manipulating the light spectrum. In particular, RB light strongly enhanced the antioxidant properties of 'Microtom' fruits, despite producing a lower number of berries per plant than FL (Table 1).

As in other crops, the total antioxidant capacity in tomato plants is due to compounds, such as carotenoids, ascorbic acid (AsA), vitamins, and polyphenols, which act as non-enzymatic defenses (*Hasan et al., 2017*; *Ntagkas et al., 2019*; *Xie et al., 2019*). AsA is considered one of the most potent scavengers in plant tissue and fruits (*Racchi, 2013*) and it has been recently demonstrated that light quantity and quality affect its production in tomato fruits (*Ntagkas et al., 2019*). Generally, the blue wavelengths of the light spectrum promote in detached tomato (*Ntagkas et al., 2019*) and strawberries fruits (*Xu et al., 2018*) an increase in AsA content

compared to white fluorescent light, red or green wavelengths. Furthermore, the pure blue or dichromatic blue-red light also stimulated the AsA content in leafy vegetables (*Ohashi-Kaneko, 2007*; *Li & Kubota, 2009*; *Ma et al., 2014*). Our data indicate that the elevated antioxidant capacity of RB compared to FL and RGB fruits is not due to AsA but rather to the highest content of phenolic compounds (Figure 7C, D, E).

Our findings agree with previous studies on the same species, which demonstrated the stimulatory role exerted by RB light on the total polyphenols and antioxidant capacity (*Xie et al., 2016*). In particular, the wavelengths in the range of red, blue and UV-light strongly affect the accumulation of polyphenols, enhancing the antioxidant capacity and the reactive oxygen species (ROS) scavenging potential in tomato fruits (*Castagna et al., 2014*; *Xie et al., 2016*; *Panjai et al., 2017*). The higher antioxidant capacity induced by the RB treatment could be related to the cryptochromes, which induces the increase of flavonoids and lycopene (*Giliberto et al., 2005*). Specifically, cryptochromes are blue-light sensing photoreceptors whose activation can be inhibited by green light (*Bouly et al., 2007*). Therefore, the green fraction in FL and even more in the RGB regime may have offset the stimulatory effect of the RB wavelengths, determining a decrease in the antioxidant capacity of RGB and FL fruits (Figure 7C).

The dichromatic RB regime also increased the scavenger enzymes SOD and CAT activity compared to FL and RGB fruits (Figure 7A, B), likely due to the incidence of oxidative stress. *Muñoz & Munné-Bosch (2018)* reported that in different species, photooxidative stress could occur in fruits during the ripening. Thus, it cannot be excluded that the growth under the RB regime through a reduction of photosynthetic and photochemical activity may have induced oxidative stress in leaves and fruits activating the scavenging systems. In such circumstances, we hypothesized that during the scavenging of H_2O_2, the ascorbate peroxidase (APX) may have used the AsA as a co-factor (*Racchi, 2013*), contributing to its reduction in fruits of RB plants.

The heatmap (Figure 8A) clustered FL and RB from RGB plants based on different physiological attributes, evidencing for RGB plants the best photosynthetic performance in terms of gas exchange and Rubisco amount. Conversely, FL regimes effectively promoted the reproductive structures (flower and fruit number). Concerning the fruits, the heatmap visualization (Figure 8B) showed that the RB light regime greatly influenced the antioxidant production, except for AsA, suggesting the RB as the best light regime to guarantee fruits with a higher nutraceutical value, despite their low production under this treatment.

Conclusions

Overall results indicate that the photosynthetic apparatus of 'Microtom' grown under RGB treatments use light more efficiently than RB treatment. In fact, under the RGB growth regime, plants showed an improvement in photosynthetic performance, evidencing the important role of the green portion of the spectrum. Furthermore, the growth under RGB induced a more compact size and increased photochemical efficiency than FL and RB regimes. The increase of A_N under RGB light treatment results from an improved mesophyll conductance due to changes in leaf

structure and the up-regulation of Rubisco expression responsible for increasing maximum carboxylation efficiency in these plants.
However, despite the reduced photosynthetic performance, RB light regime stimulates the antioxidant production in 'Microtom' tomato fruits.
This study provides valuable information for developing appropriate light cultivation protocols through light manipulation to improve tomato plant productivity in controlled environments and the nutritional value of fruit quality, promoting the synthesis of antioxidants beneficial for the human diet.

Acknowledgements

Competing Interests

The authors declare there are no competing interests.

Author Contributions

Conceptualization: V.V., T.T. and C.A.; Investigation: E.V., T.T., G.C., R.P., C.A; Data curation: E.V and C.A.; Formal analysis: T.T. and E.V.; Funding acquisition: V.V. and C.A.; Writing-original draft: E.V. and C.A; Writing-review and editing: E.V., V.V., T.T., G.C., R.P. and C.A.

References

Amitrano C, Vitale E, De Micco V, Arena C, 2018. Light Fertilization Affects Growth and Photosynthesis in Mung Bean (*Vigna radiata*) Plants. *Journal of Environmental Accounting and Management* **6**: 295-304.

Arena C, Tsonev T, Doneva D, De Micco V, Michelozzi M, Brunetti C, Centritto M, Fineschi S, Velikova V, Loreto F, 2016. The effect of light quality on growth, photosynthesis, leaf anatomy and volatile isoprenoids of a monoterpene-emitting herbaceous species (*Solanum lycopersicum* L.) and an isoprene-emitting tree (*Platanus orientalis* L.). *Environmental and Experimental Botany* **130**: 122-132.

Arena C, Vitale E, Hay Mele B, Cataletto PR, Turano M, Simoniello P, De Micco V, 2019. Sustainability of *Solanum lycopersicum* L. 'Microtom' for growth in Bioregenerative Life Support Systems: exploring the effect of high-LET ionizing radiation on photosynthesis, leaf structure and fruit traits. *Plant Biology* **21**: 615-626.

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. *Scientia Horticulturae* **198**: 277-283.

Bian ZH, Yang QC, Li T, Cheng R, Barnett Y, Lu C, 2018. Study of the beneficial effects of green light on lettuce grown under short-term continuous red and blue light emitting diodes. *Physiologia Plantarum* **164**: 226-240.

Bilger W, Björkman O, 1990. Role of xanthophyll cycle and energy dissipation in differently oriented faces of light–induced absorbance changes, fluorescence and photosynthesis in *Hedera canariensis*. *Photosynthesis Research* **25**: 173–185.

Bouly JP, 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. *The Journal of Biological Chemistry* **282**: 9383–9391.

Bradford MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**: 248-254.

Casal C, Casal JJ, 2000. Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. *Photochemistry and Photobiology* **71**: 1-11.

Castagna A, Dall'Asta C, Chiavaro E, Galaverna G, Ranieri A, 2014. Effect of Post-harvest UV-B Irradiation on Polyphenol Profile and Antioxidant Activity in Flesh and Peel of Tomato Fruits. *Food and Bioprocess Technology* **7**: 2241-2250.

