Article

Short-Term Pre-Harvest Supplemental Lighting with Different Light Emitting Diodes Improves Greenhouse Lettuce Quality

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Abstract: Winter–spring greenhouse vegetable production is limited by low-level natural light, resulting in decreased growth and quality. To investigate whether short-term pre-harvest supplemental lighting (SL) with light emitting diodes (LEDs) can address this issue, a study was conducted in a greenhouse in Dallas, Texas. Red leaf lettuce (Lactuca sativa L. ‘Red Mist’) plants grown in a hydroponic system were treated with daytime or nighttime SL with red (R) and blue (B) LEDs (RB-LED), blue and UVA LEDs (B/UVA-LED), or white LEDs (W-LED) for three days before harvest and compared to those without SL (control). All SL treatments provided a photon flux density of 167 µmol·m⁻²·s⁻¹ for 12 h daily. Compared with the control, SL treatments increased leaf thickness and greenness, antioxidant capacity, and concentrations of phytonutrients such as anthocyanins, carotenoids, and total phenolics; however, shoot fresh biomass and total leaf area were generally not affected by SL. There were no differences in all of the above traits among W-LED, RB-LED and B/UVA-LED. Compared with daytime SL, nighttime SL increased leaf greenness and carotenoid concentration. In summary, all three LEDs with different spectra were effective in improving lettuce quality as short-term pre-harvest SL sources and nighttime SL was more effective than daytime SL; however, plant fresh weight and total leaf area were not affected.

Keywords: anthocyanins; carotenoids; light quality; phenolics; phytonutrients; shoot biomass

1. Introduction

Red leaf lettuce is popular for salad mixes due to its unique color resulting from accumulated anthocyanins, which contribute to a higher nutritional value compared with green leaf lettuce [1]. For the winter–spring greenhouse production of red leaf lettuce, plants grow slowly with poor red coloration because of the lower natural light intensity and shorter daylength compared with other growing seasons. Supplemental lighting (SL) is a common approach to address low light issues. Due to the high cost of electricity, one potentially cost-effective lighting strategy is to apply SL before harvest for a few days to enhance quality [2]. The beneficial effects of the short-term pre-harvest SL on lettuce crops have been reported previously [1–5]. However, the optimal lighting spectrum and better timeframe to apply this SL strategy, and responsive plant traits are still unclear.

Light emitting diode (LED) fixtures have been increasingly used for greenhouse SL, due to their many advantages such as high lighting efficacy and easy spectral adjustment [6,7]. In lettuce, short-term pre-harvest SL with either red (R) or blue (B) LEDs was reported to increase the levels of antioxidants such as anthocyanins, thereby promoting the nutritional quality [3,5]. For SL with LEDs in greenhouse lettuce production, it was found that B LEDs resulted in a greater total anthocyanin concentration than R LEDs [8]. For lettuce grown in growth chamber, anthocyanin accumulation was lower under B LEDs than the
combination of R and B LEDs (RB-LED) [9]. For SL in greenhouse lettuce production, the LED peak wavelength across blue and ultraviolet A light (B/UVA-LED) showed a similar effect to RB-LED on anthocyanin level [2]. Possibly, a co-action with R or UVA light can strengthen the function of B light on anthocyanin synthesis [10,11]. The positive effects of RB-LED or B/UVA-LED, applied as SL for several days before harvest, on the nutritional quality (including anthocyanin concentration) or plant growth of greenhouse lettuce have been reported [1,2,4]. White (W) LEDs emit broad-spectrum light, and they are normally made from B LEDs with a phosphor coating which can transform part of the B light into longer wavelengths such as green (G), R, and sometimes far-red (FR) light [12]. It has also been found that a wide range of wavelengths from 290 to 760 nm (including UV, B, R, and FR light) are responsible for stimulating anthocyanin production [13,14]. Also, W LEDs are widely used for horticultural lighting [12]; thus, W LEDs may be a better choice for short-term pre-harvest SL than B/UVA-LED or RB-LED.

