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Published Version:
Pennisi G., Pistillo A., Orsini F., Cellini A., Spinelli F., Nicola S., et al. (2020). Optimal light intensity for sustainable water and energy use in indoor cultivation of lettuce and basil under red and blue LEDs. SCIENTIA HORTICULTURAE, 272, 1-10 [10.1016/j.scienta.2020.109508].

Availability:
This version is available at: https://hdl.handle.net/11585/776494 since: 2020-10-28

Published:
DOI: http://doi.org/10.1016/j.scienta.2020.109508

(Article begins on next page)

Optimal light intensity for sustainable water and energy use in indoor cultivation of lettuce and basil under red and blue LEDs.

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Highlights

- Optimal LED light intensity for lettuce and basil indoor growing is addressed;
- Maximum yield and leaf area is achieved at 250 μmol m^{-2} s^{-1};
- 250 μmol m^{-2} s^{-1} increased chlorophyll and improved stomatal functions in leaves;
- In lettuce, PPFD≥200 μmol m^{-2} s^{-1} raised antioxidant capacity, phenolics and flavonoids;
- Water, energy and light use efficiencies were optimized at 250 μmol m^{-2} s^{-1};

Abstract

Indoor plant cultivation systems are gaining increasing popularity because of their ability to meet the needs of producing food in unfavourable climatic contexts and in urban environments, allowing high yield, high quality, and great efficiency in the use of resources such as water and nutrients. While light is one of the most important environmental factors affecting plant development and morphology, electricity costs can limit the widespread adoption of indoor plant cultivation systems at a commercial scale. LED lighting technologies for plant cultivation are also rapidly evolving, and lamps for indoor cultivation are often designed to optimize their light emissions in the photosynthetically active spectrum (i.e. red and blue), in order to reduce energetic requirements for satisfactory yield. Under these light regimens, however, little information is available in literature about minimum photosynthetic photon flux density (PPFD) for indoor production of leafy vegetables and herbs, while existing literature often adopts light intensities from 100 to 300 μmol m^{-2} s^{-1}. This study aims at defining the optimal PPFD for indoor cultivation of basil (*Ocimum basilicum* L.) and lettuce (*Lactuca sativa* L.), by linking resource use efficiency to physiological responses and biomass production under different light intensities. Basil and lettuce plants were cultivated at 24°C and 450 μmol mol^{-1} CO_2 under red and blue light (with red:blue ratio of 3) and a photoperiod of 16 h d^{-1} of light in growth chambers using five PPFD (100, 150, 200, 250 and 300 μmol m^{-2} s^{-1}, resulting in daily light integrals, DLI, of 5.8, 8.6, 11.5, 14.4 and 17.3 mol m^{-2} d^{-1}, respectively). A progressive increase of biomass production for both lettuce and basil up to a PPFD of 250 μmol m^{-2} s^{-1} was observed, whereas no further yield increases were associated with higher PPFD (300 μmol m^{-2} s^{-1}). Despite the highest stomatal conductance associated to a PPFD of 250 μmol m^{-2} s^{-1} in lettuce and to a PPFD$\geq$200 μmol m^{-2} s^{-1} in basil, water use efficiency was maximized under a PPFD$\geq$200 μmol m^{-2} s^{-1} in lettuce and PPFD$\geq$250 μmol m^{-2} s^{-1} in basil. Energy and light use efficiencies were increased under a PPFD of 200 and 250 μmol m^{-2} s^{-1} in lettuce and under a PPFD of 250 μmol m^{-2} s^{-1} in basil. Furthermore, in lettuce grown under 250 μmol m^{-2} s^{-1} antioxidant capacity, phenolics and flavonoids were higher as compared with plants supplied with PPFD$\leq$150 μmol m^{-2} s^{-1}. Accordingly, a PPFD of 250 μmol m^{-2} s^{-1} seems suitable for optimizing yield and resource use efficiency in red and blue LED lighting for indoor cultivation of lettuce and basil under the prevailing conditions of the used indoor farming set-up.

Keywords: Photosynthetic Photon Flux Density (PPFD); Plant factory with artificial lighting (PFALs); Water Use Efficiency (WUE); Energy Use Efficiency (EUE); Light Use Efficiency (LUE); Daily Light Integral (DLI)

1 INTRODUCTION

Indoor farming systems supplied with artificial lighting are claimed to substantially decrease the pressure on natural resources, with specific potentialities in reducing water used for food production (Graamans et al., 2018). Thanks to the use of hydroponics, the improved photosynthetic efficiency under the stable lighting and climatic conditions provided by the indoor environment and the possibilities for transpiration water recovery through air dehumidification, indoor cultivation may enhance water use efficiency (WUE, commonly expressed as grams of fresh biomass produced per liter of water consumed) up to 50 times in comparison with current greenhouse systems (Kozai and Niu, 2020). On the other hand, in indoor farming, the efficiency of light assimilation is crucial not only for plant growth performances, but since it overall dramatically affects the environmental and economic sustainability of the production system (Kozai, 2015). Vegetable and aromatic crops have been extensively to date studied for their response to artificial lighting, with most promising results being associated with LED lights, which allow to maximise electricity use efficiency and reduce production costs as compared to other lighting technologies (Benke and Tomkins, 2017). Moreover, through the use of coloured diodes targeting specific regions of the light spectrum, it is possible to concentrate the light within the chlorophyll absorption peaks, which are respectively found within the red (600-700 nm) and the blue (400-500 nm) spectral regions, allowing for further improvements in the efficiency of converting electricity into photosynthetic gains (Yeh and Chung, 2009). Lettuce (*Lactuca sativa* L.) stands amongst the most studied species for indoor cultivation under LED lights (Pennisi et al., 2019a). To date, most of the research work has focused on the comparison between LED and alternative light sources (Kozai, 2016) or the comparison between monochromatic and combined colours of LED lights (Rehman et al., 2017). Energy use efficiency (EUE, expressed as grams of fresh biomass produced per kWh), was shown to increase by up to 2.5-folds when moving from fluorescent (15.9 g FW kWh^{-1}) to LED light (40.6 g FW kWh^{-1}) in lettuce (Zhang et al., 2018). More recently, EUE values up to 80 g FW kWh^{-1} were reported for lettuce grown under LED (Yan et al., 2020). Also, the role of red:blue (RB) ratio in the spectral composition used for indoor lettuce cultivation was targeted, showing that RB=3 would allow for maximum yield and resource-use efficiency (Pennisi et al., 2019a). Similarly to lettuce, the aromatic herb sweet basil (*Ocimum basilicum* L.) is a widely studied crop species for indoor cultivation. Growth of basil under LED lighting has been compared with other light sources, including high pressure sodium (Hammock, 2018) or cool fluorescent lighting (Frąszczak et al., 2014; Piovene et al., 2015). It was recently demonstrated (Pennisi et al., 2019b) that similar to lettuce the optimal red and blue spectral composition for basil cultivation and resources use efficiency stands on RB=3. Another study (Pennisi et al., 2019c) confirmed that the normalized environmental impact (based on a life cycle assessment) was reduced when RB=3 or RB≥2 were used respectively for lettuce and basil.

In indoor grown basil and lettuce, a range of optimal light intensities, ranging from 50-150 μmol m^{-2} s^{-1} (Shiga et al., 2009), to 150-250 μmol m^{-2} s^{-1} (Žukauskas et al., 2011; Cha et al., 2012; Tarakanov et al., 2012; Muneer et al., 2014; Piovene et al., 2015; Pennisi et al., 2019a, 2019b), or even above 250 (Li and Kubota, 2009; Samuoliene et al., 2009; Stutte et al., 2009; Johkan et al., 2010; Johkan et al., 2012) has been suggested. Similarly, a model for supplemental lighting in greenhouse grown lettuce adopted intensities ranging 100 to 200 μmol m^{-2} s^{-1} (Albright and Both, 2000).