Chen X, Guo WZ, Xue ZZ, Wang LC, Qiao XJ, 2014. Growth and quality responses of 'Green Oak Leaf' lettuce as affected by monochromic or mixed radiation provided by fluorescent lamp (FL) and light-emitting (LED). *Scientia Horticulturae* **172**: 168-175.

Colla G, Rouphael Y, Cardarelli M, Mazzucato A, Olimpieri I, 2007.Growth yield and reproduction of dwarf tomato grown under simulated microgravity conditions. *Plant Biosystems* **141** (1): 75-81.

Cornelissen JHC, Lavorel A, Garnier E, Dia, S, Buchmann N, Gurvich DE, Reich PB, Steege H, Morgan HD, van der Heijden MGA, Pausas JG, Poorter H, 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* **51**: 335-380.

Costanzo G, Iesce MR, Naviglio D, Ciaravolo M, Vitale E, Arena C, 2020. Comparative Studies on Different Citrus Cultivars: A Revaluation of Waste Mandarin Components. *Antioxidants* **9**(6): 517. DOI: 10.3390/antiox9060517.

Cui J, Ma ZH, Xu ZG, Zang H, Chang TT, Liu HJ, 2009. Effects of supplemental lighting with different light qualities on growth and physiological characteristics of cucumber, pepper and tomato seedlings. *Acta HorticulturaeSinica***5:** 663–670.

De Micco V, Paradiso R, Aronne G, De Pascale S, Quarto M, Arena C, 2014. Leaf anatomy and photochemical behaviour of *Solanum lycopersicum* L. plants from seeds irradiated with low-LET ionizing radiation. *The Scientific World Journal* **ID: 428141**: 1-13. DOI: 10.1155/2014/428141.

Dieleman JA, De Visser PH, Meinen E, Grit JC, Duech T, 2019. Integrating morphological and physiological responses of tomato plants to light quality to the crop level by 3D modeling. *Frontiers in Plant Science* **10**: 839. DOI: 10.3389/fpls.2019.00839.

Dougher TA, Bugbee B, 2004. Long term blue effect on histology of lettuce and soybean leaves and stems. *Journal of the American Society for Horticultural Science* **129** (4): 467-472.

Dutta-Gupta S, 2017. Light Emitting Diodes for Agriculture - Smart lighting. *Springer*, Singapore. DOI: 10.1007/978-981-10-5807-3.

Farquhar GD, von Caemmerer S, Berry JA, 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. *Planta* **149**: 78-90.

Folta KM, 2005. Green light effects on plant growth development. In: Wada M, Shimazaki K, Iino M (Eds.), Light Sensing in Plants. *Springer*, Tokio, 370.

Food and Agriculture Organization of the United Nations (FAO). 2017. The future of food and agriculture: Trends and challenges, Rome, 2017. Available at http://www.fao.org/publications/fofa.

Food and Agriculture Organization of the United Nations (FAO). **2019.** The State of the World's Biodiversity for Food and Agriculture. Bélanger J, Pilling D (Eds.), FAO Commission on Genetic Resources for Food and Agriculture Assessments, Rome, 2019, 572. http://www.fao.org/3/CA3129EN/CA3129EN.pdf.

Genty B, Briantais JM, Baker NR, 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**: 87–92.

George B, Kaur C, Khurdiya DS, Kapoor H, 2004. Antioxidants in tomato (*Lycopersicum esculentum*) as a function of genotype. *Food Chemistry* **84**: 45-51.

Giliberto L, Perrotta G, Pallara P, Weller JL, Fraser PD, Bramley PM, Fiore A, Tavazza M, Giuliano G, 2005. Manipulation of the Blue Light Photoreceptor Cryptochrome 2 in Tomato Affects Vegetative Development, Flowering Time, and Fruit Antioxidant Content. *Plant Physiology***137**: 199–208. DOI 10.1104/pp.104.051987.

Gomez C, Izzo LG, 2018. Increasing efficiency of crop production with LEDs. *AIMS Agriculture and Food* **3**(2): 135-153.

Hasan MM, Bashir T, Gosh R, Lee K, Bae H, 2017. An Overview of LEDs' Effects on the Production of Bioactive Compounds and Crop Quality. *Molecules* **22**(9): 1420.

Izzo LG, Hay Mele B, Vitale L, Vitale E, Arena C, 2020. The role of monochromatic red and blue light in tomato early photomorphogenesis and photosynthetic traits. *Environmental and Experimental Botany* **179**: 104195. DOI: 10.1016/j.envexpbot.2020.204195.

Ji Y, NuñezOcaña D, Choe D, Larsen DH, Marcelis LFM, Heuvelink E, 2020. Far-red radiation stimulates dry mass partitioning to fruits by increasing fruit sink strength in tomato. *New Phytologist* **228**: 1914-1925.

Johkan M, Shoji K, Goto F, Hahida S, Yoshihara T, 2012. Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in *Lactuca sativa*. *Environmental and Experimental Botany* **75**: 128-133.

Kalaitzoglou P, van Ieperen W, Harbinson J, van der Meer M, Martinakos S, Weerheim K, Nicole CCS, Marcelis LFM, 2021. Effects of continuous or end-of-day far-red light on tomato plant growth, morphology, light absorption, and fruit production. *Frontiers in Plant Science* **10**: 322. DOI: 10.3389/fpls.2019.00322.

Kato Y, Ozawa SY, Takahashi Y, Sakamoto W, 2015. D1 fragmentation in photosystem II repair caused by photo-damage of a two-step model. *Photosynthesis Research* **126**: 409–416.

Kitajima K, Hogan P, **2003.**Increases of chlorophylla/bratios during acclimationof tropical woody seedlings to nitrogen limitationand high light. *Plant, Cell and Environment* **26:** 857–865.

Kusuma P, Swan B, Bugbee B, 2021. Does Green Really Mean Go? Increasing the Fraction of Green Photons Promotes Growth of Tomato but Not Lettuce or Cucumber. *Plants* **10**(4): 637. DOI: 10.3390/plants10040637.

Lanoue J, Leonardos ED, Grodzinski B, 2018. Effect of light quality and intensity on diurnal patterns and rates of photo-assimilate translocation and transpiration in tomato leaves. *Frontiers in Plant Science* **9**: 756. doi: 10.3389/fpls.2018.00756.

Lester GE, 2006. Environmental regulation of human health nutrients (ascorbic acid, carotene, and folic acid) in fruits and vegetables. *HortScience* **41**: 59–64.

Li A, Li S, Wu X, Zhang J, He A, Zhao G, Yang X, 2016. Effect of light intensity on leaf photosynthetic characteristics and accumulation of flavonoids in *Lithocarpus litseifolius* (Hance) Chun. (Fagaceae). *Open Journal of Forestry* **6**: 445-459.

Li SS, Pan RC, 1994. Effect of blue light on the metabolism of carbohydrate and protein in rice (*Oryza sativa* L.) seedlings. *Acta Phytophysiologics Sinica* **21:** 22–28.

Li Q, Kubota C, 2009. Effects of supplemental light quality on growth and phytochemicals of baby lettuce. *Environmental and Experimental Botany* **67**: 9-64.

