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

3  
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17 **Highlights**

- 18 - Optimal LED light intensity for lettuce and basil indoor growing is addressed;
- 19 - Maximum yield and leaf area is achieved at  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ ;
- 20 -  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$  increased chlorophyll and improved stomatal functions in leaves;
- 21 - In lettuce,  $\text{PPFD} \geq 200 \mu\text{mol m}^{-2} \text{s}^{-1}$  raised antioxidant capacity, phenolics and flavonoids;
- 22 - Water, energy and light use efficiencies were optimized at  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ ;

23

24 **Abstract**

25 Indoor plant cultivation systems are gaining increasing popularity because of their ability to meet the needs of  
26 producing food in unfavourable climatic contexts and in urban environments, allowing high yield, high quality,  
27 and great efficiency in the use of resources such as water and nutrients. While light is one of the most important  
28 environmental factors affecting plant development and morphology, electricity costs can limit the widespread  
29 adoption of indoor plant cultivation systems at a commercial scale. LED lighting technologies for plant  
30 cultivation are also rapidly evolving, and lamps for indoor cultivation are often designed to optimize their light  
31 emissions in the photosynthetically active spectrum (i.e. red and blue), in order to reduce energetic requirements  
32 for satisfactory yield. Under these light regimens, however, little information is available in literature about  
33 minimum photosynthetic photon flux density (PPFD) for indoor production of leafy vegetables and herbs, while  
34 existing literature often adopts light intensities from 100 to 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . This study aims at defining the  
35 optimal PPFD for indoor cultivation of basil (*Ocimum basilicum* L.) and lettuce (*Lactuca sativa* L.), by linking  
36 resource use efficiency to physiological responses and biomass production under different light intensities. Basil  
37 and lettuce plants were cultivated at 24°C and 450  $\mu\text{mol mol}^{-1} \text{CO}_2$  under red and blue light (with red:blue ratio  
38 of 3) and a photoperiod of 16 h  $\text{d}^{-1}$  of light in growth chambers using five PPFD (100, 150, 200, 250 and 300  
39  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , resulting in daily light integrals, DLI, of 5.8, 8.6, 11.5, 14.4 and 17.3  $\text{mol m}^{-2} \text{d}^{-1}$ , respectively). A  
40 progressive increase of biomass production for both lettuce and basil up to a PPFD of 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was  
41 observed, whereas no further yield increases were associated with higher PPFD (300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Despite the  
42 highest stomatal conductance associated to a PPFD of 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in lettuce and to a PPFD  $\geq 200 \mu\text{mol m}^{-2}$   
43  $\text{s}^{-1}$  in basil, water use efficiency was maximized under a PPFD  $\geq 200 \mu\text{mol m}^{-2} \text{s}^{-1}$  in lettuce and PPFD  $\geq 250 \mu\text{mol}$   
44  $\text{m}^{-2} \text{s}^{-1}$  in basil. Energy and light use efficiencies were increased under a PPFD of 200 and 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in  
45 lettuce and under a PPFD of 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in basil. Furthermore, in lettuce grown under 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$   
46 antioxidant capacity, phenolics and flavonoids were higher as compared with plants supplied with PPFD  $\leq 150$   
47  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Accordingly, a PPFD of 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  seems suitable for optimizing yield and resource use  
48 efficiency in red and blue LED lighting for indoor cultivation of lettuce and basil under the prevailing conditions  
49 of the used indoor farming set-up.

50 **Keywords: Photosynthetic Photon Flux Density (PPFD); Plant factory with artificial lighting (PFALs);**  
51 **Water Use Efficiency (WUE); Energy Use Efficiency (EUE); Light Use Efficiency (LUE); Daily Light**  
52 **Integral (DLI)**  
53

## 54 1 INTRODUCTION

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

81 high pressure sodium (Hammock, 2018) or cool fluorescent lighting (Frąszczak et al., 2014; Piovene et al.,  
82 2015). It was recently demonstrated (Pennisi et al., 2019b) that similar to lettuce the optimal red and blue spectral  
83 composition for basil cultivation and resources use efficiency stands on RB=3. Another study (Pennisi et al.,  
84 2019c) confirmed that the normalized environmental impact (based on a life cycle assessment) was reduced  
85 when RB=3 or RB $\geq$ 2 were used respectively for lettuce and basil.

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

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

96 A meta-analysis of plant responses to light intensity suggests that light intensity may have strong effects on  
97 nutritional properties of plants (Poorter et al., 2019). For instance, Brazaityte et al. (2015), found that in  
98 microgreens of Brassicaceae (including mustard, red pak choi and tatsoi) grown under mixed red and blue LED  
99 lights, the accumulation of antioxidant compounds was stimulated by increasing the photosynthetic photon flux  
100 density (PPFD) from 110 to 440  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , though their concentration decreased as light intensity was further  
101 augmented to 545  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . In coriander (*Coriandrum sativum* L.), total phenolics and antioxidant capacity  
102 were increased as the intensity of a combined LED light (featuring red, white and far red LEDs) was  
103 progressively enhanced from 100 to 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Nguyen et al., 2019). In lettuce, total carotenoids were  
104 increased as PPFD increased from 60 to 140  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , but decreased when PPFD reached 220  $\mu\text{mol m}^{-2} \text{s}^{-1}$   
105 (Fu et al., 2017), although information on the spectral properties of the light source were not reported in the  
106 study. Vitamin C content in lettuce leaves was highest at 140  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , as compared with 220 and 60  $\mu\text{mol}$

107  $\text{m}^{-2} \text{s}^{-1}$  (Fu et al., 2017), while another study reported an increase in vitamin C content in lettuce in response to  
108 PPFD from 120 to 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Lin et al., 2018). However, under red and white LED lights (RB=1.2) and  
109 a photoperiod of 16  $\text{h d}^{-1}$ , it was also shown that vitamin C content was higher at PPFD of 200 as compared with  
110 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Yan et al., 2019), overall confirming an optimum response curve. On the other hand, in basil,  
111 the effect of artificial light intensity was only studied by using cool fluorescent lamps. Similarly to the previously  
112 cited studies on LEDs, antioxidant capacity was shown to increase when the PPFD was enhanced from 160  
113  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to 290  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Dou et al., 2018).

