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# Enhancing Growth and Nutritional Quality in Greenhouse-grown 'Little Gem' Lettuce using LED Supplemental Lighting

**Hossein Sheikhi[1], Mojtaba Delshad[1]*, Sasan Aliniaeifard[2], Kamahldin Haghbeen[3], Mesbah Babalar [1], Rasool Nasiri[1]**

1 Horticultural Sciences Department, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
2 Department of Horticulture, Faculty of Agricultural Technology, Aburaihan Campus, University of Tehran, Tehran, Iran
3 Department of Agricultural Biotechnology, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran

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**ABSTRACT**

Lettuce is one of the most important leafy vegetable crops. Despite lettuce being mostly grown in open fields, its greenhouse production is widely increasing. Lettuce growth comprises two distinct stages known as the seedling and head stages. The effect of supplemental lighting (SL) on lettuce growth was well studied, but the lighting requirement during the early stage of its growth and head development remained unknown. For this purpose, we evaluated nutritional qualities and growth in lettuce in response to SL in separate seedling and head development stages. The experiment involved SL with different daily light integral (DLI) provided by light-emitting diodes (LEDs). Light treatments included supplemental DLI of 8.64, 11.52, 12.96, and 17.28 mol $m^{-2}$ $d^{-1}$. The results revealed that the lighting period was more effective on lettuce biomass increase than the light intensity. Although the SL increased the photosynthetic pigment content of lettuce, its impacts on the two growth stages were not the same. In a way, the chlorophyll a, total chlorophyll, and carotenoid contents decreased under the SL conditions (DLI of 17.28 mol $m^{-2}$ $d^{-1}$ and light intensity of 300 µmol $m^{-2}$ $s^{-1}$ for 16 h). Increasing DLI caused a significant increase in the nutritional quality of lettuce, but antioxidant accumulation did not follow a similar trend in seedlings and mature plants. These findings confirmed that SL improves lettuce growth and quality, but optimal lighting requirements may vary depending on the growth stage.

## Introduction

In greenhouse systems, crop yields per cultivation area require proper management to increase, allowing sustainable intensification of food production (Stanghellini, 2014). During the last few decades, vegetable production has grown both in quantity and cultivated area, while at the same time, the number of farms has decreased, resulting in more vegetable production per farmer and grower (Petropoulou et al., 2023). In greenhouses, growers perform an integrated set of activities that result in highly dynamic production systems (Verdouw et al., 2015). Lettuce (*Lactuca sativa* L.) is one of the most cultivated greenhouse crops worldwide (Naznin et al., 2019). The production of lettuce in greenhouses is already undergoing a highly automated process. Multiple growing systems can

*Corresponding author's email: delshad@ut.ac.ir

be used for lettuce production in greenhouses, including hydroponic, aquaponic, and vertical growing systems (Petropoulou et al., 2023). This automation has led to a significant decrease in labor costs and has made higher-quality production possible. Additionally, it has made it possible to grow lettuce year-round, regardless of the weather outside. Production of lettuce in greenhouses is limited when the light requirements of this crop are not considered due to seasonal light limitations or greenhouse structural restrictions.

Plant growth and development depend on light as their main source of energy (Ghorbanzadeh et al., 2021). Plants can perceive and process information based on light signals from their surroundings to drive growth and development (Tarakanov et al., 2022). The recent development of new technologies has made it possible to manipulate the processes involved in plant growth. Light-emitting diodes (LEDs) are now used as highly efficient lighting sources to supplement natural sunlight in greenhouses and indoor cultivation systems (Zou et al., 2019). LED technology allows controlling the amount of light for greenhouse crops. LEDs are customized to match the spectrum required for the pigments and photoreceptors, leading to substantial changes in plant metabolism and morphology (Bantis et al., 2018). Studies revealed that lettuce growth happens stronger under LED lighting than in other lighting sources. These findings have been reviewed thoroughly by Bantis et al. (2018).

Low light intensities can reduce crop yield by reducing photosynthetic capacity (He et al., 2021). Plants elongate more (Hernández and Kubota, 2014) and have lower pigmentation (Matysiak, 2021) when the light intensity is lower than the photosynthetic capacity of the plants (Paucek et al., 2020). Besides light intensity, photoperiod is another crucial aspect of the lighting requirement of plants for their growth, development, and metabolism, including nutrient build-up, biomass accumulation, and pigment formation (Song et al., 2020).

In northern latitudes, providing an appropriate amount of light in greenhouses to reach a specific daily light integral (DLI) for different types of plants is a widely accepted greenhouse practice (Modarelli et al., 2022). Irrespective of the photoperiodic sensitivity of plant processes, prolonged photoperiods affect the DLI increase. Higher DLI supports more yield as well as a shorter production cycle of lettuce (Kelly et al., 2020). The DLI represents the amount of photosynthetic photon flux density (PPFD) during one day per m2; therefore, it contains both PPFD and photoperiod for the lighting requirement of the crops. In most crops, DLI (till-defined thresholds) correlates linearly with crop yield and nutrient accumulation (Dou et al., 2018). Harvestable yields in many horticultural crops reportedly increased by 0.8 to 1% by increasing the light level by 1% (Marcelis et al., 2006). Therefore, to maintain the optimal growth rates of the crops in greenhouses during seasons with insufficient sunlight, supplemental lighting (SL) is recommended.

Planting density and environmental conditions usually occur between seedling and cultivation stages in controlled-environment agriculture (Yan et al., 2019a). Additionally, the quality of seedlings affects both the growth and yield of crops after transplanting (Johkan et al., 2010). In lettuce plants, the response to light quality has been studied at different stages of growth (Chang and Chang, 2014), but no research has considered DLI. Although there were studies on the DLI requirement of lettuce, demonstrating lettuce responses to light dissemination strategy during its growth, a knowledge gap exists on the DLI requirements of seedlings compared to the latter stages of lettuce production. Thus, this study focused on the impact of SL provided by LEDs on separate stages of 'Little Gem' lettuce growth under greenhouse conditions.

## Materials and Methods

### *Plant materials and growth conditions*

The experimental duration spanned from October to December 2020 in the Research Greenhouse of the Department of Horticultural Science at the University of Tehran, Karaj, Iran (35°50′ 08″ N, 51°00′ 37″ E). Under greenhouse conditions, 'Little Gem' lettuce seeds (*Lactuca sativa* L.) were sown in 72-cell trays (53.5 × 27.5 × 4 cm) filled with 1-2 mm perlite substrate (October 29, 2020). The seeds germinated after 3 days, and with the emergence of true leaves, trays were transferred to the prepared supplemental LED lighting (November 10, 2020). Seedlings were watered every day with Yamazaki (1982) nutrient solution with the following composition (mg L): 236 $Ca(NO_3)_2.4H2O$, 404 $KNO_3$, 57 $NH_4H_2PO_4$, 123 $MgSO_4.7H_2O$, 14 Fe-EDTA, 0.615 $MnSO_4.H_2O$, 0.039 $CuSO_4.5H_2O$, 0.088 $ZnSO_4.7H_2O$, 1.127 $H_3BO_3$, 0.013 $(NH_4)6Mo_6O_{24}.4H_2O$. Seedlings grown under different SLs were harvested on November 30, 2020. The remaining healthy seedlings were transferred to plastic pots (12×12×12 cm) and then subjected to SL treatments. During lettuce growth, the same substrate and solution were used. Drip irrigation was used for the automatic delivery of nutrient solutions. The electrical conductivity (EC) and

nutrient solution pH were adjusted to 1.3 dS m and 5.8-6.1, respectively. The average relative humidity and air temperature from seed sowing to final harvest were 55-65% and 22-25 °C, respectively. A range of natural solar radiation above the plant surface at 1 p.m. was measured during the experiment, which varied from 80 to 220 µmol $m^{-2}$ $s^{-1}$.