However, it appears that studies targeting the amelioration of light intensity from productive, qualitative and resource efficiency perspectives in leafy vegetables and herbs under combined red-blue LED lighting are still lacking, while the selection of the optimal light intensity for indoor cultivation of these species still relies on other lamp typologies (e.g. fluorescent or incandescent lights, Beaman et al., 2009).

A meta-analysis of plant responses to light intensity suggests that light intensity may have strong effects on nutritional properties of plants (Poorter et al., 2019). For instance, Brazaityte et al. (2015), found that in microgreens of Brassicaceae (including mustard, red pak choi and tatsoi) grown under mixed red and blue LED lights, the accumulation of antioxidant compounds was stimulated by increasing the photosynthetic photon flux density (PPFD) from 110 to 440 μmol m^{-2} s^{-1}, though their concentration decreased as light intensity was further augmented to 545 μmol m^{-2} s^{-1}. In coriander (*Coriandrum sativum* L.), total phenolics and antioxidant capacity were increased as the intensity of a combined LED light (featuring red, white and far red LEDs) was progressively enhanced from 100 to 300 μmol m^{-2} s^{-1} (Nguyen et al., 2019). In lettuce, total carotenoids were increased as PPFD increased from 60 to 140 μmol m^{-2} s^{-1}, but decreased when PPFD reached 220 μmol m^{-2} s^{-1} (Fu et al., 2017), although information on the spectral properties of the light source were not reported in the study. Vitamin C content in lettuce leaves was highest at 140 μmol m^{-2} s^{-1}, as compared with 220 and 60 μmol m^{-2} s^{-1} (Fu et al., 2017), while another study reported an increase in vitamin C content in lettuce in response to PPFD from 120 to 150 μmol m^{-2} s^{-1} (Lin et al., 2018). However, under red and white LED lights (RB=1.2) and a photoperiod of 16 h d^{-1}, it was also shown that vitamin C content was higher at PPFD of 200 as compared with 250 μmol m^{-2} s^{-1} (Yan et al., 2019), overall confirming an optimum response curve. On the other hand, in basil, the effect of artificial light intensity was only studied by using cool fluorescent lamps. Similarly to the previously cited studies on LEDs, antioxidant capacity was shown to increase when the PPFD was enhanced from 160 μmol m^{-2} s^{-1} to 290 μmol m^{-2} s^{-1} (Dou et al., 2018).

It emerges that LED lighting technologies for plant cultivation are rapidly evolving, and lamps for indoor cultivation are often designed to optimise their light emissions in the photosynthetically active spectrum (i.e. red and blue), in order to reduce energetic requirements for satisfactory yield. Under these light regimens, however, little information is available in literature about minimum PPFD for indoor production of leafy vegetables and herbs. The aim of this paper is to assess the effects of different light intensities (e.g. ranging from 100 to 300 μmol m^{-2} s^{-1}) on plant growth, physiological response and product quality, as well as on the overall crop resource use efficiency.

2 MATERIALS AND METHODS

2.1. Plant material and growth conditions

The plants were grown in five separate compartments (0.64 m^2 surface and 0.4 m^3 volume) in a climate-controlled growth chamber (day temperature 26°C, night temperature 22°C, 55-70% relative humidity and 450 μmol mol^{-1} CO_2) at the University of Bologna (Italy) (Choi et al., 2000). Each compartment was insulated by using light opaque white walls, and equipped with fans constantly replacing internal air (hourly replacing 200 times the volume of the chamber). Lettuce plants belonging to the green typology Gentilina, commonly adopted for baby-leaf production (*Lactuca sativa* L. cv. Rebelina, Gautier, Eyragues, France), and basil plants belonging to the typology "Genovese" (*Ocimum basilicum* L. cv. Superbo, Sais seeds, Cesena, Italy) were grown. Three independent experiments were conducted for each species. A planting density of 100 plants m^{-2} and a crop cycle length of 21 days from transplant to harvest for both lettuce and basil experiments were adopted, as for previous experiments (Saha et al., 2016; Pennisi et al., 2019a, 2019b, 2019c).

Seeds were germinated in polystyrene containers filled with a mixture of peat (70%) and vermiculite (30%), under cool-white fluorescent lamps (TL-D90 De Luxe 950, Philips), providing a PPFD of 215 µmol m^{-2} s^{-1} and a photoperiod of 16 h d^{-1} of light. When plants reached a two true leaf stage (14 and 21 days after sowing - DAS - respectively for lettuce and basil), roots were washed and plantlets were transplanted into individual hydroponic systems (Pennisi et al. 2019a). Each single-plant hydroponic unit consisted of plastic jars (1 L of volume, see image in **Supplementary material S1** and further details in Pennisi et al., 2019c), filled with nutrient solution (EC = 1.6, pH = 6.5) with the following composition: N-NO_3: 14 mM; N-NH_4: 4.4 mM; P: 1.0 mM; K: 5.0 mM; S: 2.0 mM; Ca: 1.2 mM; Mg: 5.2 mM; Fe: 17.9 µM, Cu: 2.0 µM, Zn: 3.8 µM, B: 11.6 µM, Mn:18.2 µM, Mo: 0.5 µM. The nutrient solution was constantly aerated through air pumps (Airline 3, Haquoss, Turin, Italy, air exchange rate of 0.25 L min^{-1} pot^{-1}). At 14 Days After start of light Treatment (DAT), pots were replenished with 0.25 L of fresh nutrient solution.

2.2. Light treatments

Lettuce and basil plants were grown under dimmable LED lamps (Flytech s.r.l., Belluno, Italy) featuring red (peak at 669 nm) and blue (peak at 465 nm) emitting diodes. The lamps were set to supply a spectral composition with a red:blue ratio of 3 (RB=3), such ratio being calculated by the relative spectral areas within the red (600–700 nm) and the blue (400–500 nm) regions (Singh et al., 2015). The spectral distribution was measured using an illuminance spectrophotometer (CL-500A, Konica Minolta, Chiyoda, Tokyo, Japan). A photosynthetic photon flux sensor (with equal sensitivity to red and blue radiation), model QSO (Apogee instruments, Logan, UT, USA) connected with a ProCheck handheld reader (Decagon Devices Inc., Pullman, WA, USA) was used to set PPFD (µmol m^{-2} s^{-1}) over the plant canopy. Daily Light Integrals (DLI) were calculated by multiplying the PPFD (µmol m^{-2} s^{-1}) by the photoperiod (s), and expressed as mol m^{-2} d^{-1}. In order to define the lamp's efficacy of electricity-to-light conversion, the PPFD:electricity ratio (µmol J^{-1}) was estimated through flat plane integration technique as the ratio of the incident PPFD (µmol m^{-2} s^{-1}) at a set distance (40 cm, equal to the distance of the lamp from the top of the canopy during the experiments) and the light electricity power consumption (LEPC W m^{-2}, Pennisi et al., 2019a).

After transplant, 5 LED light treatments were applied, one per each compartment. Light treatments consisted of five different PPDF values of 100 (DLI: 5.8 mol m^{-2} d^{-1}, LEPC: 70 W m^{-2}, PPFD:electricity ratio: 1.44 µmol J^{-1}), 150 (DLI: 8.6 mol m^{-2} d^{-1}, LEPC: 98 W m^{-2}, PPFD:electricity ratio: 1.53 µmol J^{-1}), 200 (DLI: 11.5 mol m^{-2} d^{-1}, LEPC: 132 W m^{-2}, PPFD:electricity ratio: 1.51 µmol J^{-1}), 250 (DLI: 14.4 mol m^{-2} d^{-1}, LEPC: 164 W m^{-2}, PPFD:electricity ratio: 1.52 µmol J^{-1}) and 300 (DLI: 17.3 mol m^{-2} d^{-1}, LEPC: 197 W m^{-2}, PPFD:electricity ratio: 1.52 µmol J^{-1}) µmol m^{-2} s^{-1} (**Fig. 1**).

In each experiment, a new full randomisation of light treatments was applied. Each compartment hosted 40 plants at planting density of 100 plants m^{-2}, resembling common densities in indoor farming environments (Cha et al., 2012), and measurements were taken on the central 12 plants. Final measurements were taken 21 DAT, meaning 35 DAS for lettuce and 42 DAS for basil, at which stage the plants reached commercial harvest.

