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The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. *capitata*)

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ABSTRACT

Previous studies demonstrated that the combination of red (R) and blue (B) LED light was an effective light source for plant growth and development, and the light spectra, intensities, and durations can easily be controlled by growers in artificial growing environments. Therefore, the goal of this study was to investigate the influences of three different qualities of light on plant biomass and accumulation of chlorophylls (chl), carotenoids (car), soluble proteins and sugars, and nitrates in the leaves of lettuce (*Lactuca sativa* L. var. *capitata*). The marketable sensory characteristics (crispness, sweetness, shape, and color) of fresh plants were also evaluated. Plants were hydroponically cultured with a 16-h photoperiod at 24/20 °C (day/night), 75% relative humidity, 900 μmol mol^{-1} CO_2 level, and 210 μmol m^{-2} s^{-1} photon flux density under RB LED, RB and white (RBW) LED, and a fluorescent lamp (FL, as a control) inside growth chambers for 20 days (15 days after sowing). The shoot and root fresh and dry weights as well as the crispness, sweetness, and shape of the plants treated with RBW and FL were higher than those of plants treated with RB. The soluble sugar and nitrate contents in plants grown under RBW treatment were significantly higher and lower, respectively, compared to those under RB treatment. However, the chl, car, and soluble protein contents of lettuce leaves showed no significant differences among treatments. These results demonstrate that supplemental light quality can be strategically used to enhance the nutritional value and growth of lettuce plants grown under RBW LED lights. Precise management of the irradiance and wavelength may hold promise in maximizing the economic efficiency of plant production, quality, and nutrition potential of vegetables grown in controlled environments.

1. Introduction

Light sources such as fluorescent, metal-halide, high-pressure sodium, and incandescent lamps are generally used for plant cultivation. These sources are applied to increase photosynthetic photon flux levels but contain unnecessary wavelengths that are located outside the photosynthetically active radiation spectrum, and are of low quality for promoting growth (Kim et al., 2004a). Compared to those conventional light sources, gallium–aluminum–arsenide light-emitting diode (LED) lighting systems have several unique advantages, including the ability to control the spectral composition, a small mass and volume, durability, long operating lifetimes, wavelength specificity and narrow bandwidth, relatively cool emitting surfaces, minimum heating, and photon output that is linear with the electrical input current. These solid-state light sources are therefore ideal for use in plant lighting designs, and they allow wavelengths to be matched to plant photoreceptors to provide more-optimal production, and influence plant morphology and metabolism (Bourget, 2008; Massa et al., 2008; Morrow, 2008). Spectral light changes evoke different morphogenetic and photosynthetic responses that can vary among different plant species. Such photoresponses are of practical importance in recent plant cultivation technologies, since the feasibility of tailoring illumination spectra purposefully enables one to control plant growth, development, and nutritional quality.

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Plant development is strongly influenced by the light quality, which refers to the color or wavelength reaching a plant's surface (Johkan et al., 2010). Red (R) and blue (B) lights have the greatest impact on plant growth because they are the major energy sources for photosynthetic CO_2 assimilation in plants. Past studies examined the action spectra for photosynthesis of higher plants. It is well known that action spectra have action maxima in the B and R ranges (Cosgrove, 1981; Kasajima et al., 2008). Combined RB LED lights were proven to be an effective lighting source for producing many plant species, including lettuce, in controlled environments (Brown et al., 1995; Yanagi et al., 1996; Tanaka et al., 1998; Yorio et al., 2001; Hanyu and Shoji, 2002; Lian et al., 2002; Nhut et al., 2003; Dougher and Bugbee, 2004; Kim et al., 2004b; Lee et al., 2007; Shin et al., 2008).

