Growth, Nutritional Quality, and Energy Use Efficiency of Hydroponic Lettuce as Influenced by Daily Light Integrals Exposed to White versus White Plus Red Light-emitting Diodes

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Abstract. Few researchers examined different red light amounts added in white light-emitting diodes (LEDs) with varied daily light integrals (DLIs) for hydroponic lettuce (Lactuca sativa L.). In this study, effects of DLI and LED light quality (LQ) on growth, nutritional quality, and energy use efficiency of hydroponic lettuce were investigated in a plant factory with artificial lighting (PFAL). Hydroponic lettuce plants (cv. Ziwei) were grown for 20 days under 20 combinations of five levels of DLIs at 5.04, 7.56, 10.08, 12.60, and 15.12 mol·m⁻²·d⁻¹ and four LQs: two kinds of white LEDs with red to blue ratio (R:B ratio) of 0.9 and 1.8, and two white LEDs plus red chips with R:B ratio of 2.7 and 3.6, respectively. Results showed that leaf and root weights and power consumption based on fresh and dry weights increased linearly with increasing DLI, and light and electrical energy use efficiency (LUE and EUE) decreased linearly as DLI increased. However, no statistically significant differences were found in leaf fresh and dry weights and nitrate and vitamin C contents between DLI at 12.60 and 15.12 mol·m⁻²·d⁻¹. Also, no effects of LQ on leaf dry weight of hydroponic lettuce were observed at a DLI of 5.04 mol·m⁻²·d⁻¹. White plus red LEDs with an R:B ratio of 2.7 resulted in higher leaf fresh weight than the two white LEDs. LUE increased by more than 20% when red light fraction increased from 24.2% to 48.6%. In summary, white plus red LEDs with an R:B ratio of 2.7 at DLI 15.12 mol·m⁻²·d⁻¹ were recommended for commercial hydroponic lettuce (cv. Ziwei) production in PFALs.

Plant factories with artificial lighting have many advantages over traditional production systems (e.g., greenhouse and open-field) for leafy greens, herbs, and transplants production because of high resource use efficiency, high productivity, and its year-round cultivation (Kozai and Niu, 2016a). However, high capital investment and operation cost are still challenges and disadvantages with PFALs in commercial production. In Japan, percentages of depreciation of initial investment and electricity cost (including mainly artificial lighting and air conditioning) for total production cost in PFALs are approximately 30% and 20%, respectively (Kozai, 2018). The lighting consumption account is ~70% to 80% of total electricity consumption for year-round production in PFALs (Kozai and Niu, 2016a). Light plays an important role in affecting plant growth (Snowden et al., 2016; Son et al., 2016), photochemical biosynthesis (Dou et al., 2018; Mickens et al., 2019), and gene expression (Gilberto et al., 2005; Son et al., 2012). Therefore, lighting environment can be optimized by management of light conditions and proper design of artificial lights, thus increasing the yield and beneficial nutritional values of plants and decreasing the lighting consumption accounts. Lettuce (Lactuca sativa L.) is often used as a model crop for researchers to examine its light responses and cultivated worldwide in PFALs (Dougher and Bugbee, 2001; Li and Kubota, 2009).

Plants have varied morphological and physiological responses to specific light spectrum, and the current advancement of LEDs enables one to tailor the spectrum to obtain favorable plant growth or nutritional values (Mickens et al., 2018; Park and Runkle, 2018). Compared with other wavelengths, red and blue lights were paid more attention to lettuce (Lee et al., 2010; Son and Oh, 2015; Stutte et al., 2009; Wang et al., 2016b), cucumber seedlings (Hernández and Kubota, 2016), tomato seedlings (Hernández et al., 2016; Liu et al., 2018), Mesembryanthemum crystallinum (He et al., 2017), and sweet basil (Pennisi et al., 2019) in past and recent decades, as red and blue lights were considered more effectively absorbed by chlorophylls of plant leaves (McCree, 1971). Moreover, plants grown under the combination of red and blue lights had bigger stem diameter (Yang et al., 2018), higher net photosynthetic rate ($P_n$) (Wang et al., 2016b), anthocyanin (Lee et al., 2010; Stutte et al., 2009), and arginine (Zhang et al., 2018a) compared with those grown under monochromatic red light. In addition, red and blue LEDs had higher photosynthetic photon efficiency (Park and Runkle, 2018). As a result, red and blue LEDs are commonly applied for commercial production in PFALs. Previous studies also investigated the suitable R:B ratio created by mixture of red and blue lights for dry weight accumulation in lettuce (Wang et al., 2016b), cucumber seedlings (Hernández and Kubota, 2016), and M. crystallinum (He et al., 2017).

