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# Growth and quality responses of 'Green Oak Leaf' lettuce as affected by monochromic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED)

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ABSTRACT

Seeds of 'Green Oak Leaf' lettuce were sown and hydroponically cultured for 50 days (with no transplanting) under different light spectra from the following six lights: fluorescent light plus red LED (FLR), fluorescent light plus blue LED (FLB), monochromic red (R) or blue (B) LED, mixed red and blue LED (RB), and fluorescent light (FL), with equivalent photoperiod (14 h), PPF (133 ± 5 $\mu$mol m$^{-2}$ s$^{-1}$), and other cultivation conditions. At seedling stage, highest height-growth rate was obtained under FLR and R, plantlets under FLR showed improved morphology that was large and compact while those under monochromic R appeared sparse and fragile. Seedling growth was promoted under FLR and FLB, while inhibited the most under monochromic B as compared to FL. At harvest stage, fresh weight, dry weight and stem diameter were greatest under FLR followed by FLB and lowest under monochromatic B and RB. Chlorophyll and carotenoid contents were also significantly higher under FLR and FLB and lowest under R and FL. The soluble sugar and nitrate contents were significantly higher in plantlets cultured under FL than those under LED or mixture lights of FL and LED. When cultured under FLB, vitamin C content was significantly lower but no significant difference was observed among other treatments. In conclusion, FLR and FLB resulted in improved morphology, greater biomass and pigment contents of lettuce than monochromic R, B, FL or RB. FL mixed with R or B LED could be used as efficient light sources for hydroponic cultivation of 'Green Oak Leaf' lettuce.



## 1. Introduction

Different lights are widely used to study the effects of spectral quality on plant growth and it has been proven that more excellent growth of plants could be obtained by adjusting the spectral quality (Chung et al., 2010; Drozdova et al., 2001; Okamoto et al., 1996). Compared with fluorescent lamp (FL) that emits a wide spectrum of 400–700 nm and is commonly used in greenhouses, light-emitting diode (LED) includes several distinctive advantages like small volume, single spectrum wavelength, long life, directional light emission and little heat production (Goto, 2012; Morrow, 2008; Watanabe, 2009). LED has become a promising light source used in plant physiology research in enclosed facilities, and various studies on photobiological research including chlorophyll formation, photosynthesis and morphogenesis have been done by applying LED to various plants in which fluorescent lamps are always used as the control (Yorio et al., 2001; Nhut et al., 2003; Tang et al., 2010).

It is well known that biosynthetic wavelengths for the production of plant pigments are referred to as action spectra which have action maxima in the red and blue ranges (McCree, 1972). Red light is important for the development of the photosynthetic apparatus (Saebo et al., 1995), while blue light is important in the chloroplast development, chlorophyll synthesis, stomatal opening and photomorphogenesis (Cosgrove, 1981; Senger, 1982). Combined red and blue LED (RB) have been proven to be much effective for productions of many plant species, including lettuce (Yanagi et al., 1996; Lian et al., 2002; Nhut et al., 2003; Lee et al., 2007).

However, effects of light quality related to plant growth and development has not been clearly clarified in that responses of plants in previous experiments differ a lot. Lin et al. (2013) has proposed the hypothesis that plants would grow better under RBW (RB plus white) LED compared to RB LED and finally proved higher

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biomass and soluble sugar of plants treated with RBW and FL than those of plants treated with RB. Piao (2002) also reported that FL was a better light source than RB LED for in vitro potato, whereas Tanaka et al. (1998) reported similar biomass of cymbidium plantlets obtained under RB LED and FL, and Li et al. (2013) reported that monochromic R or B and mixture of RB LED resulted in greater biomass of rapeseed than FL, and Johkan et al. (2010) also reported greater dry weight of lettuce seedlings cultured under RB LED than FL. Moreover, significant promotion effect of R and RFr (red plus far-red LED) on stem elongation has been reported in chrysanthemum plantlets (Kim et al., 2004) while inhibition of shoot elongation was observed under red light in marigold and salvia bedding plants (Heo et al., 2002). That is, the effects of light quality differ according to plant species, stage of growth and other environmental conditions (Hahn et al., 2000; Schuerger et al., 1997).

In addition, most previous studies using LED were performed without a supplemental broad-spectrum irradiation and mainly focused on the effects of red plus blue LED compared to only red LED or only blue LED (Domurath et al., 2012; Johkan et al., 2010). Although red and blue LED have great functions in driving photosynthesis, plants in nature are adapted to utilize a wide-spectrum of light to control photomorphogenesis (Briggs, 1993). Several studies have involved mixed lights of fluorescent lamps and LEDs. Yorio et al. (2001) used red LED combined with blue fluorescent light (BF) and reported that radish and spinach grown under cool-white fluorescent lamp (CWF) or red LED + 10% BF all had significantly higher dry-weight accumulation than those grown under red LED alone. Heo et al. (2002) found that dry weight in salvia was significantly greater under fluorescent light plus blue LED (FLB) and fluorescent light plus red LED (FLR) as compared to monochromic red light (R) or blue light (B).