2.3. Growth analysis and resource use efficiency

At harvest (21 DAT), fresh weight (FW) of shoot and root was measured and dry weight was quantified after drying samples at 60°C for 72 hours. Root:shoot ratio (R:S ratio) was determined as the ratio of root dry weight to shoot dry weight. Leaf number was counted (leaves longer than 2 cm) and leaf area was determined using a leaf area meter (LI-3100C, LI-COR, Lincoln, Nebraska, USA). Specific leaf area (SLA) was calculated as the ratio between plant leaf area and leaf dry weight. For basil plants, also plant height was measured.

Water use was individually quantified for each plant during each experiment and water use efficiency (WUE) was determined as the ratio between final fresh weight of the shoot and the volume of water used, and expressed as g FW L^{-1} H_2O. Lighting energy use efficiency (EUE) was determined according to the crop cycle length and the final fresh weight of the shoot, related to the lamps' cumulated electricity absorption and expressed as g FW kWh^{-1}. Light use efficiency (LUE, g DW mol^{-1}) was calculated as the ratio of shoot dry weight production per unit surface of cultivation (g DW m^{-2}) and the light integral (mol m^{-2}), obtained by multiplying DLI values by the number of days between transplanting and harvest.

2.4. Stomatal size and density

Measurements of stomatal size and density were performed using a nail polish print of leaf abaxial sides. Imprints were taken from the middle portion of the blade between the midrib and the leaf margin, on the fourth fully expanded leaf from five plants per treatment per experiment at 14 DAT. Each imprint was placed on a microscope slide and covered with a cover slip. Image data were acquired using a brightfield biological microscope (MT4300H, Meiji Techno, Saitama, Japan) equipped with a digital camera (UK1175-C QXGA color, ABS GmbH, Jena, Germany). From each imprint, five pictures were taken in different locations. Pictures were analysed using ImageJ software (version 1.48 v, NIH, USA). For each picture, stomata number was counted and stomata size was estimated by the area of the rectangle encasing the stomata (Jensen et al., 2018).

2.5. Stomatal conductance

Measurements of stomatal conductance (mmol m^{-2} s^{-1}) were performed on the third fully expanded leaf using a leaf porometer (ΔP4, Delta-T Devices, Cambridge, UK) at 14 DAT in each experiment.

2.6. Leaf chlorophyll content

Content of chlorophyll in leaves was estimated during each experiment at 14 DAT through a leaf chlorophyll meter (YARA N-Tester, Oslo, Norway) on the third fully expanded leaf. The tool provides a numeric three-digit dimensionless value that is commonly expressed as N-Tester value and was previously used for leaf chlorophyll estimation in lettuce (Orsini et al., 2018).

2.7 Total phenolic, flavonoids and antioxidant capacity

In all experiments, leaf samples were collected at harvest (21 DAT), immersed in liquid N_2 and kept at -80°C. One gram of frozen plant tissue was extracted in a methanol:water:acetone (6:3:1, v:v:v) (Pennisi et al., 2019b). Total antioxidant capacity, phenolic and flavonoid compounds were determined on the resulting extract. The total antioxidant capacity, measured by the ferric reducing antioxidant power (FRAP) assay, was expressed as mmol Fe^{2+} kg^{-1} FW (Aaby et al., 2007). Phenolic compounds and flavonoids were quantified by Folin-Ciocalteu and aluminium chloride assays, and expressed as gallic acid and catechin equivalents, respectively (Zhishen et al., 1999; Waterhouse, 2002).

2.7. Statistical analysis

Measurements were conducted on twelve plants per light treatment (unless otherwise stated), which were surrounded by border plants. Data were analysed by one-way ANOVA considering experiments as replicates and the means were compared by Tukey's Honestly Significant Difference (HSD) test, at 5% significance level. Regression analysis was conducted on the correlation between total antioxidant capacity and phenolics and between total antioxidant capacity and total flavonoid concentration, at 5% significance level. For all statistical analyses, software used included Microsoft Excel® and SPSS package.

3 RESULTS

3.1. Effects of light intensity on lettuce and basil growth

In both lettuce and basil (**Table 1**), light intensity increased fresh (FW) and dry (DW) weights up to 250 μmol m^{-2} s^{-1}, while further increase of light intensity led to a reduction (in lettuce) or no further increase (in basil) of FW and DW. Dry matter content (DM) of lettuce plants increased with increasing PPFD, while no further change occurred when PPFD increased from 200 to 300 μmol m^{-2} s^{-1}. In basil plants, the lowest DM value was associated to the lowest light intensity level (e.g. 100 μmol m^{-2} s^{-1}), while the other treatments did not present statistically significant differences. The R:S ratio, on a dry weight basis, was not affected by light intensity in basil, whereas in lettuce it was progressively increased, reaching highest values at 250 μmol m^{-2} s^{-1}, without statistically significant differences from plants exposed to 200 μmol m^{-2} s^{-1}. The leaf number was not affected by light intensity in lettuce, whereas it reached the highest values at 250 μmol m^{-2} s^{-1} in basil, while the highest values of basil plant height was achieved under a PPFD≥200 μmol m^{-2} s^{-1}. Finally, the plant leaf area was higher in lettuce at PPFD≥200 μmol m^{-2} s^{-1} and in basil at PPFD ≥250 μmol m^{-2} s^{-1}, whereas the specific leaf area (SLA, expressed as cm^2 g^{-1} DW) was maximised at PPFD of 100 μmol m^{-2} s^{-1} in lettuce and of 100 and 150 μmol m^{-2} s^{-1} in basil (**Table 1**).

3.2. Effect of light intensity on leaf physiological functionality and anatomy

In both lettuce (**Fig. 2A**) and basil (**Fig. 2E**), light intensity increased leaf chlorophyll content up to a PPFD of 250 μmol m^{-2} s^{-1}, whereas further increases did not result in higher values of chlorophyll. Similarly, in lettuce, also stomatal conductance was positively correlated with light intensity up to 250 μmol m^{-2} s^{-1}, while a significant reduction of was observed at 300 μmol m^{-2} s^{-1} (**Fig. 2B**). In basil plants, stomatal conductance was lowest at PPFD≤150 μmol m^{-2} s^{-1} as compared with PPFD≥200 μmol m^{-2} s^{-1} (**Fig. 2F**). In lettuce, stomatal density (**Fig. 2C**) was the lowest at 100 and 150 μmol m^{-2} s^{-1} and reached the highest values at both 200 and 250 μmol m^{-2} s^{-1}. Stomatal size (**Fig. 2D**) resulted higher at 250 and 300 μmol m^{-2} s^{-1}.

In basil, stomatal density (**Fig. 2G**) reached the highest values at 200 and 250 μmol m^{-2} s^{-1}. Stomatal size (**Fig. 2H**) was the lowest at 100 μmol m^{-2} s^{-1} and the highest at 250 μmol m^{-2} s^{-1}.

3.3. Effect of light intensity on antioxidant properties

In basil, no differences in total antioxidant capacity (P=0.97), phenolics (P=0.83) and total flavonoid (P=0.66) concentrations were observed as a function of imposed light intensity (data not shown). On the other hand, total antioxidant capacity, phenolic compounds and flavonoids in lettuce were higher when PPFD≥200 μmol m^{-2} s^{-1} was supplied (**Table 2**). A significant correlation between antioxidant capacity and total flavonoids content was observed in lettuce (P=0.00025) and basil (P=0.00239), whereas no significant correlation was observed between total antioxidant capacity and phenolics (data not shown).