Nighttime SL can be economically beneficial because the electricity cost during the nighttime is usually lower than the daytime in some regions [15]. However, in most studies on greenhouse lettuce, pre-harvest SL was applied mainly in the daytime [1,3–5]. Additionally, during the daytime, the strong background sunlight may reduce plant utilization of the modest-intensity light from pre-harvest SL for photoassimilates, which are a material foundation for both plant growth and secondary metabolism [16]. However, limited information is available on the comparison of pre-harvest SL effects between daytime and nighttime application in terms of plant responses. A recent study on short-term pre-harvest SL indicated that the nighttime might be better timeframe than the daytime [2], but this needs further confirmation. For example, pre-harvest nighttime SL with B/UVA-LED increased the shoot fresh weight (FW) and dry weight (DW) by 41% and 38%, respectively, compared with daytime SL. However, this trend was not observed under pre-harvest SL with RB-LED. Thus, the optimal timeframe for short-term pre-harvest SL might vary with LED spectrum. In addition, this study [2] was conducted in the fall season with only two spectra, RB-LED and B/UVA-LED. Therefore, the effectiveness of W-LED, which are more affordable, as a short-term pre-harvest SL on boosting greenhouse lettuce quality, and possibly growth, still needs further investigation.

For red leaf lettuce, anthocyanin concentration not only determines leaf color, thus affecting appearance, but also contributes to internal quality along with other antioxidants such as carotenoids and phenolics [1,17]. In addition, for a leafy vegetable, greater plant biomass and total leaf area are directly related to faster plant growth and thus higher crop yield. Additionally, thicker and greener leaves indicate better appearance quality. Despite the few reported studies, a comprehensive knowledge of plant growth and quality traits in response to pre-harvest SL is still unavailable for red leaf lettuce. Therefore, the objectives of this study were to compare the effects of three LED spectra and SL timeframe on different crop growth and quality traits in greenhouse lettuces from winter to late spring.

2. Materials and Methods

2.1. Experimental Sites and Environmental Conditions

The study was conducted in a greenhouse at the Texas A&M AgriLife Research and Extension Center in Dallas, TX, USA (32°59′13.2″ N, 96°45′59.8″ W), and was replicated three times (three trials) from January to May 2021. The greenhouse air temperature was controlled by heating, ventilation, and an air conditioning system equipped in the adjacent office building. A datalogger (CR1000; Campbell Scientific, Logan, UT, USA), connected to thermocouple temperature sensors (Omega Engineering Inc., Norwalk, CT, USA) and quantum sensors (Apogee Instruments Inc., Logan, UT, USA), was used to record air temperature and photosynthetically active radiation (PAR) inside the greenhouse. The daily variations in natural light and average daily air temperature inside the greenhouse, throughout the growth period after transplanting, are presented in Figure 1. For Trial 1 (29 January to 19 February), Trial 2 (21 February to 11 March), and Trial 3 (23 April to
10 May), the mean DLIs were 8.0, 12.3, and 14.3 mol·m$^{-2}$·d$^{-1}$, respectively, and the mean air temperatures were 22.0, 23.1, 24.8 °C, respectively.

Figure 1. Daily light integral (DLI, mol·m$^{-2}$·d$^{-1}$) (A) and daily average air temperature (°C) (B) inside the greenhouse collected between transplanting and harvest.

2.2. Plant Materials and Maintenance

Seedlings of leaf lettuce (*Lactuca sativa* L. ‘Red Mist’) (Osborne Seed Company, Mount Vernon, WA, USA) were prepared following the methods presented by Hooks et al. [2]. Briefly, seedlings were propagated in rockwool in growth chambers under photosynthetic photon flux density (PPFD) of 132 µmol·m$^{-2}$·s$^{-1}$ with a 12 h photoperiod and irrigated with half-strength nutrient solution. When seedlings were established with three unfolded true leaves (i.e., 18–19 days after seeding), they were transplanted into deep water culture (DWC) systems for light treatment. The reservoir of the DWC system was 0.6 m wide, 1.2 m long, and 0.2 m deep. A floating raft (Beaver Plastics, Acheson, AB, Canada) supported 12 plants spaced 20 cm apart. The customized full-strength nutrient solution, which was used for post-transplanting, had an EC of 2.0 dS·m$^{-1}$ and a pH of 6.0. The mineral composition of this solution is presented in Table 1. A submersible air pump was set up to aerate the nutrient solution tank. A total of seven DWC systems were used in each of the three trials and arranged side-by-side along the west end of the greenhouse for uniform temperature and light distribution.
Table 1. Mineral composition of the full-strength, custom nutrient solution used for the experiment.