In this study, FL was not only used as a control but also mixed with LED as light treatments to meet growth requirements at different stages. Growth responses such as shoot growth, plant biomass, and accumulations of chlorophyll (Chl), carotenoids (car), soluble sugar, vitamin C and nitrate were investigated to determine the effects of monochromic red or blue and mixed radiation of fluorescent lamp with LED (red or blue) on nursery and plant growth of 'Green Oak Leaf' lettuce hydroponically cultured.

## 2. Materials and methods

### 2.1. Experimental set-up and growth conditions

Seeds of 'Green Oak Leaf' lettuce (*Lactuca sativa* var. *crispa*) were incubated at 4 °C on moistened germination gauze for 5 d and germinated seeds were sown in sponge cubes (2.0 cm × 2.0 cm × 2.0 cm) above the hydroponic boxes in an environmentally controlled growth room. Each hydroponic box (60 cm × 45 cm × 10 cm) contained 12 plants spaced 15 cm apart. All plants were grown hydroponically using modified half-strength Hoagland's solution (Hoagland and Arnon, 1950). The nutrient solution was renewed per week and adjusted to electrical conductivity 1.2–1.3 ms $cm^{-1}$ and pH 5.8–6.0. Air temperature, $CO_2$ level, and relative humidity (RH) were maintained at 24/20 °C (day/night), 900 μmol $mol^{-1}$, and 60%, respectively. The plants were irradiated with different light spectra from the following six light qualities: monochromic red (R) or blue (B) LED, mixed treatment of red and blue LED (RB), fluorescent lamp with LED of blue (FLB) or red (FLR), and pure fluorescent lamp (FL) which was considered as a control. A treatment with eight plants was considered as a replication (three replications per treatment) and plants were harvested at 50 days after sowing (DAS).

**Table 1**
The energy proportion of different light quality in per treatment.

| Treatment | PAR (μmol $m^{-2}$ $s^{-1}$) | FL/% | Blue LED/% | Red LED/% |
|---|---|---|---|---|
| FLR | 137.1 | 50.1 | / | 49.9 |
| FLB | 133.2 | 52.7 | 47.3 | / |
| R | 131.6 | / | / | 100 |
| B | 130.7 | / | 100 | / |
| RB | 132 | / | 51.8 | 48.2 |
| FL | 135.5 | 100 | / | / |

*Abbreviations*: FLR – fluorescent light plus red LED, FLB – fluorescent light plus blue LED, R – red LED, B – blue LED, RB – red LED plus blue LED (hereinafter the same).

### 2.2. Light quality treatments

Treatments in the study consisted of white fluorescent lamps and red/blue LED tubes which were provided by NERCITA, Beijing, China. Light spectra of white fluorescent light (400–700 nm), blue LED (peak at 460 nm), red LED (peak at 630 nm) and the mixed light, as determined with a spectrophotometer (OceanOptics, model-SD650, USA) in the range of 300–1000 nm, are shown in Fig. 1. Monochromic lamps of white fluorescent and red/blue LED were mounted in closely alternate arrangement to form mixed light-emitting sets over the plants, the energy proportion of different lights in mixed lamps such as fluorescent light plus red LED (FLR), fluorescent light plus blue LED (FLB) and red LED plus blue LED (RB) are shown in Table 1. However, totally radiated photosynthetic photon flux (PPF) in all treatments was set at 133 ± 5 μmol $m^{-2}$ $s^{-1}$, which was got by adjusting the distance between plants and lights and was measured with a light quantum meter (Model 1400, LI-COR, USA). The photoperiod was 14 h $d^{-1}$ for all the treatments.

### 2.3. Measurements of plant growth and morphology

Plant height was measured once every 5 days while other biometric measurements like fresh weight (FW), dry weights (DW) of shoots and roots, stem diameter, stem length, and leaf number were performed at 50 DAS. Plant samples were oven-dried at 80 °C for 50 h to determine dry weight. The ratio of shoot and root (S/R) was determined from shoot/root DW. Representative plants grown under each light treatment for 18 days and 50 days were respectively chosen as samples for morphology observation.

