



Optimal photoperiod for indoor cultivation of leafy vegetables and herbs

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Summary

In Vertical Farms with Artificial Lighting (VFALs), optimal light management is a crucial determinant of both economic and environmental viability. Applications of LED technologies to plant cultivation are still recent, and research has to date mainly targeted the definition of optimal spectral and light intensity features. On the other hand, despite the relevant implications on production costs, literature on optimal photoperiod management is to date limited. Indeed, the number of hours per day correlates with the total light supplied to the crop – expressed as Daily Light Integral (DLI) – and the associated energetic costs. The present study aims at defining how photoperiods of 16 h d⁻¹ (DLI= 14.4 mol m⁻² d⁻¹), 20 h d⁻¹ (DLI= 18 mol m⁻² d⁻¹) and 24 h d⁻¹ (DLI= 21.6 mol m⁻² d⁻¹) of light affect growth performances and resource use efficiency in leafy vegetables and herbs, represented by lettuce (*Lactuca sativa* L.), basil (*Ocimum basilicum* L.), rocket (*Eruca sativa* Mill.), and chicory (*Cichorium intybus* L.). Plants were cultivated indoor under a red (R) and blue (B) LED light (RB=3, photosynthetic photon flux density (PPFD)= 250 μmol m⁻² s⁻¹). Photoperiod variations' effects differed according to the considered plant species. In lettuce and chicory, the adoption of a DLI of 14.4 mol m⁻² d⁻¹ at 16 h d⁻¹ photoperiod resulted in a greater plants fresh biomass and leaf area, which also contributed to generally obtain higher energy use efficiency (fresh biomass per unit of electricity input), light use efficiency (dry biomass per unit of light input) and, in chicory, also water use efficiency (fresh biomass per liter of water consumed). Contrarily, although photoperiod variations did not affect basil and rocket growth parameters (e.g., fresh biomass and leaf area) and the plant capacity to transform resources (e.g., water use efficiency and light use efficiency), energy use efficiency in basil also presented a downward trend in response to growing DLI. Accordingly, the adoption of a DLI of 14.4 mol m⁻² d⁻¹ at 16 h d⁻¹ photoperiod resulted to be the optimal option among the ones tested in the presented research.

Keywords

Daily Light Integral (DLI), Plant Factory with Artificial Lighting (PFAL), vertical farm, Water Use Efficiency (WUE), Light Use Efficiency (LUE), Energy Use Efficiency (EUE)

Significance of this study

What is already known on this subject?

- LED light sustains plant growth in vertical farms. Research on plant growth and resources use in response to photoperiod management is limited.

What are the new findings?

- Fresh biomass and energy use efficiency of lettuce, chicory, basil and rocket were optimal when DLI is 14 mol m⁻² d⁻¹ at a photoperiod of 16 h d⁻¹.

What is the expected impact on horticulture?

- Using a photoperiod of up to 16 h d⁻¹ (DLI= 14 mol m⁻² d⁻¹) in chicory and lettuce cultivation may improve yield and sustainability.

Introduction

The rapid evolution in plant LED lighting technologies experienced in the last decade has resulted in revolutionary changes in the greenhouse and controlled-environment agricultural industry sector (Gómez and Izzo, 2018). From the first researches on potential applications in outer space (Massa et al., 2008), current application of LED lighting ranges from the progressive substitution of incandescent lights used in the photoperiod management for flowering species (Craig and Runkle, 2013), up to the replacement of both fluorescent lamps in growth rooms (Yeh and Chung, 2009) and high pressure sodium (HPS) lamps for supplemental lighting in greenhouses (Olle and Viršile, 2013). Indeed, in response to multiple stressors affecting the viability of global food systems in the coming decades (which include, among other, the effects of population growth and urbanization, climate change and resource scarcity), the most promising application of LED lighting in horticulture is likely in the so-called vertical farms (Al-Chalabi, 2015). In Vertical Farms with Artificial Lighting (VFALs), where radiation for photosynthesis is solely supplied through lamps, the efficiency of light assimilation is crucial for plant growth performance, as well as for the environmental and economic sustainability of the production system (Kozai, 2020). To date, however, a major share of research on VFALs has only targeted cultivation of a limited range of crops (Kozai, 2016a). First studies addressed LED applications on cereals, including wheat (Goins et al., 1997; Dougher and Bugbee, 2001; Morrow, 2008). It rapidly emerged, however, that cultivation of staple

crops would hardly meet the energetic and economic costs associated with indoor cultivation (Banerjee and Adenaueer, 2014). Alternatively, cultivation of fresh fruits and vegetables could benefit from the higher value of the products (which may also benefit from the consumer preference toward locally produced and fresher food) as well as the avoided or reduced burdens that are currently associated with post-harvest transportation and refrigeration (Al Chalabi, 2015). Accordingly, in recent years, leafy vegetables and herbs have been the major research target crops for cultivation within VFALs. As compared with fruit vegetables, they present a number of advantages, which include their small volume, the elevated harvest index (which maximises the amount of dry biomass produced per unit of light received), and the limited requirements for light intensity (Dou et al., 2018; Pennisi et al., 2020). Among these crop categories, recent studies have largely targeted applications in lettuce (*Lactuca sativa* L.) and sweet basil (*Ocimum basilicum* L.), addressing implications on metabolic and aromatic profiles, as well as the definition of their resource use efficiency (Dou et al., 2018; Pennisi et al., 2019a, b; Viršilė et al., 2019; Yan et al., 2019). Furthermore, the potential for cultivation within VFALs was also recently explored in other leafy vegetables, including rocket (*Eruca sativa* Mill.) and chicory (*Cichorium intybus* L.), whose production for the ready-to-eat sector may sustain VFALs economic viability (Taulavuori et al., 2018; Pennisi et al., 2019c; Kozai and Niu, 2020). Moreover, while research on LED light management in VFALs has mostly targeted the definition of optimal spectral composition (Carvalho et al., 2016; Clavijo-Herrera et al., 2018; Pennisi et al., 2019a, b) and recommended Photosynthetic Photon Flux Density (PPFD) (Dou et al., 2018; Viršilė et al., 2019; Yan et al., 2019; Pennisi et al., 2020), studies targeting the optimal photoperiod management under LED lighting are, to date, only limited, notwithstanding its crucial role in plants growth and development. Accordingly, photoperiod may influence several molecular mechanisms related to the leaf detection of light signal, circadian rhythm operation, transduction of light signal, which may alter growth, flowering time, abiotic and biotic stress responses, and plants metabolism (Jackson, 2009; Greenham and McClung, 2015).