### *Light treatments*

In this experiment, five light treatments, each with six replications and four plants per each experimental unit were used for growth and nutritional quality assessments of lettuce in the early (seedling) and end (head) growth stages. Light treatments included supplemental DLI of 8.64, 11.52, 12.96, and 17.28 mol $m^{-2}$ $d^{-1}$, which were obtained from a combination of supplemental light intensity at 200 and 300 µmol $m^{-2}$ $s^{-1}$ for 12 (SL200+12 and SL300+12, respectively) and 16 h (SL200+16 and SL300+16, respectively). Plants that received no SL and grew under natural greenhouse light were control specimens. The SL started at 7 a.m. and continued for 12 and 16 hours. LED panels (Iran Grow Light Company, Tehran, Iran) were placed at 30 cm on top of the plants. Since red (R) and blue (B) light spectra were recommended as proper light spectra for lettuce growth in the literature (Wojciechowska et al., 2016), these two light spectra were used as supplemental light for lettuce. The ratio of R and B light of the LED panels was 50%R:50%B. Spectral distributions of the LED fixtures were used in this experiment (Fig. 1).

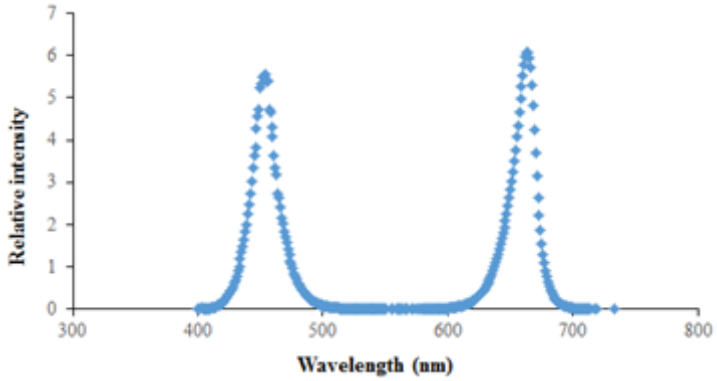


**Fig. 1.** LED spectral distribution for growing lettuce plants.

### *Sampling and morphological trait measurements*

A total of 240 plants were studied, half of them were harvested for the measurements at the seedling stage, and the other half were harvested at the head stage time. For each seedling and head stage, 60 plants were used to measure morphological traits. Another 60 plants were sampled and frozen in liquid nitrogen and quickly transferred to an −80 °C freezer for measuring biochemical traits. The fresh mass of roots and shoots (g plant) of all plants was determined using a digital electronic round pan balance (EPS, Guangdong, China), and the dried mass was determined after three days of oven drying at 65 °C. To measure leaf area, leaf length, and width (average of 4 largest leaves per plant), the leaves were scanned and then measured with Digimizer image analysis software (MedCalc Software Ltd, Belgium).

### *Chlorophylls and carotenoids*

Fresh leaves (0.2 g) were measured for chlorophyll a, b, and carotenoids. The samples were homogenized in a mortar with 80% acetone and then centrifuged at 9,500 g for 10 min. The supernatants were diluted with distilled water (total volume of 100 mL). The absorbance of the extraction at 663 nm, 645 nm, and 470 nm was read by UV-Vis spectrophotometer (Lambda EZ-201, Perkin-Elmer, Massachusetts, USA) for chlorophyll a and b, and carotenoids, respectively. Chlorophyll and carotenoid contents were calculated according to the following equations (Lichtenthaler and Wellburn, 1983):

$$\text{Chl a (mg/g)} = \frac{\left(12.72 \times A(633) - 2.59 \times A(645)\right)V}{1000W}$$

$$\text{Chl b (mg/g)} = \frac{\left(22.88 \times A(645) - 4.67 \times A(663)\right)V}{1000W}$$

$$\text{Car (mg/g)} = \frac{((1000 \times A(470) - 3.27 \times \text{Chl a} - 104 \times \text{Chl b})/229))V}{1000W}$$

where V and W are the total volume of acetone

extract (mL) and fresh mass (g) of the sample, respectively.

### *Soluble sugars*

Soluble sugar content was measured according to the method of Paquin and Lechasseur (1979). Fresh leaf tissue (0.5 g) was homogenized with 5 mL of 95% ethanol, and then 5 mL of 70% ethanol was used to wash the insoluble fraction of the extract. For 10 min, the samples were centrifuged at 10,000 rpm. Then, 0.1 mL of the extract was added to anthrone (150 mg of anthrone plus 100 mL of 72% sulphuric acid). The resulting solution was kept in a bath at 100 °C for 10 min. After cooling at room temperature, the absorbance was determined at 625 nm by a UV-vis spectrophotometer (Lambda EZ-201, Perkin-Elmer, Massachusetts, USA). Using a standard calibration curve prepared by different glucose concentrations, soluble sugar concentrations were calculated and expressed as mg g FW.

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

To determine total phenol, flavonoids, and antioxidant capacity, 0.5 g of fresh leaf tissue was homogenized with 2 mL of HCl-methanol-distilled water (1:80:19 v/v) and stored at 4 °C overnight. The products were then centrifuged at 10,000 rpm for 15 min at 4 °C. The Folin-Ciocalteu method was used to determine the total phenolic content in extracts (Meyers et al., 2003). The absorbance of the samples was read at 765 nm by a UV-vis spectrophotometer (Lambda EZ-201, Perkin-Elmer, Massachusetts, USA). Different concentrations of gallic acid were used for preparing the standard calibration curve, and total phenolic concentrations were expressed as mg gallic acid equivalent (GAE) g FW.

To determine total flavonoid content, a colorimetric method was used (Kaijv et al. 2006). To the tube containing 75 µL $NaNO_2$ solution (5%, w/v), 150 µL $AlCl_3$ solution (10%), and 500 µL NaOH solution (1 mol/L), 250 µL of the extract was added. The final volume reached 2.5 mL with distilled water and the absorbance value of the final solution was measured after 5 min at 507 nm by a UV-vis spectrophotometer (Lambda EZ-201, Perkin-Elmer, Massachusetts, USA). Different concentrations of quercetin were used for preparing the standard calibration curve, and total flavonoid concentrations were calculated and recorded as mg quercetin equivalent (QUE) g FW.

For measuring total antioxidant capacity (TAC), a 2,2-diphenyl-1-picryl-hidrazil (DPPH) radical-scavenging procedure was employed (Sánchez-Moreno et al. 1998). The absorbance values were read using a UV-vis spectrophotometer (Lambda EZ-201, Perkin-Elmer, Massachusetts, USA) at 517 nm, and the total antioxidant capacity was expressed as the percentage of DPPH radical inhibition, calculated through the following equation:

$$\text{TAC} = \frac{Ab\ sample - \text{A}b\ control}{\text{A}b\ control} \times 100$$

where TAC refers to the total antioxidant capacity and Ab refers to the absorbance at 517 nm.