| Macronutrient (mM) | Microelement (µM) |
|-------------------|------------------|
| N     | P     | K     | Ca | Mg | S    | Fe   | B    | Mn  | Zn  | Cu   | Mo   |
| 10.7  | 1.13  | 5.38  | 3.25| 1.44| 1.44 | 53.7 | 27.8 | 6.0 | 1.83| 1.10 | 0.63 |

2.3. Light Treatments

Six SL treatments and one control treatment (no SL) were set up inside the experimental greenhouse. The SL treatments consisted of three types of LEDs with different spectra (Figure 2): W-LED (PhysioSpec IndoorTM; Fluence, Austin, TX, USA), RB-LED (AnthoSpecTM; Fluence), and B/UVA-LED (UVSpecTM; Fluence). For each LED treatment, two SL timeframes (07:00–19:00 for daytime, and 19:00–07:00 for nighttime) were adopted. All SL treatments were started three days before harvest and provided a target photon flux density (PFD) of 167 µmol·m⁻²·s⁻¹ at canopy level. For the B/UVA-LED, RB-LED, and W-LED treatments, the lamps were hung at 32, 64, and 74 cm above the plants, respectively, to achieve the same PFD. The spectrum and PFD of each treatment were measured at night using a Blue Wave spectroradiometer (VIS-25; StellarNet, Tampa, FL, USA). Reflective insulation materials were hung for three days between each treatment to avoid light pollution from neighboring light treatments. For each LED treatment, actual light spectra and PFD are presented in Figure 2 and Table 2, respectively.

Figure 2. The spectral distribution of red and blue (RB) (A), blue/ultraviolet-A (B/UVA) (B), or white (W) (C) LEDs used for pre-harvest supplemental lighting.

Table 2. Total and individual waveband photon flux density (PFD) of the three commercial LED lights used for pre-harvest supplemental lighting (SL).

| LED for Pre-Harvest SL | Total PFD (µmol·m⁻²·s⁻¹) | PFD Proportion (%) in Different Wavebands |
|------------------------|--------------------------|-----------------------------------------|
|                        |                          | 340–399 (nm) | 400–499 (nm) | 500–599 (nm) | 600–699 (nm) | 700–799 (nm) |
| RB ¹                   | 167.2                    | 0            | 57           | 0            | 43           | 0           |
| B/UVA                  | 167.4                    | 27           | 73           | 0            | 0            | 0           |
| W                      | 167.8                    | 0            | 19           | 38           | 40           | 3           |

¹ RB: red and blue; B/UVA: blue and ultraviolet-A; W: white.

2.4. Growth and Quality Measurements

Due to different environment factors, at 18, 20, and 22 days after initiating Trials 1, 2, and 3, respectively, and after the pre-harvest SL treatments, 9 of the 12 plants were harvested for measurements of the following plant traits: shoot fresh weight (FW) and dry weight...
(DW), total leaf area (TLA), and specific leaf area (SLA). The relative chlorophyll content (SPAD) of mature leaves was measured immediately before harvest, using a handheld SPAD-502Plus meter (Konica Minolta, Osaka, Japan) and was taken as the average of three measurements per plant.

Plants were harvested and weighed for shoot FW. Then, all leaves were separated from the basal stem of each plant, and the TLA per plant was measured using an LI-3100C leaf area meter (LI-COR, Lincoln, NE, USA). The plants were then placed in paper bags and dried fully in an oven at 70 °C to measure shoot DW. The SLA was calculated as TLA divided by shoot DW.