Plants grown under mixed red and blue LEDs appeared purplish, thus making visual assessment of disease symptoms and growth disorder difficult to observe (Kim et al., 2004). One solution for this limitation was to apply white LEDs, alone or with blue LEDs, red LEDs, or both (Park and Runkle, 2018; Yan et al., 2019). The world’s first white LED was created in 1996 as a phosphor coating used by blue LED (Bourget, 2008) and the present white LEDs showed increased energy efficiency as the highly efficient blue-emitting diodes were invented (Pust et al., 2015). White LEDs were suggested as a substitute lighting sources compared with fluorescent lamps for lettuce production in PFALs (Park et al., 2012), and green leaf lettuce grown under white LEDs had higher leaf fresh weight (Zhang et al., 2015), root fresh weight, and phenolic concentration (Son et al., 2012) than those grown under monochromatic red LEDs. Moreover, white LEDs were used as background lighting in ‘Green Oak Leaf’ lettuce reported by Chen et al. (2016), and the effects of red, green, blue, yellow, and far red lights as supplemental lights were examined. Results indicated that lettuces grown under white light combined with supplemental red light appeared vigorous and compact compared with those grown under white LEDs alone. Mickens et al. (2018) also observed
similar results in red romaine lettuce at harvest. To identify the amounts of supplemental red lights, blue lights, or both, combination of white with different fractions of red LEDs, blue LEDs, or both were examined in sweet basil, strawberry, begonia seedings, geranium seedlings, petunia seedlings, and snapdragon seedlings (Park and Runkle, 2018; Piovene et al., 2015). The LEDs with an R:B ratio of 0.7 improved growth and nutraceutical properties in sweet basil and strawberry (Piovene et al., 2015). White LEDs had similar impacts on seedling growth and electric energy consumption of artificial lights compared with mixture of red and blue lights (Park and Runkle, 2018). Chen et al. (2016) inferred that lettuce yield would be higher with larger red light fraction when white light was applied as background light. However, proper red amounts or R:B ratios created by combination of white and red lights from sole-source LEDs for lettuce production were not yet determined by previous studies.

Daily light integral is the total amount of light received by plants in 1 d (Bruggink and Heuvelink, 1987; Kozai et al., 2018). Linear relationships were often observed between DLI and biomass of lettuce (Gent, 2014; Zhang et al., 2018b), average shoot dry weight per average internode number of Celosia seedlings (Pramuk and Runkle, 2005), and nutritional values of sweet basil (Dou et al., 2018). The suitable DLIs for hydroponic lettuce production were recommended at 12.67 mol·m⁻²·d⁻¹ (Fu et al., 2017) and 14.40 mol·m⁻²·d⁻¹ (Zhang et al., 2018b), respectively. However, few studies examined the relationships between DLI and carbohydrate accumulation under LEDs with different R:B ratios when white LEDs were used as base lighting source, and there were few bases for the spectrum design of relatively broad and wide LEDs for lettuce cultivation. In addition, few researchers focused on the energy use efficiency for lettuce production in PFALs.

The objectives of this study were to figure out suitable DLI and LQ with proper red light amounts created by white plus red light from sole-source LEDs by investigating photosynthetic characteristics, biomass, nutraceutical properties, light energy use efficiency of hydroponic lettuce, and electric energy consumption of artificial lights. The results could be used as guidelines for LEDs design and management of light conditions for lettuce production in PFALs.

Materials and Methods

**Plant materials and growth conditions.** Purple lettuce (*Lactuca sativa* L. cv. Ziwei) seeds were sown in sponge cube (23 mm × 23 mm × 23 mm) containing deionized water in plastic containers (520 mm × 360 mm × 90 mm) in a walk-in growth chamber (China Agricultural University, Beijing, China). At 7 d after sowing, lettuce seedlings were divided into 128-cell trays and cultivated for 13 d. Uniform lettuce seedlings (20 d-old) were randomly selected and transplanted to hydroponic cultivation beds (1200 mm × 600 mm × 70 mm), which were made of acrylonitrile butadiene styrene and held 28 plants. Based on the results reported by our previous study (Yan et al., 2019), the recommended photosynthetic photon flux density (PPFD) at the seedling stage was 200 μmol·m⁻²·s⁻¹ with a 16-h·d⁻¹ photoperiod provided by LEDs with an R:B ratio of 2.2 (WR-LED-16W; Beijing Lighting Valley Technology Co., Ltd., China). Air temperature and relative humidity were maintained at (22 ± 1) °C/(18 ± 1) °C and (65 ± 5)%/(75 ± 10)% at photoperiod/dark period, respectively. Carbon dioxide concentration was maintained at (800 ± 50) μmol·mol⁻¹ at photoperiod and without control at dark period. The hydroponic lettuce seedlings were transplanted at 20 d after sowing and harvest at 40 d after sowing.

Yamasaki lettuce nutrient solution was used with pH at 6.0–6.5 and EC at 1.0–1.2 mS·cm⁻¹. Tap water and 1/4 strength and 1/2 strength of the standard nutrient solution were applied at two days after sowing, cotyledon stage, and 1–2 true leaves stage, respectively. A full strength of the standard nutrient solution was used after the second true leaf expanded, and the nutrient solution was replaced once a week at the seedling stage and the cultivation stage.