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

121

## 122 **2 MATERIALS AND METHODS**

### 123 **2.1. Plant material and growth conditions**

124 The plants were grown in five separate compartments (0.64  $\text{m}^2$  surface and 0.4  $\text{m}^3$  volume) in a climate-  
125 controlled growth chamber (day temperature 26°C, night temperature 22°C, 55-70% relative humidity and 450  
126  $\mu\text{mol mol}^{-1} \text{CO}_2$ ) at the University of Bologna (Italy) (Choi et al., 2000). Each compartment was insulated by  
127 using light opaque white walls, and equipped with fans constantly replacing internal air (hourly replacing 200  
128 times the volume of the chamber). Lettuce plants belonging to the green typology Gentilina, commonly adopted  
129 for baby-leaf production (*Lactuca sativa* L. cv. Rebelina, Gautier, Eyragues, France), and basil plants belonging  
130 to the typology “Genovese” (*Ocimum basilicum* L. cv. Superbo, Sais seeds, Cesena, Italy) were grown. Three  
131 independent experiments were conducted for each species. A planting density of 100 plants  $\text{m}^{-2}$  and a crop cycle



132 length of 21 days from transplant to harvest for both lettuce and basil experiments were adopted, as for previous  
133 experiments (Saha et al., 2016; Pennisi et al., 2019a, 2019b, 2019c).  
134 Seeds were germinated in polystyrene containers filled with a mixture of peat (70%) and vermiculite (30%),  
135 under cool-white fluorescent lamps (TL-D90 De Luxe 950, Philips), providing a PPFD of 215  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and  
136 a photoperiod of 16 h  $\text{d}^{-1}$  of light. When plants reached a two true leaf stage (14 and 21 days after sowing - DAS  
137 - respectively for lettuce and basil), roots were washed and plantlets were transplanted into individual  
138 hydroponic systems (Pennisi et al. 2019a). Each single-plant hydroponic unit consisted of plastic jars (1 L of  
139 volume, see image in **Supplementary material S1** and further details in Pennisi et al., 2019c), filled with  
140 nutrient solution (EC = 1.6, pH = 6.5) with the following composition: N-NO<sub>3</sub>: 14 mM; N-NH<sub>4</sub>: 4.4 mM; P: 1.0  
141 mM; K: 5.0 mM; S: 2.0 mM; Ca: 1.2 mM; Mg: 5.2 mM; Fe: 17.9  $\mu\text{M}$ , Cu: 2.0  $\mu\text{M}$ , Zn: 3.8  $\mu\text{M}$ , B: 11.6  $\mu\text{M}$ ,  
142 Mn:18.2  $\mu\text{M}$ , Mo: 0.5  $\mu\text{M}$ . The nutrient solution was constantly aerated through air pumps (Airline 3, Haquoss,  
143 Turin, Italy, air exchange rate of 0.25 L  $\text{min}^{-1} \text{pot}^{-1}$ ). At 14 Days After start of light Treatment (DAT), pots were  
144 replenished with 0.25 L of fresh nutrient solution.

145

## 146 **2.2. Light treatments**

147 Lettuce and basil plants were grown under dimmable LED lamps (Flytech s.r.l., Belluno, Italy) featuring red  
148 (peak at 669 nm) and blue (peak at 465 nm) emitting diodes. The lamps were set to supply a spectral composition  
149 with a red:blue ratio of 3 (RB=3), such ratio being calculated by the relative spectral areas within the red (600–  
150 700 nm) and the blue (400–500 nm) regions (Singh et al., 2015). The spectral distribution was measured using  
151 an illuminance spectrophotometer (CL-500A, Konica Minolta, Chiyoda, Tokyo, Japan). A photosynthetic  
152 photon flux sensor (with equal sensitivity to red and blue radiation), model QSO (Apogee instruments, Logan,  
153 UT, USA) connected with a ProCheck handheld reader (Decagon Devices Inc., Pullman, WA, USA) was used  
154 to set PPFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) over the plant canopy. Daily Light Integrals (DLI) were calculated by multiplying  
155 the PPFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) by the photoperiod (s), and expressed as  $\text{mol m}^{-2} \text{d}^{-1}$ . In order to define the lamp's  
156 efficacy of electricity-to-light conversion, the PPFD:electricity ratio ( $\mu\text{mol J}^{-1}$ ) was estimated through flat plane  
157 integration technique as the ratio of the incident PPFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at a set distance (40 cm, equal to the

158 distance of the lamp from the top of the canopy during the experiments) and the light electricity power  
159 consumption (LEPC  $\text{W m}^{-2}$ , Pennisi et al., 2019a).

160 After transplant, 5 LED light treatments were applied, one per each compartment. Light treatments consisted of  
161 five different PPFD values of 100 (DLI:  $5.8 \text{ mol m}^{-2} \text{ d}^{-1}$ , LEPC:  $70 \text{ W m}^{-2}$ , PPFD:electricity ratio:  $1.44 \text{ } \mu\text{mol J}^{-1}$ ),  
162 150 (DLI:  $8.6 \text{ mol m}^{-2} \text{ d}^{-1}$ , LEPC:  $98 \text{ W m}^{-2}$ , PPFD:electricity ratio:  $1.53 \text{ } \mu\text{mol J}^{-1}$ ), 200 (DLI:  $11.5 \text{ mol m}^{-2}$   
163  $\text{d}^{-1}$ , LEPC:  $132 \text{ W m}^{-2}$ , PPFD:electricity ratio:  $1.51 \text{ } \mu\text{mol J}^{-1}$ ), 250 (DLI:  $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$ , LEPC:  $164 \text{ W m}^{-2}$ ,  
164 PPFD:electricity ratio:  $1.52 \text{ } \mu\text{mol J}^{-1}$ ) and 300 (DLI:  $17.3 \text{ mol m}^{-2} \text{ d}^{-1}$ , LEPC:  $197 \text{ W m}^{-2}$ , PPFD:electricity ratio:  
165  $1.52 \text{ } \mu\text{mol J}^{-1}$ )  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  (Fig. 1).

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

170

### 171 **2.3. Growth analysis and resource use efficiency**

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

177 Water use was individually quantified for each plant during each experiment and water use efficiency (WUE)  
178 was determined as the ratio between final fresh weight of the shoot and the volume of water used, and expressed  
179 as  $\text{g FW L}^{-1} \text{ H}_2\text{O}$ . Lighting energy use efficiency (EUE) was determined according to the crop cycle length and  
180 the final fresh weight of the shoot, related to the lamps' cumulated electricity absorption and expressed as  $\text{g FW}$   
181  $\text{kWh}^{-1}$ . Light use efficiency (LUE,  $\text{g DW mol}^{-1}$ ) was calculated as the ratio of shoot dry weight production per  
182 unit surface of cultivation ( $\text{g DW m}^{-2}$ ) and the light integral ( $\text{mol m}^{-2}$ ), obtained by multiplying DLI values by  
183 the number of days between transplanting and harvest.