Lichtenthaler HK, 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymology* **148**: 350-382.

Liu U, Ren X, Jeong BR, 2019. Supplementary Light Source Affects Growth, Metabolism, and Physiology of *Adenophora triphylla* (Thunb.). A.D.C. Seedlings. *Biomed Research International*. **ID 6283989**. DOI: 10.1155/2019/6283989.

Liu J, van Iersel MW, 2021. Photosynthetic Physiology of Blue, Green and Red Light: Light Intensity Effects and Underlying Mechanisms. *Frontiers in Plant Science* **12**: 619987. DOI: 10.3389/fpls.2021.619987.

Loreto F, Harley PC, Di Marco G, Sharkey TD, 1992. Estimation of mesophyll conductance to CO_2 flux by three different methods. *Plant Physiology* **98**: 1437–1443.

Loreto F, Tsonev T, Centritto M, 2009. The impact of blue light on leaf mesophyll conductance. *Journal of Experimental Botany* **60**: 2283–2290.

Ma G, Zhang L, Setiawan CK, Yamawaki K, Asai T, Nishikawa F, Maezawa S, Sato H, Hanemitsu HN, Kato M, 2014. Effect of red and blue LED light irradiation on ascorbate content and expression of genes related to ascorbate metabolisms in postharvest broccoli. *Postharvest Biology and Technology* **94**: 97-103.

Maizane D, Shipley B, 1999. Interacting determinants of specific leaf area in 22 herbaceous species: effects of irradiance and nutrient availability. *Plant, Cell & Environment* **22**: 447-459.

Massa GD, Emmerich JC, Morrow RC, Bourget CM, Mitchell CA, 2006. Plant growth lighting for Space life support: a review. *Gravitational and space biology bulletin* **19**: 19-30.

Miao YX, Wang XZ, Gao LH, Chen QY, Mei Q, 2016. Blue light is more essential than red light for maintaining the activities of photosystem II and I and photosynthetic electron transport capacity in cucumber leaves. *Journal of Integrative Agriculture* **15:** 87–100.

Muñoz P, Munné-Bosch S, 2018. Photo-Oxidative Stress during Leaf, Flower and Fruit Development. *Plant Physiology* **176**: 1004–1014.

Nanya K, Ishigami Y, Shoko H, Goto E, 2012. Effects of blue and red light on stem elongation and flowering of tomato seedlings. Acta Horticulturae **956**: 261-266.

Niinemets Ü, Díaz-Espejo A, Flexas J, Galmé J, Warren CR, 2009. Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. *Journal of Experimental Botany* **60**: 2249-2009.

Ntagkas N, Woltering E, Nicole C, Labrie C, Marcelis LFM, 2019. Light regulation of vitamin C in tomato fruit is mediated through photosynthesis. *Environmental and Experimental Botany* **158**: 180-188.

Ohashi-Kaneko K, Takase M, Kon N, Fujiwara K, Kurata K, 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. *Environmental Control in Biology* **45**: 189–198.

Okazaki M, Ezura H, 2009. Profiling of melatonin in the model tomato (*Solanum lycopersicum* L.) cultivar Micro-Tom. *Journal of Pineal Research* **46**: 338–343.

Onoda Y, Hikosaka K, Hirose T, 2005. Seasonal change in the balance between capacities of RuBP carboxylation and RuBP regeneration affects CO_2 response of photosynthesis in *Polygonum caspidatum*. *Journal of Experimental Botany* **56**: 755-763.

Pallozzi E, Tsonev T, Copolovici L, Niinemets U, Loreto F, Centritto M, 2013. Isoprenoid emissions: photosynthesis and mesophyll diffusion conductance in response to blue light. *Environmental and Experimental Botany* **95**: 50-58.

Pandey P, Irulappan V, Bagavathlannan MV, Senthll-Kumar M, 2017. Impact of Combined Abiotic and Biotic Stresses on Plant Growth and Avenues for Crop Improvement by Exploiting Physio-morphological Traits. *Frontiers in Plant Science* **8**: 537. DOI: 10.3389/fpls.2017.00537.

Panjai L, Noga G, Fiebig A, Hunsche M, 2017. Effects of continuous red light and short daily UV exposure during postharvest on carotenoid concentration and antioxidant capacity in stored tomatoes. *Scientia Horticulturae* **226**: 97-103.

Pennisi G, Blasioli S, Cellini A, Maia A, Crepaldi A, Braschi I, Spinelli F, Nicola S, Fernandez JA, Stanghellini C, Marcelis LFM, Orsini F, Giaquinto G, 2019. Unraveling the role of Red: Blue LED Lights on Resources Use Efficiency and Nutritional Properties of Indoor Grown Sweet Basil. *Frontiers in Plant Science* **10**: 305. DOI: 10.3389/fpls.2019.00305.

Poorter H, De Jong R, 1999. A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. *New Phytologist* **143**: 163-176.

Racchi ML, 2013. Antioxidant Defenses in Plant with Attention to Prunus and Citrus spp. *Antioxidants* **2**(4): 340-369. DOI:10.3390/antiox2040340.

Saito T, Ariizumi T, Okabe Y, Asamizu E, Hiwasa-Tanase K, Fukuda N, Mizoguchi T, Yamazaki Y, Aoki K, Ezura H, 2011. TOMATOMA: a novel tomato mutant database distributing Micro-Tom mutant collections. *Plant Cell Physiology* **52**(2): 283-96. DOI: 10.1093/pcp/pcr004.

Samuolienė G, Urbanovičiūte A, Brazaitytė A, Šabajevienė G, Sakalauskaitė J, Duchovskis P, 2011. The impact of LED illumination on antioxidant properties of sprouted seeds. *Central European Journal of Biology* **6**: 68-74.

Samuolienė G, Miliauskienė J, Kazlauskas A, Viršilė A, 2021. Growth Stage Specific Lighting Spectra Affect Photosynthetic Performance, Growth and Mineral Element Contents in Tomato. *Agronomy* **11**: 901. DOI:10.3390/agronomy11050901.

Scott JW, Harbaugh BH, 1989. Micro-Tom A miniature dwarf tomato. Gainesville, Florida. *Agricultural Experiment Station* **370**: 1-6.

Shahzad R, Ahmed F, Wang Z, Harlina PV, Nishawy E, Ayaad M, Manan A, Maher M, Ewas M, 2020. Comparative analysis of two phytochrome mutants of tomato (Micro-Tom cv.) reveals specific physiological biochemical and molecular responses under chilling stress. *Journal of Genetic Engineering and Biotechnology* **18**: 77.

Shikata M, Hoshikawa K, Ariizumi T, Fukuda N, Yamazaki Y, Ezura H, 2016. TOMATOMA Update: Phenotypic and Metabolite Information in the Micro-Tom Mutant Resource. *Plant and Cell Physiology* **57**(1): pe11. DOI: 10.1093/pcp/pcv194.

