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Effects of Daily Light Integral and LED Spectrum on Growth and Nutritional Quality of Hydroponic Spinach

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Abstract: To achieve clean and high-quality spinach production, the effects of daily light integral (DLI) and light spectrum on growth, nutritional quality, and energy yield of hydroponic spinach (*Spinacia oleracea* L.) were investigated in a closed plant factory under light-emitting diode (LED) lighting. The hydroponic spinach plants were grown under 16 combinations of four levels of DLI (11.5, 14.4, 17.3, and 20.2 mol m^{-2} day^{-1}) with four light spectra: LED lamps with ratio of red light to blue light (R:B ratio) of 0.9, 1.2, and 2.2 and fluorescent lamps with R:B ratio of 1.8 as control. The results show that total fresh and dry weights, energy yield, and light energy use efficiency (LUE) of harvested spinach were higher under D17.3-L1.2 treatment compared to other treatments. The higher net photosynthetic rates were shown at DLI of 17.3 mol m^{-2} day^{-1} regardless of light quality. Higher vitamin C contents of spinach in all LED treatments were obtained compared with the control. L1.2 treatments with higher fraction of blue light led to more vitamin C content, lower nitrate content, and higher LUE independent of DLI. L2.2 treatment with more fraction of red light was beneficial to reduce oxalate accumulation. Power consumption based on increased total fresh weight under LED lamps with R:B ratio of 1.2 in different DLIs was over 38% lower than that under the fluorescent lamps and 1.73 kWh per 100 g FW at DLI of 17.3 mol m^{-2} day^{-1}. In conclusion, lighting environment in DLI of 17.3 mol m^{-2} day^{-1} using LED lamps with R:B ratio of 1.2 is suggested for the design of a LED plant factory for hydroponic spinach production.

Keywords: hydroponic spinach; ratio of red light to blue light; vitamin C content; nitrate content; energy yield

1. Introduction

The nutritional quality of leafy vegetables, especially spinach, is significantly affected by environmental variables such as light condition [1,2]. Hence, higher yield and nutritional quality of leafy vegetables could be achieved through plant factory with artificial lighting (PFAL) due to controlled environments [3]. Spinach is suitable to produce in PFAL because of its shorter growth cycle, lower plant height, and higher planting density. Nevertheless, higher initial investment and production cost of PFAL are the biggest obstacles to industrialization promotion. Light-emitting diode (LED) lamps are popularly used in new PFAL construction because of the unique properties in selectable and narrow-spectrum emissions, continuous improvement of lighting efficiency, long life, closer installation to plant, and higher light energy use efficiency compared to fluorescent lamps in the last decade [4]. Generally, lighting cost accounting for 70–80% of the total electricity consumption in PFAL directly led to higher vegetable production cost [3]. Thus, it is necessary to reduce lighting cost through adopting advanced LED lamps to improve the lighting system with well-designed photosynthetic photon flux density (PPFD), photoperiod, spectrum, and control strategy.

Daily light integral (DLI) is the total amount of photosynthetic active radiation received by plants each day as a function of light intensity and photoperiod. DLI is a vital light environment factor that could significantly affect plant growth and development and therefore achieve target growth. Zhang et al. [5] and Yan et al. [6] found that the shoot fresh weights of lettuces were proportional to DLI in different varieties. However, increasing DLI beyond 16.5 mol m^{-2} day^{-1} resulted in no further increases in total fresh weight and total phenolic concentration of sweet basil [7]. Spinach plants are usually prone to accumulate nitrate and oxalate under low light intensity [8]. Nitrate content of spinach shoot decreased from 1680 to 608 mg kg^{-1} as DLI increased from 3.0 to 20.5 mol m^{-2} day^{-1} [9]. Furthermore, net photosynthetic rate of spinach leaves was higher under high light intensity in 500 µmol m^{-2} s^{-1} compared to low level in 100 µmol m^{-2} s^{-1} [10]. These results demonstrate that higher DLI is normally beneficial to growth and nutritional quality of leafy plants.

Many studies have shown that growth and phytochemical accumulation of leafy plants could be regulated by applying different light spectra. The yield photon flux curve revealed that red photons could induce 30% more photosynthesis than blue photons [11]. Spinach plants had higher dry mass accumulation under white and/or red light with a light intensity of 300 µmol m^{-2} s^{-1} than they did under blue light using fluorescent lamps [1]. The shoot dry weight of spinach under white and red light with light intensity of 250 µmol m^{-2} s^{-1} was greater than that under green light [12]. More shoot fresh weight and chlorophyll contents of spinach plants grown under LED light in combination of red and blue wavebands were achieved than under the monochromatic light [2]. Previous studies have also shown that monochromatic blue light was not proper for the spinach cultivation due to a tremendously reduced shoot dry weight [13] and its deleteriousness to the photosynthetic apparatus [2]. The dry weights of spinach plants under red LEDs, red LEDs supplemented with ultraviolet radiation, and blue LEDs supplemented with ultraviolet radiation were significantly higher than those under white LED lamps and fluorescent lamps [14]. On the contrary, total dry weight was significantly lower for spinach plants grown under red LEDs supplemented with 10% blue light using blue fluorescent lamps than those grown under cool-white fluorescent lamps [15]. Ohashi-Kaneko et al. [13] suggested that red fluorescent lamps decreased nitrate content of spinach plants compared with white fluorescent lamps. Moreover, nitrate contents in lettuce plants grown under red, blue, and white LED treatments were significantly lower compared to those under the combination of red and blue LED treatments [16]. LED application of red and blue fractions using LED lamps improved the accumulation of antioxidant phenolic compounds for lettuce plants compared with those under fluorescent lamp [17]. LED lighting treatment enhanced energy use efficiency (176%) and energy yield (9%) of lettuce plants when compared with fluorescent lamps [18,19]. No significant differences between white LEDs and cool white fluorescent lamps were found for all physiological and yield parameters of lettuce plants. However, white LEDs resulted in lower power consumption, indicating that white LEDs could efficiently substitute traditional fluorescent lamps [20].

Spinach plants tend to be commercially produced in open fields and greenhouses. Light intensity greatly differs due to cultivating in the field and greenhouse with a wide range of geographic regions and different seasons. The fluctuating light environment could influence yield and phytochemical content of spinach plants. The objectives of this study were to clarify suitable DLI and LED spectrum for photosynthetic characteristics, biomass accumulation, nutritional properties, and electric energy consumption in hydroponic spinach production under LED lighting.