3.4. Effect of light intensity on light, water and energy use efficiency

Water use presented a similar trend in both lettuce and basil plants. In lettuce, water use was increased from 0.48 L $plant^{-1}$ (100 μmol m^{-2} s^{-1}) to 0.66 L $plant^{-1}$ (150 μmol m^{-2} s^{-1}), and was the highest at PPFD≥200 μmol m^{-2} s^{-1}, featuring 0.95 L $plant^{-1}$ as mean value (data not shown). Similarly, in basil, water use grew from 0.38 L $plant^{-1}$ (100 and 150 μmol m^{-2} s^{-1}, mean value) to 0.54 L $plant^{-1}$ (200 and 300 μmol m^{-2} s^{-1}, mean value) and up to 0.69 L $plant^{-1}$ under 250 μmol m^{-2} s^{-1}. Water Use Efficiency (WUE) was progressively increased in lettuce

(**Fig. 3A**) as PPFD was augmented from 100 µmol m^{-2} s^{-1} to 200 µmol m^{-2} s^{-1}, without any further significant increase for PPFD≥200 µmol m^{-2} s^{-1}. In basil (**Fig. 3D**) plants, the highest values of WUE were obtained in plants grown under PPFD≥250 µmol m^{-2} s^{-1}. The highest energy use efficiency (EUE) values in lettuce were associated with 150, 200 and 250 µmol m^{-2} s^{-1} (**Fig. 3B**). In basil, energy use efficiency was the highest at 250 µmol m^{-2} s^{-1} (**Fig. 3E**). Light use efficiency was maximised in lettuce when PPFD was equal to 200 and 250 µmol m^{-2} s^{-1} (**Fig. 3C**), whereas lower values were observed at PPFD≤150 or above 250 µmol m^{-2} s^{-1}. In basil, LUE values were generally lower than those observed in lettuce (**Fig. 3F**), and resulted the highest at PPFD=250 µmol m^{-2} s^{-1}, as compared to all other treatments.

4 DISCUSSION

4.1. A PPFD of 250 µmol m^{-2} s^{-1} is needed for improved yield in indoor grown lettuce and basil supplied with RB=3.

Plant biomass production in response to light intensity often follows an optimum function, which reaches its maximum when light stress begins to occur (Kang et al., 2013; He et al., 2019). However, optimum light intensity for fresh biomass production in lettuce was shown to vary among cultivars (Lee et al., 2019; Viršilė et al., 2019). Also, when both temperature (e.g. from 20 to 25°C) and light intensity (from 150 to 200 µmol m^{-2} s^{-1} from red and blue LEDs with RB=3, respectively supplying DLI of 8.6 to 11.5 mol m^{-2} d^{-1}) were simultaneously increased, an increase in fresh biomass of lettuce was observed. Such an increase was not visible when temperature or light intensity alone were augmented (Okazaki and Yamashita, 2019). Similarly, the response of lettuce biomass to light intensity (400 or 700 µmol m^{-2} s^{-1}, resulting in DLI of 20.2 and 35.3 mol m^{-2} d^{-1}) was also altered by the atmospheric CO_2 (400 and 700 µmol mol^{-1} CO_2) availability (Pérez-López et al., 2013). While a synergistic effect on the promotion of biomass in two cultivars (red and green) was observed when elevate light intensity (700 µmol m^{-2} s^{-1}) and CO_2 (700 µmol mol^{-1}) were supplied, at ambient CO_2 (400 µmol mol^{-1}), elevate light intensity (700 µmol m^{-2} s^{-1}) only increased growth in green lettuce, but not in the red cultivar (Pérez-López et al., 2013). It was also shown that when photoperiod was reduced from 16 to 14 h d^{-1} of light (at T=22/18°C and 800 µmol mol^{-1} CO_2), the optimum light intensity for fresh biomass production was increased from 200 to 250 μmol m^{-2} s^{-1} under red and blue LED (with both RB=1.2 and RB=2.2) (Yan et al., 2019). Looking at daily light integrals, it was observed that under RB=1.2 higher biomass was associated with DLI≥12.6 mol m^{-2} d^{-1}, whereas under RB=2.2, biomass production decreased when DLI≥11.5 mol m^{-2} d^{-1} were adopted (Yan et al., 2019). When comparing 60, 140 and 220 μmol m^{-2} s^{-1} (DLI respectively of 3.4, 8.1 and 12.7 mol m^{-2} d^{-1}) supplied by mixed red and blue LED (RB=4), Fu et al. (2017) concluded that 220 μmol m^{-2} s^{-1} was the PPFD value allowing for the greatest lettuce growth at 23°C and 16 h d^{-1} of light. However, the lack of higher PPFD values in their study, does not allow to further define the crop growth-response function to PPFD. In basil, the highest fresh biomass was previously achieved when supplying 224 μmol m^{-2} s^{-1} (DLI=12.9 mol m^{-2} d^{-1}) through a fluorescent white light (Dou et al., 2018), although no further increase was reported when the PPFD was raised up to 310 μmol m^{-2} s^{-1} (DLI=17.8 mol m^{-2} d^{-1}). The observed biomass increases were associated with enhancement of leaf photosynthetic rates when PPFD was raised from 160 to 224 μmol m^{-2} s^{-1}, albeit no leaf photosynthetic changes were observed among treatments in which plants were grown with PPFD≥ 224 μmol m^{-2} s^{-1} (Dou et al., 2018). From the results of hereby presented research, it could be advanced that the adopted environmental (including light spectrum, photoperiod and CO_2) and plant growing (including plant density and cultivar used) features resulted in an optimum PPFD of 250 μmol m^{-2} s^{-1} (DLI=14.4 mol m^{-2} d^{-1}) (**Table 1**), while higher PPFD values (e.g. 300 μmol m^{-2} s^{-1}, DLI=17.3 mol m^{-2} d^{-1}) resulted in reduced growth in lettuce. The detrimental effects on lettuce yield associated with too elevate DLI were previously observed by Zhang et al. (2018), in experiments where an optimal DLI (when plants were grown under LED with RB=2.2 and photoperiod of 12 h d^{-1} of light) for fresh biomass accumulation was found at 10.8 mol m^{-2} d^{-1} as compared with 13.0 mol m^{-2} d^{-1}. It results that the definition of optimal light intensity is a complex scenario that can only be defined building on the combined and synergistic effects of a number of environmental and crop factors.

Dry weight production in lettuce increased when light intensity was augmented from 120 to 150 μmol m^{-2} s^{-1} (DLI respectively of 6.9 and 8.6 mol m^{-2} d^{-1}) (Lin et al., 2018) and from 60 to 220 μmol m^{-2} s^{-1} (DLI from 3.4 to 12.7 mol m^{-2} d^{-1}) (Fu et al., 2017). Contrarily, Yan et al. (2019) reported highest dry biomass in lettuce seedlings grown under LED (featuring mixed red, green and blue light with RB of 1.2 or 2.2, photoperiod 16 h d^{-1}) light supplying 200 μmol m^{-2} s^{-1} (DLI=11.5 mol m^{-2} d^{-1}) as compared with those experiencing 250 μmol m^{-}

^{2} s^{-1} (DLI=14.4 mol m^{-2} d^{-1}). Nevertheless, when photoperiod was of 14 h d^{-1}, the light intensity did not result in changes in dry biomass accumulation (Yan et al., 2019). In basil, grown under fluorescent lamps, dry weight was augmented from 160 up to 290 μmol m^{-2} s^{-1} (DLI respectively from 9.3 to 16.5 mol m^{-2} d^{-1}), while higher PPFD values did not result in a further increase (Dou et al., 2018).