### *Ascorbic acid*

Ascorbic acid was measured by the method of Kevers et al. (2007). For this purpose, 0.5 g of fresh leaf tissue was homogenized with 1.5 mL of metaphosphoric acid (5%) and centrifuged at 10,000 rpm for 15 min at 4 °C. In a tube containing 500 µL of metaphosphoric acid (10%), 300 µL of citrate buffer (pH 4.2), and 300 µL of 2,6-dichloroindophenol (DCIP, 3%), 100 µL of the obtained extract was added. After storing the sample for 45 min at 4 °C, the absorbance was read using a UV-vis spectrophotometer (Lambda EZ-201, Perkin-Elmer, Massachusetts, USA) at 520 nm. Different concentrations of ascorbic acid were used to prepare the standard calibration curve, and ascorbic acid concentrations were calculated and expressed as µg g FW.

### *Statistical analysis*

Statistical analysis of the obtained data was conducted using SAS software version 9.4 (SAS Institute, Cary, NC, USA). The data were analyzed using one-way analysis of variance (ANOVA), and then the least significant difference (LSD) test was used to compare the means ($P = 0.05$). Data are expressed as mean $\pm$ standard error (SE). Kolmogorov-Smirnov was used to test the normality of the data, and all data were analyzed under normal distribution conditions. Using R Studio 2022 software version 4.2.1 (RDC, 2010), principal component analysis (PCA) was performed, and correlations between the traits were analyzed.

## Results

### *Supplemental DLI affected lettuce growth and morphology*

Lettuce growth and morphology at both growth stages were significantly affected by supplemental light duration and intensity (Table 1). As a result of SL, the fresh and dry mass of shoots and roots was significantly increased compared to the biomass of those growing without SL in both growth stages. In the seedling stage, compared to control plants, SL with the

highest DLI (17.28 mol $m^{-2}$ $d^{-1}$) significantly increased shoot fresh mass, root fresh mass, shoot dry mass, and root dry mass by 5.3, 7.4, 10.5, and 3.7 times, respectively. A similar trend was also observed in the head stage, such that the shoot fresh mass, root fresh mass, shoot dry mass, and root dry mass increased by 11.8, 12.4, 9.5, and 15 times, respectively, compared to control plants (Table 1). Lettuce plants grown under DLI of 17.28 mol $m^{-2}$ $d^{-1}$ had the highest leaf area, leaf width, and number of leaves, while their leaf length was lower than those exposed to lower DLIs at both growth stages. There was a significant increase in leaf length where SL reception did not happen. Application of supplemental light during the low light seasons of the year in the greenhouse almost doubled the number of leaves (at the seedling), reaching 2.2 to 3 times (at the head stage) compared to the leaf count in the control seedlings (Table 1).

### *Supplemental DLI and leaf pigments*

SL significantly affected photosynthetic pigments in both growth stages. The concentration of photosynthetic pigments was higher in the seedling stage than in the head stage. In the seedling stage, with the increase of supplemental DLI from 8.64 to 12.96 mol $m^{-2}$ $d^{-1}$, the photosynthetic pigment concentrations increased. However, their concentrations decreased in response to a DLI value of 17.28 mol $m^{-2}$ $d^{-1}$. Compared to the control, in seedlings that were exposed to the SL300+12 (DLI 12.96 mol $m^{-2}$ $d^{-1}$), the concentrations of chlorophyll a, b, total, and carotenoid were increased by 1.37, 1.65, 8.24, and 1.36 times, respectively (Fig. 2A-D). In the head stage, increasing DLI to 17.28 mol $m^{-2}$ $d^{-1}$ induced the accumulation of photosynthetic pigments in the leaves. The concentration of photosynthetic pigments at the head stage was not affected by increasing DLI from 8.64 to 17.28 mol $m^{-2}$ $d^{-1}$. SL at this stage increased the concentration of chlorophyll a, b, total, and carotenoid by 1.23, 1.88, 1.39, and 1.39 times, respectively, compared to the control plants (Fig. 2A-D).

### *Supplemental DLI affected phytochemical accumulation*

Phytochemical concentrations in lettuce leaf at both seedling and head stages were positively affected by SL (Fig. 3). Except for the control plants, generally higher concentrations of soluble sugar occurred at the seedling stage compared to their concentrations at the head stage. At the seedling stage, the rise in DLI increased the soluble sugar content. At the seedling stage, the highest soluble sugar concentration was related to SL300+16 (DLI 17.28 mol $m^{-2}$ $d^{-1}$), which was approximately twice as much as the seedlings grown under natural sunlight only (Fig. 3A). At the head stage, despite significantly higher soluble carbohydrates compared to the control plants, no significant differences were observed in the concentration of soluble sugar among plants exposed to different levels of SL (Fig. 3A). At head stage, soluble sugar concentrations in plants exposed to SL increased by approximately 28% compared to soluble carbohydrate concentrations in plants grown under natural sunlight only.

Under all supplemental DLIs, the total phenolic concentration at the seedling stage was higher than its concentration at the head stage (Fig. 3B). Total phenolic concentration at both growth stages increased with higher DLI up to 12.96 mol $m^{-2}$ $d^{-1}$. However, its concentration decreased in response to a DLI of 17.28 mol $m^{-2}$ $d^{-1}$. At the seedling stage, the highest total phenol concentration was observed in SL300+12 (DLI 12.96 mol $m^{-2}$ $d^{-1}$), which showed an increase of 3.37 and 1.05 times compared to the control and SL300+16 (DLI 17.96 mol $m^{-2}$ $d^{-1}$), respectively. At head stage, the highest total phenol occurred by SL300+12, which was 2.1 and 1.1 times higher than the control and SL300+16, respectively. However, no significant difference was observed between SL300+12 and SL300+16 (Fig. 3B). Regardless of the lighting duration, higher supplemental light intensity increased the phenolic concentration significantly at both growth stages.

At seedling stage, the highest total flavonoid concentration was observed under the SL300+12 and SL300+16 (DLI 12.96 and 17.28 mol $m^{-2}$ $d^{-1}$, respectively), which was approximately six times higher than the total flavonoid concentration of control. At head stage, the total flavonoid concentration was the highest under the SL300+12, which showed a 4.81-fold increase compared to the control. An increase in DLI from 12.96 to 17.28 mol $m^{-2}$ $d^{-1}$ led to a significant decrease in total flavonoid concentration at the head stage, while this increase in DLI did not lead to substantial changes in total flavonoid concentration at the seedling stage (Fig. 3C).

**Table 1.** Analysis of variance (ANOVA) and mean value of morphological traits of 'Little Gem' lettuce under different levels of supplemental daily light integral (DLI; 0, 8.64, 11.52, 12.96, and 17.28 mol $m^{-2}$ $d^{-1}$) at seedling and head stages.