**Treatments.** Lettuce were grown under 20 treatments created by combinations of five levels of DLIs. 5.04, 7.56, 10.08, 12.60, and 15.12 mol·m⁻²·d⁻¹ (hereafter, D5.04, D7.56, D10.08, D12.60, and D15.12, respectively, in the treatments) and four kinds of LEDs. The five DLIs were created by five different PPFDs of 100, 150, 200, 250, and 300 μmol·m⁻²·s⁻¹ with a 14-h·d⁻¹ photoperiod. The four kinds of LEDs included two kinds of white LEDs (W-LED-18W; Beijing Lighting Valley Technology Co., Ltd.) with R:B ratios of 0.9 and 1.8 (L0.9 and L1.8) and two white LEDs plus red chips (WR-LED-16W; Beijing Lighting Valley Technology Co., Ltd.) with R:B ratios of 2.7 and 3.6 (L2.7 and L3.6), respectively. A fiber spectrometer (AvAField-2; Avantes Inc., The Netherlands) was used to measure spectral distributions of the LED lamps (Fig. 1) with wavebands ranging from 300 nm to 800 nm at 15 cm below the lamps. LEDs were installed surrounded by mirror-like stainless steel plate (1200 mm × 600 mm × 0.4 mm), whereas the ground acted as hydroponic cultivation bed as mentioned previously, and the wall was made of aluminum plastic plate with small holes for ventilation. Photon flux of ultraviolet (ultraviolet, 300–399 nm), blue (B, 400–499 nm), green (G, 500–599 nm), red (R, 600–699 nm), and far red (FR, 700–800 nm) lights were integrated for calculating R:B or R:FR ratio according to spectral distributions (Table 1). Fractions of ultraviolet, blue, green, red, and far red lights were photon flux based. There were six replications in each treatment, and eight plants were considered as a replication.

**Measurement of net photosynthetic rate (Pn).** In each treatment, six uniform plants were randomly selected at harvest. The third fully expanded leaf from apical shoot was exposed to light source with PPFDs of 100, 150, 200, 250, and 300 μmol·m⁻²·s⁻¹ provided by a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE) to measure Pn. Leaf temperature and CO₂ concentration were controlled at 22 °C and 800 μmol·mol⁻¹ in the leaf chamber with red and blue LEDs, respectively.

**Measurements of plant morphology and growth characteristics.** In each treatment, six uniform plants were randomly selected at 20 d after transplanting for following measurement. The maximum leaf blade (third to fifth fully expanded leaf counted from top of the plants) of each lettuce was selected to measure leaf length (LL) and leaf width (LW). The fresh weights of leaves and roots were measured by an electronic analytical balance (FA1204B; BioonGroup, Shanghai, China) and then the leaves and roots were oven-dried at 105 °C for 3 h and then set to 80 °C until constant weights for measuring the dry weights.

**Determinations of nutritional values.** Samples were randomly selected and excised from fresh leaves of six plants in each treatment. A whole fragment of lettuce leaf was cut into small pieces and mixed, and then the fresh tissue was used for the following measurements. Coloration method of sulfo-salicylic acid (Cataldo et al., 1975), 2,6-dichlorophenol indophenol titration method (Li, 2000), and spectrometric method (Cao et al., 2007) were used to measure nitrate, vitamin C, and anthocyanin contents of the sampled lettuce leaf, respectively. Absorbances of sample’s solutions were measured by ultraviolet-VIS spectrophotometer (UV3150; Shimatsu Productions Co., Japan) at a wavelength of 410 nm for nitrate content and at wavelengths of 530 and 600 nm for anthocyanin contents, respectively.

**Determinations of energy use efficiencies.** Light energy use efficiency and electric energy use efficiencies were calculated according to Kozai and Niu (2016b), where LUE = f × D/PAR and EUE = ℎ × LUE. In this equation, f is the conversion coefficient from dry weight to chemical energy (≈20 MJ·kg⁻¹), D is the dry weight increase rate of plants (kg·m⁻²·h⁻¹), PAR is the PAR (MJ·m⁻²·h⁻¹), h is the conversion factor from electrical energy to PAR energy, ℎ = D/PAR, D/PAR = 0.9, 0.635, 0.636, 0.637, 0.638, and 0.638; LEDs with an R:B ratio of 1.8 were 0.682, 0.679, 0.680, 0.680, and 0.680; LEDs with an R:B ratio of 2.7 were 0.638, 0.637, 0.511, 0.533, and 0.550; and LEDs with an R:B ratio of 3.6 were 0.661, 0.662, 0.660, 0.658, and 0.658, respectively. Smart metering (TP9004; Shenzhen Northmeter Co., Ltd., Shenzhen, China) was applied to measure the power consumption of LED lights in each treatment. Power consumption was calculated as total power consumption of LED lights in each
treatment divided by 100 g fresh weight and 1 g dry weight accumulated during cultivation stage, which was increased from transplanting day to harvest day.

**Statistical analysis.** Statistical analysis was conducted using the SPSS 18.0 (IBM, Inc., Chicago, IL). Analysis of variance was performed to test the significance of main factors of DLI and light quality. Duncan’s multiple range test was conducted to compare the means between treatments (*P* < 0.05). The results were reported as the mean ± standard deviation values (*n* = 6). Regressions between DLI and growth of lettuce and energy use efficiency were performed using Microsoft Excel 2010 software.