Most of the available research on indoor leafy vegetables cultivation adopted long days photoperiod (e.g., 16 hours of light per day) (Shimizu et al., 2011), building on the evidence that shorter days would result in lower plant growth and biomass production (Lee and Kim, 2013). Furthermore, some crops (including lettuce) have shown to feasibly stand also continuous light conditions (Ikeda et al., 1987; Velez-Ramirez et al., 2011). These results were also confirmed in a more recent study, where fluorescent lamps supplied either 12, 18 or 24 h d⁻¹ of light, achieving the larger growth when lettuce plants were continuously irradiated (Park et al., 2012). In another study (Kang et al., 2013), it was shown that lettuce would present higher shoot fresh weight when grown under two cycles of 9:3 (light:dark) hours per day, as compared with a single cycle of 18:6, which on the other hand resulted in higher leaf dry weight, suggesting that changes in the plant circadian rhythms would also alter leaf morphological parameters and assimilates allocation. However, in VFALs, where solar radiation is not used, the length of the day is directly related to the energetic costs. Therefore, the need of identifying the optimal photoperiod must build on the mutual relationship between yield and energy costs in response to day length increases.

Building on this ground, the present research aims at

identifying the morphological and physiological responses of four vegetable and herb crops (lettuce, basil, rocket and chicory) to variations in the supply of Daily Light Integrals (DLIs) through photoperiod management. The study also explores on how photoperiod alters resource use efficiency, particularly with reference to water, energy and light.

Materials and methods

Plant material and growth conditions

Plants were grown in three separate compartments (2 m² surface and 3.6 m³ volume) in a climate-controlled growth chamber (24 ± 2°C air temperature, 55–70% relative humidity and 450 μmol mol⁻¹ CO₂) at the University of Bologna (Italy). Each compartment was insulated by using light opaque white walls, and equipped with fans constantly replacing internal air (hourly exchange rate of 35 v:v). Three independent experiments were conducted on four plant species, namely lettuce (*Lactuca sativa* L. 'Rebelina', belonging to the typology Gentilina), basil (*Ocimum basilicum* L. 'Superbo', belonging to the typology Genovese), rocket (*Eruca sativa* Mill., 'Coltivata') and chicory (*Cichorium intybus* L. 'Bionda a foglie larghe'). In all three experiments and four crop species, a planting density of 100 plants m⁻² and a crop cycle length of 21 days after transplant (DAT) to harvest, were adopted. Seeds were germinated in polystyrene containers filled with a mixture of peat (70%) and vermiculite (30%), under fluorescent lamps (using 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 days after sowing for lettuce, rocket and chicory, and 21 days after sowing for basil), roots were washed, and plantlets were transplanted into a hydroponic deep water culture system. Each growing unit was composed by a plastic tray (5 L of volume), covered by a polypropylene panel hosting 6 plants and filled with nutrient solution (EC = 1.6, pH = 6.5) with the following composition: N-NO₃: 14 mM; N-NH₄⁺: 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 DAT, trays were replenished with 2 L of fresh nutrient solution. In each experiment, each compartment hosted three plastic trays per species, for a total of 12 trays.

Light treatments

After transplanting, plants were grown under dimmable LED lamps (Flytech s.r.l., Belluno, Italy) featuring hyper 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), and a photosynthetic photon flux density of 250 μmol m⁻² s⁻¹. Light electricity power consumption was 164 W m⁻². The spectral distribution was measured using an illuminance spectrophotometer (CL-500A, Konica Minolta, Chiyoda, Tokyo, Japan). A PAR 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 Photosynthetic Photon Flux Density (PPFD, μmol m⁻² s⁻¹) over the plant canopy. Electric power consumption (expressed in W = J s⁻¹) was measured using a multimeter (Fluke 189, Fluke Corporation, Everett, WA, USA). In order to define the lamp's ef-

ficacy of electricity-to-light conversion, the Photosynthetic Photon Efficacy (PPE) (expressed in $\mu\text{mol J}^{-1}$) was calculated as the ratio between PPFD and electric power consumption, resulting to be $1.52 \mu\text{mol J}^{-1}$.

Three LED light treatments were applied, one per each compartment, characterised by different DLIs (namely 14.4 , 18 and $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$) obtained by changing the photoperiod (respectively 16 , 20 and 24 h d^{-1} of light).

Growth analysis and resource use efficiency

At harvest (21 DAT), fresh biomass, also referred to as fresh weight (FW), of shoot and root was measured in all experiments and dry biomass, also referred to as dry weight (DW), was quantified after drying samples at 60°C for 72 hours. Root-shoot ratio (RS) was determined as the ratio of root dry biomass to shoot dry biomass. Leaf number was counted, 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 biomass. For basil plants, also plant height was measured.

Water use was quantified for each tray during each experiment and Water Use Efficiency (WUE) was determined as the ratio between fresh weight and the volume of water used, and expressed 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 the final fresh weight of the shoot, related to the lamps' cumulated electricity absorption (measured using a multimeter model Fluke 189, Fluke Corporation, Everett, WA, United States) and expressed as g FW kWh^{-1} . Light use

efficiency (LUE) was calculated as the ratio of shoot dry weight production per unit surface of cultivation (g DW m^{-2}) and the light integral (obtained by multiplying DLI values per the number of days between transplanting and harvest, expressed as mol m^{-2}), and expressed as g DW mol^{-1} .

Stomatal conductance

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

Leaf chlorophyll content

Concentration of chlorophyll in leaves was estimated during each experiment at 14 DAT through a leaf chlorophyll meter (SPAD 502, Spectrum Technologies Inc., Aurora, IL, USA) on the third fully expanded leaf.