184

#### 185 **2.4. Stomatal size and density**

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

194

#### 195 **2.5. Stomatal conductance**

196 Measurements of stomatal conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) were performed on the third fully expanded leaf using a  
197 leaf porometer ( $\Delta P4$ , Delta-T Devices, Cambridge, UK) at 14 DAT in each experiment.

198

#### 199 **2.6. Leaf chlorophyll content**

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

204

#### 205 **2.7 Total phenolic, flavonoids and antioxidant capacity**

206 In all experiments, leaf samples were collected at harvest (21 DAT), immersed in liquid  $\text{N}_2$  and kept at  $-80^\circ\text{C}$ .  
207 One gram of frozen plant tissue was extracted in a methanol:water:acetone (6:3:1, v:v:v) (Pennisi et al., 2019b).  
208 Total antioxidant capacity, phenolic and flavonoid compounds were determined on the resulting extract. The  
209 total antioxidant capacity, measured by the ferric reducing antioxidant power (FRAP) assay, was expressed as  
210  $\text{mmol Fe}^{2+} \text{ kg}^{-1} \text{ FW}$  (Aaby et al., 2007). Phenolic compounds and flavonoids were quantified by Folin-Ciocalteu

211 and aluminium chloride assays, and expressed as gallic acid and catechin equivalents, respectively (Zhishen et  
212 al., 1999; Waterhouse, 2002).

213

## 214 **2.7. Statistical analysis**

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

221

## 222 **3 RESULTS**

### 223 **3.1. Effects of light intensity on lettuce and basil growth**

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

237

### 238 **3.2. Effect of light intensity on leaf physiological functionality and anatomy**

239 In both lettuce (**Fig. 2A**) and basil (**Fig. 2E**), light intensity increased leaf chlorophyll content up to a PPFD of  
240  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ , whereas further increases did not result in higher values of chlorophyll. Similarly, in lettuce,  
241 also stomatal conductance was positively correlated with light intensity up to  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ , while a  
242 significant reduction of was observed at  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  (**Fig. 2B**). In basil plants, stomatal conductance was  
243 lowest at  $\text{PPFD} \leq 150 \mu\text{mol m}^{-2} \text{s}^{-1}$  as compared with  $\text{PPFD} \geq 200 \mu\text{mol m}^{-2} \text{s}^{-1}$  (**Fig. 2F**). In lettuce, stomatal  
244 density (**Fig. 2C**) was the lowest at 100 and  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  and reached the highest values at both 200 and  $250$   
245  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Stomatal size (**Fig. 2D**) resulted higher at 250 and  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ .  
246 In basil, stomatal density (**Fig. 2G**) reached the highest values at 200 and  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Stomatal size (**Fig.**  
247 **2H**) was the lowest at  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  and the highest at  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

248

### 249 **3.3. Effect of light intensity on antioxidant properties**

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

256

### 257 **3.4. Effect of light intensity on light, water and energy use efficiency**

258 Water use presented a similar trend in both lettuce and basil plants. In lettuce, water use was increased from  
259  $0.48 \text{ L plant}^{-1}$  ( $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) to  $0.66 \text{ L plant}^{-1}$  ( $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), and was the highest at  $\text{PPFD} \geq 200 \mu\text{mol m}^{-2}$   
260  $\text{s}^{-1}$ , featuring  $0.95 \text{ L plant}^{-1}$  as mean value (data not shown). Similarly, in basil, water use grew from  $0.38 \text{ L}$   
261  $\text{plant}^{-1}$  ( $100$  and  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ , mean value) to  $0.54 \text{ L plant}^{-1}$  ( $200$  and  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , mean value) and up  
262 to  $0.69 \text{ L plant}^{-1}$  under  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Water Use Efficiency (WUE) was progressively increased in lettuce

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

271

## 272 4 DISCUSSION

### 273 4.1. A PPFD of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ is needed for improved yield in indoor grown lettuce and basil supplied 274 with RB=3.

275 Plant biomass production in response to light intensity often follows an optimum function, which reaches its  
276 maximum when light stress begins to occur (Kang et al., 2013; He et al., 2019). However, optimum light  
277 intensity for fresh biomass production in lettuce was shown to vary among cultivars (Lee et al., 2019; Viršilė et  
278 al., 2019). Also, when both temperature (e.g. from 20 to 25°C) and light intensity (from 150 to 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$   
279 from red and blue LEDs with RB=3, respectively supplying DLI of 8.6 to 11.5  $\text{mol m}^{-2} \text{d}^{-1}$ ) were simultaneously  
280 increased, an increase in fresh biomass of lettuce was observed. Such an increase was not visible when  
281 temperature or light intensity alone were augmented (Okazaki and Yamashita, 2019). Similarly, the response of  
282 lettuce biomass to light intensity (400 or 700  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , resulting in DLI of 20.2 and 35.3  $\text{mol m}^{-2} \text{d}^{-1}$ ) was  
283 also altered by the atmospheric  $\text{CO}_2$  (400 and 700  $\mu\text{mol mol}^{-1} \text{CO}_2$ ) availability (Pérez-López et al., 2013). While  
284 a synergistic effect on the promotion of biomass in two cultivars (red and green) was observed when elevate  
285 light intensity (700  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and  $\text{CO}_2$  (700  $\mu\text{mol mol}^{-1}$ ) were supplied, at ambient  $\text{CO}_2$  (400  $\mu\text{mol mol}^{-1}$ ),  
286 elevate light intensity (700  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) only increased growth in green lettuce, but not in the red cultivar (Pérez-  
287 López et al., 2013). It was also shown that when photoperiod was reduced from 16 to 14  $\text{h d}^{-1}$  of light (at  
288  $T=22/18^\circ\text{C}$  and 800  $\mu\text{mol mol}^{-1} \text{CO}_2$ ), the optimum light intensity for fresh biomass production was increased