2. Materials and Methods

2.1. Seedling Materials and Growth Conditions

Seeds of spinach (*Spinacia oleracea* L. cv. BJC009) were soaked in warm water at 50 °C for 2 h, and then at room temperature for 22 h to fully absorb water before sowing. The seeds of spinach were sown in sponge cube (23 mm × 23 mm × 23 mm) filled with deionized water in plastic containers (520 mm × 360 mm × 90 mm). The seedling environments were with a temperature of 20 ± 1 °C in photoperiod and 15 ± 1 °C in dark period, relative humidity of 70 ± 5%, and CO_2 concentration of 800 μmol mol^{-1} in photoperiod and no control in dark period. Fluorescent lamps (FL-T5-28W, Shanghai Flower and Biology Lighting Co., Shanghai, China) with color temperature of 4200 K were provided to raise seedlings in light intensity of 200 μmol m^{-2} s^{-1} with photoperiod of 12 h day^{-1}. Standard nutrient solution based on Yamazaki formula was provided by following components (mg L^{-1}): $Ca(NO_3)_2·4H_2O$, 472; KNO_3, 808; $MgSO_4·7H_2O$, 492; $NH_4H_2PO_4$, 152; Fe-DTPA (7%), 28.5; $MnSO_4·H_2O$, 0.615; $CuSO_4·5H_2O$, 0.039; $ZnSO_4·7H_2O$, 0.088; H_3BO_3, 1.127; and $(NH_4)_6Mo_6O_{24}·4H_2O$, 0.013. The 1/2 strength of the standard nutrient solution with pH of 6.5 and EC of 1.0 mS cm^{-1} was used after cotyledon expanding. After the first true leaf expanded, the nutrient solution was changed to standard strength with pH of 6.5 and EC of 2.0 mS cm^{-1}. The nutrient solution was replaced once a week at the seedling stage and the later cultivation stage.

Fourteen days after sowing, spinach seedlings with two expanded true leaves were transplanted to hydroponic cultivation beds (1200 mm × 900 mm × 70 mm) (Figure 1A). Each bed held 54 plants. The air temperature, relative humidity, and nutrient solution at the cultivation stage were controlled at the same level as at seedling stage. Considering the response of net photosynthetic rate of spinach leaves to intercellular CO_2 concentration, the CO_2 concentration was set at 800 μmol mol^{-1} in photoperiod (Figure 2). The hydroponic spinach plants were harvested at20 days after transplanting (Figure 1B). The experiments after the transplanting were conducted in three small PFAL rooms located in water college building of China Agricultural University, China.

Figure 1. Hydroponic spinach cultivation at 0 (**A**) and 20 (**B**) days after transplanting.

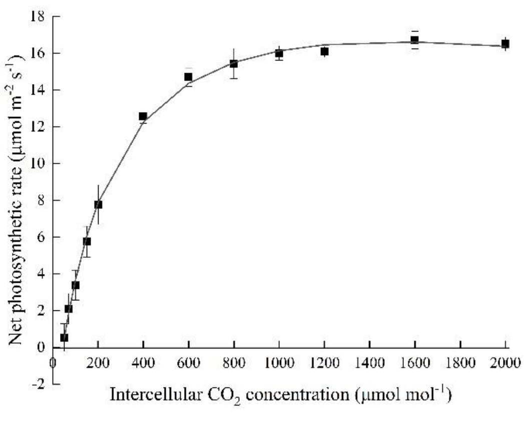

Figure 2. Net photosynthetic rate of spinach leaves responded to intercellular CO_2 concentration.

2.2. LED Lighting Treatments

Spinach seedlings were transplanted in 16 light combination treatments by four kinds of artificial lamps at four DLI levels of 11.5, 14.4, 17.3, and 20.2 mol m^{-2} day^{-1} (Table 1). The three kinds of LED lamps including one white lamp and two white plus red lamps with ratio of red light to blue light (R:B ratio) of 0.9, 1.2 and 2.2, respectively, and one kind of white fluorescent lamps in color temperature of 4200 K with R:B ratio of 1.8 as control (Beijing Lighting Valley Technology Co., Beijing, China) were used for four lighting spectrum designs. The four DLIs were delivered by four different light intensities of 200, 250, 300, and 350 µmol m^{-2} s^{-1} with a photoperiod of 16 h day^{-1}. Spectral distribution of above lighting environments with wavelengths ranging from 300 to 800 nm were scanned at 15 cm below the lamps in DLI treatments of 14.4 mol m^{-2} day^{-1} using a fiber spectrometer (AvaSpec-ULS2048, Avantes Inc., Apeldoorn, The Netherlands) (Table 2).

Table 1. Lighting treatments created by four levels of daily light integral (D) provided by white LEDs and white plus red LEDs (L) with different R:B ratio, and fluorescent lamps (F) as control for hydroponic spinach growth.

Treatment Symbols	DLI ^{x} (mol m^{-2} day^{-1})	R:B Ratio ^{y}	Light Intensity (µmol m^{-2} s^{-1})	Photoperiod (h day^{-1})	Light Source
D11.5-F1.8	11.5	1.8	200	16	White fluorescent lamp in color temperature of 4200 K
D14.4-F1.8	14.4		250		
D17.3-F1.8	17.3		300		
D20.2-F1.8	20.2		350		
D11.5-L0.9	11.5	0.9	200	16	LED lamp with white chip in color temperature of 6500 K
D14.4-L0.9	14.4		250		
D17.3-L0.9	17.3		300		
D20.2-L0.9	20.2		350		
D11.5-L1.2	11.5	1.2	200	16	LED lamp with white and red chips in 5:1 ratio, the white chip same to above and red chip in 660 nm
D14.4-L1.2	14.4		250		
D17.3-L1.2	17.3		300		
D20.2-L1.2	20.2		350		
D11.5-L2.2	11.5	2.2	200	16	LED lamp with white and red chips in 5:3 ratio, the white and red chips same to above
D14.4-L2.2	14.4		250		
D17.3-L2.2	17.3		300		
D20.2-L2.2	20.2		350		

^{x} DLI (mol m^{-2} day^{-1}) = light intensity (µmol m^{-2} s^{-1}) × photoperiod (h day^{-1}) × 3600 (s h^{-1}) × 10^{-6}. ^{y} R:B ratio is abbreviated for ratio of red light to blue light.

Table 2. Spectral distribution of lighting environments with DLI at 14.4 mol m^{-2} day^{-1} provided by three LED lamps with R:B ratio of 0.9, 1.2, and 2.2, respectively (L0.9, L1.2, and L2.2), and fluorescent lamps with R:B ratio of 1.8 (F1.8) as control.

Parameter	Spectral Fraction of Light Source (%)			
	F1.8	L0.9	L1.2	L2.2
Photon flux (300–800 nm)	100.0 ^{z}	100.0	100.0	100.0
Ultraviolet light (300–399 nm)	1.4	0.0	0.0	0.0
Blue light (400–499 nm)	20.3	27.0	25.9	20.4
Green light (500–599 nm)	39.0	46.9	41.1	33.9
Red light (600–699 nm)	35.8	24.2	31.4	44.1
Far-red light (700–800 nm)	3.5	1.9	1.6	1.6
R:B ratio ^{y}	1.8	0.9	1.2	2.2

^{z} Data are fractions of integral photon flux ranging from 300 to 800 nm in ultraviolet, blue, green, red, and far red lights. ^{y} R:B ratio is abbreviated for ratio of red light to blue light.