Statistical analysis

Measurements were conducted on 18 plants per light treatment per species per experiment, 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 HSD test, at 5% significance level. Before the analysis, all data were checked for normality and homogeneity of the variance. The linear response to levels of photoperiod was assessed by the orthogonal polynomial contrast. Significance level was set at $P < 0.05$. For all statistical analyses, R (v. 3.3.2) software was used.

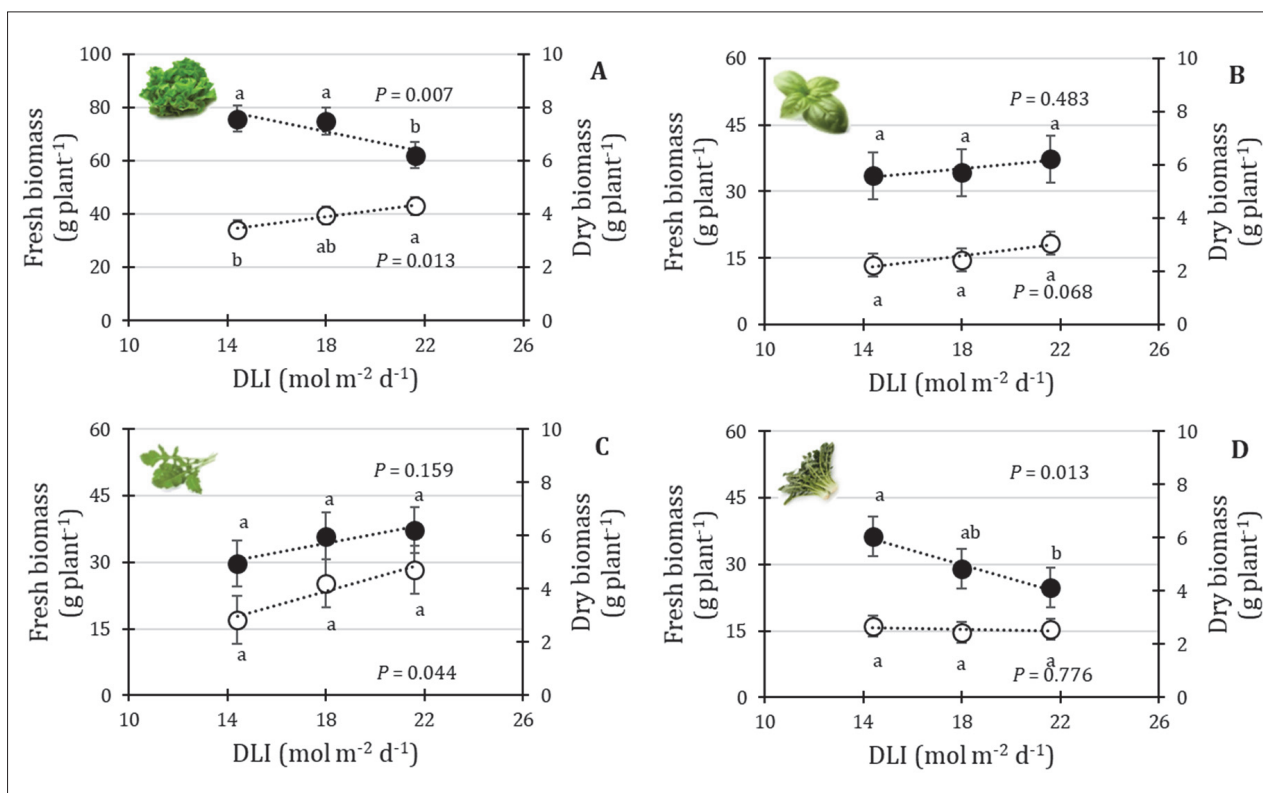


FIGURE 1. Fresh biomass (closed symbols) and dry biomass (open symbols) of (A) lettuce, (B) basil, (C) rocket and (D) chicory plants grown under different DLI obtained by changing photoperiod, respectively at 14.4 (16 h d^{-1}), 18 (20 h d^{-1}) and $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$ (24 h d^{-1}). Each value is the mean of three experiments, each with 18 replicate plants. Vertical bars represent SE. Different letters indicate statistically significant differences compared by Tukey's HSD test, at 5% significance level. P values represent the significance of the linear component.

Results

Plant growth parameters and leaf gas exchanges

Fresh biomass production of lettuce plants linearly decreased ($P=0.007$) as a result of DLI increase, with plants grown under the highest DLI ($21.6 \text{ mol m}^{-2} \text{ d}^{-1}$) presenting the lowest fresh biomass (-18%) as compared to plants grown under a DLI of 14.4 or $18 \text{ mol m}^{-2} \text{ d}^{-1}$ (Figure 1A). Contrarily, a linear ($P=0.013$) significant increase in lettuce dry biomass (+26%) was observed when DLI was increased from 14.4 to $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$ (Figure 1A). In basil (Figure 1B) and rocket (Figure 1C) plants, lighting regimes did not statistically affect fresh biomass. When considering dry biomass, a significant ($P=0.044$) linear increase was associated with DLI increase in rocket plants (Figure 1C), while dry biomass did not respond to variation of DLI in basil (Figure 1B) and chicory (Figure 1D) plants. As in the case of lettuce plants, fresh biomass of chicory plants linearly decreased ($P=0.013$) in response to DLI increase, with higher (+47%) fresh biomass detected in plants grown under 14.4 as compared with those supplied with $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$ (Figure 1D).

Dry matter content linearly increased with the increase of DLI, resulting the highest in lettuce (Figure 2A), basil (Figure 2B) and chicory (Figure 2D) plants grown at $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$. In rocket plants, dry matter was not significantly affected by variation in DLI (Figure 2C). RS ratio was not affected by lighting treatments in the four studied species (data not shown). Leaf number was not affected by DLI in basil, chicory and rocket, whereas a linear decrease ($P=0.025$) in response to growing DLI was observed only in lettuce (data not shown).

Leaf area of lettuce and chicory and specific leaf area of the four studied species linearly decreased with the increase of DLI (Figure 3). Accordingly, in lettuce and chicory, higher leaf area (+32% and +81%, respectively) and specific leaf area (+64% and 70%, respectively) were observed in plants grown under 14.4 as compared with $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$ (Figure 3A, D). In basil and rocket, although no changes occurred in leaf area, specific leaf area was higher (+30% and +45%, respectively) in plants grown under 14.4 as compared with $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$ (Figure 3B, C).