Singh D, Basu C, Meinhardt-Wollweber M, Roth B, 2015. LEDs for energy efficient greenhouse lighting. *Renewable & Sustainable Energy Reviews* **49**: 139-147.

Smith HL, Mc Ausland L, Murchie EH, 2017. Don't ignore the green light: exploring diverse role in plant processes. *Journal of Experimental Botany* **68**: 2099-2110.

Su N, Wu Q, Shen ZG, Xia K, Cu J, 2014. Effects of light quality on the chloroplastic ultrastructure and photosynthetic characteristics of cucumber seedlings. *Plant Growth Regulation* **73**: 227-235.

Terashima I, Fujita T, Inoue T, Chow WS, Oguchi R, 2009. Green light drives leaf photosynthesis more efficiently than red lighting strong white light: Revisiting the enigmatic question of why leaves are green. *Plant Cell Physiology* **50**: 684 – 697.

Tomás M, Flexas J, Copolovici L, Galmés J, Hallik L, Medrano H, Ribas-Carbó M, Tosens T, Vislap V, Niinemets U, 2013. Importance of leaf anatomy in determining mesophyll diffusion conductance to CO_2 across species: quantitative limitations and scaling up by models. *Journal of Experimental Botany* **64**: 2269–2281.

Urbonavičiūte A, Pinho P, Samuolienė G, Duchovskis P, Vitta P, Stonkus A, Tamulaitis G, Zukauskas A, Halonen L, 2007. Effect of Short-wavelength Light on Lettuce Growth and

Nutritional Quality. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture. *Sodininkysteir Daržininkyste* **26**: 157–165.

Vitale L, Vitale E, Guercia G, Turano M, Arena C, 2020. Effects of different light quality and biofertilizers on structural and physiological traits of spinach plants. *Photosynthetica* **58**: 932-943. DOI:10.32615/ps.2020.039.

Vitale E, Vitale L, Costanzo G, Velikova V, Tsonev T, Simoniello P, De Micco V, Arena C, 2021. Light Spectral Composition Influences Structural and Eco-Physiological Traits of *Solanum lycopersicum* L. cv. 'Microtom' in Response to High-LET Ionizing Radiation. *Plants* **10**: 1752. DOI: 10.3390/plants10081752.

Von Caemmerer S, Farquhar GD, 1981. Some relationship between the biochemistry of photosynthesis and the gas exchanges of leaves. *Planta* **153**: 376-387.

Wang W, Vignani R, Scali M, Crest, M, 2006. A universal and rapid protocol for protein extraction from recalcitrant plant tissue for proteomic analysis. *Electrophoresis* **27**: 2782–2786.

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*. *Journal of Photochemistry and Photobiology* **96**: 30-37.

Wang Y, Folta KM, 2013. Contributions of green light to plant growth and development. *American Journal of Botany* **100**: 70-78.

Wheeler RM, Morrow RC, 2010. Physiological disorders in closed, controlled environment crops. *NASA*: Technical Reports.

Xiaoying L, Shirong G, Taotao C, Zhigang X, Tezuka T, 2012. Regulation of the growth and photosynthesis of cherry tomato seedlings by different light irradiations of light emitting diodes (LED). *African Journal of Biotechnology* **11**: 6169-6177.

Xie B, Song S, Liu H, Sun G, Chen R, 2016. Effect of Light Quality on The Quality Formation of Tomato Fruits. Proceedings of the International Conference on Biological Engineering and Pharmacy (*BEP 2016*). DOI: 10.2991/bep-16.2017.3.

Xie B, Wei JJ, Zhang YT, Song SW, Sun GW, Hao YW, Liu HC, 2019. Supplemental blue and red light promote lycopene synthesis in tomato fruits. *Journal of Integrative Agriculture* **18**: 590-598.

Xu F, Shi L, Chen W, Cao S, Su X, Yang Z, 2018. Effect of blue light treatment on fruit quality, antioxidant enzymes and radical-scavenging activity in strawberry fruit. *Scientia Horticulturae* **175**: 181–186.

Yang LY, Wang LT, Ma JH, Ma ED, Li JY, Gong M, 2017. Effect of light quality on growth and development, photosynthetic characteristics and content of carbohydrates in tobacco (*Nicotiana tabacum* L.) plants. *Photosynthetica* **55**: 467-477.

Zhang T, Folta KM, 2012. Green light signaling and adaptive response. *Plant Signaling & Behavior* **7**: 75-78.

750 **Zheng L, Van Labeke MC, 2017**. Long-Term Effects of Red- andBlue-Light Emitting Diodes
751 on LeafAnatomy and PhotosyntheticEfficiency of Three Ornamental Pot Plants. *Frontiers in*
752 *Plant Science* **8**:917. doi: 10.3389/fpls.2017.00917.
753

Figure 1

Spectral distributions in the relative energy of FL (white fluorescent light), RB (Red-Blue) and RGB (Red-Green-Blue) treatments.

Spectral distributions in the relative energy of the white fluorescent tubes and LEDs panels recorded for FL (white fluorescent light), RB (Red-Blue) and RGB (Red-Green-Blue) treatments at the top of the plant canopy.

		Light quality regimes		
Color	Wavelenght range	FL (Fluorescent)	RB (LED)	RGB (LED)
Violet	400-450 nm	7.0%	12.5%	15.5%
Blue	450-485 nm	5.8%	7.9%	11.1%
Cyan	485-500 nm	3.6%	0.3%	6.3%
Green	500-565 nm	26.1%	0.0%	15.6%
Yellow	565-590 nm	21.1%	0.7%	1.0%
Orange	590-625 nm	23.5%	42.8%	28.6%
Red	625-700 nm	10.0%	34.3%	20.4%
FR	700-800 nm	2.9%	1.5%	1.4%

Photosynthetic photon flux density: 300 μmol photons $m^{-2}s^{-1}$
Irradiance range: 400-800nm

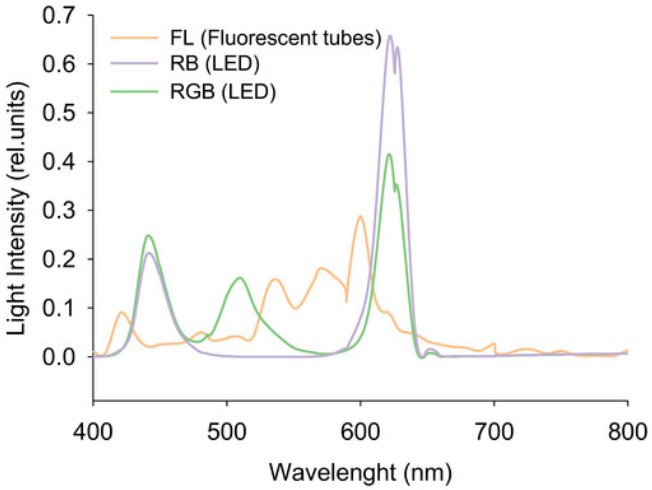

Figure 2

‘Microtom’ plants grown under three different light quality regimes: fluorescent light (FL), red-green-blue (RGB) and red-blue (RB)

Representative view of *Solanum lycopersicum* L. ‘Microtom’ plants grown under three light quality regimes: white fluorescent light (FL), red-green-blue (RGB) and red-blue (RB). Scale bar = 2.5 cm.