2.3. Measurement Indexes and Methods

2.3.1. Plant Morphological and Growth Characteristics

Six uniform plants were randomly selected at 20 days after transplanting for following growth measurement in each treatment. The leaf length, width, and petiole length of mature leaves in the same part of samples were measured with a ruler. The petiole diameter was measured with a digital vernier caliper (573–605, Japan Mitustoyo Precision Measuring Instrument, Kanagawa, Japan). The mature leaves of spinach were scanned with a scanner (LiDE-110, Canon, Tokyo, Japan) for leaf area measurement by Photoshop image processing (Adobe Photoshop CS6, Adobe System Inc., San Jose, CA, USA). Electronic balance (YP402, Shanghai Precision Science Instrument, Shanghai, China) was used to measure the fresh weight of the spinach shoots and roots. The shoots and roots were dried in a ventilated oven at 105 °C for 3 h and then at 80 °C for over 72 h until no weight reduction. The dry weights of the spinach shoots and roots were measured by electronic balance (FA1204B, Shanghai Precision Science Instrument, Shanghai, China).

2.3.2. Photosynthetic Characteristics and Nutritional Indices

The fifth fully expanded leaf from apical shoot was exposed to LED lamps with light intensity of 300 μmol m^{-2} s^{-1} supplied by a portable photosynthesis system (LI-6400XT, LI-COR Inc., Lincoln, NE, USA) to measure net photosynthetic rate (μmol m^{-2} s^{-1}), stomatal conductance (mmol m^{-2} s^{-1}), intercellular CO_2 concentration (μmol mol^{-1}), and transpiration rate (mmol m^{-2} s^{-1}). Leaf temperature and CO_2 concentration were maintained at 20 °C and 800 μmol mol^{-1} in the leaf chamber, respectively. Nitrate, vitamin C, and oxalate contents as the spinach nutritional indices were measured by the following methods. A whole fragment of spinach leaf was cut into small pieces and mixed for the nutritional index measurement. Coloration method of sulfosalicylic acid [21], 2,6-dichlorophenol indophenol titration method [22], and potassium permanganate by titration method [23] were used to measure nitrate, vitamin C, and oxalate contents of spinach leaves, respectively.

2.3.3. Energy Yield and Light Energy Use Efficiency

The power consumptions of light sources in each treatment were measured by a power monitor (T8006, Shenzhen BeiDian Instrument Co., Shenzhen, China). Energy yield defined as increased shoot fresh weight divided by power consumption of light source during cultivation stage is used for evaluation of LED lamps adaptability in hydroponic plant production [19]. Light energy use efficiency (LUE) is calculated according to Kozai and Niu [3] as LUE = $f \times D$/PAR, where f is the conversion coefficient from dry mass to chemical energy (approximately 20 MJ kg^{-1}), D is the dry mass increase rate of plants (kg m^{-2}), and PAR is the photosynthetically active radiation (MJ m^{-2}). To evaluate power consumption level of hydroponic spinach cultivation more intuitively and provide quantitative basis for future commercial production, power consumption based on increased total fresh weight is the power consumption measured on a cultivation bed divided by the actual total fresh weight (the total fresh weight in the harvest period minus the total fresh weight in the transplanting period). This index is known as the estimated power consumption per 100 g of fresh weight per kilowatt hour (kWh per 100 g FW), as 80–100 g of fresh spinach shoots are often used for commercial packaging.

2.4. Statistical Analysis

Statistical analysis was implemented using SPSS 18.0 software (IBM, Inc., Chicago, IL, USA). Statistical significance was performed by two-way ANOVA with Duncan's multiple range test ($p <$ 0.05) for determining significant effects of DLI and light spectrum. The results are reported as the mean ± standard deviation values ($n = 6$). The regression analysis between biomass accumulation and DLI was carried out using OriginPro 2018 software (OriginLab Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. Photosynthetic and Morphological Characteristics of Hydroponic Spinach

Net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate of hydroponic spinach leaf were significantly influenced by DLI and light spectrum (Table 3). No significant differences in net photosynthetic rates were observed in different DLI levels provided by LEDs with R:B ratio of 0.9 and 2.2, but significant decreases were shown when DLI was from

17.3 to 20.2 mol m^{-2} day^{-1} in treatments of F1.8 and L1.2. The treatments in DLI at 17.3 mol m^{-2} day^{-1} showed higher net photosynthetic rate regardless of light quality. The net photosynthetic rate of spinach leaf under D14.4-L0.9 treatment was 14.4 ± 0.5 µmol m^{-2} s^{-1}, 8%, 13%, and 16% greater than that under treatments of D14.4-F1.8, D14.4-L1.2 and D14.4-L2.2, respectively, but there was no significant difference compared to D17.3-L1.2 treatment. Stomatal conductance and transpiration rate increased significantly with DLI increasing from 11.5 to 17.3 mol m^{-2} day^{-1} and then decreased regardless of light quality. Intercellular CO_2 concentrations were lower in DLI of 11.5 mol m^{-2} day^{-1} compared with other DLI treatments regardless of light quality. The change trend of transpiration rates was consistent with stomatal conductance in different light spectrum treatments.

Table 3. Net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate of hydroponic spinach grown under lighting treatment at four daily light integrals (D11.5, D14.4, D17.3, and D20.2) provided by fluorescent or LED lamps with R:B ratio of 1.8, 0.9, 1.2, and 2.2 (F1.8, L0.9, L1.2, and L2.2), respectively, for 20 days after transplanting.

Treatments	Net Photosynthetic Rate (µmol m^{-2} s^{-1})	Stomatal Conductance (mmol m^{-2} s^{-1})	Intercellular CO_2 Concentration (µmol mol^{-1})	Transpiration Rate (mmol m^{-2} s^{-1})
D11.5-F1.8	13.3 ± 0.6 ab [z]	407 ± 21 f	712 ± 3 d	4.1 ± 0.1 f
D14.4-F1.8	13.3 ± 0.6 ab	583 ± 72 e	724 ± 8 c	5.9 ± 0.4 d
D17.3-F1.8	13.5 ± 0.8 ab	650 ± 70 cd	726 ± 6 bc	6.2 ± 0.2 c
D20.2-F1.8	11.5 ± 0.9 c	593 ± 64 e	731 ± 6 b	5.6 ± 0.6 de
D11.5-L0.9	13.2 ± 0.7 ab	573 ± 23 e	722 ± 3 c	6.1 ± 0.1 c
D14.4-L0.9	14.4 ± 0.5 a	870 ± 79 ab	731 ± 8 b	7.9 ± 0.4 a
D17.3-L0.9	13.6 ± 0.6 ab	837 ± 38 b	729 ± 4 b	7.8 ± 0.2 a
D20.2-L0.9	13.8 ± 0.5 ab	683 ± 81 cd	730 ± 8 b	6.6 ± 0.4 bc
D11.5-L1.2	13.0 ± 0.7 b	603 ± 35 d	727 ± 9 c	6.2 ± 0.2 c
D14.4-L1.2	12.8 ± 0.6 b	717 ± 118 c	729 ± 7 b	6.4 ± 0.5 bc
D17.3-L1.2	13.4 ± 0.3 ab	937 ± 32 a	738 ± 2 ab	6.9 ± 0.4 b
D20.2-L1.2	11.7 ± 0.9 c	577 ± 46 e	730 ± 9 b	4.8 ± 0.1 e
D11.5-L2.2	13.0 ± 0.4 b	560 ± 40 e	727 ± 2 bc	4.4 ± 0.1 ef
D14.4-L2.2	12.4 ± 0.6 bc	700 ± 20 cd	737 ± 1 ab	4.9 ± 0.3 e
D17.3-L2.2	13.3 ± 0.5 ab	827 ± 75 b	745 ± 3 a	5.8 ± 0.2 d
D20.2-L2.2	12.6 ± 0.8 bc	633 ± 15 cd	728 ± 5 b	4.8 ± 0.3 e
DLI	*	*	*	*
LQ	*	*	*	*
DLI × LQ	*	*	NS	*