The absence of univocal recommendations on the optimal PPFD may be associated to the elevate variability among the lighting technologies and spectral properties and overall environmental conditions used in the cited literature. In the present study an optimized LED spectral composition (RB=3) was used, and a PPFD of 250 μmol m^{-2} s^{-1} (DLI=14.4 mol m^{-2} d^{-1}) in lettuce and of 250 and 300 μmol m^{-2} s^{-1} (DLI of 14.4 mol m^{-2} d^{-1} and 17.3 mol m^{-2} d^{-1}, respectively) in basil allowed for maximum fresh and dry yields (**Table 1**). The increase in dry biomass production in response to augmented light intensity was previously associated to increased photosynthate accumulation (Kang et al., 2013; Lin et al., 2018), as a consequence of larger photosynthetic rates (Fu et al., 2017; Dou et al., 2018). Similarly, higher values of shoot fresh and dry weight (g $plant^{-1}$) upon PPFD of 250 μmol m^{-2} s^{-1} (DLI=14.4 mol m^{-2} d^{-1}) and dry matter content upon PPFD≥200 μmol m^{-2} s^{-1} (DLI≥11.5 mol m^{-2} d^{-1}) were observed in lettuce (**Table 1**). Similar trend was also observed in basil shoots for both fresh and dry biomass production with higher values being found in plants grown upon PPFD≥250 μmol m^{-2} s^{-1} (DLI≥14.4 mol m^{-2} d^{-1}), although significant differences in dry matter content could only be found between PPFD≤150 μmol m^{-2} s^{-1} and PPFD≥150 μmol m^{-2} s^{-1} (**Table 1**).

The greater plant growth at 250 μmol m^{-2} s^{-1} (DLI=14.4 mol m^{-2} d^{-1}) was also consistent with a larger leaf area in both crops and, in basil, also to increased plant height and leaf number (**Table 1**). Increased leaf area and number were previously observed in lettuce plants, when PPFD was increased from 260 to 290 μmol m^{-2} s^{-1} (DLI from 16.8 to 18.8 mol m^{-2} d^{-1}) from LED featuring mixed red, blue and white light (RB=8) (Kang et al., 2013). Similarly, in basil, an increase in leaf area and plant height were also observed after 21 days of light treatment when PPFD≥224 μmol m^{-2} s^{-1} (DLI=12.9 mol m^{-2} d^{-1}) was supplied (Dou et al., 2018). Despite the higher shoot biomass in response to growing PPFD, functional changes in dry biomass partitioning to roots were also observed, overall altering the plant R:S ratio in lettuce (**Table 1**). In some other studies on lettuce, R:S ratio was either reported to increase or not to change (Fu et al., 2017) or even decrease (Lin et al., 2018) in response to growing PPFD. Possibly, an optimum function may be hereby demonstrated (**Table 1**), with 200 and 250 μmol m^{-2} s^{-1} resulting in the highest R:S ratio. In a previous study on lettuce, the R:S ratio was shown to increase as light intensity increased, when moving from 200 (DLI=13.0 mol m^{-2} d^{-1}, R:S ratio=0.15) to 230 μmol m^{-2} s^{-1} (DLI=14.9 mol m^{-2} d^{-1}, R:S ratio=0.21), but then decrease as light intensity reached 260 μmol m^{-2} s^{-1} (DLI=16.8 mol m^{-2} d^{-1}, R:S ratio=0.18) (Kang et al., 2013). According to the functional equilibrium hypothesis, as irradiance increases, plants fix larger amounts of carbon in photosynthesis and show higher allocation to roots at the expenses of shoots, while as light leads to stress in leaves the R:S ratio will not increase anymore (Poorter et al., 2012).

The changes in leaf area and plant dry biomass production in response to varying light intensity regimes also altered the leaf structure. The observed reduction of SLA (**Table 1**) in response to increased PPFD was previously associated in basil with more compact mesophyll cells (higher dry matter content) and thicker and larger leaves (Dou et al., 2018). Besides, light intensity may also result in functional adaptations of leaf anatomy and physiology as described in the following section.

4.2. Leaf adaptation mechanisms to increased PPFD.

Light intensity was previously shown to alter leaf anatomical and physiological features in both basil and lettuce grown in greenhouse (Orsini et al., 2018) and indoor farming (Dou et al., 2018; Kang et al., 2013) environments. Leaf chlorophyll content was reported to be lower in basil plants grown under PPFD≥224 μmol m^{-2} s^{-1} (DLI≥12.9 mol m^{-2} d^{-1}) as compared with those grown under PPFD≤200 μmol m^{-2} s^{-1} (DLI≤11.5 mol m^{-2} d^{-1}) (Dou et al., 2018). However, in the same work, leaf chlorophyll was not reported to vary between plants grown under 224 (DLI=12.9 mol m^{-2} d^{-1}) and 310 μmol m^{-2} s^{-1} (DLI=17.8 mol m^{-2} d^{-1}). Similarly, in lettuce, no differences in chlorophyll content could be observed in plants grown under PPFD ranging 200 to 290 μmol m^{-2} s^{-1} (DLI from 13.0 to 18.8 mol m^{-2} d^{-1}) (Kang et al., 2013) or when plants were grown under either 150 to 200 μmol m^{-2} s^{-1} (DLI respectively of 8.6 and 11.5 mol m^{-2} d^{-1}) (Okazaki and Yamashita, 2019). The observed behaviour (**Fig. 2A** and **2E**) is consistent with the hypothesis that under either non-optimal radiation intensity, leaf chlorophyll content is reduced, as previously described in lettuce (Fu et al., 2012; Orsini et al., 2018). It should be noted that such a reduction in chlorophyll may also result in lighter green colour of the leaves, a trait that was previously associated with reduced consumer preference in fresh vegetable products (Rouphael et al., 2012).

Alongside with the role played by leaf chlorophyll content, photosynthesis in leaves is regulated by stomatal features, as evidenced in basil (Mancarella et al., 2016). Stomatal opening is a general response of plants to high light intensity, facilitating both CO_2 uptake for photosynthesis and evaporative cooling of the leaf undergoing elevate radiative heat loads (Matsuda, 2016). Two mechanisms are mainly associated with the light-induced stomatal response (Shimazaki et al., 2007), one of them supposedly driven by the photosynthetic activity of both guard and mesophyll cells, the other induced by blue light triggering the response of the photoreceptor phototropin (Hiyama et al., 2017). Accordingly, the light spectral composition was shown not only to alter biomass growth, but also to modify stomatal functionality and overall water use in both lettuce and basil plants (Pennisi et al., 2019a and 2019b). Stomatal conductance was previously reported to increase in lettuce when PPFD was raised from 60 to 220 μmol m^{-2} s^{-1} (DLI from 3.4 to 12.7 mol m^{-2} d^{-1}) (Fu et al., 2017) or from 200 to 230 μmol m^{-2} s^{-1} (DLI from 13.0 to 14.9 mol m^{-2} d^{-1}) (Kang et al., 2013), while was decreased at higher PPFD values (Kang et al., 2013). Similarly, in basil, Dou et al. (2018) reported stomatal conductance to increase from 160 (DLI=9.3 mol m^{-2} d^{-1}) up to 224 μmol m^{-2} s^{-1} (DLI=12.9 mol m^{-2} d^{-1}), while becoming stable upon higher PPFD. Accordingly, in the hereby presented study, in lettuce plants stomatal conductance reached the highest values at 250 μmol m^{-2} s^{-1} (DLI=14.4 mol m^{-2} d^{-1}), and then decreased for greater values of light intensity (**Fig. 2B**), while in basil plants stomatal conductance resulted stable in plants grown under PPFD≥200 μmol m^{-2} s^{-1} (DLI≥11.5 mol m^{-2} d^{-1}) (**Fig. 2F**). Changes in stomatal conductance were previously associated with modifications in stomatal size and/or density in both lettuce (Pennisi et al., 2019a) and basil (Barbieri et al., 2012). Similarly, stomatal density followed an optimum function showing higher values at 200 and 250 μmol m^{-2} s^{-1} (DLI of 11.5 and 14.4 mol m^{-2} d-1, respectively) in both lettuce and basil (**Fig. 2C** and **2G**). Moreover, stomatal size resulted to be increased by growing PPFD up to 250 μmol m^{-2} s^{-1}, in both species (**Fig. 2D** and **2H**). These changes in stomatal size and density were also consistent with the response in stomatal conductance (Fig. **2B** and **2F**), which was highest at PPFD=250 and PPFD≥200 μmol m^{-2} s^{-1}, in lettuce and basil, respectively.

The observed changes in leaf morphology and physiology are likely responsible of the overall plant water relations and secondary metabolism, as targeted in the following sections.