Figure 3

Gas exchanges of Microtom plants under different light quality regimes

A) Net CO_2 assimilation (A_N), B) stomatal conductance (g_s), C) mesophyll conductance (g_m), D) maximum rate of Rubisco carboxylation (V_{cmax}) in plants of *Solanum lycopersicum* L. 'Microtom' grown under three light quality regimes: white fluorescent light (FL), red-green-blue (RGB) and red-blue (RB). Data are expressed as mean ± standard error (n=5). Different letters indicate statistically significant differences among light treatments ($P<0.05$) according to one-way ANOVA.

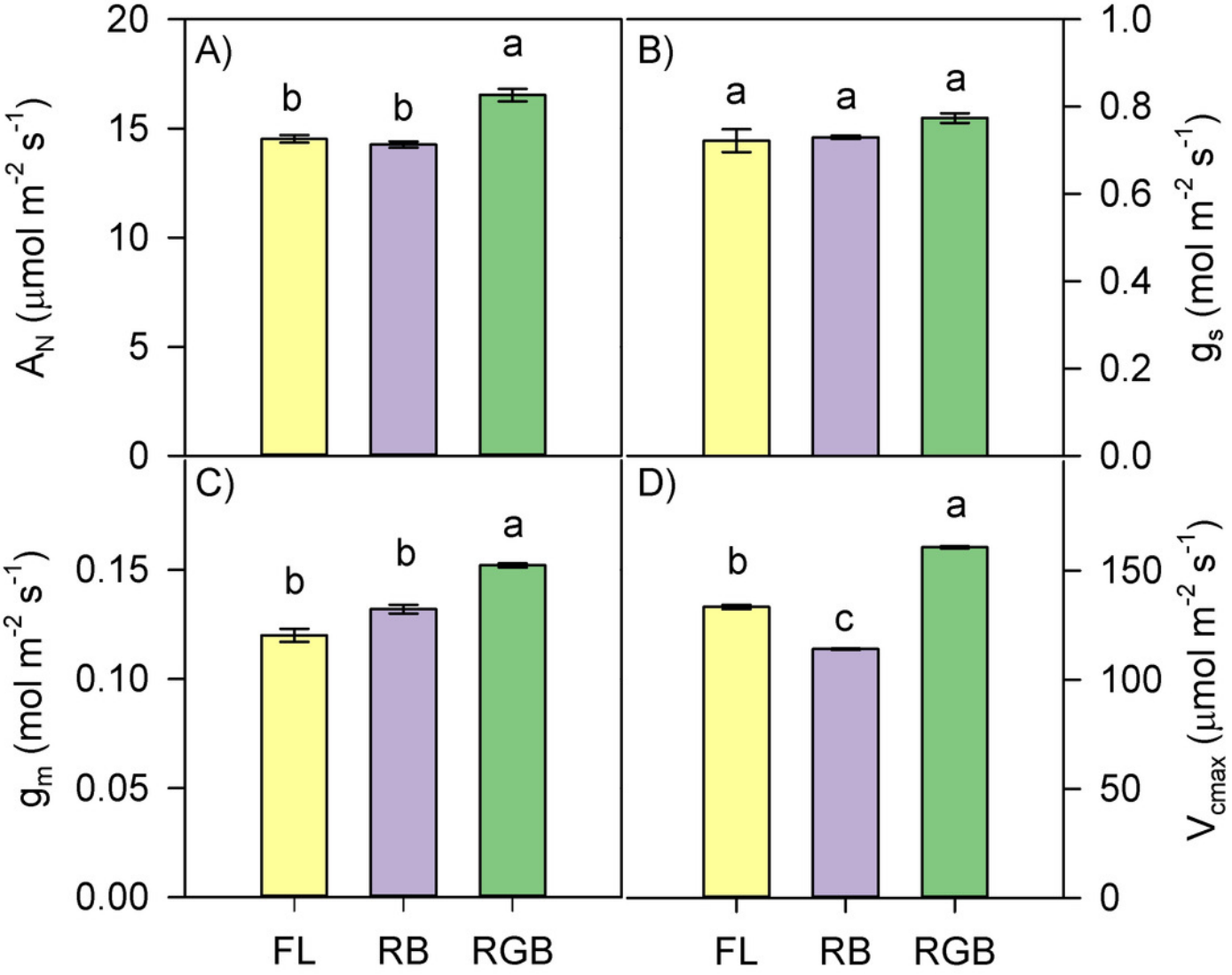
A)
B)
C)
D)
A_N (µmol m^{-2} s^{-1})
g_s (mol m^{-2} s^{-1})
g_m (mol m^{-2} s^{-1})
V_{cmax} (µmol m^{-2} s^{-1})
FL
RB
RGB

Figure 4

PSII photochemistry of Microtom plants under different light quality regimes

A) Quantum yield of PSII electron transport (Φ_{PSII}), B) non-photochemical quenching (NPQ), and C) maximum PSII photochemical efficiency (F_v/F_m) in plants of *Solanum lycopersicum* L. 'Microtom' grown under three light quality regimes: white fluorescent light (FL), red-blue (RB) and red-green-blue (RGB). Data are expressed as mean ± standard error (n=5). Different letters indicate statistically significant differences among light treatments ($P<0.05$) according to one-way ANOVA.