[z] Different letters in the same column indicate significant differences based on two-way ANOVA with Duncan's multiple range test (n = 6) at $p \leq 0.05$. NS and * represent no significant difference or significant difference at $p \leq 0.05$, respectively.

Matsuda et al. [10] found that spinach grown under white light had higher net photosynthetic rate than those grown under blue-deficient light with the same light intensity at 500 µmol m^{-2} s^{-1}. Similar trends were also observed in lettuce reported by Yan et al. [6]. Song et al. [24] found that cucumber seedlings grown under white LEDs with 27% blue light had higher transpiration rate than those grown under fluorescent lamps with 14–19% blue light, indicating that H_2O exchange rate of plants was promoted by higher fraction of blue light. In this study, white LEDs led to higher transpiration rate of hydroponic spinach than white plus red LEDs, and this might arise from the different fraction of blue light contained in LEDs. The lighting environments in more blue and green light combined with less red light led to higher stomatal opening and transpiration, but no differences were found in net photosynthetic rate. Therefore, the results of photosynthetic properties show that DLI from 14.4 to 17.3 mol m^{-2} day^{-1} would be more suitable for photosynthesis of hydroponic spinach leaf, but the influence of light spectra needs further analysis.

Morphological characteristics of hydroponic spinach leaf were significantly affected by DLI and LED spectrum (Table 4). In general, fluorescent lamps resulted in more leaf numbers of hydroponic spinach compared with those grown under LEDs, especially white LED. For example, leaf numbers in treatments of L0.9 and L2.2 were 10–17% and 7–20% lower compared to D11.5-F1.8 treatment, respectively. However, the same numbers of leaves in treatments of D14.4-L1.2 and D17.3-L1.2 were harvested as D17.3-F1.8 treatment. The higher petiole length and diameter were also harvested in treatments of D14.4-L1.2 and D17.3-L1.2 compared to other treatments. From the results in leaf length and width, leaf area, and specific leaf area, hydroponic spinach growth in D17.3-L1.2 treatment was greater than that in D14.4-L1.2 treatment. The decrease of petiole length was found with increasing DLI in F1.8 treatments; however, the LED treatments showed that petiole length increased first and then decreased with the increase of DLI. This result could be explained by shade avoidance response due to auxin and transcription factors of the phytochrome interacting factor class [25]. Similarly, Ohashi-Kaneko et al. [13] reported that small petiole length of spinach leaf was observed under monochromatic blue light treatment compared with other treatments. A similar trend response to blue light at higher PPFD in 500 μmol m^{-2} s^{-1} in petiole length was also observed in tomato, cucumber, and radish [26,27]. Previous studies have demonstrated that leaf area of lettuce [26,28], cucumber seedlings [29], and spinach [13] decreased as blue light fraction increased. These results indicate that leaf expansion associated with blue light was a cryptochrome-mediated response, and the blue light percent of total light intensity could better predict leaf area [30]. Specific leaf area influenced canopy expansion and growth through light interception and light use efficiency and determined how much new leaf area to deploy for each unit of biomass produced. The specific leaf area in L1.2 treatments in this study decreased by 27–39% when DLI increased from 14.4 to 20.2 mol m^{-2} day^{-1}. These results are consistent with those of Dou et al. [7] who discovered that thickness of basil leaves significantly increased with higher DLI. However, no significant differences were found in specific leaf area among DLIs under white LEDs with an R:B ratio of 0.9. From the morphological indices of hydroponic spinach leaves, greater growth in D17.3-L1.2 treatment might have resulted from more photosynthesis brought by appropriate blue light ratio.

3.2. Biomass Accumulation of Hydroponic Spinach

The biomass accumulation data were not very different for different light spectrum treatments in DLI in 11.5 mol m^{-2} day^{-1}, but the differences were more and more obvious with the increase of DLI, especially when DLI was in 17.3 mol m^{-2} day^{-1} (Figure 3). When DLI increased from 11.5 to 14.4, 17.3, and 20.2 mol m^{-2} day^{-1}, biomass accumulations in L1.2 treatments were significantly greater than in other light spectrum treatments and especially when DLI was in 17.3 mol m^{-2} day^{-1}. Compared to treatments of L0.9 and L2.2, shoot, root, and total fresh weights of hydroponic spinach in F1.8 treatments were greater, but the same trend was not found in dry weight (Figure 3A,C,E). Among the L1.2 treatments, shoot, root, and total fresh weights of hydroponic spinach grown under DLI in 17.3 mol m^{-2} day^{-1} were 42.0, 10.8, and 52.7 g per plant, respectively, and were over 1.2 times more than those with DLI in 14.4 and 20.2 mol m^{-2} day^{-1} and 1.5 times more than those with DLI in 11.5 mol m^{-2} day^{-1}. The shoot and total dry weights were over 1.3 and 1.7 times more compared to those in the above two treatment groups (Figure 3B,D,F). These results indicate that excessive DLI would inhibit spinach growth and DLI in 17.3 mol m^{-2} day^{-1} was beneficial for hydroponic spinach cultivation.

A previous study reported that shoot fresh and dry weights of spinach (“Manyoh” and “Okame”) increased as DLI increased from 4.3 to 13.0 mol m^{-2} day^{-1} [1]. However, Gent [9] observed that total fresh weight of spinach decreased as DLI increased from 4.0 to 14.0 mol m^{-2} day^{-1} when the temperature increased from 16.1 to 20.1 °C. More blue and green lights and less red light in L0.9 treatments did not lead to more biomass accumulation due to excessive stomatal opening and transpiration consuming too much energy. Those results were consistent with the findings reported by Ohashi-Kaneko et al. [13] who observed that the lowest shoot dry weights of spinach were found under monochromatic blue light. Generally, increasing blue light component could inhibit cell division and leaf expansion, thus reducing leaf area and resulting in photon capture reduction. White plus red LEDs with R:B ratio of 1.2 in L1.2 treatments led to higher shoot and root fresh weights compared with those grown under F1.8 treatments when DLI was over 11.5 mol m^{-2} day^{-1}. Contrarily, total dry weight of spinach was significantly lower under red LEDs supplemented with 10% blue light than those grown under cool-white fluorescent lamps [15]. This phenomenon might mainly result from different varieties and experimental conditions.