4.3. In lettuce, PPFD affects antioxidant capacity

The content of flavonoid compounds and the overall antioxidant capacity of lettuce and basil (**Table 2** and data not shown) were closely related, suggesting that flavonoids may be the main compounds responsible for radical scavenging in these species. Despite the large variety of commercial cultivars among basil and lettuce species (presenting different secondary metabolite concentrations and responsiveness to environmental cues), the role of flavonoids on radical-scavenging is a well-established assumption (Ouzounis et al, 2015).

In basil, red wavelengths (Piovene et al., 2015; Pennisi et al., 2019b) have also been implicated in the increased biosynthesis of phenolic and flavonoid compounds, while light shading is probably responsible for their reduced content (Stagnari et al., 2018). However, in this work, light intensity did not affect antioxidant capacity, phenolics and total flavonoid concentration in basil (data not shown), suggesting that the spectral composition and/or the intensity of radiation at wavelength not considered here (e.g. UV), but not light intensity *per se*, may underlie the stimulation of antioxidants biosynthesis in this crop.

In contrast, lettuce responded to light intensity, showing the highest antioxidant activity and concentrations of phenolics and flavonoids between 200 and 300 μmol m^{-2} s^{-1} PPFD (**Table 2**), resembling previously reported values for the same crop species (Msilini et al., 2013; Ouhibi et al., 2014) and confirming the hypothesis of a PPFD-related effect on the plant antioxidant profile (Poorter et al., 2019). The finding of an optimum intensity value, rather than a proportional relation, suggests that antioxidant capacity and both flavonoids and phenolics concentrations may be determined as a trade-off between different processes with opposite effects. For instance, the finding of a lower stomatal conductance (i.e., potentially higher accumulation of O_2) at 300 vs 250 μmol m^{-2} s^{-1} PPFD, associated with comparable chlorophyll contents, suggests a higher risk of oxygen radical formation as a result of electron leakage from photosynthetic machinery (Anjum et al., 2011).

4.4. Toward efficient resource use in indoor lettuce and basil cultivation: the role of light intensity.

Reducing water use while preserving satisfactory yield is a target priority for agricultural production (Fernández et al., 2018). The increased yield associated with 250 µmol m^{-2} s^{-1} PPFD (DLI=14.4 mol m^{-2} d^{-1}) in lettuce and with PPFD≥250 µmol m^{-2} s^{-1} (DLI≥14.4 mol m^{-2} d^{-1}) in basil (**Table 1**), compensated for the increase in stomatal conductance (**Fig. 2B** and **2F**), overall leading to greater water use efficiency (WUE, **Fig. 3A** and **3D**). The observed values for WUE (reaching up to 60 g FW L^{-1} H_2O and 38 g FW L^{-1} H_2O, respectively in lettuce and basil, **Fig. 3A** and **3D**), are extremely impressive when compared with reported values for traditional cultivation. Accordingly, from data on open-field and greenhouse cultivation, WUE of lettuce was respectively defined at 4 g FW L^{-1} H_2O and 50 g FW L^{-1} H_2O (Barbosa et al., 2015), whereas basil respectively performed 3 g FW L^{-1} H_2O and 22 g FW L^{-1} H_2O in open-field (Ekren et al., 2012) and greenhouse (Montesano et al., 2018) systems. Similarly, the balance between increased electricity needs at growing PPFD and greater plant biomass achieved in response to higher light intensities, altered the crop Energy Use Efficiency (**Fig. 3B** and **3E**). From such equilibrium, maximum EUE was achieved under 200 to 250 µmol m^{-2} s^{-1} PPFD (corresponding to DLI of 11.5 and 14.4 mol m^{-2} d^{-1}, respectively) in lettuce and at 250 µmol m^{-2} s^{-1} PPFD (DLI=14.4 mol m^{-2} d^{-1}) in basil. The achieved EUE values under 250 µmol m^{-2} s^{-1} PPFD (110 and 45 g FW kWh^{-1}, in lettuce and basil, respectively) are already higher than those reached under comparable environmental conditions at lower intensities (215 µmol m^{-2} s^{-1} PPFD and DLI=12.4 mol m^{-2} d^{-1}) in both lettuce (91 g FW kWh^{-1}, Pennisi et al., 2019a) and basil (33 g FW kWh^{-1}, Pennisi et al., 2019b).

In the hereby presented experiments, LUE was highest respectively at 200 and 250 µmol m^{-2} s^{-1} (DLI of 11.5 and 14.4 mol m^{-2} d^{-1}, respectively) in lettuce (LUE=1.03 g DW mol^{-1}, **Fig. 3C**) and at 250 µmol m^{-2} s^{-1} in basil (LUE=0.70 g DW mol^{-1}, **Fig. 3F**), as compared with all other light intensities. Janssen et al. (2019) reported values of LUE ranging from 15 to 30 g FW mol^{-1} in lettuce (around 0.75-1.50 g DW mol^{-1} considering 5% of dry matter content) and from 8 to 12 g FW mol^{-1} in basil (around 0.60-0.96 g DW mol^{-1} considering 8% of dry matter content) in indoor systems with artificial lighting, in a range of experiments where they tested the effects of temperature (ranging 22 to 30°C), CO_2 supply (400 to 1600 µmol mol^{-1}), photoperiod (14 to 18 h d^{-1} of light) and light intensity (180 to 400 µmol m^{-2} s^{-1}). Graamans et al. (2018) simulated a LUE of 0.37 g DW mol^{-1} for lettuce production in a plant factory (PPFD=500 µmol m^{-2} s^{-1}, photoperiod=16 h d^{-1}, DLI=28.8 mol m^{-2} d^{-1},

CO_2=1200 µmol mol^{-1}). In lettuce plants grown in a growth chamber under HPS lamps (PPFD=420 µmol m^{-2} s^{-1}, photoperiod=16 h d^{-1}, DLI=24.2 mol m^{-2} d^{-1}, CO_2=370-410 µmol mol^{-1}), lower values of LUE were reported (0.15-0.18 g DW mol^{-1}, El-Nachel et al., 2019). In greenhouses, however, reported LUE values were even lower and ranged 0.33-1.39 g DW MJ^{-1}, which would correspond (considering a conversion factor of 4.6 mol MJ^{-1}) to LUE value as little as 0.07 g DW mol^{-1} (Wheeler et al., 1993). When greenhouse values were referred to the actually absorbed PAR instead, De Pinheiro Henriques and Marcelis (2000) reported LUE to range 3.5 to 4.9 g DW MJ^{-1}, which would correspond to 0.8 to 1.1 g DW mol^{-1}.

5 CONCLUSIONS

The research confirmed that an optimum response curve exists between light intensity and plant growth, with 250 µmol m^{-2} s^{-1} (DLI=14.4 mol m^{-2} d^{-1}) resulting in improved fresh and dry biomass production as well as larger plant leaf area under the prevailing conditions of red and blue light (RB=3), a photoperiod of 16 h d^{-1} of light, 24°C, 450 µmol mol^{-1} CO_2 and a plant density of 100 plants m^{-2}. At this light intensity regime and following the functional equilibrium hypothesis, an increased R:S ratio was also observed, altogether with reductions in SLA, possibly as a consequence of functional leaf adaptations. Consistently, leaves of plants grown under 250 µmol m^{-2} s^{-1} (DLI=14.4 mol m^{-2} d^{-1}) presented denser and larger stomata, which allowed for improved stomatal conductance and higher leaf chlorophyll content. On the contrary, lower light intensities reduced leaf functionality (in terms of stomatal features and chlorophyll content), which also resulted in reduced nutritional content in lettuce, where antioxidant capacity, phenolics and flavonoids concentrations were lower.