In basil, rocket and chicory, chlorophyll content linearly increased ($P=0.012$, $P=0.038$ and $P=0.012$, respectively) in response to growing DLI (Table 1). Contrarily, no responses to DLI variations were observed in chlorophyll content of lettuce plants (Table 1). Moreover, when DLI was augmented, leaf stomatal conductance linearly decreased in lettuce ($P=0.011$) and basil ($P=0.005$) plants, while it did not vary in rocket and chicory (Table 1).

Input use efficiency

From a resources use perspective, water use per plant was not significantly affected by light regimes in lettuce, basil, rocket and chicory (data not shown), with mean values of 1.03, 0.97, 0.81 and $0.86 \text{ L plant}^{-1}$, respectively. Accordingly, with regard to the capacity of plants to transform resources, water use efficiency (WUE) did not vary among lighting treatments in lettuce, basil and rocket plants (mean value of 64.2, 36.0 and $31.4 \text{ g FW L}^{-1} \text{ H}_2\text{O}$, for these three species respectively) (Figure 4A). Contrarily, in chicory WUE linearly ($P=0.015$) decreased with the increase of DLI, being WUE 33% higher in plants grown under $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$ as compared to plants grown under $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$ (Figure 4A).

Changes in DLI resulted in variations in energy use along the crop cycle, which respectively resulted in daily electricity requirements of $2.6 \text{ kWh m}^{-2} \text{ d}^{-1}$ (under $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$), $3.3 \text{ kWh m}^{-2} \text{ d}^{-1}$ (under $18 \text{ mol m}^{-2} \text{ d}^{-1}$) and $3.9 \text{ kWh m}^{-2} \text{ d}^{-1}$ (under $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$). Accordingly, in lettuce and chicory crops, energy use efficiency (EUE; fresh biomass per electricity use) was significantly reduced (respectively by 83 and 120%) when DLI of $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$ was used as compared with $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$ (Figure 4B). A linear downward trend ($P=0.011$) in response to rise in DLI was also evidenced in basil plants (Figure 4B). Contrarily, energy use efficiency in rocket plants did not vary with variation of DLI (mean value 50 g FW kWh^{-1}) (Figure 4B).

In chicory, light use efficiency (LUE; dry biomass per light integral) resulted the highest in plants grown under $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$ with an increase of 57% as compared to plants grown under higher DLI (Figure 4C). Similarly, a linear downward trend ($P=0.028$) in response to increasing DLI was observed in lettuce (Figure 4C). On the other hand, in basil and rocket plants no changes in LUE occurred (Figure 4C).

Discussion

Crop species respond differently to DLI variations

It is a well-documented phenomenon that variations of DLI influence vegetative growth of plants (Poorter et al., 2019). To date, several researches addressed the effect of variation of DLI through changes in light intensity in several indoor-grown crops, including lettuce (Nájera and Urrestarazu, 2019; Meng and Runkle, 2019; Yan et al., 2019), kale and spinach (Baumbauer et al., 2019), basil (Beaman et al., 2009; Dou et al., 2018; Meng and Runkle, 2019), rocket, endive, radish, and beetroot (Nájera and Urrestarazu, 2019). On the other hand, the consequences of changing DLI through modifications of photoperiod in indoor cultivation

TABLE 1. Effect of DLI (obtained by changing photoperiod from 16 to 24 h d^{-1} light) on leaf chlorophyll content (estimated by SPAD-value) and stomatal conductance (measured by leaf porometer) of lettuce, basil, rocket and chicory plants after three weeks of growth at a light intensity of $250 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

DLI ($\text{mol m}^{-2} \text{ d}^{-1}$)	Chlorophyll content (SPAD-value)				Stomatal conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$)			
	Lettuce	Basil	Rocket	Chicory	Lettuce	Basil	Rocket	Chicory
14.4 (16 h d^{-1})	34.33	44.06 b	64.74	39.95 b	236.4 a	190.0 a	292.0	224.8
18 (20 h d^{-1})	35.17	46.90 ab	65.75	42.25 ab	230.9 b	178.5 ab	289.8	218.6
21.6 (24 h d^{-1})	35.22	48.89 a	70.55	44.11 a	198.8 c	164.2 b	280.2	222.2
<i>P</i> value	0.562	0.012	0.038	0.012	0.011	0.005	0.443	0.847

Each value is the mean of three experiments, each with 18 replicate plants.

Different letters indicate significant differences compared by Tukey's HSD test, at 5% significance level.

P values represent the significance of the linear component.