Table 4. Morphological characteristics of hydroponic spinaches grown under lighting treatment at four daily light integrals (D11.5, D14.4, D17.3, and D20.2) provided by fluorescent or LED lamps with R:B ratio of 1.8, 0.9, 1.2, and 2.2 (F1.8, L0.9, L1.2, and L2.2), respectively, for 20 days after transplanting.

Treatments	Leaf Number	Leaf Length (cm)	Leaf Width (cm)	Petiole Length (cm)	Petiole Diameter (mm)	Leaf Area (cm^2)	Specific Leaf Area (m^2 kg^{-1})
D11.5-F1.8	10.0 ± 0.8 b [z]	12.6 ± 0.2 bc	5.7 ± 0.6 c	7.7 ± 1.1 ab	4.4 ± 0.4 ab	53.6 ± 5.0 d	4.40 ± 0.91 c
D14.4-F1.8	10.3 ± 0.6 b	11.9 ± 1.9 c	5.8 ± 0.4 c	5.6 ± 1.5 cd	3.9 ± 0.5 bc	51.2 ± 7.2 d	5.16 ± 1.31 b
D17.3-F1.8	11.3 ± 1.0 a	12.0 ± 0.7 c	5.9 ± 0.3 c	5.1 ± 1.6 cd	4.3 ± 0.5 ab	51.5 ± 2.5 d	2.73 ± 0.37 d
D20.2-F1.8	10.6 ± 0.9 ab	10.5 ± 0.9 d	5.6 ± 0.4 c	4.4 ± 1.8 d	4.1 ± 0.7 b	42.1 ± 2.2 e	2.28 ± 0.15 d
D11.5-L0.9	9.0 ± 0.8 c	10.3 ± 0.6 d	6.1 ± 0.5 c	4.5 ± 1.5 d	4.1 ± 0.5 b	47.5 ± 5.1 de	4.34 ± 0.54 c
D14.4-L0.9	8.3 ± 0.6 cd	11.1 ± 0.2 cd	6.6 ± 1.0 bc	6.0 ± 1.1 c	3.5 ± 0.3 c	55.1 ± 7.4 cd	4.92 ± 0.92 bc
D17.3-L0.9	8.3 ± 0.6 cd	12.8 ± 1.5 bc	5.6 ± 0.3 c	6.4 ± 0.6 c	3.2 ± 0.5 c	52.9 ± 12.4 d	4.80 ± 1.14 bc
D20.2-L0.9	8.8 ± 0.5 cd	11.2 ± 0.8 cd	6.0 ± 0.6 c	5.8 ± 0.6 cd	3.7 ± 0.1 bc	49.3 ± 9.5 d	4.35 ± 0.69 c
D11.5-L1.2	8.3 ± 0.6 cd	12.2 ± 0.6 bc	5.9 ± 0.3 c	7.0 ± 1.6 b	4.1 ± 0.5 b	53.2 ± 3.1 d	4.76 ± 1.68 bc
D14.4-L1.2	10.7 ± 0.6 ab	13.5 ± 1.0 b	7.3 ± 0.3 b	8.4 ± 0.6 a	5.0 ± 0.4 a	70.7 ± 7.2 b	3.47 ± 0.83 cd
D17.3-L1.2	11.0 ± 1.0 a	15.3 ± 0.7 a	8.7 ± 0.9 a	8.6 ± 0.8 a	5.1 ± 0.6 a	98.3 ± 5.6 a	3.10 ± 0.57 d
D20.2-L1.2	9.3 ± 0.6 c	13.5 ± 0.6 b	7.9 ± 0.9 ab	6.3 ± 0.3 c	4.4 ± 0.6 ab	75.8 ± 9.5 b	2.89 ± 0.65 d
D11.5-L2.2	8.8 ± 0.5 cd	11.9 ± 1.0 c	6.9 ± 0.7 bc	4.5 ± 0.9 d	4.2 ± 1.4 b	60.8 ± 5.7 c	4.69 ± 0.89 bc
D14.4-L2.2	8.0 ± 1.4 d	12.1 ± 1.4 bc	6.8 ± 0.7 bc	5.3 ± 1.2 cd	3.8 ± 0.5 bc	61.6 ± 10.1 c	6.69 ± 1.18 a
D17.3-L2.2	9.3 ± 0.6 c	12.4 ± 1.8 bc	7.4 ± 0.7 b	5.7 ± 1.1 cd	4.6 ± 0.2 ab	70.5 ± 16.8 b	4.86 ± 2.16 bc
D20.2-L2.2	8.8 ± 0.4 cd	10.8 ± 0.6 d	6.8 ± 0.2 bc	4.4 ± 1.1 d	4.3 ± 0.7 ab	53.1 ± 1.8 d	4.20 ± 0.67 c
DLI	*	*	*	*	*	*	*
LQ	*	*	*	*	*	*	*
DLI × LQ	*	*	*	*	NS	*	*

[z] Different letters in the same column indicate significant differences based on two-way ANOVA with Duncan's multiple range test ($n = 6$) at $p \leq 0.05$. NS and * represent no significant difference or significant difference at $p \leq 0.05$, respectively.