### Results

**Photosynthetic characteristics of hydroponic lettuce leaves.** *Pn* of hydroponic lettuce leaves was significantly influenced by DLI and light quality (Fig. 2). Increased DLI and decreased R:B ratio led to increasing *Pn*, whereas no statistically significant differences were observed among different LQs under a DLI of 5.04 mol·m⁻²·d⁻¹. *Pn* was highest at a DLI of 15.12 mol·m⁻²·d⁻¹ under white LEDs with an R:B ratio of 0.9 among all treatments, which was 114% higher than that at a DLI of 15.12 mol·m⁻²·d⁻¹ under white LEDs with an R:B ratio of 0.9. No statistically significant differences were found in leaf dry weight between R:B ratios of 0.9 and 1.8 under white LEDs. Root fresh and dry weights were highest in the treatment with a DLI of 12.60 mol·m⁻²·d⁻¹ provided by LEDs with an R:B ratio of 2.7. No statistically significant differences were found in leaf dry weight and root fresh weight among light qualities at lower DLIs (5.04 mol·m⁻²·d⁻¹).

**N utritional values of hydroponic lettuce.** DLI and light quality had remarkable effects on nitrate and anthocyanin contents of hydroponic lettuce. Vitamin C content was significantly influenced by DLI (Table 3). Higher DLI led to lower nitrate content, higher anthocyanin and vitamin C contents.

Lettuce grown under a DLI of 5.04 mol·m⁻²·d⁻¹ had highest nitrate content, which was 109% higher than those grown under a DLI of 15.12 mol·m⁻²·d⁻¹ provided by white LEDs with an R:B ratio of 0.9. No statistically significant differences were observed in nitrate and vitamin C contents as DLI increased from 10.08 to 15.12 mol·m⁻²·d⁻¹, regardless of light quality. Nitrate and anthocyanin contents were higher under LEDs with an R:B ratio of 0.9, and no significant differences were found in anthocyanin and vitamin C contents between LEDs with R:B ratios of 2.7 and 3.6 with the same DLI.

**Energy use efficiency of LED lighting for hydroponic lettuce production.** Total power consumptions in treatment of D5.04, D7.56, D10.08, D12.60, and D15.12 provided by LEDs with an R:B ratio of 0.9 were 10.3, 15.4, 20.5, 25.6, and 30.7 kWh; LEDs with an R:B ratio of 2.7 were 9.6, 14.5, 24.1, 28.8, and 33.5 kWh; and LEDs with an R:B ratio of 0.9 were 10.3, 15.4, 20.5, 25.6, and 30.7 kWh; and LEDs with an R:B ratio of 1.8 were 9.2, 13.9, 18.5, 23.1, and 27.8 kWh; LEDs with an R:B ratio of 2.7 were 9.6, 14.5, 24.1, 28.8, and 33.5 kWh; and LEDs with an R:B ratio of 3.6 were 9.1, 13.7, 18.3, 22.9, and 27.5 kWh, respectively. Power consumption based on fresh and dry weights was influenced by DLI and light quality remarkably. Increased DLI led to increasing power consumption linearly (Fig. 4). Under each LED source, there were no significant differences in power consumption as DLI increased from 5.04 to 7.56 mol·m⁻²·d⁻¹. Power consumption based on fresh weight at a DLI of 15.12 mol·m⁻²·d⁻¹ provided by white LEDs was 47% higher than that at a DLI of 5.04 mol·m⁻²·d⁻¹.
DLI of 5.04 mol·m⁻²·d⁻¹ with the same LEDs. Power consumption based on fresh and dry weight under white plus red LEDs with an R:B ratio of 2.7 decreased by more than 13% and 7%, respectively, compared with white LEDs with an R:B ratio of 0.9.

Both DLI and LQ affected LUE and EUE significantly; LUE and EUE decreased linearly with increasing DLI (Fig. 4). In general, LUE was higher as lettuce grown under white plus red LEDs with an R:B ratio of 2.7 than those grown under white LEDs with R:B ratios of 0.9 and 1.8. LUE and EUE under white plus red LEDs with an R:B ratio of 2.7 was greater than 22% and 7%, respectively, higher than those grown under white LEDs with an R:B ratio of 0.9. The highest LUE and EUE were 0.038 and 0.022, respectively, with an R:B ratio of 0.9.

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Discussion

DLI and light quality influenced \( P_n \) leaf morphology, and yield of hydroponic lettuce. DLI exhibited positive effects on \( P_n \) of hydroponic lettuce (Fig. 2), and similar trends were observed in previous studies (Fu et al., 2017; Lu et al., 2017). Fan et al. (2013) found that \( P_n \) of tomato plants leaves increased as DLI increased from 2.16 to 12.96 mol·m⁻²·d⁻¹, as higher DLI led to higher palisade cells and leaf thickness, which were associated with photosynthetic capacity. \( P_n \) significantly decreased as DLI was higher than 12.96 mol·m⁻²·d⁻¹ (Fan et al., 2013), indicating that excess DLI (with higher PPFD) had detrimental impacts on the photosynthetic organelles and even caused physiological disorders. Similarly, Dou et al. (2018) reported that no significant differences were found in \( P_n \) of sweet basil at higher DLIs (12.9, 16.5, and 17.8 mol·m⁻²·d⁻¹), which might be interpreted by the possession of chloroplasts adapted to higher DLI.