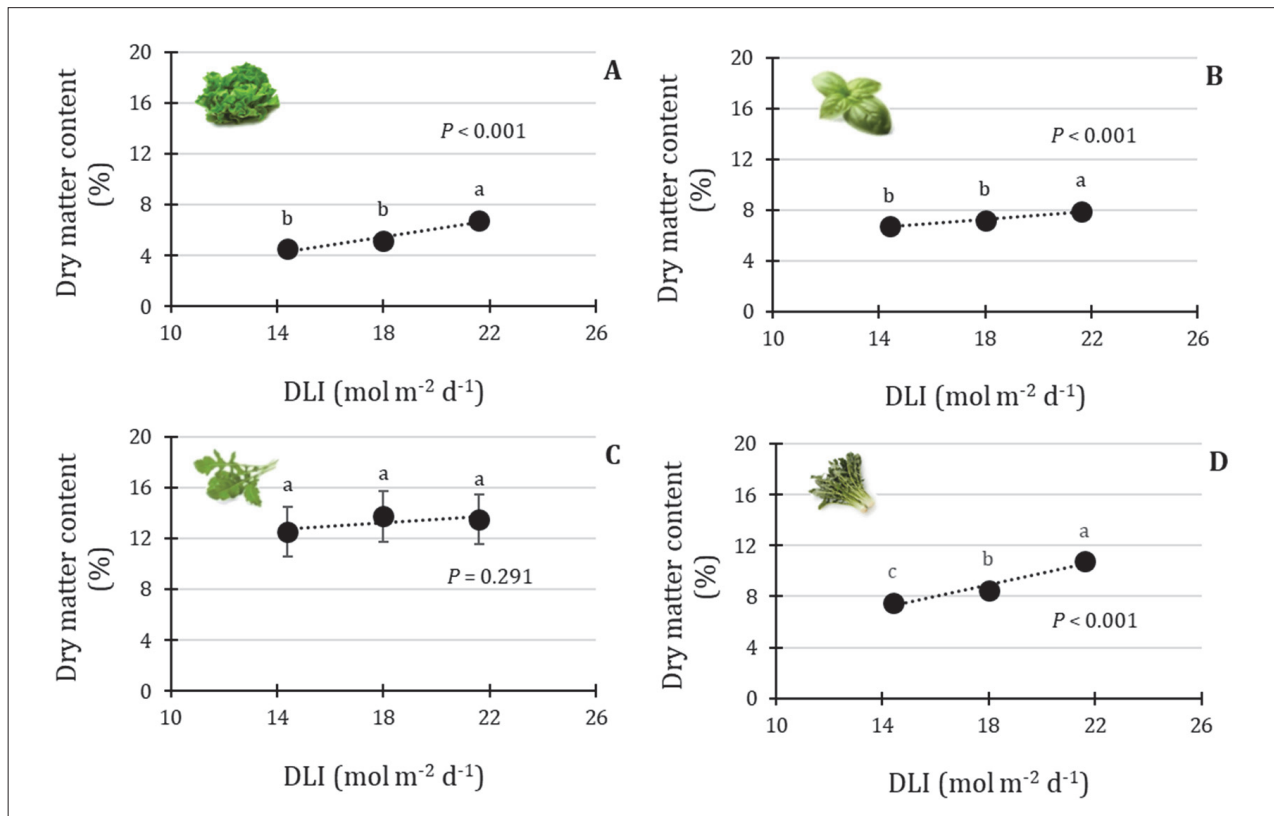


FIGURE 2. Dry matter content of (A) lettuce, (B) basil, (C) rocket and (D) chicory plants grown under different DLI obtained by changing photoperiod, respectively at 14.4 (16 h d⁻¹), 18 (20 h d⁻¹) and 21.6 mol m⁻² d⁻¹ (24 h d⁻¹). Each value is the mean of three experiments, each with 18 replicate plants. Vertical bars represent SE. Different letters indicate statistically significant differences compared by Tukey’s HSD test, at 5% significance level. *P* values represent the significance of the linear component.

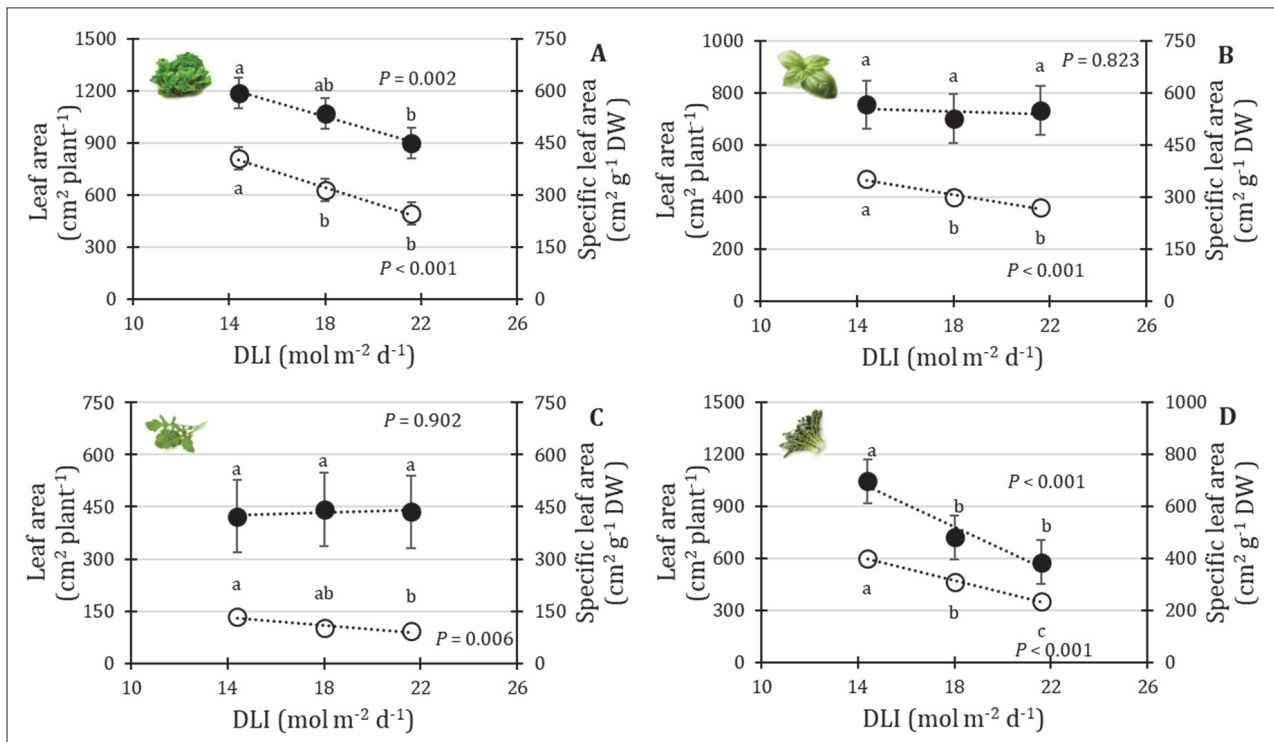


FIGURE 3. Leaf area (closed symbols) and specific leaf area (open symbols) of (A) lettuce, (B) basil, (C) rocket and (D) chicory plants grown under different DLI obtained by changing photoperiod, respectively at 14.4 (16 h d⁻¹), 18 (20 h d⁻¹) and 21.6 mol m⁻² d⁻¹ (24 h d⁻¹). Each value is the mean of three experiments, each with 18 replicate plants. Vertical bars represent SE. Different letters indicate statistically significant differences compared by Tukey’s HSD test, at 5% significance level. *P* values represent the significance of the linear component.