For the remaining three plants of each treatment in each of the three trials, three representative mature leaves were selected per plant for measuring phytonutrient concentrations. Fresh tissue samples (1 g) were immediately frozen in liquid nitrogen and stored in a freezer at −80 °C for later analysis. During analysis, for a full and effective extraction, these samples were ground into fine powders with a mortar and pestle using liquid nitrogen and subsequently extracted in methanol. To avoid sample degradation and metabolite interconversion, the whole storage and extraction process were maintained at a low temperature. The extracted samples were analyzed for anthocyanins, carotenoids, total phenolic compounds (TPC), and Trolox equivalent antioxidant capacity (TEAC) using the methods by Silva et al. (2017) [17], Wellburn (1994) [18], Ainsworth and Gillespie (2007) [19], and Arnau et al. (2001) [20], respectively. The procedures are briefly described as follows.

For anthocyanins, the absorbance of the extract was measured at 530 nm using a spectrophotometer (Genesys 10S ultraviolet/Vis, Thermo Fisher Scientific, Madison, WI, USA), and anthocyanin concentration was expressed as micrograms of cyanidin-3-glucoside equivalent per gram of the FW of lettuce leaves using a molar extinction coefficient of 29,600. For carotenoids, absorbance of the extract was measured at 470 nm using a spectrophotometer (Genesys 10S ultraviolet/Vis), and carotenoid concentration was expressed as $\mu g g^{-1}$ FW. For TPC, a modified Folin–Ciocalteau reagent method was used to determine the TPC of lettuce leaves. A mixture of 100 $\mu$L extraction sample, 200 $\mu$L 1/10 dilution Folin–Ciocalteau reagent, and 800 $\mu$L 7.5% Na$_2$CO$_3$ (sodium carbonate) was incubated at room temperature for two hours, and the absorbance was measured at 725 nm using a microplate reader (EL-800, BioTek, Winooski, VT, USA). The results are presented as micrograms of gallic acid equivalent per gram of the FW of lettuce leaves. For TEAC, a mixture of 100 $\mu$L of the extracted sample and 1 mL of ABTS+ solution was measured at 734 nm using a spectrophotometer (Genesys 10S ultraviolet/Vis). The results are presented as micrograms of Trolox equivalent antioxidant capacity per gram of the FW of lettuce leaves.

2.5. Experimental Design and Statistical Analysis

The experiment was arranged in a randomized complete block design with 7 treatments, 3 blocks (trials), and 12 plants (subsamples) in each trial. An experimental unit consisted of one hydroponic system with 12 plants. One-way analysis of variance (ANOVA) was used to determine the effects of light treatment. For each plant trait, the means were separated using Tukey’s honest significant difference (HSD) test at $\alpha = 0.05$. All data were analyzed using JMP 14 (SAS, Cary, NC, USA).

3. Results

3.1. Shoot Biomass

Pre-harvest SL treatments did not affect shoot FW (Figure 3A). Compared with the control (no SL), RB-D, RB-N, B/UVA-N and W-N increased shoot DW by 20%, 25%, 18% and 28%, respectively (Figure 3B). The other light treatments also tended to increase shoot DW but did not differ significantly from the control. There was no difference in shoot DW both among different LEDs when applied at the same timeframe and between daytime and nighttime SL for the same LED.
3.2. Leaf Characteristics

Although TLA was not affected by light treatments (Figure 4A), SLA decreased by 12–19% under SL treatments except for RB-D and B/UVA-D compared with the control (Figure 4B). There was no difference in SLA both among different LEDs when applied at the same timeframe and between daytime and nighttime SL for the same LED.

SPAD increased by 19% and 20% under RB-N and W-N, respectively, compared with the control (Figure 4C). Compared with daytime SL, SPAD under nighttime SL treatments increased by 11–12%. There was no difference among the three LEDs when applied at the same SL timeframe.