Generally, \( P_n \) of lettuce leaf increased with the decrease in R:B ratio at higher DLIs (Fig. 2), which suggested that photosynthetic performance of lettuce was efficiently improved with higher fraction of blue light. This was due to that blue light inhibited photosynthesis and stimulated stomatal opening to enhance CO₂ uptake for photosynthesis (Chen et al., 2016; Shimazaki et al., 2007). Under the same DLI, blue LEDs (with 8% blue light) were added to red and white LEDs (with 21% blue light), resulting in significant increase of \( P_n \) of lettuce leaf (Son et al., 2016). Similar effects were found in lettuce by Wang et al. (2016b), who observed that \( P_n \) increased as blue light fraction increased from 7.7% up to 50.0%. However, Kang et al. (2016) observed that \( P_n \) was suppressed as blue light fraction increased from 20% to 30%. These differences might arise from other experimental conditions or cultivars.

Linear relationships are often found between DLI and crop yield (Albright et al., 2000; Dou et al., 2018; He et al., 2019). Fresh and dry weights of leaves and roots increased linearly as DLI increased. There were no significant differences in fresh and dry weights of leaf as DLI increased from 12.60 to 15.12 mol·m⁻²·d⁻¹ (Table 2), showing that excessive DLI did not improve carbohydrate accumulation (Dou et al., 2018; Fan et al., 2013), and even reduced dry weight accumulation (Colonna et al., 2016).

No effects of light quality on growth and morphology were observed under lower DLI, which may indicate that light quantity had bigger effects than light quality in lettuce production. This was probably due to the light amounts received by leaves and there were no remarkable differences found in \( P_n \) among treatments at lower DLIs. Thus, the comparison of plants grown under different LEDs should be conducted under a sufficient DLI or light intensity. Previous studies also reported that a higher proportion of red light often promoted plant growth (Son and Oh, 2013; Wang et al., 2018). However, it was of interest that lettuce grown under higher red light fraction had higher yield with lower \( P_n \) which indicated that dry weight accumulation was not only related to instantaneous \( P_n \) of single leaf directly but also related to leaf morphology indirectly (Kang et al., 2016; Park and Runkle, 2017; Wang et al., 2016b).

A higher fraction of red light resulted in bigger leaves (Table 2), thus increasing carbohydrate accumulation of lettuce. This was because red light increased light capture by leaf expansion. Net CO₂ exchange amounts of lettuce plants may increase because of bigger leaves and more leaf numbers, although instantaneous \( P_n \) of single lettuce leaf decreased as R:B ratio increased. This may be the reason why higher \( P_n \) did not lead to higher fresh weight. \( P_n \) of whole canopies would be more correlated to biomass of lettuce. Wang et al. (2016b) observed that leaf area, leaf number, and leaf dry weight of lettuce increased with \( P_n \) decreasing as R:B ratio increased from 1 to 12. This phenomenon also agreed with prior study reported by Swan and Bugbee (2017). No statistically significant differences were found in \( P_n \) at dry weight between LEDs with R:B ratios of 0.9 and 1.8, although red light fraction increased from 24.2% to 33.9%, suggesting that plant responses to red light may also be associated with remaining spectral composition. Kim et al. (2004) observed that 24% green light added to red and blue lights promoted lettuce leaf fresh. This may be due to that green light can penetrate plant canopy easily compared with red and blue lights, thus increasing photosynthesis of lower leaves. However, when green light proportion accounted for more than 40% of spectral composition, no significant differences were observed as R:B ratio fluctuated. The green light fraction may be the main reason affecting lettuce growth between LEDs with R:B ratios of 0.9 and 1.8. Snowden et al. (2016) found that no remarkable differences were observed in lettuce dry weight between two white LEDs with 45.6% and 48.0% green light, respectively. Cope and Bugbee (2013) observed that no significant differences were found in total dry weight in radish, soybean, and wheat grown under three types of white LEDs with 23% to 49% red light and 41% to 50% green light. Similar results were reported by Cope et al. (2014) in lettuce. However, Nozue et al. (2018) demonstrated that white LEDs containing 48% to 57% green light fraction led to higher fresh weight of lettuce than those grown under white LEDs with 58% to 63% green light fraction, suggesting that excessive green light reduced plant growth and caused energetic waste, which agreed with the study reported by Kim et al. (2004). In addition, LEDs with an R:B ratio of 3.6 (with 52.2%
red light) resulted in lower leaf fresh and dry weights of hydroponic lettuce than those grown under LEDs with an R:B ratio of 2.7 (with 48.6% red light) at higher DLIs (Table 2), demonstrating that excess red light had negative impacts on dry weight accumulation as these two LEDs had similar fraction of green light. As a consequence, less blue light decreased Pn of lettuce leaf, thus leading to the imbalance of Pn and leaf area. Liu et al. (2018) observed that dry weight of cherry tomato seedlings increased first as red fraction increased from 0% to 50%, and then decreased as red fraction increased from 50% to 75%. Similar trends were also found in spinach (Agarwal et al., 2018). Spinach fresh weights increased as red fraction increased from 0% to 50% and then decreased as red fraction increased from 50% to 75%. Furuyama et al. (2014) observed that no significant differences were found in total dry weight of red leaf lettuce as red fraction increased from 75.2% to 81.3% at lower DLI (5.76 mol·m⁻²·d⁻¹); however, total dry

Table 2. Growth and morphology of hydroponic lettuce grown for 20 d after transplanting at five daily light integrals (D) provided by LEDs (L) with red to blue ratios of 0.9, 1.8, 2.7, and 3.6.