### 2.4. Determination of soluble sugar

Soluble sugar was extracted using the method of Dubois et al. (1956). A total of 0.5 g lettuce tissue (shoot FW) were homogenized with 2 mL of 80% ethanol solution, and the homogenate were heated in a 75 °C water bath for 10 min. The precipitate were removed by centrifuging at 5,000 × *g* for 10 min. The residues were washed again with 2 mL of 80% ethanol at 75 °C and re-centrifuged. The supernatants were mixed and dried under a stream of hot air, and the residue was resuspended in 1 mL of distilled water and desalted through a column of ion-exchange resin (Shanghai Resin Factory Co., Ltd). The soluble sugar concentrations were determined using the filtrate with a spectrophotometer (UV-1200, MAPADA, Shanghai, China) at 485 nm according to the phenol-sulphuric acid method.

### 2.5. Determination of nitrate

Nitrate content was measured according to the method of Cataldo et al. (1975). A total of 2.0 g lettuce samples (shoot FW) were cut into small pieces and mixed with 10 mL deionized water. After heating in a 80 °C water bath for 30 min, the precipitate were removed by centrifuging at 13,000 × *g* for 10 min, and 0.2 mL of the supernatant was mixed with 19 mL of 4 M NaOH and 0.8 mL of 5%

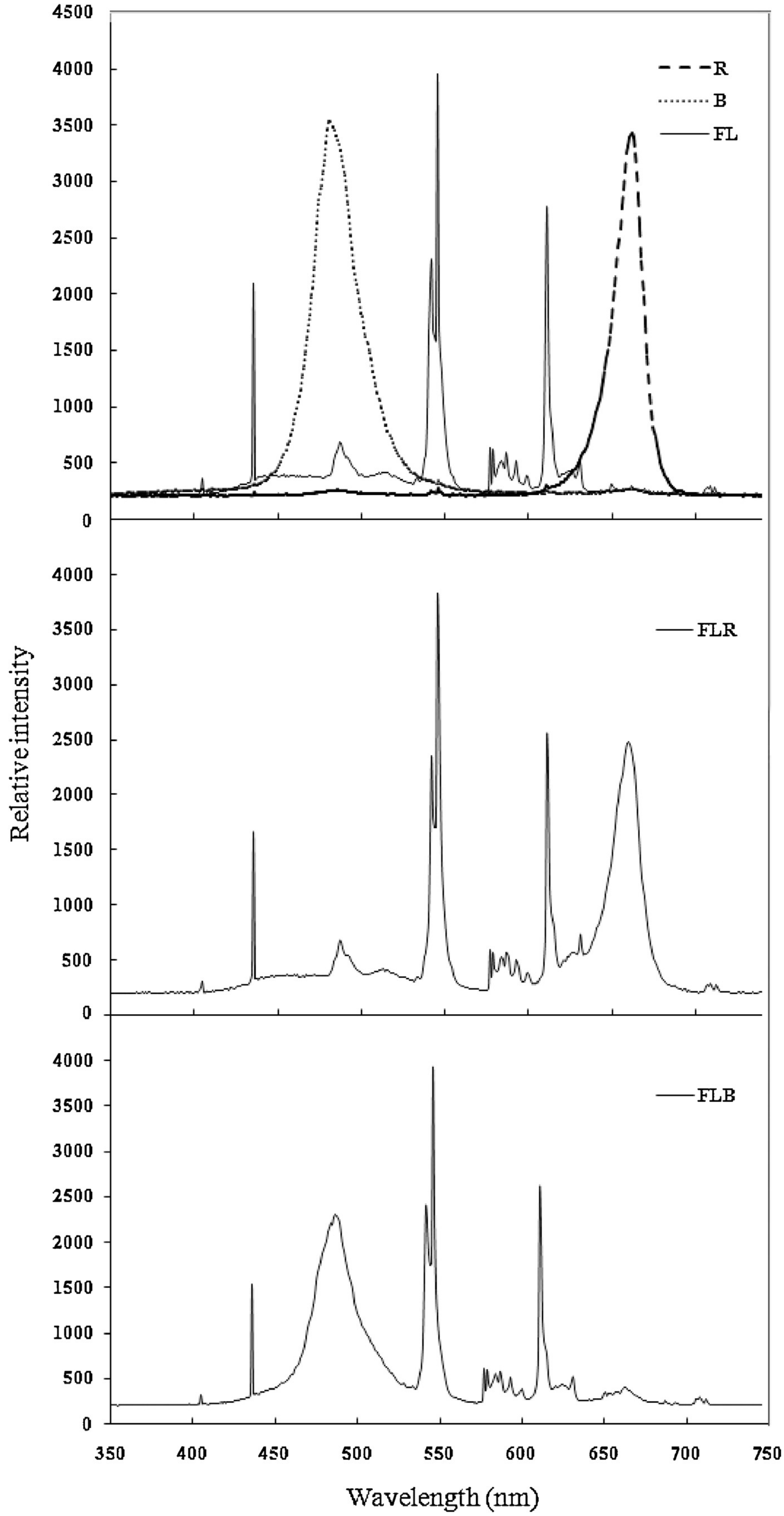


**Fig. 1.** Light spectra of monochromic or mixed lights used in the experiments, the spectra of RB is the superposition of R and B. *Abbreviations*: R – red LED, B – blue LED, FL – fluorescent light, RB – red LED plus blue LED, FLR – fluorescent light plus red LED, FLB – fluorecent light plus blue LED (hereinafter the same).