Despite the higher water requirements and the higher electricity needs experienced when a PPFD of 250 µmol m^{-2} s^{-1} was supplied as compared with lower light intensities, the yield gain allowed for improved water (WUE), energy (EUE) and light (LUE) use efficiencies. On the other hand, additional light intensity (e.g. up to 300 µmol m^{-2} s^{-1}) did not allow for additional yield and therefore WUE, EUE and LUE were not further improved. From the study it may be concluded that under a mixed red and blue LED light (featuring RB=3) and a photoperiod of 16 h d^{-1} of light, indoor cultivation of both lettuce and basil may be improved when DLI=14.4 mol m^{-2} d^{-1} and PPFD=250 µmol m^{-2} s^{-1} are supplied. The novelty proposed therefore stands in the optimization of radiation intensity in a specific spectral environment (RB=3) that was recently shown to improve productivity and resource use efficiency in basil and lettuce (Pennisi et al., 2019a, b). The research also elaborates on physiological changes associated with stomatal response to light, that result in viable strategies for maximising water, energy and light use efficiencies in the studied crops.

Author Contributions

Giuseppina Pennisi designed and performed all experiments and drafted the manuscript. Alessandro Pistillo managed the experiments and performed measurements. Francesco Orsini and Leo Marcelis contributed to the experimental design and the drafting of the manuscript. Francesco Spinelli and Antonio Cellini performed the analyses of antioxidant compounds and contributed to the manuscript preparation. Andrea Crepaldi coordinated the manufacturing of the lamps used in the experiment. Silvana Nicola, Juan Fernandez and Giorgio Gianquinto supervised the research and critically revised the manuscript.

Acknowledgments

This research was partially funded by a grant of the Fundacion Séneca (reference 20555/IV/18, Call for Fellowships for Guest Researcher Stays at Universities and OPIS of the Region of Murcia).

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Tables

Table 1. Effect of different DLI (obtained by changing light intensity from 100 to 300 µmol m^{-2} s^{-1}) on morphological parameters of indoor grown lettuce and basil plants at 21 DAT. Each value is based on 3 experiments, each with 12 replicate plants. Different letters indicate significant differences at $P \leq 0.05$.

DLI	PPFD	Shoot FW		Shoot DW		DM		R:S ratio		Plant height		Leaf number		Leaf area		SLA	
mol m^{-2} d^{-1}	*µmol m^{-2} s^{-1}*	*g $plant^{-1}$*		*g $plant^{-1}$*		*%*				*cm*		*n*		*cm^2*		*cm^2 g^{-1} DW*	
Lettuce										-							
5.8	100	20.1	d	0.87	d	4.41	b	0.09	d	-		13.9		680	c	883	a
8.6	150	30.7	c	1.39	c	4.51	b	0.12	cd	-		14.1		751	bc	572	b
11.5	200	48.2	b	2.36	b	4.93	ab	0.16	ab	-		14.8		875	ab	381	bc
14.4	250	61.1	a	3.26	a	5.35	a	0.19	a	-		15.2		1020	a	343	c
17.3	300	50.9	b	2.61	b	5.13	a	0.15	bc	-		15.3		937	a	373	bc
P value		***<0.001***		***<0.001***		***<0.001***		***<0.001***		-		***ns***		***<0.001***		***<0.001***	
Basil																	
5.8	100	7.4	c	0.52	c	7.27	b	0.30		18.03	b	9.06	d	231	c	437	a
8.6	150	9.3	c	0.71	c	8.04	ab	0.18		18.83	b	11.83	cd	286	bc	395	a
11.5	200	14.1	b	1.17	b	8.43	a	0.21		21.41	ab	15.00	bc	378	b	316	b
14.4	250	25.0	a	2.12	a	8.57	a	0.23		26.01	a	21.83	a	625	a	296	b
17.3	300	21.0	a	1.76	a	8.37	a	0.22		25.32	a	18.11	b	530	a	303	b
P value		***<0.001***		***<0.001***		***0.002***		***ns***		***<0.001***		***<0.001***		***<0.001***		***<0.001***	

FW= Fresh Weight; DW= Dry Weight; DM= Dry Matter content; R:S ratio=Root-to-shoot ratio; SLA=Specific Leaf Area.

Table 2. Effect of different DLI (obtained by changing light intensity from 100 to 300 µmol m^{-2} s^{-1}) on antioxidant properties of indoor grown lettuce plants at 21 DAT. Each value is the mean of 12 independent measures. Different letters indicate significant differences at $P \leq 0.05$.

DLI	**PPFD**	**Total Antioxidant capacity (FRAP)**		**Phenolics**		**Total flavonoid concentration**	
mol m^{-2} d^{-1}	***µmol m^{-2} s^{-1}***	***mmol Fe^{2+} kg^{-1} FW***		***mg GA g^{-1} FW***		***mg CE g^{-1} FW***	
Lettuce							
5.8	100	6.50	b	0.21	bc	0.17	b
8.6	150	5.41	b	0.18	c	0.14	b
11.5	200	8.64	ab	0.37	ab	0.20	ab
14.4	250	11.61	a	0.62	a	0.30	a
17.3	300	8.72	ab	0.47	ab	0.22	ab
P value		***<0.05***		***<0.001***		***<0.001***	

GA = Gallic Acid; CE= Catechin equivalents.

Figures

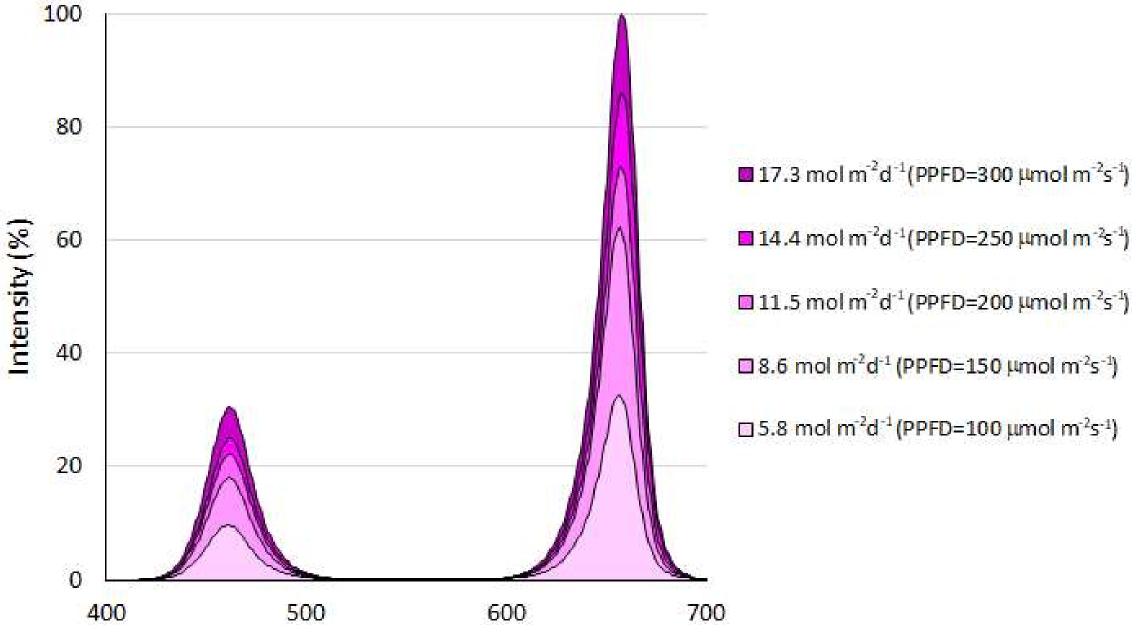

Figure 1. Light spectra of the five light treatments used in the experiments. The chart is based on relative values based on the maximum red peak (obtained when 17.3 mol d^{-1} were supplied).