| Treatment | Leaf length (cm) | LL/LW* | Leaf number | Leaf fresh wt g per plant | Root fresh wt g per plant | Leaf dry wt g per plant | Root dry wt g per plant |
|-----------|------------------|--------|-------------|---------------------------|--------------------------|------------------------|-------------------------|
| L0.9-D5.04| 13.1 ± 0.8 bc    | 1.11 ± 0.08 ab | 6.5 ± 0.5 c | 14.44 ± 2.27 f | 3.43 ± 0.61 f | 0.73 ± 0.09 c | 0.11 ± 0.02 g |
| L0.9-D7.56| 13.4 ± 1.1 bc    | 1.07 ± 0.13 ab | 7.8 ± 0.4 b | 20.76 ± 3.63 c | 6.22 ± 0.82 de | 0.96 ± 0.22 de | 0.22 ± 0.03 ef |
| L0.9-D10.08| 13.3 ± 1.0 bc   | 1.01 ± 0.04 b  | 7.5 ± 0.8 b | 25.92 ± 3.59 d | 6.86 ± 0.77 d | 1.18 ± 0.20 cd | 0.27 ± 0.04 de |
| L0.9-D12.60| 12.4 ± 1.1 cd   | 0.95 ± 0.11 bc | 8.2 ± 0.4 ab | 29.39 ± 3.33 cd | 8.39 ± 0.80 e | 1.32 ± 0.16 cd | 0.29 ± 0.05 d |
| L0.9-D15.12| 12.4 ± 0.3 cd   | 0.92 ± 0.07 bc | 8.3 ± 0.5 ab | 33.83 ± 1.86 c | 8.91 ± 0.50 bc | 1.39 ± 0.14 c | 0.35 ± 0.02 cd |
| L1.8-D5.04| 12.7 ± 1.1 bc   | 1.04 ± 0.05 b  | 5.8 ± 0.4 c  | 14.00 ± 3.69 f | 3.93 ± 0.61 f | 0.64 ± 0.17 c | 0.17 ± 0.03 f |
| L1.8-D7.56| 13.0 ± 0.9 bc   | 1.07 ± 0.05 ab | 6.0 ± 0.6 c  | 17.73 ± 2.78 ef | 5.42 ± 0.80 e | 0.79 ± 0.15 c | 0.22 ± 0.05 c |
| L1.8-D10.08| 12.9 ± 0.2 c    | 0.98 ± 0.03 bc | 7.3 ± 0.5 b  | 22.13 ± 2.32 de | 6.51 ± 0.73 de | 1.01 ± 0.26 de | 0.22 ± 0.03 c |
| L1.8-D12.60| 12.8 ± 0.6 c    | 0.94 ± 0.05 bc | 7.2 ± 0.4 b  | 25.27 ± 3.65 de | 7.42 ± 1.18 cd | 1.22 ± 0.02 cd | 0.30 ± 0.08 d |
| L1.8-D15.12| 11.5 ± 0.4 d    | 0.90 ± 0.05 c  | 8.3 ± 1.0 ab | 26.30 ± 1.76 d | 7.53 ± 0.50 cd | 1.15 ± 0.12 cd | 0.28 ± 0.02 d |
| L2.7-D5.04| 15.4 ± 0.8 a    | 1.13 ± 0.16 a  | 7.5 ± 1.0 b  | 20.79 ± 3.76 c | 4.36 ± 0.71 ef | 0.85 ± 0.23 de | 0.18 ± 0.03 ef |
| L2.7-D7.56| 15.4 ± 1.0 a    | 1.03 ± 0.12 ab | 8.2 ± 0.4 ab | 27.01 ± 5.42 d | 6.23 ± 1.11 de | 1.04 ± 0.27 de | 0.27 ± 0.07 de |
| L2.7-D10.08| 13.7 ± 1.0 b    | 0.99 ± 0.08 bc | 8.5 ± 0.5 a  | 42.03 ± 4.42 b | 9.43 ± 0.89 bc | 1.61 ± 0.23 bc | 0.37 ± 0.05 c |
| L2.7-D12.60| 12.8 ± 1.4 c    | 0.93 ± 0.11 bc | 8.3 ± 0.5 ab | 48.67 ± 3.95 a | 11.44 ± 2.01 a | 2.05 ± 0.47 a | 0.52 ± 0.04 a |
| L2.7-D15.12| 12.5 ± 1.3 cd   | 0.94 ± 0.03 bc | 8.2 ± 0.4 ab | 45.46 ± 6.23 ab | 11.35 ± 1.51 a | 2.02 ± 0.41 ab | 0.49 ± 0.04 a |
| L3.6-D5.04| 13.8 ± 1.3 b    | 1.12 ± 0.13 a  | 7.2 ± 1.2 b  | 19.09 ± 4.99 c | 3.97 ± 0.72 f | 0.79 ± 0.16 e | 0.18 ± 0.03 ef |
| L3.6-D7.56| 14.8 ± 0.9 ab   | 0.98 ± 0.08 bc | 8.3 ± 0.5 ab | 27.61 ± 1.38 d | 6.52 ± 1.20 de | 1.09 ± 0.09 d | 0.26 ± 0.04 de |
| L3.6-D10.08| 14.3 ± 2.0 ab   | 0.99 ± 0.13 bc | 8.8 ± 0.4 a  | 30.85 ± 3.01 cd | 7.75 ± 1.22 cd | 1.28 ± 0.07 cd | 0.31 ± 0.03 d |
| L3.6-D12.60| 13.4 ± 0.9 bc   | 0.90 ± 0.07 c  | 8.5 ± 0.5 ab | 32.91 ± 2.36 cd | 8.29 ± 1.60 e | 1.55 ± 0.29 bc | 0.35 ± 0.04 cd |
| L3.6-D15.12| 13.3 ± 0.8 bc   | 0.85 ± 0.07 c  | 8.7 ± 0.8 a  | 38.52 ± 8.58 bc | 9.93 ± 1.46 b | 1.76 ± 0.17 b | 0.43 ± 0.06 b |