(w/v) salicylic acid (in pure $H_2SO_4$), the nitrate content was measured with a spectrophotometer (UV-1200, MAPADA, Shanghai, China) at a wavelength of 410 nm.

### 2.6. Determination of vitamin C

Vitamin C content was determined by the method of Gahler et al. (2003). A total of 20 mg lettuce samples (shoot FW) were mixed with 1.5 mL of 4.5% aqueous phosphoric acid. After shaking at 300 rpm for 30 min in the darkness, the mixtures were centrifuged at 16,000 × g for 10 min. The supernatants were used to determine the concentration of Vitamin C with the HPLC system equipped with C18 column (Restek USA, Bellefonte, PA, USA) at 30 °C. Extract was eluted with mobile phase (0.21% phosphoric acid) at a flow rate of 0.8 mL/min, and the concentrations were determined at 254 nm against ascorbic acid standards (Standard Substance Center, China).

### 2.7. Determination of chlorophyll and carotenoid

Chlorophyll (Chl) were extracted from the mature leaves of eight plantlets within each treatment. A total of 20 mg lettuce samples (shoot FW) were ground in a mortar. The ground were washed using 80% acetone and subsequently filtered, the residues were washed again with 80% acetone and re-filtered until the leaf turned white. Both filtrates were mixed, and distilled water was added to a total volume of 10 mL. The optical density of the extract was measured with a UV-1200 spectrophotometer (MAPADA, Shanghai, China) at 663 nm (OD 663) for chlorophyll a (Chl a), 645 nm (OD 645) for chlorophyll b (Chl b), and 470 nm (OD 470) for carotenoid (Car). Concentrations of the chlorophyll and carotenoid were determined using the following equations (Lichtenthaler and Wellburn, 1983):

$$\mathrm{Chl\,a\,(mg/g)} = \frac{(12.72 \times \mathrm{OD}_{663} - 2.59 \times \mathrm{OD}_{645})V}{1000W}$$

$$\mathrm{Chl\,b\,(mg/g)} = \frac{(22.88 \times \mathrm{OD}_{645} - 4.67 \times \mathrm{OD}_{663})V}{1000W}$$

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

where $V$ is the total volume of acetone extract (mL) and $W$ is the fresh weight (g) of the sample.

### 2.8. Statistical analysis

All measurements were analyzed by Tukey's multiple range test for significance at the 0.05 significance level using SPSS 11.0 software (SPSS Inc., Chicago, USA).

## 3. Results

### 3.1. Plant morphology

As shown in Fig. 2, during the first half growth period (0–25 DAS), the average height-growth rate of seedlings from FLR and R treatments were respectively 6.59 and 5.23 mm d$^{-1}$, significantly higher than the others. However, shoot growth under monochromic R was fragile due to excessive elongation. Height growth was restrained under B with an average speed of 2.08 mm d$^{-1}$ which was the lowest among all the light sources (Fig. 2). Large-sized and compact seedlings with dark green leaves were detected under FLR and FLB while seedlings with long but thin stems and light green leaves were obtained under monochromic R. Moreover, seedlings under monochromatic R looked weak and creeping, while those under monochromatic B appeared dwarfed and small similar to those under RB, normal appearance were observed under FL (Fig. 3).

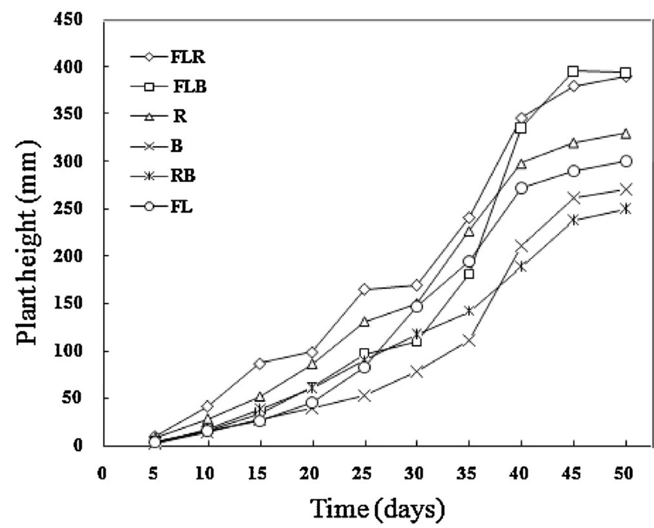


**Fig. 2.** Height growth of lettuce cultivated under monochromic light and mixed radiation from fluorescent lamp and LEDs of blue or red for 50 days.