| | Treatments | Supplemental DLI (mol $m^{-2}$ $d^{-1}$) | (g plant) | | | | Leaf area ($cm^2$) | cm | | N. of leaves |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Shoot fresh mass | Root fresh mass | Shoot dry mass | Root dry mass | | Leaf length | Leaf width | |
| Seedling stage | SL200+12 | 8.64 | 2.02 ± 0.11$^{c}$ | 0.46 ± 0.06$^{b}$ | 0.11 ± 0.008$^{b}$ | 0.016 ± 0.001$^{c}$ | 65.45 ± 3.4$^{b}$ | 7.51 ± 0.18$^{bc}$ | 3.34 ± 0.08$^{c}$ | 8.16 ± 0.24$^{b}$ |
| | SL200+16 | 11.52 | 2.33 ±0.16$^{bc}$ | 0.61 ± 0.06$^{b}$ | 0.14 ± 0.008$^{b}$ | 0.031 ± 0.001$^{b}$ | 82.79 ± 8.4$^{a}$ | 7.58 ± 0.2$^{b}$ | 3.69 ± 0.08$^{b}$ | 7.83 ± 0.16$^{b}$ |
| | SL300+12 | 12.96 | 2.52 ± 0.18$^{b}$ | 0.56 ± 0.04$^{b}$ | 0.14 ± 0.01$^{b}$ | 0.02 ± 0.001$^{c}$ | 53.53 ± 9$^{b}$ | 6.91 ± 0.14$^{d}$ | 2.57 ± 0.07$^{b}$ | 8.33 ± 0.22$^{ab}$ |
| | SL300+16 | 17.28 | 3.43 ± 0.17$^{a}$ | 0.82 ± 0.1$^{a}$ | 0.21± 0.01$^{a}$ | 0.037 ± 0.002$^{a}$ | 84.38 ± 2.8$^{a}$ | 7.02 ± 0.18$^{cd}$ | 3.92 ± 0.07$^{a}$ | 8.75 ± 0.17$^{a}$ |
| | 0 | - | 0.64 ± 0.00$^{d}$ | 0.11 ± 0.00$^{c}$ | 0.02 ± 0.00$^{c}$ | 0.01 ± 0.000$^{d}$ | 23.77 ± 0.24$^{c}$ | 9.2 ± 0.18$^{a}$ | 2.29 ± 0.04$^{d}$ | 4.75 ± 0.17$^{c}$ |
| Significance | | | **** | **** | **** | **** | **** | **** | **** | **** |
| Head stage | SL200+12 | 8.64 | 48.49 ± 2.18$^{d}$ | 5.38 ± 0.30$^{c}$ | 2.12 ± 0.09$^{c}$ | 0.21 ± 0.01$^{c}$ | 838.1 ± 26$^{c}$ | 13.14 ± 0.21$^{c}$ | 7.55 ± 0.13$^{b}$ | 22.08 ± 0.33$^{c}$ |
| | SL200+16 | 11.52 | 74.47 ± 2.12$^{b}$ | 7.43 ± 0.34$^{b}$ | 3.04 ± 0.08$^{b}$ | 0.29 ± 0.01$^{b}$ | 1073.8 ± 29.5$^{ab}$ | 13.72 ± 0.19$^{b}$ | 8.34 ± 0.05$^{a}$ | 29.16 ± 0.67$^{a}$ |
| | SL300+12 | 12.96 | 68.56 ± 1.89$^{c}$ | 7.83 ± 0.14$^{b}$ | 2.87 ± 0.07$^{b}$ | 0.32 ± 0.01$^{b}$ | 1012.5 ± 16.2$^{b}$ | 13.15 ± 0.13c | 8.18 ± 0.14$^{a}$ | 27.41 ± 0.87$^{b}$ |
| | SL300+16 | 17.28 | 88.49 ± 2.27$^{a}$ | 11.33 ± 0.2$^{a}$ | 3.62 ± 0.11$^{a}$ | 0.45 ± 0.01$^{a}$ | 1116.7 ± 31.6$^{a}$ | 11.93 ± 0.14$^{d}$ | 8.37 ± 0.13$^{a}$ | 30.33 ± 0.43$^{a}$ |
| | 0 | - | 7.50 ± 0.25$^{e}$ | 0.91 ± 0.08$^{d}$ | 0.38 ± 0.01$^{d}$ | 0.03 ± 0.00$^{d}$ | 195.2 ± 10$^{d}$ | 14.39 ± 0.23$^{a}$ | 4.31 ± 0.16$^{c}$ | 10 ± 0.27$^{d}$ |
| Significance | | | **** | **** | **** | **** | **** | **** | **** | **** |

**** indicates significance ($p < 0.0001$). Significant differences in the same column are indicated by different letters (LSD, $p = 0.05$, $N = 6$). Values are expressed as mean ± SE.

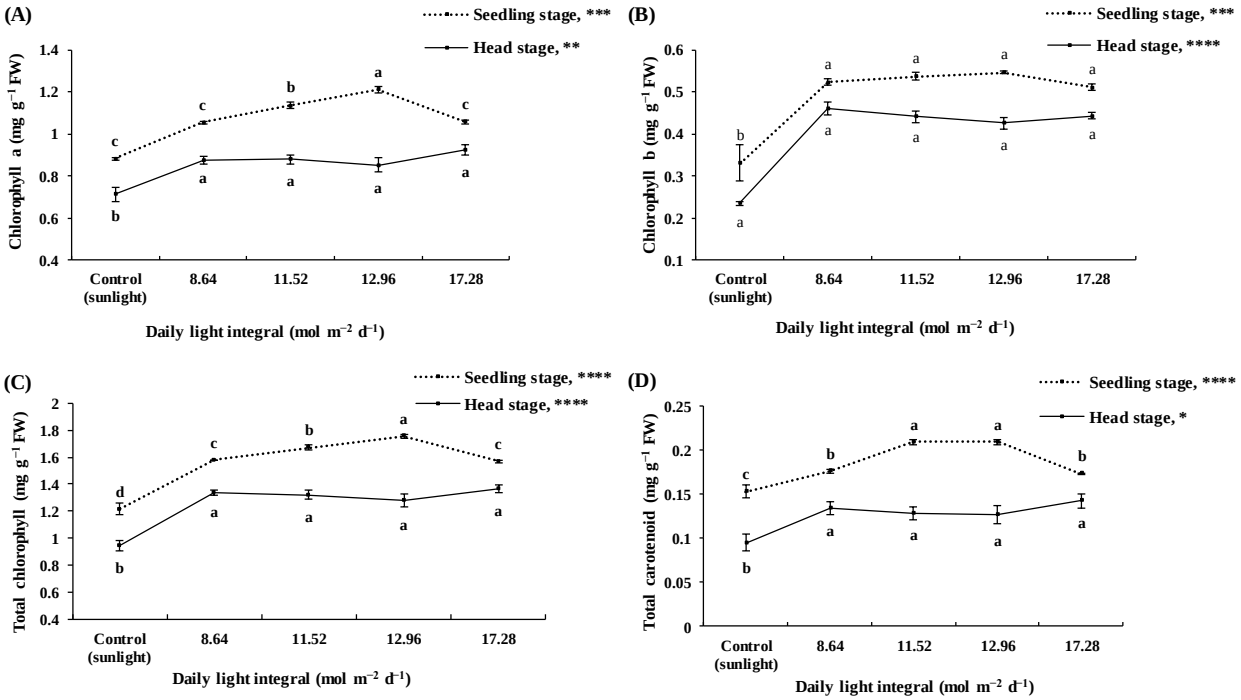


**Fig. 2.** Photosynthetic pigments of 'Little Gem' lettuce under different levels of supplemental daily light integral (DLI). Chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoid are indicated by A, B, C, and D, respectively. *, **, ***, and **** indicate significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively). Differences between mean values with the same letter are not significant (LSD, $p = 0.05$, $N = 3$). Values are expressed as mean ± SE.

At both growth stages, an increase in DLI up to 12.96 mol $m^{-2}$ $d^{-1}$ was accompanied by an increase in antioxidant capacity, and after that, it was statistically stable. At the seedling stage, the highest antioxidant capacity occurred by the SL300+12 and SL300+16, which caused a 2.6% and 2.2% increase compared to the control, respectively. At head stage, this increase in antioxidant capacity occurred under the SL300+12 and SL300+16, which was 3.6% and 4.1% higher than the control, respectively (Fig. 3D).