Fig. 3. Relationships between daily light integrals (DLIs) and fresh and dry weights of hydroponic lettuce grown for 20 d after transplanting in PFALs.
The weight of red leaf lettuce decreased as red fraction increased from 75.2% to 81.3% at higher DLIs (17.28 mol \cdot m^{-2} \cdot d^{-1}). Previous studies also indicated that no significant differences were found in fresh weight and dry weight of lettuce as red fraction increased from 50% to 75% (Xu et al., 2015) and 89% to 92% (Wang et al., 2016b), respectively, which confirmed that proper balance of light wavelengths would be beneficial to plants.

Nutritional values of hydroponic lettuce influenced by DLI and light quality. Higher DLI increased anthocyanin and vitamin C contents, and decreased nitrate contents, which were consistent with previous studies (Gent, 2014; He et al., 2019; Kang et al., 2013; Wang et al., 2016a). However, no significant differences were found in anthocyanin content between higher DLIs. Similar trends were found in sweet basil reported by Dou et al. (2018). Previous studies investigated effects of monochrome or combination of red and blue lights on anthocyanin and vitamin C contents of plants, indicating that monochrome blue light led to higher anthocyanin content of Chinese kale sprouts (Qian et al., 2016) and vitamin C contents of lettuce (Ohashi-Kaneko et al., 2007; Zhang et al., 2018a) than monochrome red light. However, no significant differences were observed

### Table 3. Nitrate, anthocyanin, and vitamin C contents of hydroponic lettuce grown for 20 d after transplanting at five daily light integrals (D) provided by LEDs (L) with red to blue ratios of 0.9, 1.8, 2.7, and 3.6, respectively.

| Treatment | Nitrate content (g kg^{-1} FW) | Anthocyanin content (\Delta OD/g FW) | Vitamin C content (mg/100 g FW) |
|-----------|---------------------------------|-------------------------------------|---------------------------------|
| L0.9-D5.04 | 6.15 ± 0.50 a*                  | 0.77 ± 0.13 c                      | 15.2 ± 0.9 bc                   |
| L0.9-D7.56 | 4.04 ± 0.38 b                   | 1.35 ± 0.24 bc                     | 23.3 ± 6.5 bc                   |
| L0.9-D10.08| 3.64 ± 0.60 bc                  | 2.17 ± 0.17 ab                     | 27.4 ± 7.1 ab                   |
| L0.9-D12.60| 3.46 ± 0.77 bc                  | 2.36 ± 0.23 a                      | 28.7 ± 2.5 ab                   |
| L0.9-D15.12| 2.94 ± 0.89 cd                  | 2.39 ± 0.41 a                      | 25.7 ± 2.9 ab                   |
| L1.8-D5.04 | 3.04 ± 0.36 cd                  | 0.78 ± 0.27 c                      | 16.0 ± 4.7 bc                   |
| L1.8-D7.56 | 3.31 ± 0.45 c                   | 0.87 ± 0.30 c                      | 20.9 ± 4.8 bc                   |
| L1.8-D10.08| 2.39 ± 0.28 d                   | 1.25 ± 0.39 c                      | 23.6 ± 5.1 b                    |
| L1.8-D12.60| 2.33 ± 0.16 d                   | 1.95 ± 0.67 ab                     | 30.7 ± 2.2 ab                   |
| L1.8-D15.12| 2.22 ± 0.21 d                   | 2.30 ± 0.61 ab                     | 29.9 ± 7.9 ab                   |
| L2.7-D5.04 | 3.25 ± 0.22 c                   | 0.30 ± 0.08 d                      | 14.4 ± 2.4 c                    |
| L2.7-D7.56 | 2.63 ± 0.52 d                   | 0.54 ± 0.07 d                      | 23.3 ± 4.7 b                    |
| L2.7-D10.08| 2.25 ± 0.44 d                   | 1.18 ± 0.41 c                      | 25.6 ± 7.5 b                    |
| L2.7-D12.60| 2.17 ± 0.48 d                   | 1.26 ± 0.38 c                      | 27.4 ± 1.3 ab                   |
| L2.7-D15.12| 2.13 ± 0.70 d                   | 1.61 ± 0.29 b                      | 31.2 ± 8.5 a                    |
| L3.6-D5.04 | 3.60 ± 0.16 bc                  | 0.19 ± 0.05 d                      | 18.8 ± 5.4 bc                   |
| L3.6-D7.56 | 3.42 ± 0.16 bc                  | 0.61 ± 0.17 d                      | 20.7 ± 7.2 bc                   |
| L3.6-D10.08| 2.90 ± 0.63 cd                  | 1.00 ± 0.28 c                      | 24.6 ± 8.7 ab                   |
| L3.6-D12.60| 2.75 ± 0.30 cd                  | 1.14 ± 0.05 c                      | 30.8 ± 5.4 ab                   |
| L3.6-D15.12| 2.60 ± 0.17 d                   | 1.83 ± 0.57 b                      | 27.9 ± 3.5 ab                   |

**Table 3.** Nitrate, anthocyanin, and vitamin C contents of hydroponic lettuce grown for 20 d after transplanting at five daily light integrals (D) provided by LEDs (L) with red to blue ratios of 0.9, 1.8, 2.7, and 3.6, respectively.