289 from 200 to 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  under red and blue LED (with both RB=1.2 and RB=2.2) (Yan et al., 2019).  
290 Looking at daily light integrals, it was observed that under RB=1.2 higher biomass was associated with  
291  $\text{DLI} \geq 12.6 \text{ mol m}^{-2} \text{ d}^{-1}$ , whereas under RB=2.2, biomass production decreased when  $\text{DLI} \geq 11.5 \text{ mol m}^{-2} \text{ d}^{-1}$  were  
292 adopted (Yan et al., 2019). When comparing 60, 140 and 220  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (DLI respectively of 3.4, 8.1 and 12.7  
293  $\text{mol m}^{-2} \text{ d}^{-1}$ ) supplied by mixed red and blue LED (RB=4), Fu et al. (2017) concluded that 220  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was  
294 the PPFD value allowing for the greatest lettuce growth at 23°C and 16 h  $\text{d}^{-1}$  of light. However, the lack of higher  
295 PPFD values in their study, does not allow to further define the crop growth-response function to PPFD. In basil,  
296 the highest fresh biomass was previously achieved when supplying 224  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ( $\text{DLI} = 12.9 \text{ mol m}^{-2} \text{ d}^{-1}$ )  
297 through a fluorescent white light (Dou et al., 2018), although no further increase was reported when the PPFD  
298 was raised up to 310  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ( $\text{DLI} = 17.8 \text{ mol m}^{-2} \text{ d}^{-1}$ ). The observed biomass increases were associated with  
299 enhancement of leaf photosynthetic rates when PPFD was raised from 160 to 224  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , albeit no leaf  
300 photosynthetic changes were observed among treatments in which plants were grown with  $\text{PPFD} \geq 224 \mu\text{mol m}^{-2}$   
301  $\text{s}^{-1}$  (Dou et al., 2018). From the results of hereby presented research, it could be advanced that the adopted  
302 environmental (including light spectrum, photoperiod and  $\text{CO}_2$ ) and plant growing (including plant density and  
303 cultivar used) features resulted in an optimum PPFD of 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ( $\text{DLI} = 14.4 \text{ mol m}^{-2} \text{ d}^{-1}$ ) (Table 1),  
304 while higher PPFD values (e.g. 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $\text{DLI} = 17.3 \text{ mol m}^{-2} \text{ d}^{-1}$ ) resulted in reduced growth in lettuce.  
305 The detrimental effects on lettuce yield associated with too elevate DLI were previously observed by Zhang et  
306 al. (2018), in experiments where an optimal DLI (when plants were grown under LED with RB=2.2 and  
307 photoperiod of 12 h  $\text{d}^{-1}$  of light) for fresh biomass accumulation was found at 10.8  $\text{mol m}^{-2} \text{ d}^{-1}$  as compared with  
308 13.0  $\text{mol m}^{-2} \text{ d}^{-1}$ . It results that the definition of optimal light intensity is a complex scenario that can only be  
309 defined building on the combined and synergistic effects of a number of environmental and crop factors.  
310 Dry weight production in lettuce increased when light intensity was augmented from 120 to 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$   
311 (DLI respectively of 6.9 and 8.6  $\text{mol m}^{-2} \text{ d}^{-1}$ ) (Lin et al., 2018) and from 60 to 220  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (DLI from 3.4  
312 to 12.7  $\text{mol m}^{-2} \text{ d}^{-1}$ ) (Fu et al., 2017). Contrarily, Yan et al. (2019) reported highest dry biomass in lettuce  
313 seedlings grown under LED (featuring mixed red, green and blue light with RB of 1.2 or 2.2, photoperiod 16 h  
314  $\text{d}^{-1}$ ) light supplying 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ( $\text{DLI} = 11.5 \text{ mol m}^{-2} \text{ d}^{-1}$ ) as compared with those experiencing 250  $\mu\text{mol m}^{-2}$

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

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

332 The greater plant growth at 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (DLI=14.4 mol  $\text{m}^{-2} \text{d}^{-1}$ ) was also consistent with a larger leaf area  
333 in both crops and, in basil, also to increased plant height and leaf number (Table 1). Increased leaf area and  
334 number were previously observed in lettuce plants, when PPFD was increased from 260 to 290  $\mu\text{mol m}^{-2} \text{s}^{-1}$   
335 (DLI from 16.8 to 18.8 mol  $\text{m}^{-2} \text{d}^{-1}$ ) from LED featuring mixed red, blue and white light (RB=8) (Kang et al.,  
336 2013). Similarly, in basil, an increase in leaf area and plant height were also observed after 21 days of light  
337 treatment when PPFD $\geq$ 224  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (DLI=12.9 mol  $\text{m}^{-2} \text{d}^{-1}$ ) was supplied (Dou et al., 2018). Despite the  
338 higher shoot biomass in response to growing PPFD, functional changes in dry biomass partitioning to roots were  
339 also observed, overall altering the plant R:S ratio in lettuce (Table 1). In some other studies on lettuce, R:S ratio  
340 was either reported to increase or not to change (Fu et al., 2017) or even decrease (Lin et al., 2018) in response



341 to growing PPFD. Possibly, an optimum function may be hereby demonstrated (**Table 1**), with 200 and 250  
342  $\mu\text{mol m}^{-2} \text{s}^{-1}$  resulting in the highest R:S ratio. In a previous study on lettuce, the R:S ratio was shown to increase  
343 as light intensity increased, when moving from 200 ( $\text{DLI}=13.0 \text{ mol m}^{-2} \text{d}^{-1}$ , R:S ratio=0.15) to 230  $\mu\text{mol m}^{-2} \text{s}^{-1}$   
344 ( $\text{DLI}=14.9 \text{ mol m}^{-2} \text{d}^{-1}$ , R:S ratio=0.21), but then decrease as light intensity reached 260  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ( $\text{DLI}=16.8$   
345  $\text{mol m}^{-2} \text{d}^{-1}$ , R:S ratio=0.18) (Kang et al., 2013). According to the functional equilibrium hypothesis, as  
346 irradiance increases, plants fix larger amounts of carbon in photosynthesis and show higher allocation to roots  
347 at the expenses of shoots, while as light leads to stress in leaves the R:S ratio will not increase anymore (Poorter  
348 et al., 2012).