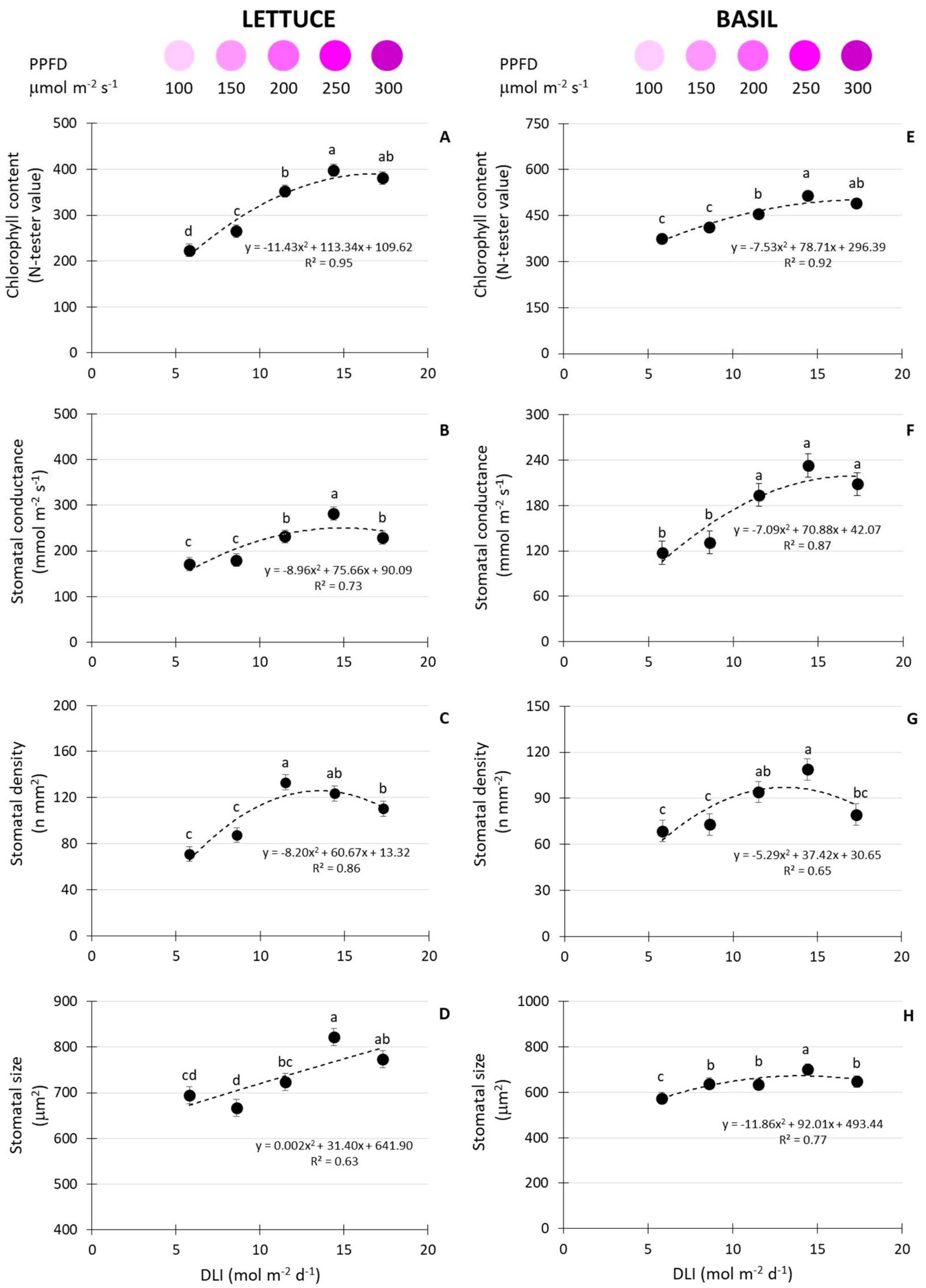

Figure 2. Chlorophyll content, stomatal conductance, stomatal density and stomatal size in leaves of lettuce (A, B, C and D) and basil (E, F, G and H) from plants grown under different DLI (obtained by changing light intensity from 100 to 300 µmol m^{-2} s^{-1}) at 14 DAT. Each value is the mean of 3 experiments, each with 12 replicate plants. Vertical bars represent standard errors. Different letters indicate significant differences at $P \leq 0.05$.

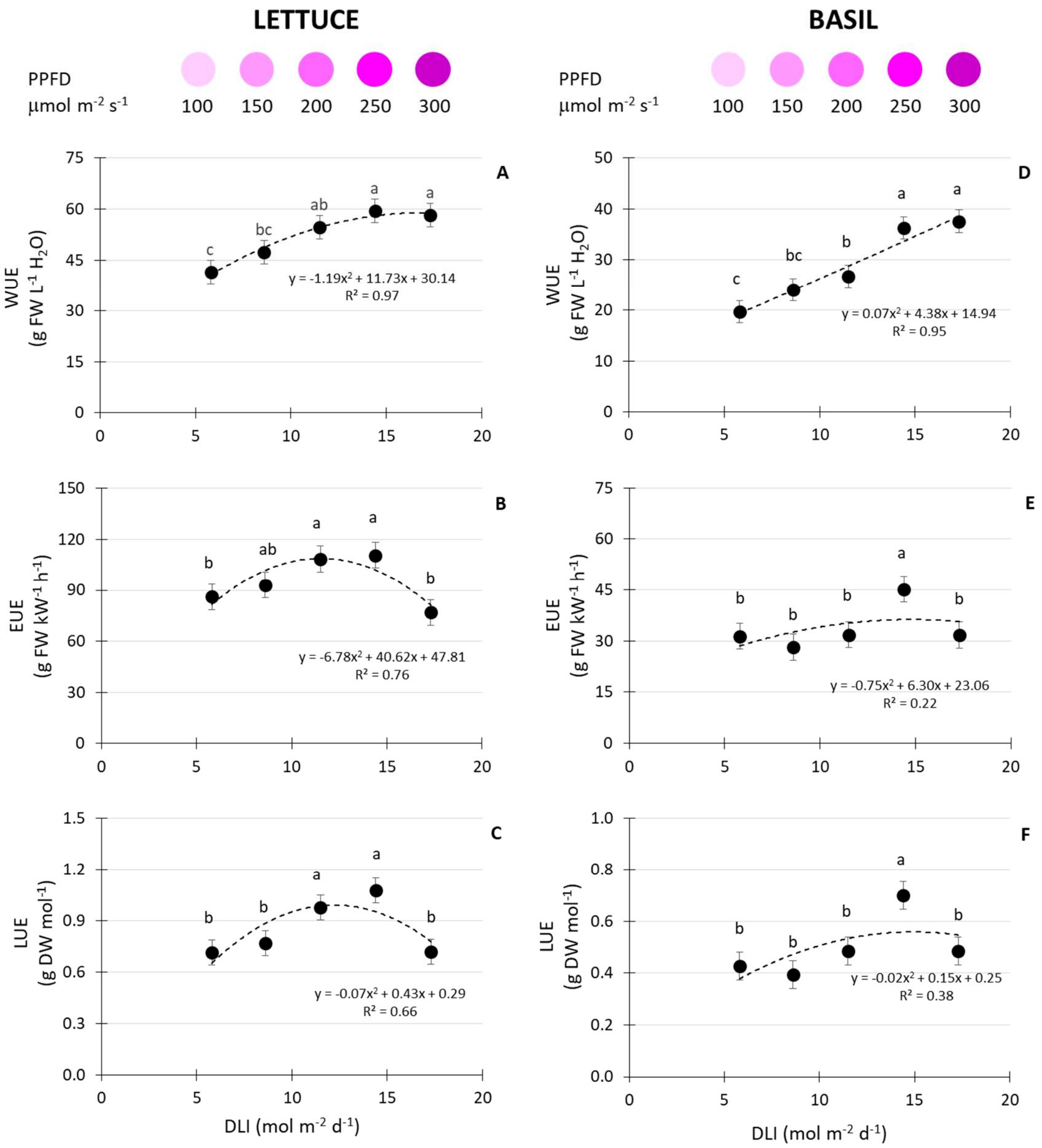

Figure 3. Water Use Efficiency (WUE), Energy Use Efficiency (EUE) and Light Use Efficiency (LUE) of lettuce (A, B and C) and basil (D, E and F) plants grown under different DLI (obtained by changing light intensity from 100 to 300 µmol m^{-2} s^{-1}) at 21 DAT. Each value is the mean of 36 independent measures. Vertical bars represent standard errors. Different letters indicate significant differences at $P \leq 0.05$.