Different letters in the same column indicate significant differences at the 5% level, according to Duncan’s multiple range test (n = 6). NS and * represent no significant or significant difference at the 5% level, respectively.

Fig. 4. Relationships between daily light integrals (DLI) and power consumption based on fresh and dry weights (FW and DW), light energy use efficiency (LUE), and electrical energy use efficiency (EUE) of LED lighting for hydroponic lettuce production in PFALs.
between monochromic red and blue lights in vitamin C content of ‘Green Oak Leaf’ lettuce reported by Chen et al. (2014). In this study, compared with white plus red LEDs (with 14% to 18% blue light), lettuce grown under white LEDs (with 19% to 27% blue light) had higher anthocyanin contents, indicating that LEDs with higher fraction of blue light had higher anthocyanin contents, as anthocyanin biosynthesis was related to blue light through cryptochromes (Cashmore, 1999).

Leafy vegetables often accumulate excessive nitrate that can be converted to nitrite in human body, which is considered as a potential threat to human health. In this study, higher DLI led to lower nitrate content, this might be because higher DLI promoted the activity of nitrate reductase, thus decreasing nitrate accumulation. Generally, lettuce grown under white plus red LEDs (with 49% to 52% red light) had lower nitrate content than those grown under white LEDs with an R:B ratio of 0.9 (with 24% red light). Similar results were reported by Chen et al. (2016) when 18% red light was added to white LEDs. These results indicated that red light might be effective for stimulating the activity of nitrate reductase.

Impacts of DLI and light quality on energy use efficiency of LED lighting for lettuce production. Previous researchers examined EUE, LUE, or both with different definitions based on fresh weight, and LUE and EUE were closely correlated with specific spectra of LEDs and plant cultivars. Son et al. (2016) defined LUE and EUE as shoot fresh weight and total energy consumption divided by PPFD, respectively. They found that treatment with higher proportion of red light (RGB treatment with 78% red lights) resulted in higher LUE, and lowest power consumption combined with highest EUE were obtained in the treatment with highest portion of white LEDs, as with the same PPFD, RGB treatments led to highest shoot fresh weight. EUE was also defined as final fresh weight divided by artificial lights’ power consumption. Piovene et al. (2015) observed that EUE decreased as R:B ratio increased from 0.7 to 5.5 (red light fraction increased from 25% to 40%) in basil leaves, whereas no significant differences were found in EUE between LEDs in strawberry leaves. Pennisi et al., (2019) used similar definition as Piovene et al. (2015) did in sweet basil, suggesting that sweet basil grown under LEDs with an R:B ratio of 2.0–4.0 (62% to 75% red light) resulted in higher EUE than those grown under LEDs with an R:B ratio of 0.5–1.0 (30% to 46% red light), as higher yield was observed in the treatment with higher red light fraction.

LUE could be improved by using LEDs increasing the ratio of light energy received by leaves and controlling environmental factors (Kozai, 2013). One vital way for improving LUE and EUE is to figure out suitable lighting environment. In this study, power consumption based on fresh and dry weights increased linearly and LUE and EUE decreased linearly as DLI increased, which were not reported by previous studies. When 24% red light was added in white LEDs with an R:B ratio of 0.9, LUE and EUE increased by more than 20% and 7%, respectively. To reduce operational cost of LED lighting for lettuce production and improve LUE, EUE, or both, Wang et al. (2016a, 2018) investigated PPFD and R:B ratio of artificial lights. Results showed that PPFD at 200 and 300 mmol·m⁻²·s⁻¹ led to higher LUE and EUE, which were 0.042 and 0.0095, respectively. Increasing R:B ratio from 1 to 12 resulted in increased LUE and EUE with highest value of 0.017 and 0.0049, respectively. This was because higher fraction of red light resulted in higher leaf area index and thus increased light capture.

Conclusions

Growth and nutritional values of hydroponic lettuce were associated with both relative and absolute amounts of red, blue, or both lights. Impacts of light quality on plant growth were insignificant at low DLI light intensity. It was suggested that DLI should not be less than 7.56 mol·m⁻²·d⁻¹ for light quality comparison in lettuce production. Red light added in white LEDs was beneficial for improving LUE, which was also related to the proportion and remaining spectral composition. In conclusion, DLI at 12.60 mol·m⁻²·d⁻¹ provided by LEDs with an R:B ratio of 2.7 was beneficial for energy saving and carbohydrate accumulation of hydroponic lettuce, whereas higher blue fraction was positive for accumulation of antioxidant phenolic compounds (anthocyanin content). Our results have demonstrated a practical way of tailoring a crop-specific optimal light spectrum by adding different LEDs to a white light to create a “friendly” light environment to human eyes.

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