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

354

#### 355 **4.2. Leaf adaptation mechanisms to increased PPFD.**

356 Light intensity was previously shown to alter leaf anatomical and physiological features in both basil and lettuce  
357 grown in greenhouse (Orsini et al., 2018) and indoor farming (Dou et al., 2018; Kang et al., 2013) environments.  
358 Leaf chlorophyll content was reported to be lower in basil plants grown under  $\text{PPFD} \geq 224 \mu\text{mol m}^{-2} \text{s}^{-1}$   
359 ( $\text{DLI} \geq 12.9 \text{ mol m}^{-2} \text{d}^{-1}$ ) as compared with those grown under  $\text{PPFD} \leq 200 \mu\text{mol m}^{-2} \text{s}^{-1}$  ( $\text{DLI} \leq 11.5 \text{ mol m}^{-2} \text{d}^{-1}$ )  
360 (Dou et al., 2018). However, in the same work, leaf chlorophyll was not reported to vary between plants grown  
361 under 224 ( $\text{DLI}=12.9 \text{ mol m}^{-2} \text{d}^{-1}$ ) and 310  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ( $\text{DLI}=17.8 \text{ mol m}^{-2} \text{d}^{-1}$ ). Similarly, in lettuce, no  
362 differences in chlorophyll content could be observed in plants grown under PPFD ranging 200 to 290  $\mu\text{mol m}^{-2}$   
363  $\text{s}^{-1}$  ( $\text{DLI}$  from 13.0 to 18.8  $\text{mol m}^{-2} \text{d}^{-1}$ ) (Kang et al., 2013) or when plants were grown under either 150 to 200  
364  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ( $\text{DLI}$  respectively of 8.6 and 11.5  $\text{mol m}^{-2} \text{d}^{-1}$ ) (Okazaki and Yamashita, 2019). The observed  
365 behaviour (**Fig. 2A** and **2E**) is consistent with the hypothesis that under either non-optimal radiation intensity,  
366 leaf chlorophyll content is reduced, as previously described in lettuce (Fu et al., 2012; Orsini et al., 2018). It

367 should be noted that such a reduction in chlorophyll may also result in lighter green colour of the leaves, a trait  
368 that was previously associated with reduced consumer preference in fresh vegetable products (Rouphael et al.,  
369 2012).

370 Alongside with the role played by leaf chlorophyll content, photosynthesis in leaves is regulated by stomatal  
371 features, as evidenced in basil (Mancarella et al., 2016). Stomatal opening is a general response of plants to high  
372 light intensity, facilitating both CO<sub>2</sub> uptake for photosynthesis and evaporative cooling of the leaf undergoing  
373 elevate radiative heat loads (Matsuda, 2016). Two mechanisms are mainly associated with the light-induced  
374 stomatal response (Shimazaki et al., 2007), one of them supposedly driven by the photosynthetic activity of both  
375 guard and mesophyll cells, the other induced by blue light triggering the response of the photoreceptor  
376 phototropin (Hiyama et al., 2017). Accordingly, the light spectral composition was shown not only to alter  
377 biomass growth, but also to modify stomatal functionality and overall water use in both lettuce and basil plants  
378 (Pennisi et al., 2019a and 2019b). Stomatal conductance was previously reported to increase in lettuce when  
379 PPFD was raised from 60 to 220  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (DLI from 3.4 to 12.7  $\text{mol m}^{-2} \text{d}^{-1}$ ) (Fu et al., 2017) or from 200 to  
380 230  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (DLI from 13.0 to 14.9  $\text{mol m}^{-2} \text{d}^{-1}$ ) (Kang et al., 2013), while was decreased at higher PPFD  
381 values (Kang et al., 2013). Similarly, in basil, Dou et al. (2018) reported stomatal conductance to increase from  
382 160 (DLI=9.3  $\text{mol m}^{-2} \text{d}^{-1}$ ) up to 224  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (DLI=12.9  $\text{mol m}^{-2} \text{d}^{-1}$ ), while becoming stable upon higher  
383 PPFD. Accordingly, in the hereby presented study, in lettuce plants stomatal conductance reached the highest  
384 values at 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (DLI=14.4  $\text{mol m}^{-2} \text{d}^{-1}$ ), and then decreased for greater values of light intensity (Fig.  
385 2B), while in basil plants stomatal conductance resulted stable in plants grown under PPFD $\geq$ 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$   
386 (DLI $\geq$ 11.5  $\text{mol m}^{-2} \text{d}^{-1}$ ) (Fig. 2F). Changes in stomatal conductance were previously associated with  
387 modifications in stomatal size and/or density in both lettuce (Pennisi et al., 2019a) and basil (Barbieri et al.,  
388 2012). Similarly, stomatal density followed an optimum function showing higher values at 200 and 250  $\mu\text{mol}$   
389  $\text{m}^{-2} \text{s}^{-1}$  (DLI of 11.5 and 14.4  $\text{mol m}^{-2} \text{d}^{-1}$ , respectively) in both lettuce and basil (Fig. 2C and 2G). Moreover,  
390 stomatal size resulted to be increased by growing PPFD up to 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , in both species (Fig. 2D and  
391 2H). These changes in stomatal size and density were also consistent with the response in stomatal conductance  
392 (Fig. 2B and 2F), which was highest at PPFD=250 and PPFD $\geq$ 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , in lettuce and basil, respectively.

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

395

### 396 **4.3. In lettuce, PPFD affects antioxidant capacity**

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

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

408 In contrast, lettuce responded to light intensity, showing the highest antioxidant activity and concentrations of  
409 phenolics and flavonoids between 200 and 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD (**Table 2**), resembling previously reported  
410 values for the same crop species (Msilini et al., 2013; Ouhibi et al., 2014) and confirming the hypothesis of a  
411 PPFD-related effect on the plant antioxidant profile (Poorter et al., 2019). The finding of an optimum intensity  
412 value, rather than a proportional relation, suggests that antioxidant capacity and both flavonoids and phenolics  
413 concentrations may be determined as a trade-off between different processes with opposite effects. For instance,  
414 the finding of a lower stomatal conductance (i.e., potentially higher accumulation of  $\text{O}_2$ ) at 300 vs 250  $\mu\text{mol m}^{-2}$   
415  $\text{s}^{-1}$  PPFD, associated with comparable chlorophyll contents, suggests a higher risk of oxygen radical formation  
416 as a result of electron leakage from photosynthetic machinery (Anjum et al., 2011).