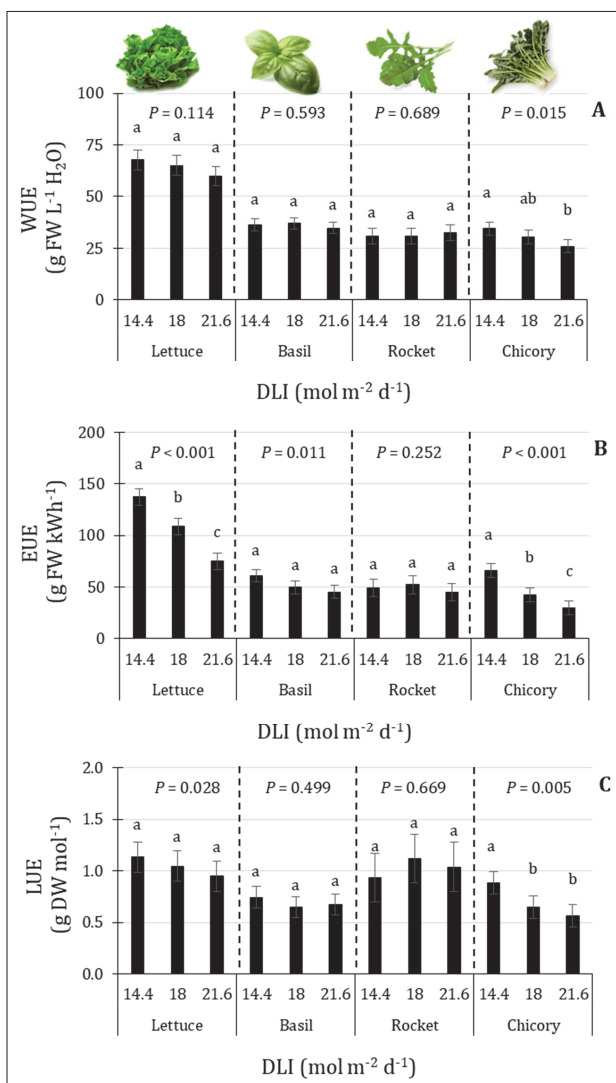


FIGURE 4. (A) Water use efficiency, (B) energy use efficiency, and (C) light use efficiency of lettuce, basil, rocket and chicory plants grown under different DLI obtained by changing photoperiod, respectively at 14.4 (16 h d⁻¹), 18 (20 h d⁻¹) and 21.6 mol m⁻² d⁻¹ (24 h d⁻¹). Each value is the mean of three experiments, each with 18 replicate plants. Vertical bars represent SE. Different letters indicate statistically significant differences compared by Tukey's HSD test, at 5% significance level. *P* values represent the significance of the linear component.

under LED light were, to date, targeted in few researches, mainly focussing on lettuce (Park et al., 2012; Zhang et al., 2018; Viršilė et al., 2019; Yan et al., 2019).

Previous studies on photoperiod management have shown that plant species differently react to 24 h d⁻¹ photoperiod (Velez-Ramirez et al., 2011). In several crops, including eggplant (Murage and Masuda, 1997), tomato (Demers et al., 1998), and onion (Van Gestel et al., 2005), continuous light led to leaf necrosis and chlorosis and reduced photosynthetic performances. On the other hand, continuous light was shown to increase biomass production in *Arabidopsis* (Lepistö et al., 2009) and lettuce (Gaudreau et al., 1994). In the present research, crop-specific responses to changes in DLI were observed. Accordingly, fresh biomass production and leaf area in lettuce (Figures 1A and 3A) and chicory (Figures 1D and 3D) were higher in plants grown under 14.4 mol m⁻² d⁻¹ (photoperiod of 16 h d⁻¹) as compared to 21.6 mol

m⁻² d⁻¹ (photoperiod of 24 h d⁻¹). Contrarily, in basil and rocket no statistically significant changes occurred in fresh biomass (Figure 1B, C) and leaf area (Figure 3B, C), even if in rocket a 37% increase in fresh biomass production of plants grown under the highest DLI as compared to plants grown under the lowest was observed (Figure 1C). Fresh weight of green and red lettuce was previously shown not to vary in response to DLI ranging 13 to 26 mol m⁻² d⁻¹ (obtained with photoperiod variations from 12 to 24 h d⁻¹, and under constant light intensity of 300 μmol m⁻² s⁻¹ supplied by red, blue and far red diodes) (Viršilė et al., 2019). However, the same study identified optimal DLI for leaf area development at, respectively, 17 and 19.5 mol m⁻² d⁻¹ in green and red lettuce plants (Viršilė et al., 2019). Fresh weight and leaf area of lettuce were also increased when DLI rose from 4.3 to 8.6 mol m⁻² d⁻¹ (obtained by varying photoperiod from 12 to 24 h d⁻¹ with a light intensity of 100 μmol m⁻² s⁻¹) in plants cultivated under fluorescent or a white LED lighting system (Park et al., 2012). In Yan et al. (2019), the factorial combination of light intensity and photoperiod was studied by targeting DLI ranging from 10.1 (with 200 μmol m⁻² s⁻¹ and 14 h d⁻¹), to 11.5 (with 200 μmol m⁻² s⁻¹ and 16 h d⁻¹), 12.6 (with 250 μmol m⁻² s⁻¹ and 14 h d⁻¹), and 14.4 (with 250 μmol m⁻² s⁻¹ and 16 h d⁻¹) mol m⁻² d⁻¹. Under these conditions, the authors suggested that a DLI of 11.5 would promote fresh biomass production in lettuce plants grown under fluorescent or LED lighting systems featuring a RB ratio of 1.1 or 2.1. Furthermore, lettuce biomass production was altered by variations of DLI (obtained by changes in photoperiod from 12 to 16 h d⁻¹) with significant interactions with other light parameters (spectrum and light intensity) (Zhang et al., 2018). When lettuce plants were grown under fluorescent lamps, a longer photoperiod (ranging 12 to 16 h d⁻¹) led to higher biomass production independently of the light intensity. Contrarily, in plants grown under LED lights featuring a RB ratio of 1.2, a photoperiod of 16 h d⁻¹ resulted in a greater biomass production with PPFD ≥ 250 μmol m⁻² s⁻¹ compared to a PPFD ≤ 200 μmol m⁻² s⁻¹ (Zhang et al., 2018). A great variability emerges among the observed results of different studies, which may be attributed to the elevated variability in terms of light quality compared (e.g., fluorescent lamps or white LED system in Park et al. [2012], and red blue and far red LEDs system in Viršilė et al. [2019]) or range of DLI tested (e.g., ranging 4.3 to 8.6 mol m⁻² d⁻¹ in Park et al. [2012], or 13 to 26 mol m⁻² d⁻¹ in Viršilė et al. [2019]). Furthermore, also environmental factors (e.g., temperature and CO₂ concentration) may play a fundamental role in plant responses to changes of light parameters, resulting in combined and synergistic effects (Pennisi et al., 2020). Within our former research (Pennisi et al., 2020), where lettuce and basil plants were grown in the same environmental condition as compared to the hereby reported experiments, under red and blue LEDs lighting systems supplying DLIs ranging 5.8 to 17.3 mol m⁻² d⁻¹ (obtained by varying PPFD from 100 to 300 μmol m⁻² s⁻¹), a DLI of 14.4 mol m⁻² d⁻¹ improved plants fresh weight, while a further increase of DLI (up to 17.3 mol m⁻² d⁻¹) reduced (in lettuce) or did not change (in basil) the fresh biomass production. Merging these results with the results hereby presented, possibly, an optimum function may be demonstrated, with a DLI of 14.4 mol m⁻² d⁻¹ resulting to be the optimal solution for lettuce and basil fresh biomass production.