During the rest cultivation period (25–50 DAS), height growth of plants under FLR and R became considerably slower and the most rapid height growth were detected under FLB, followed by B and FL treatments (Fig. 2). Plants with continuous radiation of monochromatic R became strong and compact with tortuous leaves while those from monochromatic B were loose and scattered with straight leaves. Plants radiated with FLR and FLB showed improved morphology containing vigorous and large-sized shoot (Fig. 3), higher number of leaves, and greater stem diameter (Table 2).

### 3.2. Growth characteristics

The fresh and dry weight of plants were the highest under FLR. The fresh weight of shoot increased by 159%, 100% and 46%, respectively under FLR, FLB and R treatments compared with FL (Table 2). In contrast, growth was severely inhibited under monochromatic B or RB lights, showing the lowest fresh and dry weight. The stem diameter was significantly higher under the mixture of FL and R or B LED than that under monochromatic R or B. The highest stem length was observed in R plants, followed by FLR, while the lowest was observed in B plants. The number of leaves was the smallest under B but not significantly different among other treatments. Plants grown under FL and B showed the lowest S/R ratio while the highest S/R ratio was obtained under FLB followed by R.

### 3.3. Chlorophyll and carotenoid contents

As shown in Fig. 4, the Chl a and total Chl content were significantly higher with FLR than other treatments, plants treated with FLR showed the highest total Chl content of 1.31 mg g$^{-1}$ followed by FLB and RB, while those grown under monochromic R showed the lowest total Chl content of 0.64 mg g$^{-1}$. Additionally, the lowest content of Chl a, Chl b and Car were all detected under monochromic R treatment. No significant differences were observed in Chl b and Car content irrespective of monochromic R, B, FL or RB light sources, but Chl b and Car content of plants irradiated with FL plus R or B LED were significantly increased compared with the others.

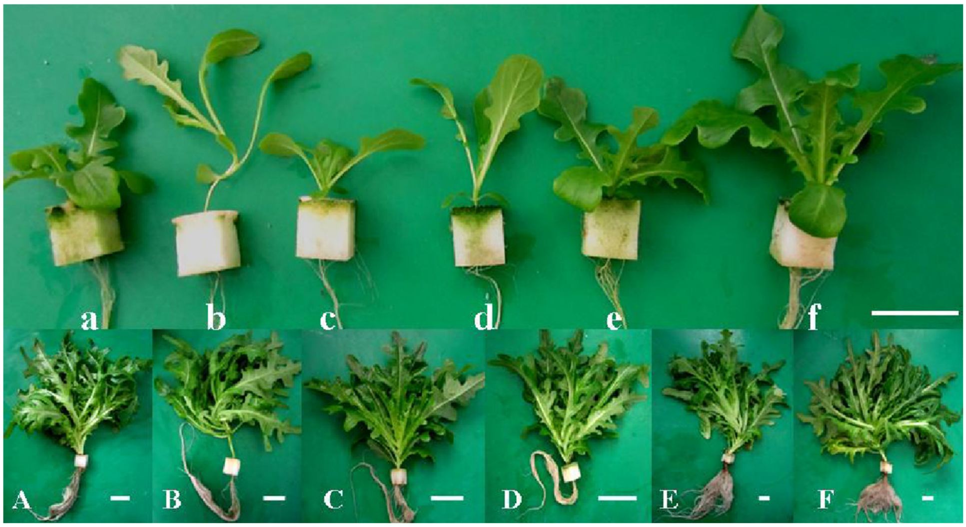


**Fig. 3.** Morphology of 'Green Oak Leaf' lettuce under different light sources of a/A (FL), b/B (R), c/C (B), d/D (RB), e/E (FLB), and f/F (FLR). Lowercase and uppercase letters respectively indicate 18 and 50 DAS. The bar on the right of 'f' indicates 4 cm.