Regarding ascorbic acid, increasing the DLI at both stages of lettuce growth increased the ascorbic acid level. However, there was no significant difference in the concentration of ascorbic acid in response to DLI from 11.52 to 17.28 mol $m^{-2}$ $d^{-1}$ at the seedling stage and from 12.96 to 17.28 mol $m^{-2}$ $d^{-1}$ at the head stage. At the seedling stage, plants grown under SL200+16, SL300+12, and SL300+16 had the highest ascorbic acid concentration. They produced approximately two times more ascorbic acid than plants grown under natural sunlight only. At the head stage, the SL300+12 and SL300+16 increased the ascorbic acid concentration by almost 2.5-fold compared to the control (Fig. 3E).

## *Principal component analysis*

PCA resulted in a comprehensive overview and interpretation of morphological and qualitative characteristics in lettuce at the seedling and head stages in response to SL under greenhouse conditions (Fig. 4 and 5). At the seedling stage, the first principal component (PC1) accounted for 83.2% of the cumulative variance, while PC2 explained 9.6% of the total variance (Fig. 4). The SL300+16 was positioned on the positive side of PC1 in the upper right quadrant of the individuals-PCA (Fig. 4A), as it produced plants with higher shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM), root dry mass (RDM), leaf width (LW), leaf area (LA), number of leaves (NoL), and soluble sugar (SS) (Fig. 4B). Moreover, the SL200+16 and SL300+12 were located on the negative side of PC1 in the lower right quadrant of the individuals-PCA (Fig. 4A), as they produced plants with higher concentrations of chlorophyll a (Chla), b (Chlb), and total (TChl) and carotenoid (Car) as well as antioxidant capacity (AC), total phenolic (TPh), total flavonoid (TF), and ascorbic acid (AsA) (Fig. 4B). On the other hand, the control treatment was located on the upper left quadrant (Fig. 4A), characterized only by a higher leaf length (LL) (Fig. 4B). At the head stage, the first two

principal components described 89.9% and 5.2% of the data variability, respectively (Fig. 5). The SL300+12 and SL300+16 were positioned on the positive side of PC1 in the upper right quadrant of the individuals-PCA (Fig. 5A). These treatments were characterized by higher concentrations of TF, TPh, AC, RDM, SFM as well as RFM (Fig. 5B). According to Fig. 5A, the SL200+12 and SL200+16 were located on the negative side of PC1 in the lower right quadrant of the individuals-PCA. These treatments also produced plants with higher SDM, NoL, LA, LW, TChl, Chla, Chlb, and Car (Fig. 5B). Furthermore, the control group occurred in the upper left quadrant (Fig. 5A), characterized only by a higher LL (Fig. 5B).

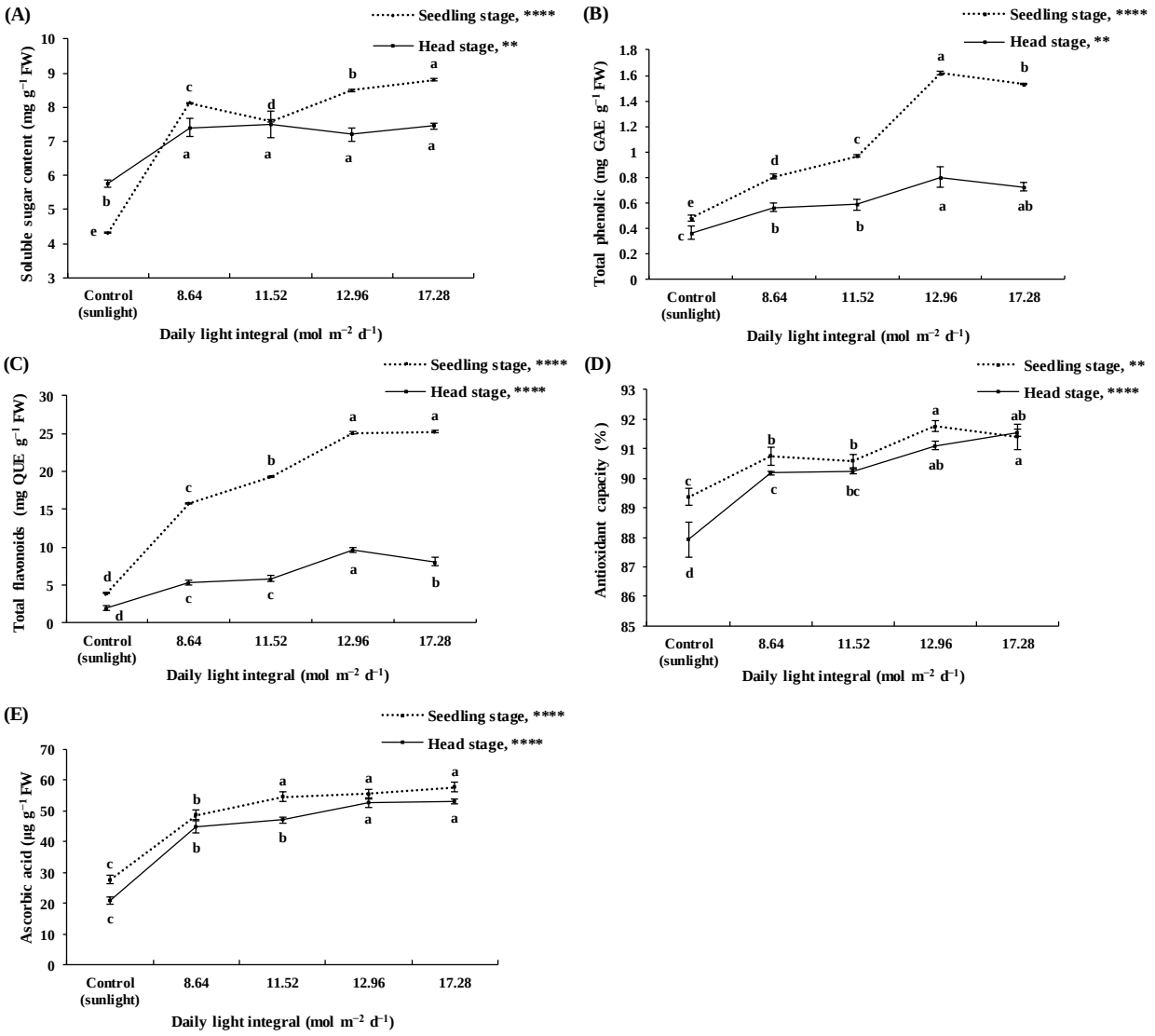


**Fig. 3.** Phytochemical contents of 'Little Gem' lettuce under different levels of supplemental daily light integrals (DLI). Soluble sugar, total phenolic, total flavonoids, antioxidant capacity, and ascorbic acid are indicated by A, B, C, D, and E, respectively. ** and **** indicate significance at a probability level of $p < 0.01$ and $p < 0.0001$, respectively. Differences between means with the same letter are not significant (LSD, $p = 0.05$, $N = 3$). Values are expressed as mean ± SE.