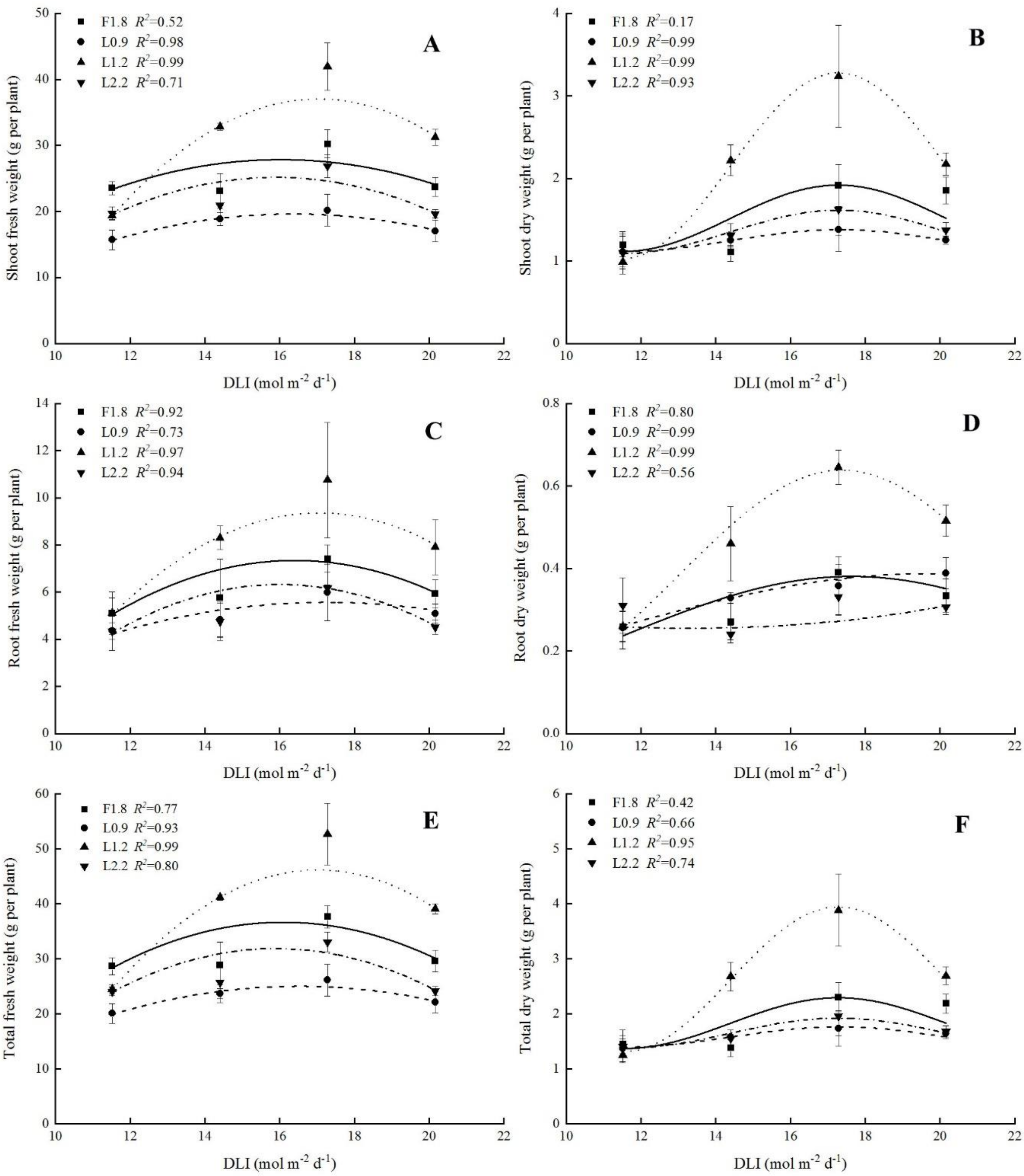

Figure 3. Relationships between daily light integral (DLI) and shoot fresh weight (**A**), shoot dry weight (**B**), root fresh weight (**C**), root dry weight (**D**), total fresh weight (**E**) and total dry weight (**F**)biomass accumulation of hydroponic spinach grown under different light spectra for 20 days after transplanting.

3.3. Nutritional Quality of Hydroponic Spinach

Light intensity and spectrum are critical factors in regulating vitamin C biosynthesis and its accumulation in higher plants [31]. The vitamin C contents of hydroponic spinach cultivated in F1.8 treatments were significantly lower than those in all LED treatments, and there was no significant difference regardless of DLI (Figure 4A). In treatments of L0.9 and L1.2, no significant differences in vitamin C contents were shown at DLI of 14.4, 17.3 and 20.2 mol m^{-2} day^{-1}, but it was 20% higher than that of 11.5 mol m^{-2} day^{-1}. Among L2.2 treatments, there was no significant difference in vitamin C content when DLI were 11.5, 14.4, and 17.3 mol m^{-2} day^{-1}, but it was 20% lower than that of 20.2 mol m^{-2} day^{-1}. When DLI was at 11.5 mol m^{-2} day^{-1}, no significant difference was shown regardless of LED spectrum, but there was significant difference when DLI was up to over 14.4 mol m^{-2} day^{-1}, which showed that vitamin C contents in L1.2 treatments were significantly higher than those in L0.9 treatments, and L0.9 treatments was significantly higher than L2.2 treatments when DLI was in 14.4 and 17.3 mol m^{-2} day^{-1}, while no significant difference was found when DLI increased to 20.2 mol m^{-2} day^{-1}. Similar results were reported by Yan et al. [6], where vitamin C of hydroponic lettuce increased with increasing DLI from 5 to 10 mol m^{-2} day^{-1}. It could be interpreted that higher light intensity increased whole plant photosynthetic capacity and then promoted the vitamin C synthesis and accumulation [31]. Compared with white fluorescent lamps (with 20% blue light fraction) and white LED lamps with R:B ratio of 2.2 (with 20% blue light fraction) in this study, spinach grown under LEDs with R:B ratio of 0.9 and 1.2 (with 27% and 26% blue light fraction) had higher vitamin C contents. This was consistent with the findings of Ohashi-Kaneko et al. [13], where vitamin C content of lettuce and komatsuna increased under blue fluorescent lamps or red and blue combined fluorescent lamps compared with those grown under white fluorescent lamps. Similarly, vitamin C content of the 28-day-old spinach under blue LED light was significantly higher compared with that under fluorescent lamps and red LEDs [14]. These results demonstrate that a higher fraction of blue light could result in more vitamin C content.