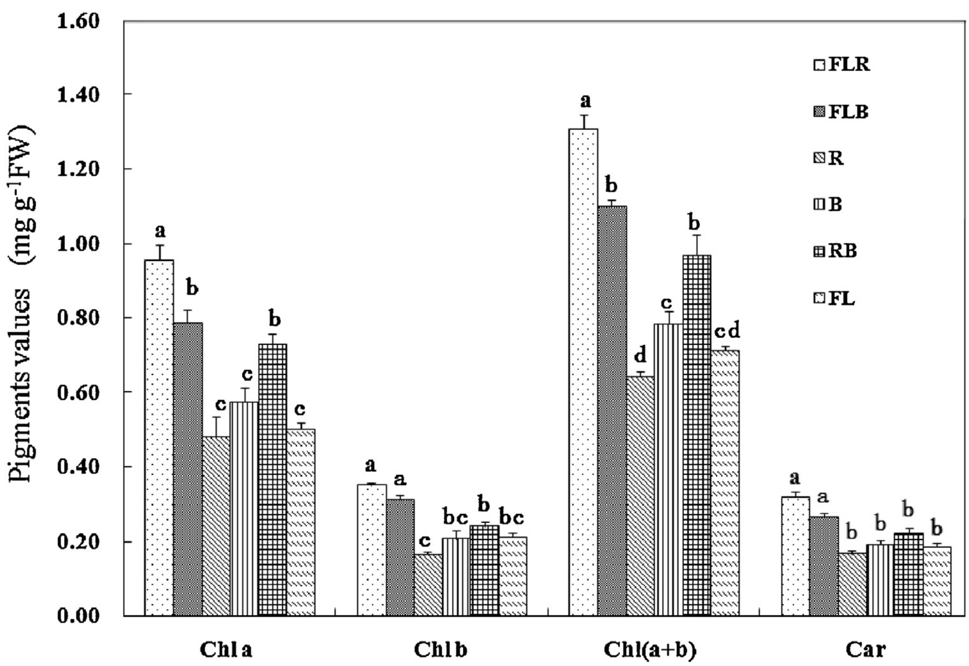


**Fig. 4.** Chlorophyll and carotenoid contents of plants grown under monochromic light and mixed radiation from fluorescent lamp and LEDs of blue or red for 50 days. Different letters for the same parameter indicate significant differences at the 5% level, according to the Tukey's test ($n = 3$). The bars represent the standard deviation.

**Table 2**
Influence of light quality on plant height, fresh weight (FW), dry weight (DW), stem diameter, stem length, leaf number and shoot: root ratio (S/R) at 50 DAS.

| Light treatment | Plant height (mm) | FW (g) | | DW (g) | | Stem diameter (mm) | Stem length (mm) | Leaf number | S/R |
|---|---|---|---|---|---|---|---|---|---|
| | | Shoot | Root | Shoot | Root | | | | |
| FLR | 390a* | 83.26a | 8.05a | 5.07a | 0.38a | 9.12a | 33.1ab | 42a | 13.34b |
| FLB | 393a | 64.43b | 4.45ab | 4.25ab | 0.22ab | 8.37a | 24.1b | 38a | 19.32a |
| R | 330ab | 46.92bc | 3.90b | 3.44b | 0.18b | 6.72b | 37.2a | 34a | 19.11a |
| B | 270b | 23.46c | 3.12bc | 1.36c | 0.16b | 5.62c | 20.1b | 25b | 8.50c |
| RB | 250b | 24.39c | 2.11c | 1.53c | 0.09c | 4.36c | 22.4b | 32a | 17.00a |
| FL | 300ab | 32.14bc | 6.83a | 1.97c | 0.34a | 7.81b | 24.7b | 35a | 5.79c |

* Values for the same parameter with different letters significantly differ at the 5% level (by Tukey's test, $n = 3$).