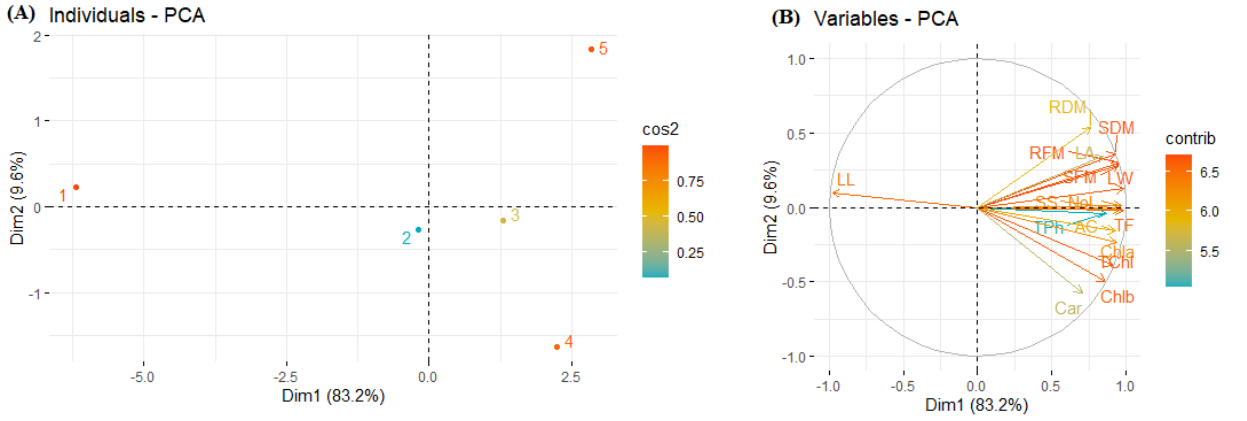


**Fig. 4.** Principal component analysis of morphological and biochemical traits of 'Little Gem' lettuce plants at the seedling stage under different levels of supplemental light intensity and duration. The location of the treatments (1, control; 2, SL200+12; 3, SL200+16; 4, SL300+12; 5, SL300+16) is presented in the individuals-PCA (A) and the traits are shown in the variables-PCA (B). Shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM), root dry mass (RDM), leaf width (LW), leaf length (LL), leaf area (LA), number of leaves (NoL), chlorophyll a (Chla), b (Chlb), and total (TChl), carotenoid (Car), soluble sugar (SS), total phenolic (TPh), total flavonoids (TF), antioxidant capacity (AC), and ascorbic acid (AsA).

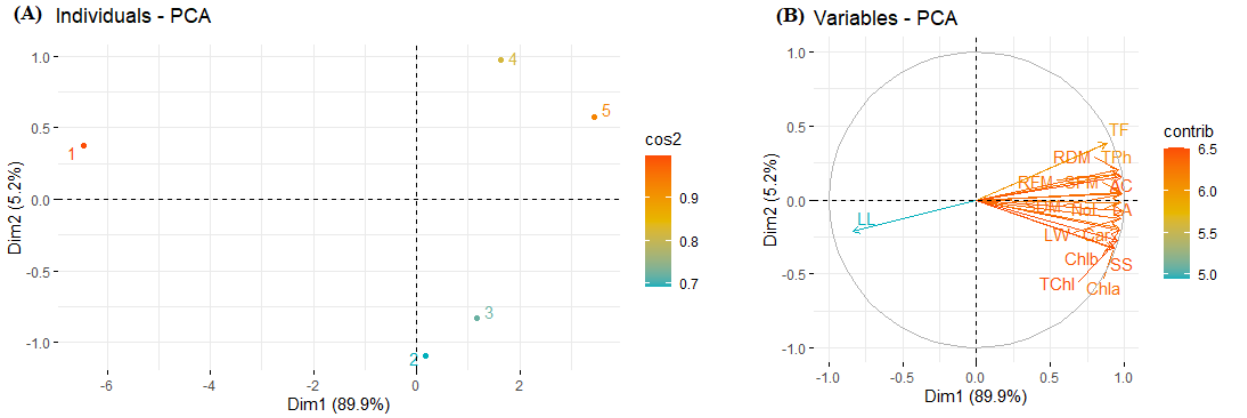


**Fig. 5.** Principal component analysis of morphological and biochemical traits of 'Little Gem' lettuce plants at head stage under different levels of supplemental light intensity and duration. The location of the treatments (1, control; 2, SL200+12; 3, SL200+16; 4, SL300+12; 5, SL300+16) is presented in the individuals-PCA (A) and the traits are shown in the variables-PCA (B). Shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM), root dry mass (RDM), leaf width (LW), leaf length (LL), leaf area (LA), number of leaves (NoL), chlorophyll a (Chla), b (Chlb), and total (TChl), carotenoid (Car), soluble sugar (SS), total phenolic (TPh), total flavonoids (TF), antioxidant capacity (AC), and ascorbic acid (AsA).

## *Correlation Analysis*

Correlation analysis among the measured traits showed that at both growth stages, LL correlated significantly and negatively with the other measured traits (Fig. 6A and B). At the seedling stage, there was a significant positive correlation among the traits (without LL), except for a non-significant positive correlation between RDM with TChl, Chla, Chlb, Car, and AsA as well as among LA, Car, TPh, and AC. In addition, Car correlated insignificantly but positively with RFM, SFM, and SDM (Fig. 6A). At the head stage, except for the LL, which significantly and negatively correlated with the other traits, most of the traits correlated significantly and positively with each other. Only the correlation between TPh and SS was not significant (Fig. 6B).

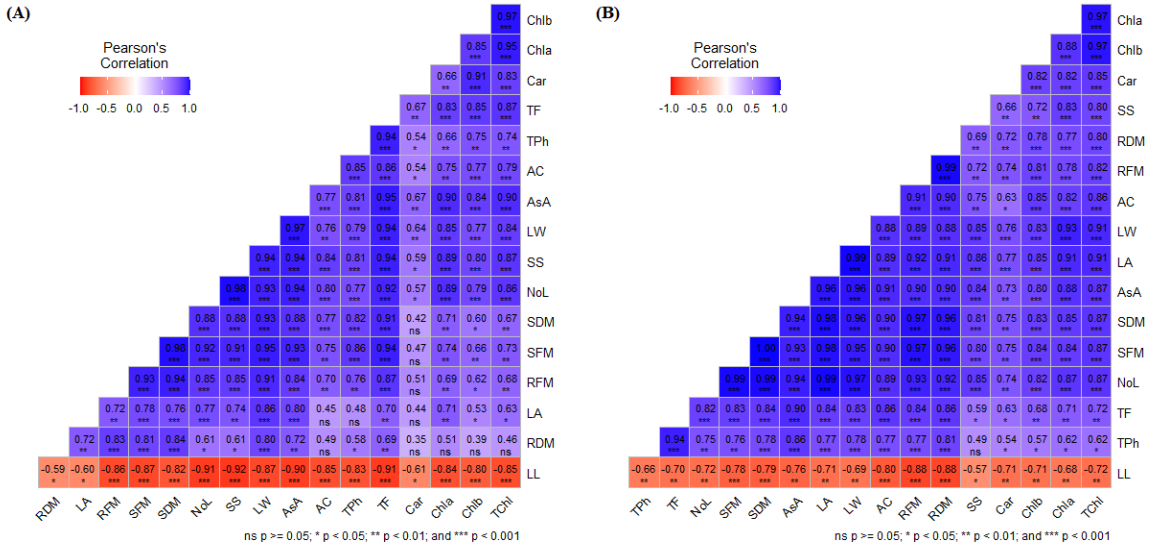


**Fig. 6.** Pearson's correlation coefficients among studied traits in 'Little Gem' lettuce plants under different levels of supplemental light intensity and duration at seedling (A) and head (B) stages. Shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM), root dry mass (RDM), leaf width (LW), leaf length (LL), leaf area (LA), number of leaves (NoL), chlorophyll a (Chla), b (Chlb), and total (TChl), carotenoid (Car), soluble sugar (SS), total phenolic (TPh), total flavonoids (TF), antioxidant capacity (AC), and ascorbic acid (AsA).