417

### 418 **4.4. Toward efficient resource use in indoor lettuce and basil cultivation: the role of light intensity.**

419 Reducing water use while preserving satisfactory yield is a target priority for agricultural production (Fernández  
420 et al., 2018). The increased yield associated with 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD (DLI=14.4  $\text{mol m}^{-2} \text{d}^{-1}$ ) in lettuce and  
421 with PPFD $\geq$ 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (DLI $\geq$ 14.4  $\text{mol m}^{-2} \text{d}^{-1}$ ) in basil (Table 1), compensated for the increase in stomatal  
422 conductance (Fig. 2B and 2F), overall leading to greater water use efficiency (WUE, Fig. 3A and 3D). The  
423 observed values for WUE (reaching up to 60  $\text{g FW L}^{-1} \text{H}_2\text{O}$  and 38  $\text{g FW L}^{-1} \text{H}_2\text{O}$ , respectively in lettuce and  
424 basil, Fig. 3A and 3D), are extremely impressive when compared with reported values for traditional cultivation.  
425 Accordingly, from data on open-field and greenhouse cultivation, WUE of lettuce was respectively defined at 4  
426  $\text{g FW L}^{-1} \text{H}_2\text{O}$  and 50  $\text{g FW L}^{-1} \text{H}_2\text{O}$  (Barbosa et al., 2015), whereas basil respectively performed 3  $\text{g FW L}^{-1}$   
427  $\text{H}_2\text{O}$  and 22  $\text{g FW L}^{-1} \text{H}_2\text{O}$  in open-field (Ekren et al., 2012) and greenhouse (Montesano et al., 2018) systems.  
428 Similarly, the balance between increased electricity needs at growing PPFD and greater plant biomass achieved  
429 in response to higher light intensities, altered the crop Energy Use Efficiency (Fig. 3B and 3E). From such  
430 equilibrium, maximum EUE was achieved under 200 to 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD (corresponding to DLI of 11.5  
431 and 14.4  $\text{mol m}^{-2} \text{d}^{-1}$ , respectively) in lettuce and at 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD (DLI=14.4  $\text{mol m}^{-2} \text{d}^{-1}$ ) in basil. The  
432 achieved EUE values under 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD (110 and 45  $\text{g FW kWh}^{-1}$ , in lettuce and basil, respectively)  
433 are already higher than those reached under comparable environmental conditions at lower intensities (215  $\mu\text{mol}$   
434  $\text{m}^{-2} \text{s}^{-1}$  PPFD and DLI=12.4  $\text{mol m}^{-2} \text{d}^{-1}$ ) in both lettuce (91  $\text{g FW kWh}^{-1}$ , Pennisi et al., 2019a) and basil (33  $\text{g}$   
435  $\text{FW kWh}^{-1}$ , Pennisi et al., 2019b).

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

445  $\text{CO}_2=1200 \mu\text{mol mol}^{-1}$ ). In lettuce plants grown in a growth chamber under HPS lamps (PPFD=420  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ,  
446 photoperiod=16 h  $\text{d}^{-1}$ , DLI=24.2  $\text{mol m}^{-2} \text{d}^{-1}$ ,  $\text{CO}_2=370\text{-}410 \mu\text{mol mol}^{-1}$ ), lower values of LUE were reported  
447 (0.15-0.18 g DW  $\text{mol}^{-1}$ , [El-Nachel et al., 2019](#)). In greenhouses, however, reported LUE values were even lower  
448 and ranged 0.33-1.39 g DW  $\text{MJ}^{-1}$ , which would correspond (considering a conversion factor of 4.6  $\text{mol MJ}^{-1}$ ) to  
449 LUE value as little as 0.07 g DW  $\text{mol}^{-1}$  ([Wheeler et al., 1993](#)). When greenhouse values were referred to the  
450 actually absorbed PAR instead, [De Pinheiro Henriques and Marcelis \(2000\)](#) reported LUE to range 3.5 to 4.9 g  
451 DW  $\text{MJ}^{-1}$ , which would correspond to 0.8 to 1.1 g DW  $\text{mol}^{-1}$ .

452

## 453 5 CONCLUSIONS

454 The research confirmed that an optimum response curve exists between light intensity and plant growth, with  
455 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (DLI=14.4  $\text{mol m}^{-2} \text{d}^{-1}$ ) resulting in improved fresh and dry biomass production as well as  
456 larger plant leaf area under the prevailing conditions of red and blue light (RB=3), a photoperiod of 16 h  $\text{d}^{-1}$  of  
457 light, 24°C, 450  $\mu\text{mol mol}^{-1} \text{CO}_2$  and a plant density of 100 plants  $\text{m}^{-2}$ . At this light intensity regime and following  
458 the functional equilibrium hypothesis, an increased R:S ratio was also observed, altogether with reductions in  
459 SLA, possibly as a consequence of functional leaf adaptations. Consistently, leaves of plants grown under 250  
460  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (DLI=14.4  $\text{mol m}^{-2} \text{d}^{-1}$ ) presented denser and larger stomata, which allowed for improved stomatal  
461 conductance and higher leaf chlorophyll content. On the contrary, lower light intensities reduced leaf  
462 functionality (in terms of stomatal features and chlorophyll content), which also resulted in reduced nutritional  
463 content in lettuce, where antioxidant capacity, phenolics and flavonoids concentrations were lower.

464 Despite the higher water requirements and the higher electricity needs experienced when a PPFD of 250  $\mu\text{mol}$   
465  $\text{m}^{-2} \text{s}^{-1}$  was supplied as compared with lower light intensities, the yield gain allowed for improved water (WUE),  
466 energy (EUE) and light (LUE) use efficiencies. On the other hand, additional light intensity (e.g. up to 300  $\mu\text{mol}$   
467  $\text{m}^{-2} \text{s}^{-1}$ ) did not allow for additional yield and therefore WUE, EUE and LUE were not further improved. From  
468 the study it may be concluded that under a mixed red and blue LED light (featuring RB=3) and a photoperiod  
469 of 16 h  $\text{d}^{-1}$  of light, indoor cultivation of both lettuce and basil may be improved when DLI=14.4  $\text{mol m}^{-2} \text{d}^{-1}$  and  
470 PPFD=250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  are supplied. The novelty proposed therefore stands in the optimization of radiation

471 intensity in a specific spectral environment (RB=3) that was recently shown to improve productivity and  
472 resource use efficiency in basil and lettuce (Pennisi et al., 2019a, b). The research also elaborates on  
473 physiological changes associated with stomatal response to light, that result in viable strategies for maximising  
474 water, energy and light use efficiencies in the studied crops.