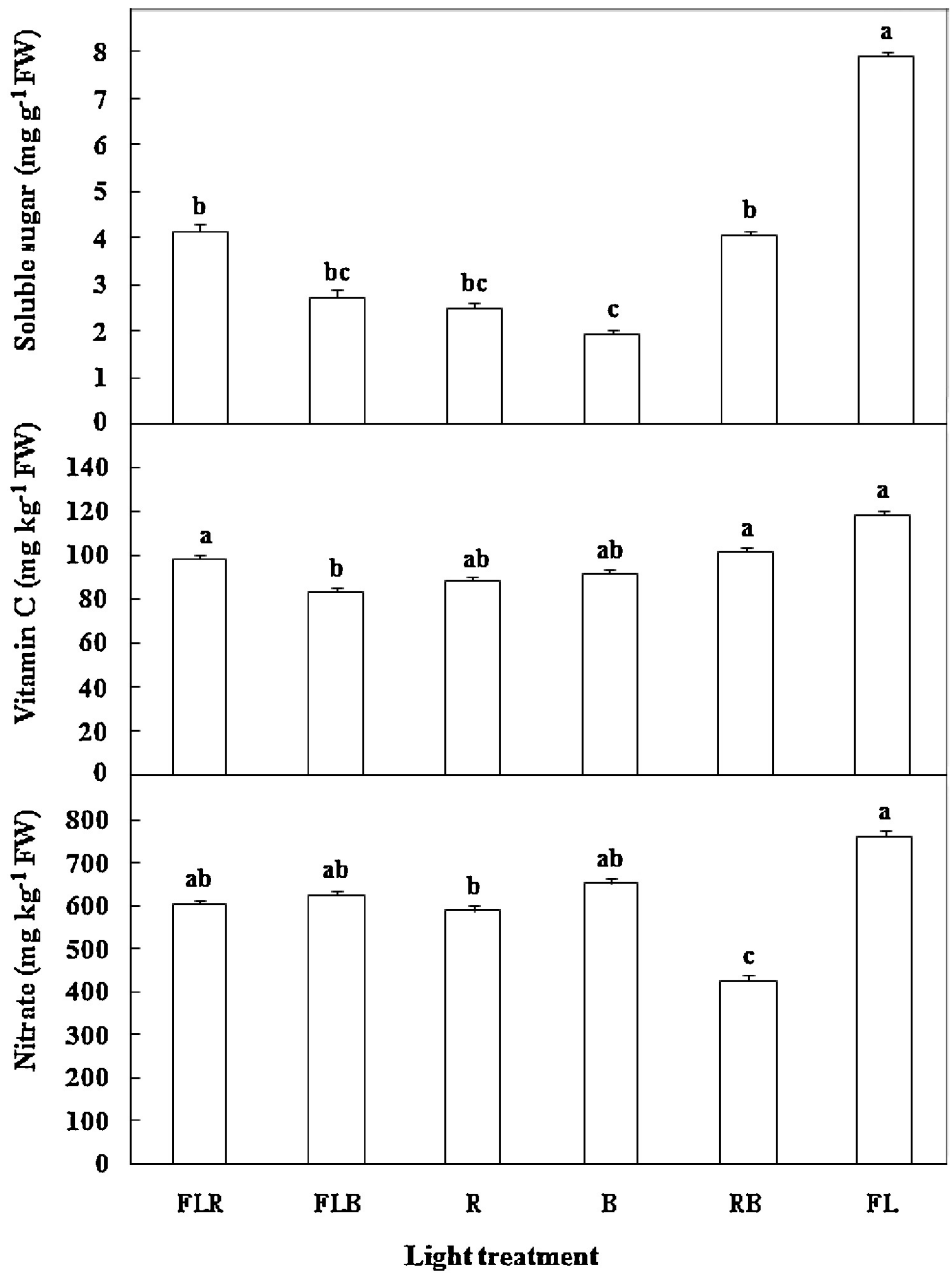


**Fig. 5.** Soluble sugar, vitamin C and nitrate contents of plants grown under monochromic light and mixed radiation from fluorescent lamp and LEDs of blue or red for 50 days. Different letters for the same parameter indicate significant differences at the 5% level, according to the Tukey's test ($n = 3$). The bars represent the standard deviation.

### *3.4. Soluble sugar, vitamin C and nitrate contents*

As shown in Fig. 5, plants grown under FL had maximum soluble sugar, vitamin C and nitrate content of 7.9 mg $g^{-1}$, 118 mg $kg^{-1}$ and 760 mg $kg^{-1}$ respectively, while the lowest contents of the three substances were respectively obtained under B, FLB, and RB lights. The difference in soluble sugar content reached significant level ($p \leq 0.05$) among FL, RB, and B treatments. Vitamin C content of plants in FLB was significantly lower than that in FLR, RB and FL, however no significant difference existed among other treatments. Plants from RB light source had the lowest nitrate content of 424 mg $kg^{-1}$, which decreased by 28–44% compared with other treatments.

## 4. Discussion

It has been reported that red and blue light spectrum strongly shape plant morphology and R light resulted in elongation of hypocotyls and leaf blades and increase of leaves (Hoenecke et al., 1992; Johkan et al., 2010), while B light is important for leaf expansion and inhibition of hypocotyls (Li et al., 2010; Hogewoning et al., 2010; Johkan et al., 2012). Lettuce has very short stem so that hypocotyl is generally considered as stem. In our study, plants under monochromatic R appeared to have long stems and tortuous leaves while lettuce leaves with radiation of monochromatic B looked upright and flat. Plants under FLR and R treatments showed significantly higher height-growth rate especially at seedling stage compared with other light treatments (Figs. 2 and 3), both stem length and plant height were the highest at seedling stage (0–25 DAS) (Table 2). By harvest stage, the highest stem length was obtained in FLR and R plants, while the plant height of lettuce grown under FLR and R was a little inferior to those grown under FLB which may be due to the tortuousness of leaves. Kim et al. (2004) reported similar results for in vitro multiplication of potato in which stem length was the highest under R and RFr but shoot growth was fragile due to excessive elongation. In the present experiments, plants under monochromic R was also fragile and thin at seedling stage, but it is noteworthy that, during the later growth period, plants treated with R became relatively vigorous and developed normally like those under FL. At 50 DAS, stem diameter of plants treated with R was even increased by 20% and 54% than that under B and RB respectively. These results indicate that responses of plant growth to light quality are perhaps not only related to species or cultivar, but also the growth period, since post transplant success after nursery stage is strongly influenced by plant morphology (Lazcano et al., 2009), monochromic red is not suitable for nursery stage.