The light spectra in many reported experiments, which were produced by LEDs or fluorescent lamps, were inconsistent, and the light intensity was non-uniform because the investigators were unable to precisely modulate and quantify the spectral energy parameters (Liu et al., 2011). Furthermore, the experimental results may have been influenced in part by differences in the light intensity, and this often presents a problem when comparing results from experiments conducted under inconsistent light parameters. While it is widely understood that light intensity can positively affect photochemical accumulation (Li and Kubota, 2009; Fu et al., 2012), the effects of light quality are more complex, and mixed results were often reported. In order to apply the findings to lettuce quality and production, we considered it important to investigate the light-quality effects when provided as supplemental light rather than as the sole source of light. In this regard, the white (W) LED, broad-spectrum light, was supplied to an RB LED system (RBW LEDs) to meet different purposes. Therefore, the hypothesis of this study was that plants would grow better under RBW LED lighting-generated spectra of uniform intensity compared to RB LEDs. Hence in this study, the growth, development, nutritional quality, and edible quality of lettuce hydroponically grown under RB LED with and without supplemental W LED lighting at the same light intensity were investigated to determine the efficacy of this promising radiation source. The final goal of the research was to develop a year-round and rapid production system for fresh, high-quality, pesticide-free, and economically feasible hydroponic lettuce that is produced close to the final retail market. An optimal strategy of light quality regulation will help in designing the growth chamber or greenhouse light environment to obtain maximum economic benefits for vegetable growers.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of Boston lettuce (*Lactuca sativa* L. var. *capitata*) were germinated in rockwool cubes (2.5 cm × 2.5 cm × 3.0 cm) and hydroponically grown for 15 days in an environmentally controlled growth room. The temperature was at a constant 24 °C under a light intensity of approximately 100 μmol m^{-2} s^{-1} photon flux density (PFD) for 14 h with cool white fluorescent lamps. Uniform-sized seedlings of lettuce at the 5-leaf stage were individually raised in a polystyrene foam cube, then mounted into a Styrofoam plate with eight holes, and placed in a container (59 cm × 48 cm × 9.7 cm) filled with continuously aerated complete nutrient solution (Gul et al., 2005) in a commercial growth chamber (CH-202-A, CHIN-HSIN, Taipei, Taiwan). The nutrient solution was renewed every week and adjusted to pH 6 and an electrical conductivity of 1.1 mS cm^{-1}. The photoperiod was maintained at 16 h. The air temperature, relative humidity, and CO_2 levels for all treatments were respectively maintained throughout the experiment at 24/20 °C (day and night), 75%, and 900 μmol mol^{-1} in the growth chamber.

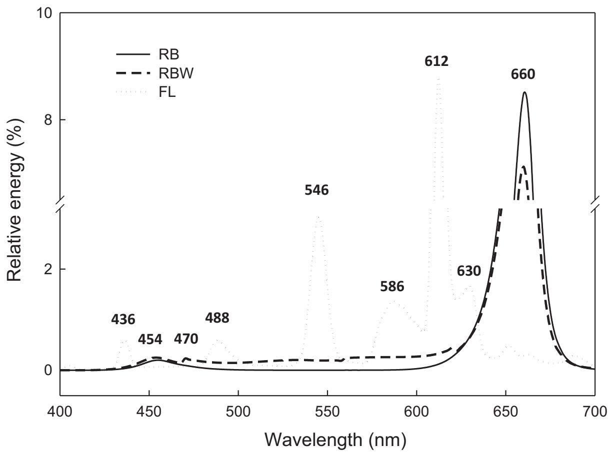

Fig. 1. Spectral distributions in the relative energy of the LEDs and fluorescent lamps. Spectral scans were recorded at the top of the plant canopy with a spectroradiometer.