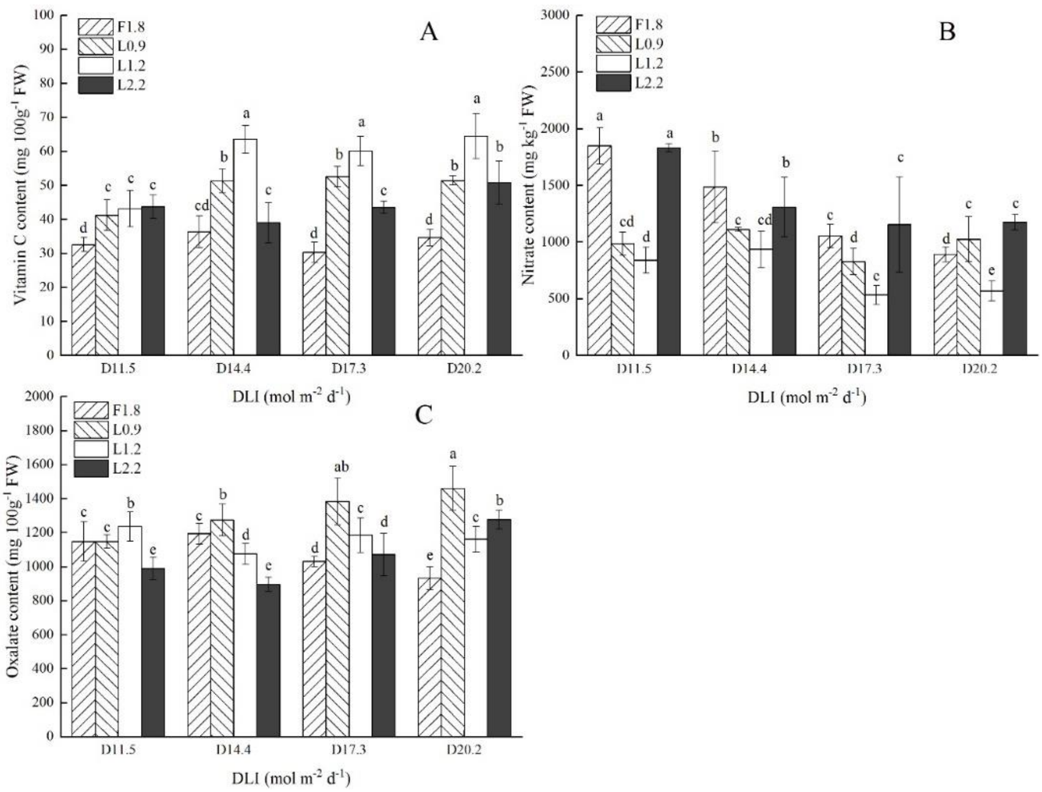

Figure 4. Vitamin C (**A**), nitrate (**B**) and oxalate (**C**) contents of hydroponic spinach leaves grown under lighting treatments at four daily light integrals (D11.5, D14.4, D17.3, and D20.2) provided by fluorescent or LED lamps with R:B ratio of 1.8, 0.9, 1.2, and 2.2 (F1.8, L0.9, L1.2, and L2.2), respectively, for 20 days after transplanting. Different letters correspond to significant differences ($p \leq 0.05$) by two-way ANOVA with Duncan's multiple range test (n = 6).

Light environments can directly influence nitrate synthesis of plants and leafy vegetables generally tend to accumulate nitrate under low light intensities. Higher light intensity contributed to reduction of nitrate contents of lettuce [32]. Similarly, more nitrate contents of spinach grown under low light intensity at 200 μmol m^{-2} s^{-1} were harvested compared to high level in 800 μmol m^{-2} s^{-1} [8,33]. Nitrate content of hydroponic spinach decreased with DLI increasing from 11.5 to 20.2 mol m^{-2} day^{-1}

provided by fluorescent lamps with R:B ratio of 1.8 in this study (Figure 4B), which was consistent with previous study [9]. The nitrate contents in treatments of L1.2 and L2.2 had similar changing trend, and were obtained in low level at treatments of D14.4-L1.2 and D17.3-L1.2, which were 535.3 ± 83.4 and 569.8 ± 88.2 mg kg^{-1} FW, respectively. However, the nitrate contents in L0.9 treatments did not show a certain change rule with increasing DLI. Returning to the data of L1.2 and L2.2 treatments, it could be found that, when DLI increased from 17.3 to 20.2 mol m^{-2} day^{-1}, nitrate content did not decrease any more. It also meant that the much higher light intensity did not necessarily lead to lower nitrate content because stimulation of nitrate reductase activity by light intensity was limited. Therefore, DLI in 17.3 mol m^{-2} day^{-1} might be the threshold of nitrate regulation in hydroponic spinach under LED lighting and higher fraction of blue light might be conducive to nitrogen reduction of hydroponic spinach.

Oxalate exits primarily as soluble oxalate, insoluble oxalate, or the combination of these two forms in plants. Many studies have revealed that the immediate precursor of oxalate is ascorbic acid [34], glycolate, and glyoxylate [35,36]. It had also been reported that ascorbic acid was not a precursor of oxalic acid [37]. These previous results indicate oxalate synthesis pathway varied with plant species. With increasing DLI, oxalate content of hydroponic spinach cultivated in F1.8 treatments decreased significantly, but it increased significantly in treatments of L0.9 and L2.2, while L1.2 treatments also showed a downward trend (Figure 4C). Compared with D20.2-F1.8 treatment at low oxalate level, oxalate contents in treatments of D17.3-L2.2 and D20.2-L2.2 also reached the same low level, which were 989.5 ± 64.8 and 897.0 ± 41.0 mg kg^{-1} FW, respectively. When DLI was in 11.5 and 14.4 mol m^{-2} day^{-1}, oxalate contents in L2.2 treatments were 16–42% lower than those in treatments of L1.8, L0.9, and L1.2. However, when DLI increased to 17.3 mol m^{-2} day^{-1}, it was 11–29% lower than that in treatments of L0.9 and L1.2, but there was no significant difference compared to F1.8 treatment. When DLI increased to up to 20.2 mol m^{-2} day^{-1}, the oxalate content in L2.2 treatment was 15% lower than that in L0.9 treatment, but 9–27% higher than that in F1.8 and L1.2 treatments. It could be concluded that LED quality with more fraction of red light is beneficial to inhibit oxalate accumulation, and light spectral adjustment might replace light intensity regulation in hydroponic spinach production. Proietti et al. [8] found that the oxalate content of spinach leaves was 25% lower under high light intensity in 800 μmol m^{-2} s^{-1} than that under low level at 200 μmol m^{-2} s^{-1}. The oxalate oxidase activity of oxalate synthesis increased with increasing light intensity [35]. This indicates that high light intensity affected the oxalate accumulation by improving oxalate oxidase activity. Leaf is the main part of oxalate synthesis and red light might help oxalate transport from leaf to other parts. The oxalate contents of hydroponic spinach grown in L2.2 treatments with more red light components (44% red light fraction) were 15–40% lower than those in L0.9 treatment (24% red light fraction) regardless of DLI and were 16–33% lower than those in treatments of F1.8 and L1.2 (36% and 31% red light fraction) at DLI of 11.5 and 14.4 mol m^{-2} day^{-1}. Similarly, Qi et al. [38] found that oxalate content in spinach leaves under red light was 47.6% lower than that under white light. Under the light condition using fluorescent lamps, the oxalate content could be effectively reduced by increasing the light intensity, but the LED lighting technology might be realized by adjusting the spectral composition.

3.4. Energy Yield and Light Energy Use Efficiency

Higher electric power consumption is a bottleneck restricting the industrialization of plant factory technology. Therefore, improving energy yield and LUE as a recent hot research is focused on providing technical supply in agricultural engineering area. Compared to F1.8 treatments, power consumptions based on increased total fresh weight in LED treatments were significantly reduced, while energy yield and LUE were significantly improved, especially in L1.2 treatments (Table 5). When DLI was 11.5, 14.4, and 17.3 mol m^{-2} day^{-1} in F1.8 treatments, there were no significant differences in energy consumption and LUE, but when DLI increased to 20.2 mol m^{-2} day^{-1}, the power consumption based on increased total fresh weight increased by 50% and the energy yield decreased by more than 25%, but LUE did not decrease much. The change trend in L0.9 treatments was similar to that with

F1.8 treatments. In L2.2 treatments, power consumption based on increased total fresh weight and LUE decreased with the increase of DLI, and the energy yield did not change much, but, when DLI increased up to 20.2 mol m^{-2} day^{-1}, the power consumption based on increased total fresh weight increased by more than 40%, and the energy yield and LUE decreased by more than 38%. The results show that LUE in L1.2 treatments was significantly higher than that in other treatments, but there was no significant difference among different DLI levels. When DLI in 14.4 and 17.3 mol m^{-2} day^{-1}, the power consumption based on increased total fresh weight and energy yield in L1.2 treatments were significantly lower and higher than those when DLI in 11.5 and 20.2 mol m^{-2} day^{-1}, and the best performance was obtained at D17.3-L1.2 treatment, which were 1.73 ± 0.19 kWh per 100 g FW and 46.82 ± 3.99 g FW kWh^{-1}, respectively. These results show that both higher and lower DLI would reduce energy yield and increase power consumption based on increased total fresh weight, but an optimized spectral formula in LED lamps could effectively promote LUE in plant factory.