3.3. Phytonutrients Concentrations

The anthocyanin concentration under SL treatments increased by 19–34% compared with control (Figure 5A). There was no difference in anthocyanin concentration both among different LEDs when applied at the same timeframe and between daytime and nighttime SL for the same LED.
Figure 4. Total leaf area (TLA) (A), specific leaf area (SLA) (B), and relative chlorophyll content (SPAD) (C) of hydroponic lettuce treated with pre-harvest supplemental lighting. Different letters indicate significant differences and “ns” indicate no significant differences according to Tukey’s HSD test ($p < 0.05$).

Carotenoid concentration under RB-N, B/UVA-N, and W-N increased by 43%, 23%, and 34% relative to control (Figure 5B). Compared with daytime SL, nighttime SL increased carotenoid concentration by 22% and 17% for RB-LED and W-LED, respectively. For either nighttime SL or daytime SL, the three LEDs did not differ from each other in carotenoid concentration.

Under SL treatments except for RB-D or W-D, TPC increased by 41–61% relative to the control (Figure 5C). There was no difference in TPC both among different LEDs when applied at the same timeframe and between daytime and nighttime SL for the same LED.

Under RB-N, B/UVA-N, and W-N treatments, TEAC increased by 80%, 82%, 75%, respectively, relative to control (Figure 5D). There was no difference in TEAC both among different LEDs when applied at the same timeframe and between daytime and nighttime SL for the same LED.
Figure 5. Anthocyanins (ANT) (A), carotenoids (CAR) (B), total phenolic content (TPC) (C), and Trolox equivalent antioxidant capacity (TEAC) (D) of leaf tissues sampled from hydroponic lettuce treated with pre-harvest supplemental lighting. Different letters indicate significant differences and “ns” indicates no significant differences among treatments according to Tukey’s HSD test ($p < 0.05$).

4. Discussion

4.1. Short-Term Pre-Harvest SL Improves Crop Quality Rather than Growth

In the present study, all SL treatments relative to the control did not increase shoot fresh biomass and total leaf area, showing little effect on crop growth and potential yield. Previous studies indicated that SL applied throughout the production cycle increased both lettuce growth and yield when natural DLI levels were low [21,22]. Differing from whole-cycle SL, pre-harvest SL was applied for only three days or nights before harvest. In this case,
although the dry biomass increased or tended to increase under the short-term pre-harvest SL, this did not contribute in a timely manner to increases in fresh biomass and total leaf area. On one hand, in a short time scale there is a trade-off between assimilate accumulation from photosynthesis and water loss from transpiration, since the stomata are a common gate for the two physiological processes [23]. On the other hand, photosynthesis might have adapted faster to the SL than plant water balance and leaf expansion growth [24,25].

Unlike plant growth, crop quality benefited from pre-harvest SL, demonstrated by the greater leaf thickness and greenness, and higher concentrations of phytonutrients depending on different LEDs and SL timeframe. These results suggest that crop quality traits are more sensitive than plant growth in response to short-term pre-harvest SL, since plants normally change faster at the micro-level (e.g., biochemical and cellular) than at the macro-level (e.g., whole plant) to adapt to modifications to environmental factors including light amount [24,25]. Under pre-harvest SL, the development of photosynthetic tissues and apparatus such as mesophylls and chloroplasts might have been promoted in plant leaves to acclimate and capture more light [26,27]. This increased photo-assimilates under pre-harvest SL, providing a material foundation for improved biosynthesis of the secondary metabolites [16], which include many phytonutrients. Similar beneficial effects on phytonutrients from pre-harvest SL have been reported in previous studies [2,3,5]. Among the phytonutrients, anthocyanins are natural pigments that produce red, blue, and purple colors in many plants, including red leaf lettuce [28]. In the present study, lettuce did develop more red coloration under the pre-harvest SL, as demonstrated in leaf appearance (Supplemental Figure S1). In addition, anthocyanins are important antioxidants and have been increasingly playing an important role in our diet and health [29].