## Discussion

### *Biomass accumulation*

In the present study, the lettuce biomass enhanced at the seedling and head growth stages under the applied SL programs. However, the highest increase was observed with the supplemental DLI of 17.28 mol $m^{-2}$ $d^{-1}$. In previous research, regardless of the light spectrum, increasing the DLI from 5 to 15 mol $m^{-2}$ $d^{-1}$ (Yan et al., 2019b) or 6 to 17 mol $m^{-2}$ $d^{-1}$ (Zhang et al., 2018) approximately doubled the fresh mass of lettuce. Kelly et al. (2020) also reported that, regardless of light intensity and photoperiod combination, the fresh and dry mass of 'Rex' and 'Roxai' lettuces increased as the DLI increased. Nonetheless, the authors did not provide any recommendations concerning the seedling phase of lettuce plants since they considered only the lettuce maturity stage. The findings of the present study are consistent with those of Givens et al. (2023), indicating that an increase of DLI from 5.2 to 51.8 mol $m^{-2}$ $d^{-1}$ (60 to 600 µmol $m^{-2}$ $s^{-1}$) increased fresh and dry mass in 'Rex' seedlings by 475% and 1050%, respectively. Our results showed that the lighting duration was more effective than the light intensity in increasing lettuce biomass. At the seedling stage, the SL200+16 (DLI 11.52 mol $m^{-2}$ $d^{-1}$) produced the same shoot fresh mass, root fresh mass, and shoot dry mass, while higher root dry mass compared to the SL300+12 (DLI 12.96 mol $m^{-2}$ $d^{-1}$). At the head stage, lettuce plants grown under SL200+16 had higher shoot fresh mass (74.47 g $plant^{-1}$) than those grown under SL300+12 (68.56 g $plant^{-1}$). However, no significant difference was observed in their root fresh mass, shoot fresh mass, and root dry mass (Table 1). Under specific light intensities, lower DLI can be compensated by low light intensity but by increasing the lighting duration. Kelly et al. (2020) stated that at a DLI of 15.6 mol $m^{-2}$ $d^{-1}$, lower PPFD with longer photoperiod produced greater lettuce fresh and dry mass than higher PPFD with shorter photoperiod. Weaver and van Iersel (2020) examined the greenhouse-grown lettuce 'Little Gem' under SL with a DLI of 17 mol $m^{-2}$ $d^{-1}$. They reported that a longer photoperiod led to an increase in photosynthetic efficiency and stimulated plant growth. Abdullah et al. (2023) indicated that the growth rates of plants in the presence of LED grow lights are most significantly influenced by the duration of exposure. It is possible that a longer light duration with the same DLI results in a higher daily photochemical integral and quantum yield of photosystem II (ΦPSII) (Elkins and van Iersel, 2020). Increasing light intensity leads to the closure of a larger fraction of the PSII reaction center, resulting in a decrease in ΦPSII of lettuce (Elkins and van Iersel, 2020). However, a high light intensity can reduce the efficiency of photosynthesis by increasing flux density until the light saturation point, where further increases in PPFD do not induce photosynthesis, which does not convey its effects on the induction of plant growth and

biomass (Kelly et al., 2020). Theoretically, only the increase in the photoperiod elevates net daily photosynthesis once it reaches light saturation (Kelly et al., 2020). However, in a normal photoperiod, it has been well established that plants tend to increase their growth rate when the light intensity increases within a specific range (Esmaili et al., 2021a, 2020b; Ghorbanzadeh et al., 2021; Kang et al., 2013).

At both growth stages of lettuce, the highest leaf area, leaf width, and number of leaves were obtained under supplemental DLI of 17.28 mol $m^{-2}$ $d^{-1}$ (Table 1). The increase in biomass of lettuce plants grown under DLI of 17.28 mol $m^{-2}$ $d^{-1}$ compared to other treatments can also be related to a higher number of leaves and leaf area. Increasing DLI generally increases the number of leaves with a broader surface (Kang et al., 2013; Kelly et al., 2020; Sago, 2016). According to Givens et al. (2023), leaf numbers increased by 60% at harvest as the DLI changed from 5.2 to 34.6 mol $m^{-2}$ $d^{-1}$, confirming our results. At the same time, plants receiving SL had shorter leaf lengths, and those grown under natural sunlight showed shade avoidance responses (Table 1) that typically produced thinner and longer leaves (Spalholz et al., 2020). In the seedlings stage, the number of leaves and leaf width were statistically the same for the SL200+16 and the SL300+12, but the leaf length and the leaf area were higher at SL200+16, which can be attributed to the strategy of plants for capturing light. In the head stage, the light duration affected the number of leaves more. In general, SL200+16 (DLI 11.52 mol $m^{-2}$ $d^{-1}$) produced more leaves than SL300+16 (DLI 17.96 mol $m^{-2}$ $d^{-1}$) (Table 1). Under low light intensity, plants tend to have larger, thinner leaves (making higher specific leaf area) for better light capture when the photon spectrum is maintained at a constant level (Evans and Poorter, 2001). In support of our results, cucumber seedlings grown under long SL duration with low light intensity showed shade avoidance responses (Yan et al., 2021).

## *DLI threshold for pigment accumulation*

Lettuce plants grown under supplemental DLI at both growth stages exhibited higher concentrations of photosynthetic pigment than their concentrations in plants grown under natural sunlight only (Fig. 2A-D). However, at the seedling stage, the supplemental DLI of 17.28 mol $m^{-2}$ $d^{-1}$ decreased the concentration of photosynthetic pigments compared to lower supplemental DLIs (8.64, 11.52, and 12.96 mol $m^{-2}$ $d^{-1}$). However, this trend did not occur at the head stage, and all supplemental DLIs were at the same statistical level (Fig. 2A-D). Fu et al. (2012) showed that chloroplast size and density are affected by low-light conditions, indicating fewer chloroplast accumulation than under high-light conditions. Plants grown in low-light environments appeared to have denser chloroplasts than plants grown in higher-light environments (Baumbauer et al., 2019). Inadequate light exposure interferes with chlorophyll and its ability to perform its full potential, which leads to yellow leaves and eventual death (Nájera et al., 2023). Yan et al. (2021) reported that in cucumber seedlings, supplemental light intensity increased chlorophyll content. Considering our study, the red (R) and blue (B) LEDs (50%R:50%B) functioned as complementary lighting. Thus, the effect of these spectra can be another explanation for the increase in the concentration of photosynthetic pigments in lettuce plants. Chen et al. (2016) reported that supplemental R and B lights are more efficient in chlorophyll and carotenoid accumulation in lettuce plants than other light spectra. Other researchers indicated similar results (Heo et al., 2002; Mizuno et al., 2009; Johkan et al., 2012). The synthesis of plant pigments depends on the exposed wavelengths, specifically the R and B ranges of the spectrum (McCree, 1971). R light stimulates the development of the photosynthetic apparatus (Sæbø et al., 1995), while B light stimulates the generation of chloroplasts and the synthesis of chlorophyll in plants (Cosgrove, 1981; Senger, 1982). However, Ghorbanzadeh et al. (2021) reported that higher light intensity accelerates the development of the photosynthetic system in lettuce. The concentration of photosynthetic pigments was higher at the seedling stage than at the head stage. A high chlorophyll concentration occurred in young leaves, per an earlier study (Sabir et al. 2010).