Table 5. Energy yield and light energy use efficiency (LUE) of hydroponic spinach production under fluorescent or LED lamps with R:B ratio of 1.8, 0.9, 1.2, and 2.2 (F1.8, L0.9, L1.2, and L2.2) providing four daily light integrals (D11.5, D14.4, D17.3, and D20.2).

Treatments	Power Consumption Based on Increased Total Fresh Weight (kWh per 100 g FW)	Energy Yield (g FW kWh^{-1})	LUE
D11.5-F1.8	4.50 ± 0.11 cd [z]	17.91 ± 1.21 de	0.018 ± 0.001 b
D14.4-F1.8	4.82 ± 0.43 c	15.17 ± 1.75 e	0.016 ± 0.003 bc
D17.3-F1.8	4.86 ± 0.17 c	15.92 ± 0.82 e	0.022 ± 0.002 ab
D20.2-F1.8	7.12 ± 0.37 a	11.21 ± 0.54 f	0.015 ± 0.001 bc
D11.5-L0.9	4.05 ± 0.32 d	21.23 ± 2.08 d	0.014 ± 0.003 c
D14.4-L0.9	3.82 ± 0.28 de	21.46 ± 1.10 d	0.013 ± 0.003 c
D17.3-L0.9	3.55 ± 0.31 de	20.13 ± 2.40 d	0.013 ± 0.004 c
D20.2-L0.9	5.65 ± 0.40 b	14.13 ± 0.87 e	0.012 ± 0.003 c
D11.5-L1.2	2.73 ± 0.10 f	29.77 ± 1.00 c	0.022 ± 0.008 ab
D14.4-L1.2	2.07 ± 0.03 g	39.16 ± 0.62 b	0.026 ± 0.003 a
D17.3-L1.2	1.73 ± 0.19 h	46.82 ± 3.99 a	0.025 ± 0.003 a
D20.2-L1.2	2.77 ± 0.07 f	29.25 ± 1.16 c	0.021 ± 0.006 ab
D11.5-L2.2	2.83 ± 0.06 f	29.91 ± 1.72 c	0.020 ± 0.003 ab
D14.4-L2.2	3.12 ± 0.30 e	26.71 ± 2.47 cd	0.013 ± 0.002 c
D17.3-L2.2	2.77 ± 0.15 f	29.98 ± 1.92 c	0.015 ± 0.002 bc
D20.2-L2.2	4.38 ± 0.04 cd	18.66 ± 0.59 de	0.012 ± 0.002 c
DLI	*	*	NS
LQ	*	*	*
DLI × LQ	*	*	NS

[z] Different letters in the same column indicate significant differences based on two-way ANOVA with Duncan's multiple range test (n = 6) at $p \leq 0.05$. NS and * represent no significant difference or significant difference at $p \leq 0.05$, respectively.

Yan et al. [6] reported that LED with R:B ratio of 1.2 led to higher energy yield and LUE, lower power consumption based on increased shoot fresh weight compared with LEDs with R:B ratios of 0.9 and 2.2 in hydroponic lettuce production. Similarly, energy yield of red lettuce increased by 114% as R:B ratio of LED lighting increased from 0.3 to 4 [19]. Additionally, energy yield of lettuce increased by 44% as R:B ratio increased from 0.5 to 3.0 [18]. This demonstrates that higher fraction of red light promotes plant growth to some extent and growers should select optimal R:B ratio of LED lamps in plant factory. In this study, energy yields in all LED treatments were significantly higher compared to those in fluorescent lamps treatments. This was consistent with a previous study on lettuce, where energy yields of all LED treatments were dramatically higher than those with fluorescent treatments [18]. Many researchers had investigated on methods of improving LUE. Li et al. [39] found that movable LED at a shorter distance (10 cm) above the seedlings could both improve LUE and save electric power consumption. LED replacing fluorescent lamps could increase LUE by 60% [40]. In this study, although the energy yield and power consumption of LED lamps were obviously better than those under fluorescent lamps, the LUEs of LED treatments were not higher. Therefore, the appropriate combination of DLI and light spectrum can significantly improve the LUE of hydroponic spinach cultivation. Without considering DLI, LUEs in L1.2 treatments are at a higher level, which is equivalent to the highest level of fluorescent lamps. The results show that the LED lamp with R:B ratio of 1.2 (fractions of red, blue, and green light in 31%, 26%, and 41%, respectively) is suitable for hydroponic spinach production in plant factory. According to the comprehensive evaluation of biomass accumulation, quality index and energy yield, DLI of 17.3 mol m^{-2} day^{-1} was the best selection of light environment design for spinach production.

4. Conclusions

This study revealed that LEDs with different R:B ratios had significant impacts on growth, and nutritional component accumulation, and energy yield in hydroponic spinach. The adoption of DLI at 17.3 mol m^{-2} day^{-1} emitted by LEDs with an R:B ratio of 1.2 maximized spinach shoot yield (42 g per plant) and minimized nitrate content (535 mg kg^{-1} FW), resulting in lower power consumption based on increased total fresh weight (1.7 kWh per 100 g FW) and greater energy yield (46.8 g FW kWh^{-1}). Higher LUE (0.025–0.026) was also ensured by LED lighting with R:B ratio of 1.2 (red, blue, and green light fractions of 31%, 26%, and 41%, respectively). Higher fraction of red light was beneficial to inhibit oxalate accumulation and higher fraction of blue light was conducive to vitamin C accumulation and oxalate reduction in hydroponic spinach. The light spectral adjustment by using LED lamps might replace light intensity regulation by using fluorescent lamps in PFAL for spinach production. Suitable fractions of blue and red lights such as L1.2 treatment could improve photosynthesis and stomatal opening therefore lead to more biomass accumulation and formation of nutritional quality. Those results demonstrate that DLI at 17.3 mol m^{-2} day^{-1} provided by LED lamp with an R:B ratio of 1.2 could be a practical alternative for hydroponic spinach production under controlled environment. The power consumption of LED lamps adopted in this study was still very high and there is much more space to improve the performance.

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