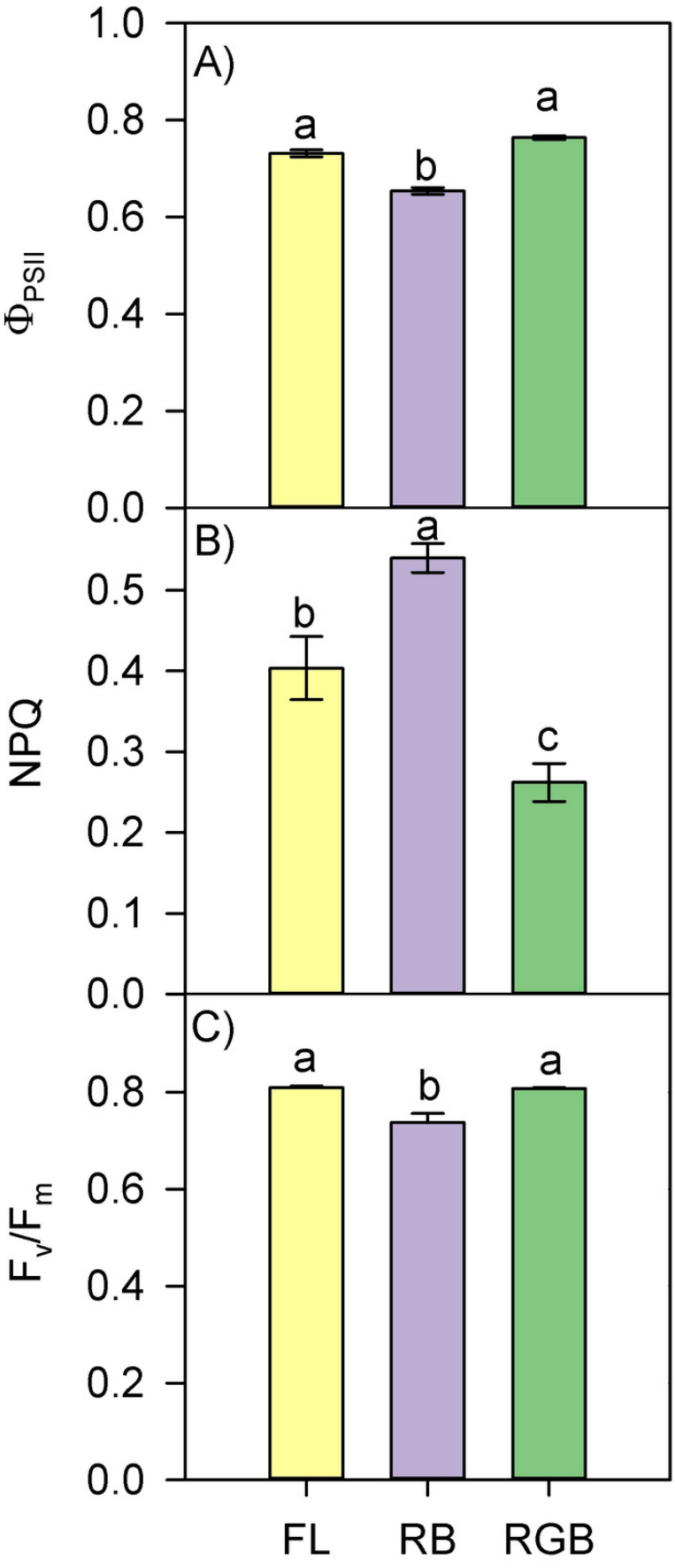
A)
B)
C)
Φ_{PSII}
NPQ
F_v/F_m
1.0
0.8
0.6
0.4
0.2
0.0
0.5
0.3
0.1
a
b
c
FL
RB
RGB

Figure 5

Western Blot and densitometric analysis of the D1 protein and Rubisco in ‘Microtom’ plants grown under white fluorescent (FL), red-blue (RB) and red-green-blue (RGB) light regimes.

Western Blot and densitometric analysis of the photosynthetic proteins D1 (A) and Rubisco (B) in *Solanum lycopersicum*L. ‘Microtom’ plants grown under three light quality regimes: white fluorescent light (FL), red-blue (RB) and red-green-blue (RGB). The bar diagrams represent pixel volumes expressed in arbitrary units of each band of D1 protein and Rubisco. Data are expressed as mean ± standard error (n=3). Different letters indicate statistically significant differences among light regimes (P<0.05) according to one-way ANOVA.

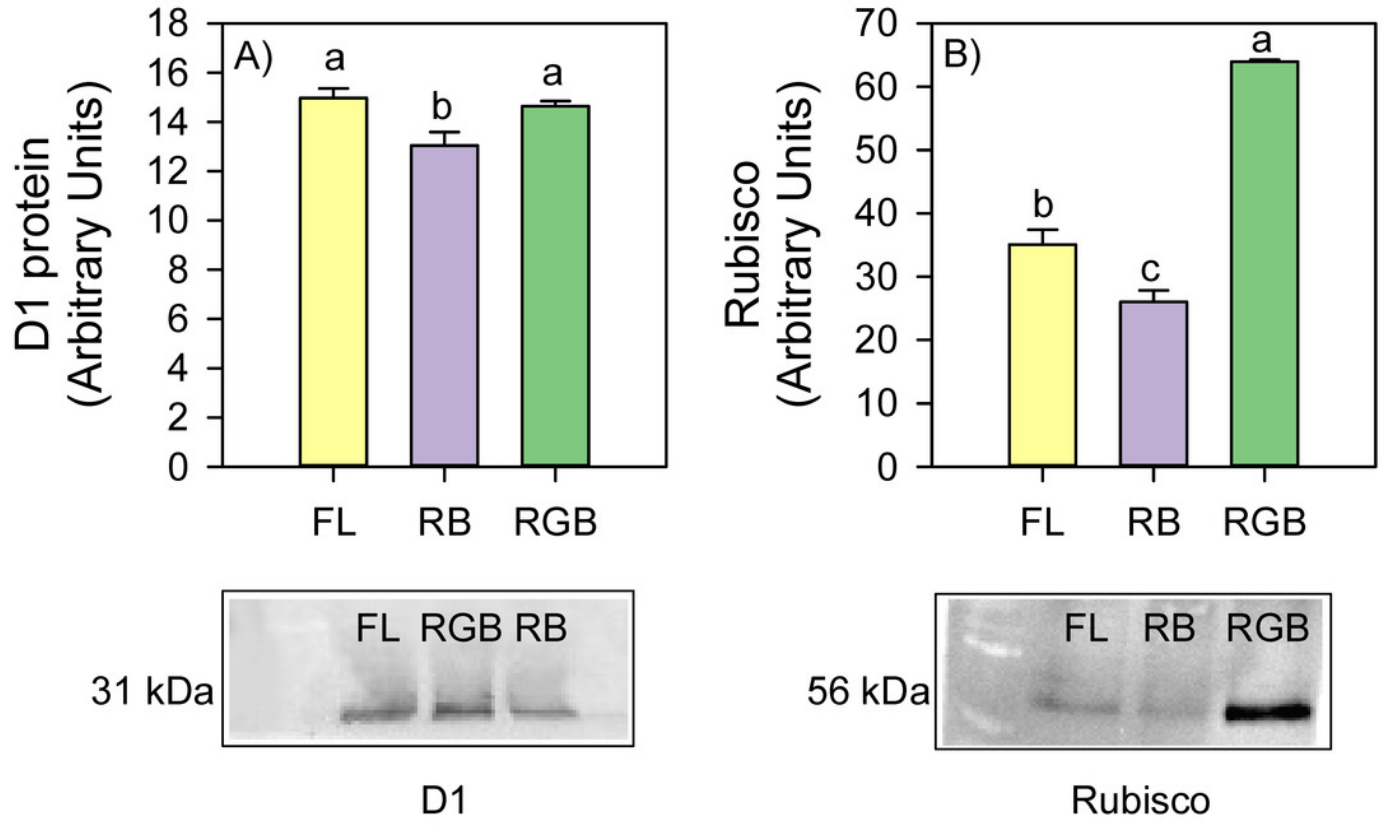

Figure 6

Total chlorophylls (*a*+*b*), total carotenoids (*x*+*c*), and ratio between chlorophyll *a* and chlorophyll *b* (Chl *a*/*b*), in 'Microtom' plants grown under white fluorescent (FL), red-blue (RB) and red-green-blue (RGB) light regimes.