In the present research, a linear increase in dry weight in response to rising DLI was observed in lettuce and rocket (Figure 1A, C). Similar response to DLI – although at lower DLI values, e.g., 4.3 to 8.6 mol m⁻² d⁻¹ – was previously ob-

served in lettuce (Park et al., 2012). In Yan et al. (2019), optimal DLI for increased lettuce dry weight was defined to be $11.5 \text{ mol m}^{-2} \text{ d}^{-1}$. Contrarily, Viršilė et al. (2019) did not observe variations in lettuce dry weight in response to changes in DLI between 13 and $26 \text{ mol m}^{-2} \text{ d}^{-1}$. Greater dry biomass production due to a prolonged photoperiod was previously associated with increase of leaf chlorophyll content in geranium, impatiens, pansy and petunia (Langton et al., 2003), and in both lettuce and tsukena (Fukuda et al., 2002). Similarly, in the present research, chlorophyll content linearly increased in response to DLI in basil, rocket and chicory (Table 1).

The rise of dry weight in lettuce plants (Figure 1A) in response to higher DLI resulted in increased dry matter content (Figure 2A), as also observed in basil (Figure 2B) and chicory (Figure 2D). The increase in dry matter content in plants undergoing longer days was previously related with higher carbohydrate production through photosynthesis (Zhang et al., 2018), and considered a qualitative marker (Scuderi et al., 2011). This hypothesis is further substantiated by the observed trends for SLA, which in all the studied crops presented a linear negative response to increase of DLI (Figure 3). SLA decreases in response to higher DLI are, however, mainly to be associated with decrease in leaf area in chicory, increase of dry weight in rocket and the combination of both trends in lettuce. These trends confirm the formerly reported difficulties in finding univocal interpretations of plant response to photoperiodic variations (Adams and Langton, 2005) across different plant species. The increase in dry weight (Figure 1A) and dry matter (Figure 2A) in lettuce plants exposed to long photoperiods is consistent with the hypothesis that longer days resulted in increased net assimilation ratio (NAR, e.g., the plant growth rate per unit of leaf area, closely related to the leaf photosynthetic rate minus respiration), rather than increased leaf area ratio (LAR, e.g., the ratio between plant leaf area and total plant biomass), as evident from the observed decrease in both leaf area and SLA (Figure 3A). While there is not a universal agreement on the mechanisms involved in photoperiodic responses, gains in dry biomass accumulation have been generally associated with either leaf area, chlorophyll content or photosynthetic efficiency (Langton et al., 2003; Adams and Langton, 2005). Accordingly, despite the fact that longer days never resulted in increased leaf area (Figure 3), they significantly reduced stomatal conductance in lettuce and basil while fostering increased chlorophyll content in basil and chicory (Table 1), ultimately resulting in increased dry matter content at highest DLI in lettuce (Figure 2A), basil (Figure 2B) and chicory (Figure 2D).

Alteration of plant circadian rhythm due to longer photoperiod was previously suggested to affect stomatal functionality (Arve et al., 2013), through modifications of endogenous hormone (e.g., abscisic acid) levels in plant tissues (Tallman, 2004). In the present research, increase in DLI created by prolonging the photoperiod resulted in linear decreases of stomatal conductance in both lettuce and basil (Table 1). The induction of stomatal closure by long photoperiod was previously observed in peanuts (*Arachis hypogea*) (Rowell et al., 1999), and associated with decrease of photosynthetic rates with modification of the Rubisco enzyme from oxygenation to carboxylation in plants grown under continuous light. The downregulation of photosynthesis in plants under continuous light was also claimed to be caused by saturation of starch and sugar concentrations in plant tissues (Van Gestel et al., 2005), overall also leading to reduced stomatal opening (Velez-Ramirez et al., 2011).

A DLI of $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$ (16 h d^{-1} photoperiod) enhances energy and light use efficiency in indoor cultivation of leafy vegetables and herbs

Indoor plant cultivation with artificial lighting is claimed to allow for massive water saving thanks to the use of closed-loop hydroponics, the recovery of the water transpired by plants and the possibility to adjust environmental conditions to the plant needs, with WUE values up to 50-times higher as compared to traditional cultivation systems (e.g., open field or greenhouse) (Kozai, 2013). Accordingly, observed WUE values reached up to 68 (lettuce), 37 (basil), 32 (rocket) and $34 \text{ g FW L}^{-1} \text{ H}_2\text{O}$ (chicory) (Figure 4A), resulting in increases up to 20-folds as compared to traditional cultivation systems (Orsini et al., 2020).