475

#### 476 **Author Contributions**

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

483

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683

684 **Tables**

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

<b>DLI</b>	<b>PPFD</b>	<b>Shoot FW</b>		<b>Shoot DW</b>		<b>DM</b>		<b>R:S ratio</b>		<b>Plant height</b>	<b>Leaf number</b>		<b>Leaf area</b>		<b>SLA</b>		
<i>mol m<sup>-2</sup> d<sup>-1</sup></i>	<i><math>\mu\text{mol m}^{-2} \text{s}^{-1}</math></i>	<i>g plant<sup>-1</sup></i>		<i>g plant<sup>-1</sup></i>		<i>%</i>				<i>cm</i>	<i>n</i>	<i>cm<sup>2</sup></i>		<i>cm<sup>2</sup> g<sup>-1</sup> DW</i>			
<b>Lettuce</b>										-							
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		
<b><i>P value</i></b>		<b><i>&lt;0.001</i></b>		<b><i>&lt;0.001</i></b>		<b><i>&lt;0.001</i></b>		<b><i>&lt;0.001</i></b>		-	<b><i>ns</i></b>	<b><i>&lt;0.001</i></b>		<b><i>&lt;0.001</i></b>			
<b>Basil</b>																	
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
<b><i>P value</i></b>		<b><i>&lt;0.001</i></b>		<b><i>&lt;0.001</i></b>		<b><i>0.002</i></b>		<b><i>ns</i></b>		<b><i>&lt;0.001</i></b>		<b><i>&lt;0.001</i></b>		<b><i>&lt;0.001</i></b>		<b><i>&lt;0.001</i></b>	

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



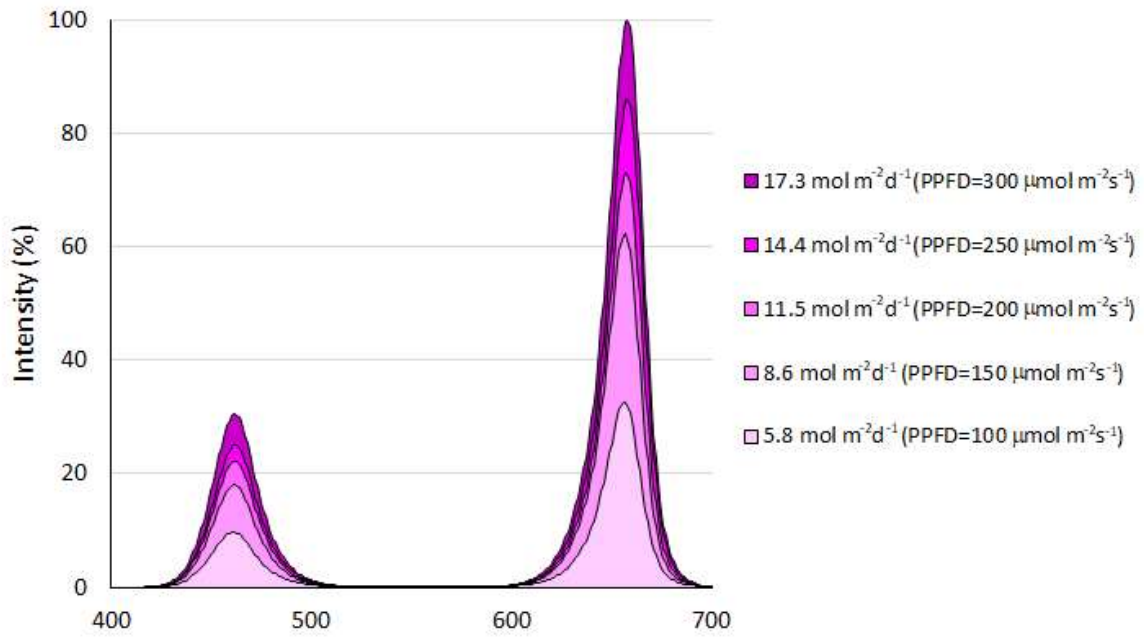
688 **Table 2.** Effect of different DLI (obtained by changing light intensity from 100 to 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) on  
 689 antioxidant properties of indoor grown lettuce plants at 21 DAT. Each value is the mean of 12 independent  
 690 measures. Different letters indicate significant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

<b>DLI</b>	<b>PPFD</b>	<b>Total Antioxidant capacity (FRAP)</b>		<b>Phenolics</b>		<b>Total flavonoid concentration</b>	
<i>mol m<sup>-2</sup> d<sup>-1</sup></i>	<i><math>\mu\text{mol m}^{-2} \text{s}^{-1}</math></i>	<i>mmol Fe<sup>2+</sup> kg<sup>-1</sup> FW</i>		<i>mg GA g<sup>-1</sup> FW</i>		<i>mg CE g<sup>-1</sup> FW</i>	
<b>Lettuce</b>							
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
<b><i>P value</i></b>		<b><i>&lt;0.05</i></b>		<b><i>&lt;0.001</i></b>		<b><i>&lt;0.001</i></b>	