A) Total chlorophylls (*a*+*b*), B) total carotenoids (*x*+*c*), C) ratio between chlorophyll *a* and chlorophyll *b* (Chl *a*/*b*), in *Solanum lycopersicum* L. 'Microtom' plants grown under three light quality regimes: white fluorescent light (FL), red-blue (RB) and red-green-blue (RGB). Data are expressed as mean ± standard error (n=5). Different letters indicate statistically significant differences among light regimes ($P<0.05$) according to one-way ANOVA.

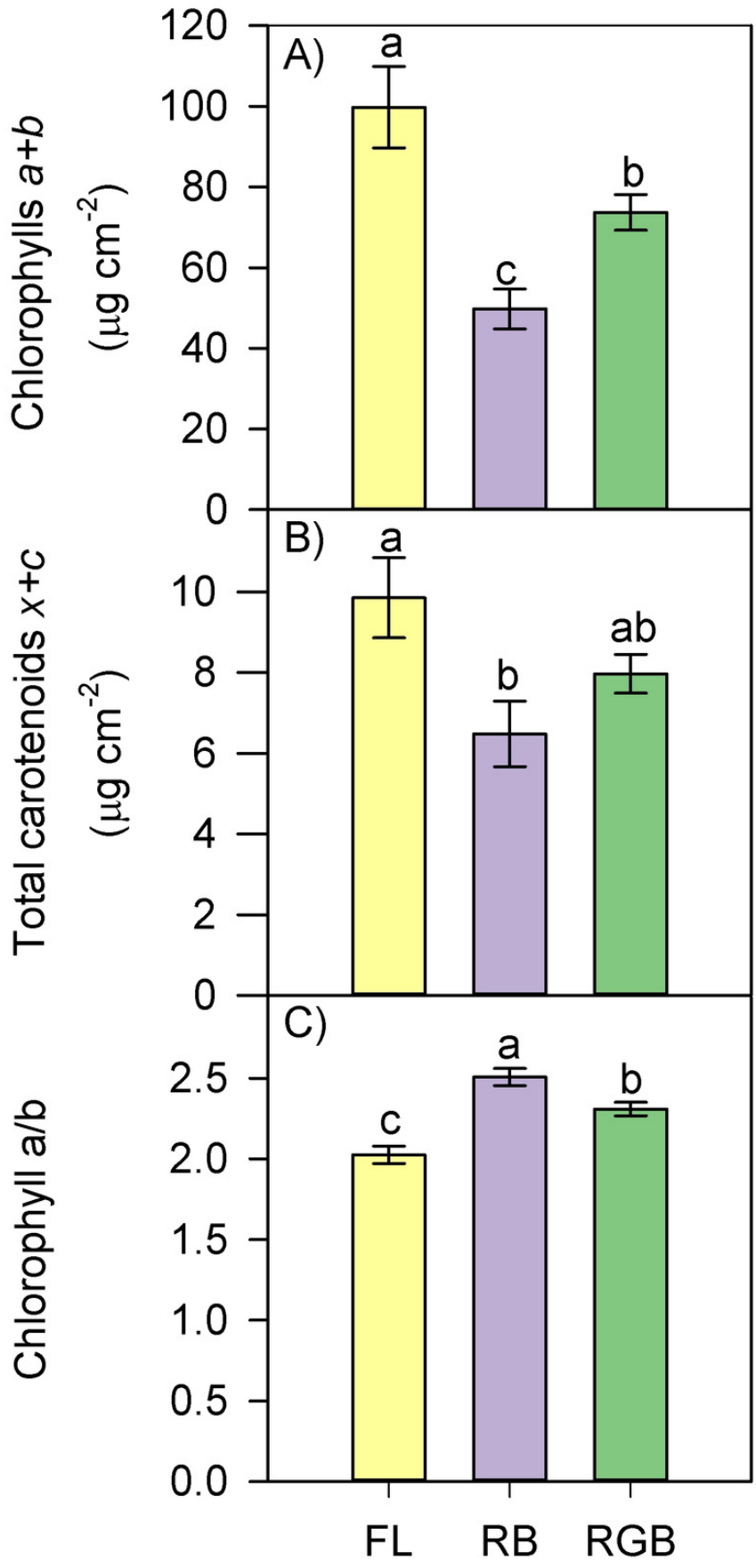
A)
B)
C)
Chlorophylls a+b (µg cm-2)
Total carotenoids x+c (µg cm-2)
Chlorophyll a/b
120
100
80
60
40
20
0
10
8
6
4
2
0
2.5
2.0
1.5
1.0
0.5
0.0
a
b
c
a
b
ab
c
a
b
FL
RB
RGB

Figure 7

SOD and CAT activity, antioxidant capacity, ascorbic acid concentration, and total polyphenols in fruits of 'Microtom' plants grown under white fluorescent (FL), red-blue (RB) and red-green-blue (RGB) light regimes.

A) SOD activity, B) CAT activity, C) antioxidant capacity, D) ascorbic acid concentration, E) total polyphenols in fruits of *Solanum lycopersicum* L. 'Microtom' plants grown under three light quality regimes: white fluorescent light (FL), red-blue (RB) and red-green-blue (RGB). Data are expressed as mean ± standard error (n=5). Different letters indicate statistically significant differences among light regimes (P<0.05) according to one-way ANOVA.