To date, stomatal behaviour and photosynthetic functionality were reported to vary in response to changes in DLI (Arve et al., 2013). When DLI was increased from 5.8 to $17.3 \text{ mol m}^{-2} \text{ d}^{-1}$ by means of growing light intensity (PPFD ranging 100 to $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$), WUE in lettuce resulted to grow up to a DLI of $11.5 \text{ mol m}^{-2} \text{ d}^{-1}$, while no further variations occurred when higher DLI values were supplied (Pennisi et al., 2020). In the same study, WUE in basil was shown to increase from 5.8 to $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$, while it did not vary between 14.4 and $17.3 \text{ mol m}^{-2} \text{ d}^{-1}$ (Pennisi et al., 2020). In the present research, WUE was not affected by changes in DLI in lettuce, basil and rocket, whereas in chicory plants, it was higher at the lowest DLI (Figure 4A). It may be advanced that when DLI increases, a decrease in photosystem efficiency in chicory occurred (Van Gestel et al., 2005), which overall resulted in reduced plant fresh biomass (Figure 1D) and therefore in WUE decrease (Figure 4A).

Energetic requirements are the most important factors limiting the expansion of indoor cultivation worldwide (Kozai, 2016b). In the present research, varying DLI altered both the electricity needs and the crop fresh biomass production in lettuce and chicory (Figure 1A, D), resulting in highest EUE values when plants were grown under $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$ (Figure 4B). Interestingly, an optimum function in EUE response to DLI changes (obtained through modifications in light intensity) was also recently reported for both lettuce and basil crops, where highest EUE values were observed at $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$, as compared with higher DLI (Pennisi et al., 2020). To further comprehend how DLI affected EUE in the studied crops, a comparison against previous researches (Pennisi et al., 2019a, b, c) under similar experimental conditions (RB=3, DLI of $12.4 \text{ mol m}^{-2} \text{ d}^{-1}$ obtained by applying $215 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for 16 h d^{-1}) is hereby proposed. Accordingly, while the current research yielded EUE in lettuce up to $138 \text{ g FW kWh}^{-1}$ (Figure 4B), in the previous experience it was limited to 91 g FW kWh^{-1} (Pennisi et al., 2019b). Similarly, EUE in basil plants was hereby found to reach 61 g FW kWh^{-1} (Figure 4B), against previous reports of 33 g FW kWh^{-1} (Pennisi et al., 2019a). Finally, while EUE in chicory and rocket reached 65 and 50 g FW kWh^{-1} (Figure 4B), previous experiences reported values of 19 and 25 g FW kWh^{-1} for these two crops, respectively (Pennisi et al., 2019c). The substantial increases in EUE brought by the current study are to be mainly associated with changes in intrinsic characteristics of the light source, and specifically the lamp energy consumption (W m^{-2}) and, consequently, its Photosynthetic Photon Efficacy (PPE, expressed as $\mu\text{mol J}^{-1}$) (Kusuma et al., 2020). Accordingly, the higher EUE should be associated with a decrease in lamp energy consumption ($164 \text{ vs. } 219 \text{ W m}^{-2}$) and a concurrent increase in PPE ($1.52 \text{ vs. } 0.98 \mu\text{mol J}^{-1}$) in the lamps used for the current research as com-

pared with those adopted in the previous experiments (Pennisi et al., 2019a, b, c). Indeed, lamps in all these experiments were supplied by the same manufacturer, and changes in the lamp performances over time shall mainly be associated with the rapid technological evolution that the sector is facing (Kusuma et al., 2020). Accordingly, LUE may turn out as a more stable indicator as compared with EUE on the plant response to different DLI. Significant response of LUE to DLI increase was detected in lettuce and chicory, where a linear negative trend was observed (Figure 4C). The absence of effects on LUE to changes in DLI in rocket (Figure 4C) should be related to a positive effect on dry biomass, which indeed resulted increased (+66%) in plants grown under the highest DLI as compared to those grown under the lowest (Figure 1C). Light spectral composition and the overall growing environment may also affect plant LUE (Ibaraki, 2016; Cocetta et al., 2017). Nevertheless, the hereby obtained LUE value for lettuce are also impressive when compared with LUE values ($0.37 \text{ g DW mol}^{-1}$) estimated by Graamans et al. (2018), in a study where a greenhouse lettuce growth model (Van Henten, 1994) was adapted to indoor farming conditions. Similarly, previous indication of low LUE (ranging 0.15 to $0.18 \text{ g DW mol}^{-1}$) in a growth chamber supplied with HPS lamps may have been affected by the wider spectrum of the light source (El-Nakhel et al., 2019). On the other hand, when similar light spectral features and environmental conditions were used (Pennisi et al., 2020), LUE values reached similar figures (e.g., up to 1.08 and $0.70 \text{ g DW mol}^{-1}$, in lettuce and basil, respectively).

Conclusions

The hereby presented study highlighted species-specific changes in plant morphology and physiological response to DLI as varied by photoperiod. The adoption of a DLI of $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$ (16 h d^{-1} photoperiod) enabled to obtain a greater fresh biomass production and leaf area in lettuce and chicory plants as compared to plants grown under a DLI of $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$ (24 h d^{-1} photoperiod). Furthermore, dry matter content in lettuce, basil and chicory was enhanced by the increase of DLI. Looking at the capacity to transform resources, energy use efficiency (EUE), expressed in grams of fresh biomass produced per unit of energy (in lettuce, basil and chicory plants), and light use efficiency (LUE), expressed in grams of dry biomass per light integral (in lettuce and chicory plants), linearly decreased with the increase of DLI, while EUE remained constant in rocket and LUE in basil and rocket. Furthermore, WUE of chicory plants resulted greater under a DLI of $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$ as compared to $21.6 \text{ mol m}^{-2} \text{ d}^{-1}$. From the study it may be concluded that indoor cultivation (using RB= 3 LED lamps providing PPFD = $250 \mu\text{mol m}^{-2} \text{ s}^{-1}$) of lettuce and chicory may be improved supplying a photoperiod of 16 h d^{-1} resulting in a DLI of $14.4 \text{ mol m}^{-2} \text{ d}^{-1}$.

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