Pepper biomass decreased when plants were grown under monochromic red LED compared to plants supplemented with blue light (Brown et al., 1995). Yorio et al. (2001) reported that higher dry matter weight was obtained in lettuce grown under RB light than those grown under monochromic R. In contrast to the findings, in our study, biomass of lettuce from R was higher than those from RB, while biomass from B was the lowest. The inconsistencies may due to different R/B ratio, like Dougher and Bugbee (2001) reported that, biomass was much sensitive to the R/B ratio, lettuce dry mass increased when as little as 2% blue was added but decreased when increasing the fraction of blue photons. Another possible reason may be the long-duration of our experiments, which may have allowed time for all red light effects to be fully manifested. Lettuce in early growth may be more sensitive to the disadvantages of monochromic red light such as excessive hypocotyls elongation and fragile shoot than the favorable effects such as biomass enhancement. The favorable effects of red light became more highlighted as the plants matured. Among the mixed light, biomass of lettuce shoots significantly increased with FLR and FLB treatment compared to RB treatment. It may because that white light of broad spectrum from FL might better penetrate the plant canopy than RB LED light for photosynthesis, R or B supplemented with FL may have achieved a balanced spectral environment which led to more leaves and greater light interception. Similar result has been reported by Lin et al. (2013) in which greater biomass of lettuce was obtained under RBW treatment than RB. This indicated that R or B light responses may also depended on the remaining spectral composition, and light effect differed when other parts of the spectrum vary. Moreover, although FLB treatment had a low total plant dry weight compared to FLR treatment, it had a significantly higher S/R ratios, that is, the lettuce plants under FLB treatment partitioned more of its dry weight into the shoots which were the marketable part of lettuce. The observation indicates that root induction is probably also dependent on the light spectrum.

We detected higher Chl and Car content under B than under R in the study, it may because that blue light was more effective than red light in the induction of Chl and Car synthesis in leaves (Bukhov et al., 1992), however, among mixed light treatments, the Chl and Car content all appeared to be the highest in FLR, followed by FLB and RB. This indicates that the remaining spectrum besides blue and red which were known as the absorption spectra (Wang et al., 2009) might also have effects on the induction of pigments. Moreover, Chl a is the indispensable molecule for photosynthesis and it has a wider absorption spectrum than Chl b (Calatayud and Barreno, 2004). In our study, Chl a content of plants appeared to be more than Chl b content in all light treatments. In addition, plants under R seemed to use the Chl more efficiently compared to B, RB and FL treatments, since they had the lowest pigment content but the highest biomass among these treatments.

The amounts of Vitamin C showed no significant differences among most treatments, which suggested that response in Vitamin C was not so sensitive as to assess the nutritional quality of lettuce plants grown under different light spectrum. Reduction of nitrate content is beneficial for human health, the FL spectrum possibly stimulated vital activities of nitrate uptake, for which lettuce under FL had the highest nitrate content. In addition, among R, RB and B treatments, we observed that content of nitrate and sugar had the opposite trend, that is, increase of B light enhanced the accumulation of sugar and simultaneously degraded the nitrate content. It may because sugar can elicit an increase in nitrate reductase messenger RNA accumulation (Lillo, 1994), however, when FL was involved, the trend were not the same, which may due to some other regulatory mechanism.

Improved morphology (high number of leaves, greater stem diameter) and greatest biomass of 'Green Oak Leaf' lettuce were obtained under the mixed radiation of fluorescent lamp with red or blue LED, while the highest content of soluble sugar and the lowest content of nitrate were respectively obtained under FL and RB treatments. Monochromic R was not appropriate for nursery stage but significantly enhanced lettuce growth during the mature period. That is, a specific spectral combination was not the best for all growth parameters nor the whole growth period. This indicates that using different light treatments during different segments of the plant's life cycle is possible. For example, using FLR or FLB for the first half of the cycle and then switching to FL or RB. Appropriate light combinations should be selected according to the growth stage and the planting purposes.

## 5. Conclusion

Light quality effects on lettuce growth are related to growth stage, FLR promoted the seedling growth and shortened the breeding cycle of lettuce while FLB played a more significant role in promoting lettuce growth during the maturity period. FL mixed with R or B LED resulted in improved morphology, greater biomass and pigments content of lettuce than monochromic R, B, FL or RB. The mixed light sources of fluorescent lamps and red or blue LED were beneficial for cultivations of 'Green Oak Leaf' lettuce.

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