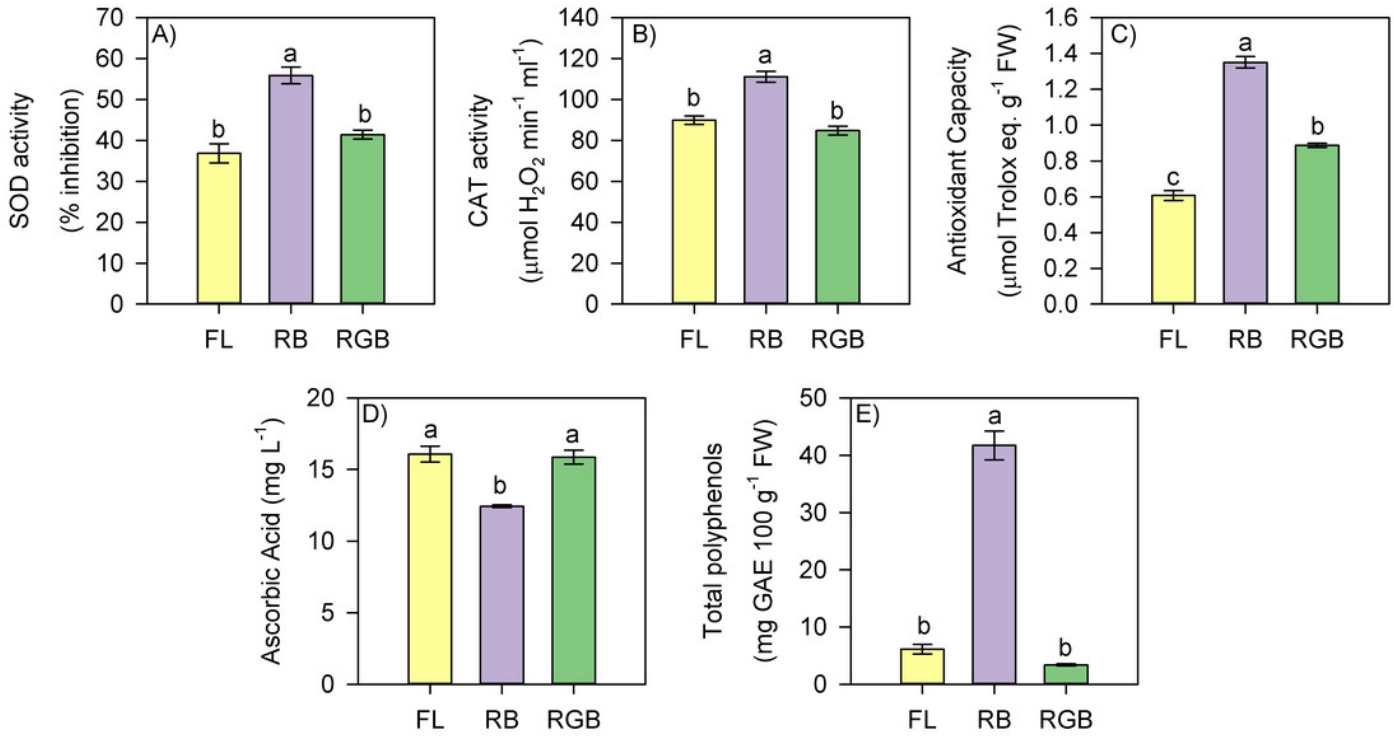

Figure 8

Heatmaps showing plant morphological, physiological and biochemical traits and fruit characteristics of Microtom under white fluorescent (FL), red-blue (RB) and red-green-blue (RGB) light regimes.

Cluster heatmap analysis summarizing plant morphological, physiological and biochemical traits (A) and fruit characteristics (B) of *Solanum lycopersicum* L. 'Microtom' plants cultivated under white fluorescent light (FL), red-blue (RB) and red-green-blue (RGB) light regimes. The color scale shows numeric differences within the data matrix: red and blue indicate increasing and decreasing values. Parameters are clustered in the rows; sample groups are clustered in the Light Quality factor columns.

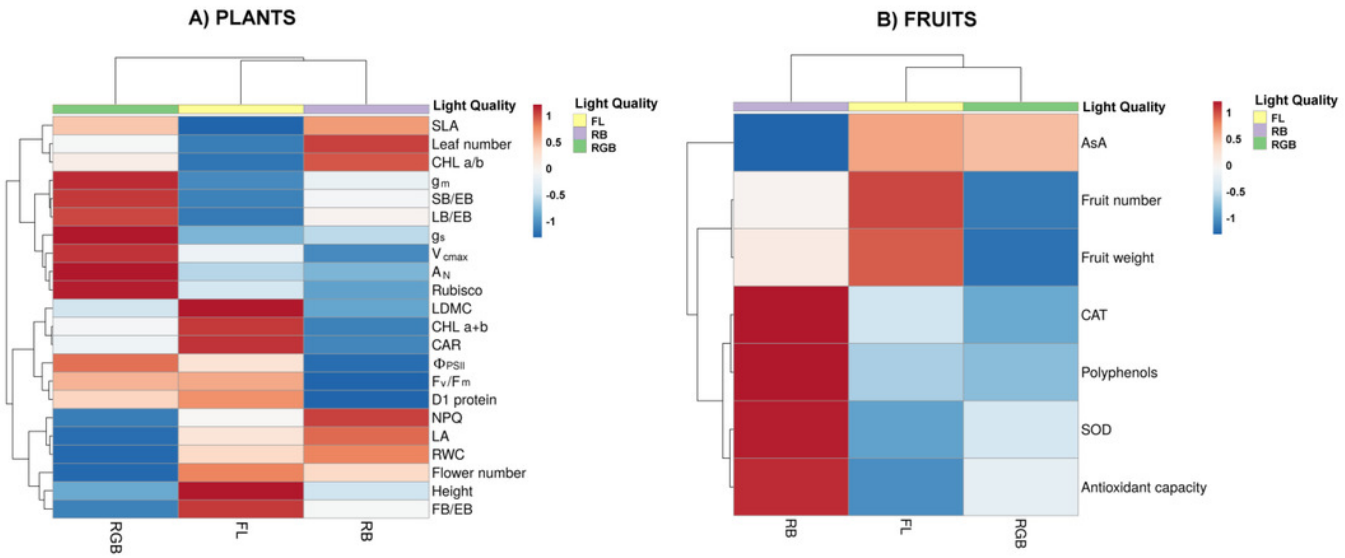

Table 1(on next page)

Morphological parameters and leaf functional traits of Microtom plants

Morphological parameters and leaf functional traits of *S. lycopersicum* L. cv. 'Microtom' plants cultivated under white fluorescent (FL), red-blue (RB) and red-green-blue (RGB) light regimes. Data are mean (n=5) ± standard error. Different letters indicate statistically significant differences among light treatments ($P<0.05$) according to one-way ANOVA.

Table 1. Morphological parameters and leaf functional traits of *S. lycopersicum* L. cv. 'Microtom' plants cultivated under white fluorescent (FL), red-blue (RB) and red-green-blue (RGB) light regimes. Data are mean (n=5) ± standard error. Different letters indicate statistically significant differences among light treatments (P<0.05) according to one-way ANOVA.

	Light quality regimes		
	FL	RB	RGB
Morphological parameters			
Height (cm)	15.66±0.658 [a]	12.16±0.556 [b]	11.26±0.370 [b]
Leaf number	22.60±0.980 [c]	37.60±1.860 [a]	30.0±0.837 [b]
Flower number	50.00±2.280 [a]	47.00±3.302 [a]	36.60±1.364 [b]
Fruit number	18.60±1.939 [a]	14.60±0.872 [b]	10.00±0.632 [c]
Fruit weight (g)	34.78±0.820 [a]	28.75±3.400[a]	19.26±0.692[b]
SB/EB	0.234±0.004 [c]	0.250±0.001 [b]	0.272±0.006 [a]
LB/EB	0.168±0.006 [c]	0.244±0.010 [b]	0.309±0.003 [a]
FB/EB	0.598±0.010 [a]	0.506±0.011 [b]	0.420±0.004 [c]
Leaf functional traits			
LA (cm^2)	14.07±0.494 [a]	15.62±0.588 [a]	10.84±0.433 [b]
SLA ($cm^2\ g^{-1}$)	321.5±11.25 [b]	409.3±8.900 [a]	399.7±9.824 [a]
RWC (%)	81.97±0.736 [a]	82.89±0.850 [a]	78.83±1.080 [a]
LDMC ($g\ g^{-1}$)	0.101±0.003 [a]	0.082±0.001 [b]	0.085±0.003 [b]

SB/EB: Stem biomass/epigeal biomass, LB/EB: leaf biomass/epigeal biomass, FB/EB: fruit biomass/epigeal biomass, LA: leaf area, SLA: specific leaf area, RWC: relative water content, LDMC: leaf dry matter content