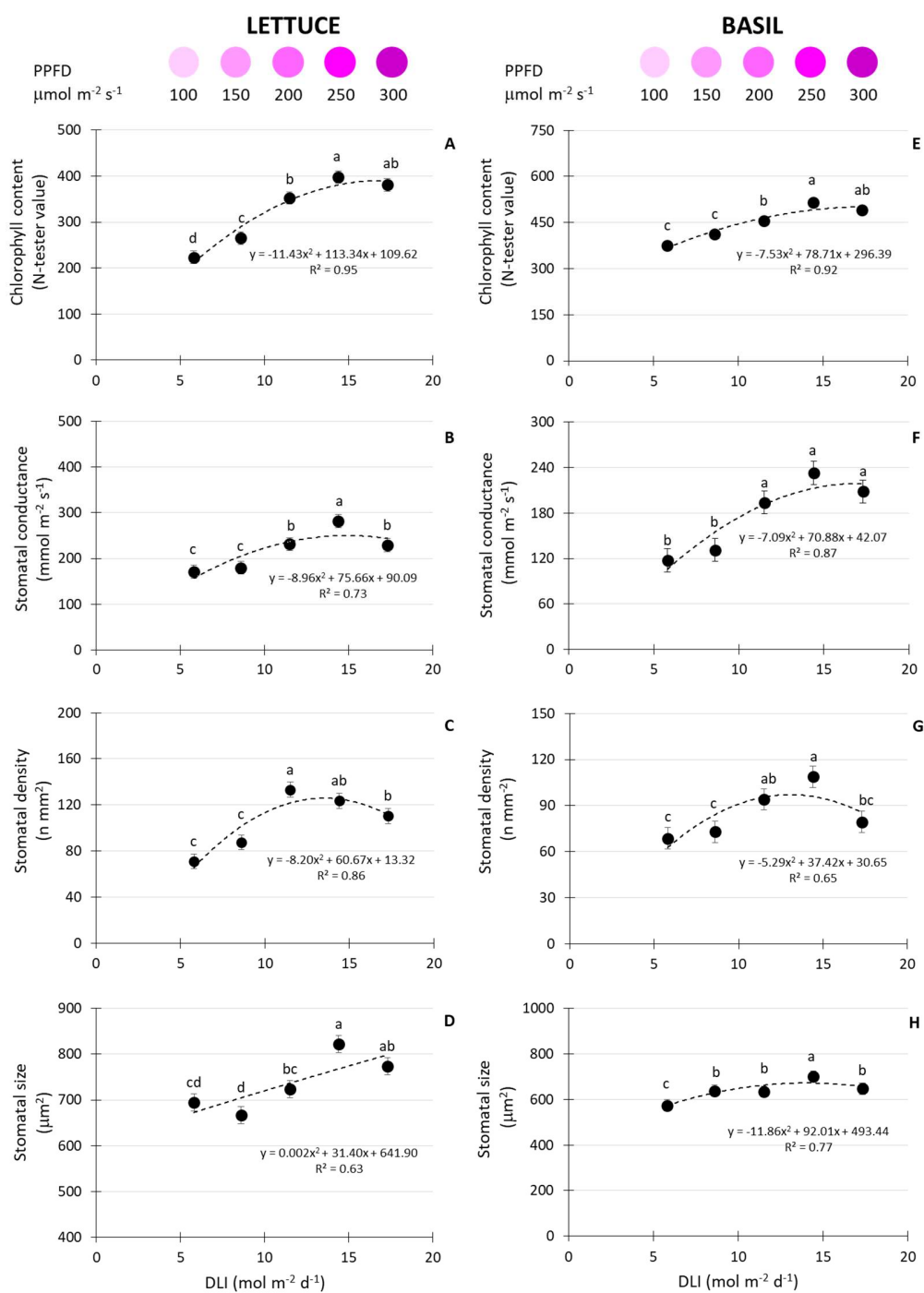
691 GA = Gallic Acid; CE= Catechin equivalents.

692

693 **Figures**

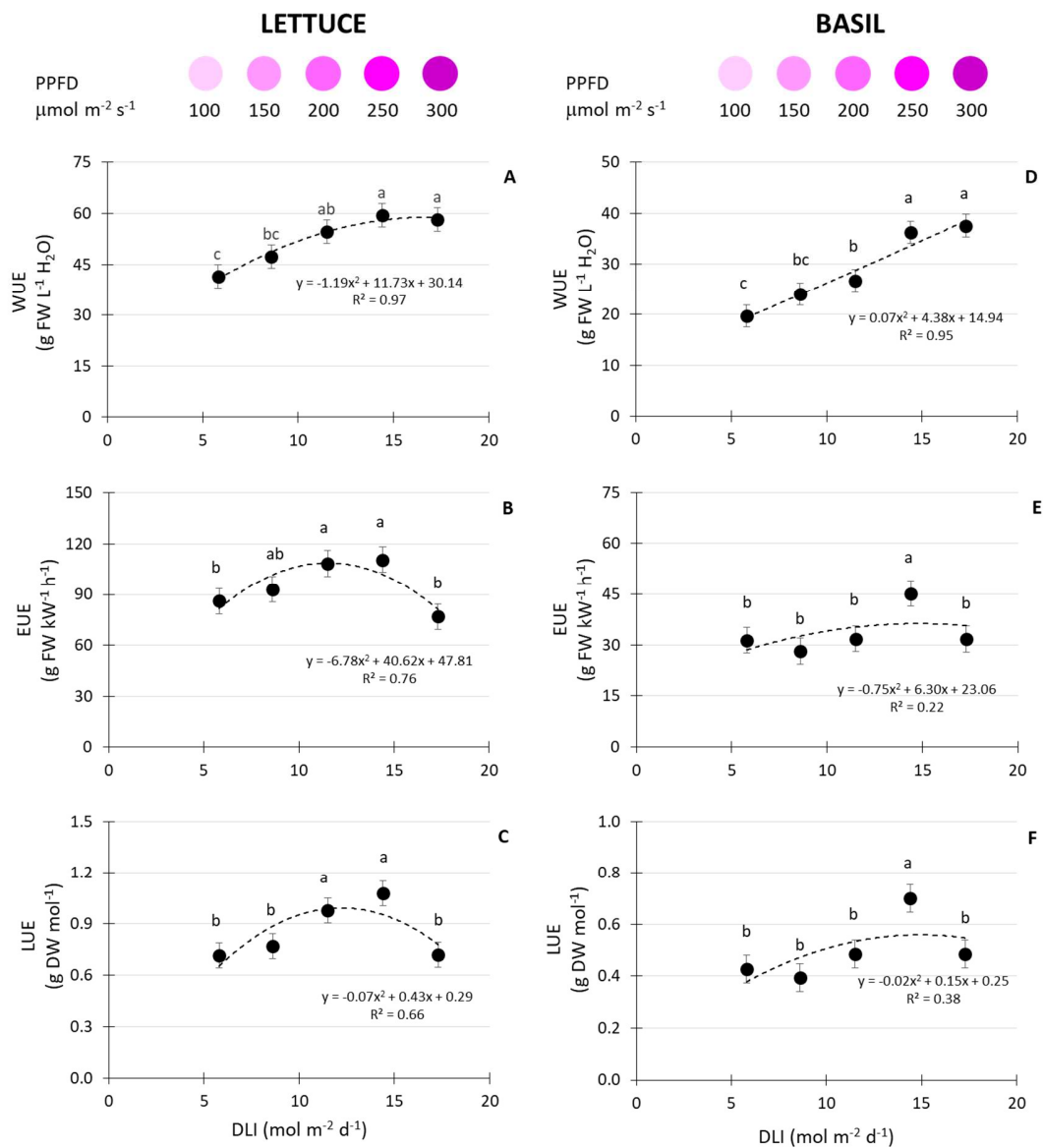


694  
695 **Figure 1.** Light spectra of the five light treatments used in the experiments. The chart is based on  
696 relative values based on the maximum red peak (obtained when  $17.3 \text{ mol d}^{-1}$  were supplied).  
697



698

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



704

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

709