4.2. Different LEDs Similarly Affect Lettuce Growth and Quality

For plant biomass and total leaf area, there were no differences among the three LEDs, suggesting that they had a similar effect on plant growth. It has been reported that RB-LED can increase photosynthesis compared to monochromatic light such as B-LED [30,31]. Also, W-LED have some advantages over RB-LED partly through the function of G light (500–600 nm), since G light contributes to whole plant photosynthesis due to its deeper canopy penetration ability than R and B light [32]. However, in the present study, plant dry biomass under SL was similar among the three LEDs, indicating a similar photoassimilate accumulation. Possibly, it takes a longer time for photosynthesis to respond to modification of the light spectrum than the amount of light. In this case, the short timeframe of pre-harvest SL might not fully show the difference in the three LEDs’ contribution to photosynthesis and thus plant growth.

All quality traits, including leaf thickness and greenness, and concentrations of phytonutrients benefited similarly from the three LEDs when applied at the same SL timeframe. Leaf thickness, greenness, and carotenoid concentration are directly related to photosynthetic function of plants [26,27]. Compared with B/UVA-LED, plants being exposed to wider-spectrum light emitted from RB-LED or W-LED might have contributed to more efficient plant photosynthetic function, since plants have developed a response system to adapt to a broad-spectrum light in natural conditions [33]. However, the advantages of RB-LED and W-LED on the photosynthetic function over B/UVA-LED were not observed in the present study, possibly due to a short treatment time. Among the phytonutrients, biosynthesis of anthocyanins can be promoted by more wavelengths such as R or FR from RB-LED or W-LED, besides B/UVA light [13,14], and phenolics’ accumulation in plants can be stimulated specifically by strong B and UVA light [11]. However, there were no differences in these traits among the three LEDs, again possibly due to the short period of time that plants were treated.

4.3. Pre-Harvest SL at Nighttime Is Better than Daytime for Some Crop Quality

Nighttime SL had similar effects as daytime SL on plant growth, as demonstrated by similar plant biomass and total leaf area. The reason for this may lie in the fact that plant
growth depends on the total daily light amount they received [34,35], since daytime SL had the same daily light integral as nighttime SL. This also supports the important role played by daily light integral in winter–spring greenhouse production [36].

Differing from plant growth, some lettuce quality traits such as leaf greenness and carotenoid concentrations were generally better under nighttime SL than daytime SL for each LED. This would be demonstrated by greener leaves, as well as higher phytonutrient contents. Possibly, nighttime SL acted as a sole-source lighting during the totally dark period, so light from SL could be fully sensed and used by plants. However, during the daytime, some light might be saturated for plants and even wasted when natural light was strong at midday [37]. It may be more economical for SL to be applied at night, since the nighttime electricity price is normally lower in some regions and crops benefit more from nighttime SL [15,38,39].

5. Conclusions

In summary, for the winter–spring greenhouse production of lettuce, short-term pre-harvest SL with W-LED, RB-LED, or B/UVA-LED increased leaf thickness and greenness, and concentrations of phytonutrients, compared with no SL. However, plant growth was generally not affected by SL treatment except for an increase in dry biomass under nighttime SL. Plant growth and quality did not differ from each other under the three LEDs. For any LED, nighttime SL resulted in better crop quality than daytime SL, as demonstrated by greater leaf greenness and higher carotenoid concentrations. In conclusion, W-LED, RB-LED, or B/UVA-LED are similarly effective for pre-harvest SL to improve crop quality, and the beneficial effects were greater when SL is applied at nighttime than in the daytime.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8050435/s1, Figure S1: Lettuce plants that received a three-day pre-harvest supplemental lighting with red and blue (RB), blue/ultraviolet-A (B/UVA), or white (W) LEDs at daytime (D) or nighttime (N) during winter–spring greenhouse production. The control plants did not receive any supplemental lighting. Pictures were taken on the final day of each trial. The dates for the three trials were 29 January to 19 February (Trial 1, A), 21 February to 11 March (Trial 2, B), and 23 April to 10 May (Trial 3, C) in 2021.

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