The results showed that SL300+16 (17.28 mol $m^{-2}$ $d^{-1}$) reduced the concentration of photosynthetic pigments at the seedling stage compared to lower supplemental DLIs (Fig. 2A-D). A high light intensity had a destructive effect on the structure of chlorophylls and carotenoids. The relative chlorophyll concentration in lettuce plants grown at a high PPFD of 800 µmol $m^{-2}$ $s^{-1}$ was reportedly lower than that of lower PPFDs (100-400 µmol $m^{-2}$ $s^{-1}$), suggesting that severe light stress can adversely affect chlorophyll content (Fu et al., 2012). Supplemental DLI of 17.28 mol $m^{-2}$ $d^{-1}$ did not adversely affect photosynthetic pigments at the head stage of 'Little Gem' lettuce, which may indicate that chlorophyll synthesis in 'Little Gem' lettuce responds positively to high DLI. However, its seedlings did not show the same behavior.

Some reports show different behaviors of various plant species to high DLI regarding photosynthetic pigments. For example, Lefsrud et al. (2006) reported that the total chlorophyll concentration of kale increased with increasing DLI from 11 to 21.6 mol $m^{-2}$ $d^{-1}$. In contrast, Fu et al. (2012) reported that as the DLI increased from 4 to 14 mol $m^{-2}$ $d^{-1}$, chlorophyll a and b concentrations decreased in the lettuce.

Yao et al. (2017) stated that the chlorophyll concentration of rape seedlings (Brassica napus L.) was not affected by DLI (8.6 and 12.96 mol $m^{-2}$ $d^{-1}$). Thus, the plant species, cultivar, and growth stage respond differently to DLI regarding chlorophyll and carotenoid concentrations.

### *Lettuce soluble sugar and antioxidant compounds induced by higher DLIs*

SL significantly increased the lettuce soluble sugar concentration at both growth stages compared to plants that did not receive SL (Fig. 3A). Lettuce plants are generally tastier when they contain high soluble sugar content (Lin et al., 2013). Light drives photosynthesis, which is the engine for carbohydrate production. Wojciechowska et al. (2015) reported similar findings in greenhouses when lamb's lettuce plants experienced SL exposure. An increase in soluble sugars by applying R and B LEDs as SL has reportedly been beneficial (Wanlai et al., 2013). At the seedling stage, the increase of supplemental DLI up to 17.28 mol $m^{-2}$ $d^{-1}$ (300 µmol $m^{-2}$ $s^{-1}$ for 16 h) was effective on soluble sugar accumulation, while at the head stage, there was no difference among different levels of supplemental DLI (Fig. 3A). Lin et al. (2018) reported that higher light intensity (150 versus 120 µmol $m^{-2}$ $s^{-1}$) increased soluble sugars in lettuce. In another study, Bian et al. (2015) reported that light intensity higher than 300 µmol $m^{-2}$ $s^{-1}$ induced soluble sugar accumulation in plants. The increase in soluble sugars under SL can be related to its effects on photosynthesis. Photosynthesis occurs in chloroplasts of mesophyll cells, and more light enables chlorophylls to absorb more photons, resulting in higher photosynthesis rates. Furthermore, since photosynthesis produces organic material containing sugar, a higher photosynthetic rate within a specific range can produce more sugars in a shorter timeframe (Lin et al., 2018).

Low light intensities provide fewer excitations in the electron transport system and reduce the photosynthetic rate, leading to the down-regulation of photosynthetic product synthesis (Feng et al., 2019). Thus, it is not entirely surprising when soluble sugar levels increase under SL conditions. Regarding the differences in soluble sugar content at the two growth stages, young leaves usually have a greater net photosynthetic rate than old leaves (Lawlor, 1995), so they use light more efficiently and produce more carbohydrates and photosynthetic products.

Our results demonstrated that SL in both growth stages correlated positively with total phenolic and flavonoid concentrations, both antioxidant compounds, with antioxidant capacity and ascorbic acid (Fig. 3B-E). SL has been recommended as an effective way to make lettuce plants more visually appealing and nutritionally valuable, especially in situations with low DLI (Zhang et al., 2019). Photochemical changes can occur in response to light (Matysiak et al., 2022) and may involve phenolic acid production and flavonoid synthesis that protect against solar radiation (Gude et al., 2021). In agreement with our results, Zha et al. (2019) found that higher light intensity enhanced antioxidant capacity, as reflected in larger ascorbate and glutathione pools, and more efficient ascorbate synthesis. In a study on sweet basil, Dou et al. (2018) reported that total phenols and flavonoid content positively correlated with DLIs, and the antioxidant capacity was 73% higher in response to the DLI value of 17.8 mol $m^{-2}$ $d^{-1}$ than in the DLI value of 9.3 mol $m^{-2}$ $d^{-1}$. There is no significant difference between the SL300+12 and SL300+16 in terms of total phenol and flavonoid concentrations, antioxidant capacity, and ascorbic acid at both stages of lettuce growth (Gavhane et al., 2023), which is consistent with the findings of the present study. A noteworthy observation is that the total phenolic concentration at the seedling stage and the total flavonoid concentration at the head stage were higher under SL300+12 than under SL300+16, which shows that the light intensity is a more stimulating factor for the production of phenol and flavonoids than its duration. However, it is also possible to enhance phytonutrient concentrations (e.g., anthocyanins, beta-carotene, lutein, and phenolic compounds) by lengthening the light duration (Lefsrud et al., 2006; Mou, 2005). The antioxidant compounds were generally more concentrated at the seedlings stage than at the head stage in this study. For example, the decrease in the concentration of phenolic compounds may result from gallic acid converting into tartaric acid esters during maturation (Tian et al., 2009), thereby decreasing the amount of antioxidant compounds in the product. Young leaves demand more photoprotection than mature leaves. Thus, phenolic compounds in young leaves support the

photoprotection hypothesis (Zhang et al., 2018). This requirement in young leaves results from chloroplast immaturity (Choinski Jr et al., 2003; Li et al., 2015) and the lowered capability of the photosynthetic apparatus to utilize absorbed photons (Hughes et al., 2007; Ranjan et al., 2014).

## Conclusions

Adding SL to natural sunlight in greenhouse conditions during low-light seasons of the year can significantly increase the yield and quality of 'Little Gem' lettuce. When supplemental DLI reached 17.28 mol $m^{-2}$ $d^{-1}$, the growth and biomass of lettuce increased, and the response was the same for both growth stages. There was also a positive effect on photosynthetic pigments in lettuce leaves at both growth stages when using supplemental DLI. However, the growth stage showed a different response to supplemental DLI, so the different levels of supplemental DLI caused no significant differences at the head stage. However, at the seedling stage, photosynthetic pigment concentrations were highest in response to the DLI of 12.96 mol $m^{-2}$ $d^{-1}$. Additionally, when supplemental DLI increased, the response of both growth stages to SL exhibited the same pattern, improving the nutritional quality of lettuce. SL300+12 and SL300+16 produced the highest soluble sugar, total phenol, flavonoid, antioxidant capacity, and ascorbic acid in both growth stages of lettuce. In general, the SL improved lettuce growth and nutritional quality, but the lighting requirements at different growth stages in lettuce plants may differ depending on the growth stage.

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### *Author contributions*

HS was involved in investigation, implementation, methodology, conceptualization, data curation and analysis, and writing the original draft. MD was involved in conceptualization, methodology, supervision, review, and editing. SA was involved in conceptualization, review, and editing. KH was involved in conceptualization, review, and editing. MB was involved in conceptualization, review, and editing. RN was involved in investigation and implementation. All authors have read and approved of the final manuscript.

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### *Data availability*

All data generated or analyzed during this study are included herein.

## Conflict of interest

The authors indicate no conflict of interest for this work.

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