2.2. Light treatments

Treatments with eight replicates consisted of three commercially available light sources: (1) RB LEDs, (2) RBW LEDs, and (3) fluorescent lamps (FL, as the control). All light sources were provided by Ting-Mao Technology, Taipei, Taiwan. The peak emissions of the B (454 nm) and R (660 nm) LEDs closely coincide with the absorption peaks of chlorophylls a and b, and the reported wavelengths are at their respective maximum photosynthetic efficiency (McCree, 1972). The spectral energy distribution scans were recorded at 400–700 nm with 2-nm steps of the LEDs and FL (Fig. 1) with a calibrated spectroradiometer (LI-COR1800, Lincoln, NE, USA) placed horizontally in the cabinets used for the experiments, with the sensor covered by the glass lid of the vessel. Each treatment was run in a growth chamber, and the spectrum was recorded at the top of the plant canopy. The RBW LEDs had peak outputs in B and R regions with a supplemental broad spectral energy of 500–600 nm. All treatments maintained a 16/8-h light/dark photoperiod and the same light intensity expressed as photosynthetic PFD of 210 μmol m^{-2} s^{-1} which was measured daily above the plant canopy and maintained by adjusting the distance of the LEDs to the plant canopy. Plants were harvested at 35 days after sowing (DAS). The experiment was independently performed twice for a randomized design of growth conditions, and plant measurements represent means of 16 plants (two reps consisting of eight plants each).

2.3. Plant growth measurements

Measurements included shoot fresh weight (FW), root FW, shoot dry weight (DW), root DW, shoot/root (S/R) DW, leaf area (LA), and specific LA (SLA). Plant tissue samples were dried in a drying oven for 48 h at 70 °C before weighing. The LA (cm^2) of every plant was measured by an LA meter (LI-3100, LI-COR). A standard growth analysis was used to calculate the LA index (LA/shoot DW).

2.4. Chlorophyll (chl) and carotenoid (car) contents

Chl and car were eluted from the shoot DW samples (0.05 g) with 1 mL 80% acetone at 4 °C overnight and determined by the methods of Porra et al. (1989) and Holm (1954), respectively. The sample was then centrifuged at 13,000 × *g* for 5 min. The supernatant was applied to determinate the absorbances of chl a, chl b, and car in acetone, as measured with a spectrophotometer (U-2000, Hitachi, Tokyo, Japan), at respective wavelengths of 663, 645, and 470 nm. Concentrations ($g\,g^{-1}$ DW) of chl a, chl b, and car were determined from the following equations: chl a = $12.72 \times OD_{663} - 2.59 \times OD_{645}$; chl b = $22.88 \times OD_{645} - 4.67 \times OD_{663}$; and car = $(1000 \times OD_{470} - 3.27 \times \text{chl a} - 104 \times \text{chl b})/229$.

2.5. Soluble sugar determination

The content of soluble sugars was measured by the method of Fairbairn (1953). Samples (0.05 g shoot DW) were put into a test tube, to which 5 ml of distilled water was added and mixed. After 30 min in a water bath at 85 °C, the supernatant was collected. This step was repeated twice, and then distilled water was added to a volume of 10 ml. The soluble sugar content was determined with the sulfuric acid anthrone method at a wavelength of 620 nm.

2.6. Soluble protein content

Soluble proteins were measured by the Bradford (1976) method. Samples (0.05 g shoot DW) were ground up in a mortar with liquid nitrogen, to which 3 ml of a phosphate-buffered solution (pH 7.0) was added. The extract was centrifuged at 13,000 × *g* for 15 min at 4 °C, and 0.1 ml of the supernatant was combined with 4.9 ml of a Coomassie brilliant blue G-250 solution ($0.1\,g\,L^{-1}$). After 2 min, the soluble protein content was determined at a wavelength of 595 nm.

2.7. Nitrate determination

The nitrate content was measured by the method of Cataldo et al. (1975). Briefly, samples (0.05 g shoot DW) were ground up, and then 10 ml of hot distilled, deionized water was added. After 30 min in a water bath at 80 °C, the extract was centrifuged at 13,000 × *g* for 10 min, and 0.2 ml of the supernatant was mixed with 0.8 ml of 5% (w/v) salicylic acid (in pure H_2SO_4) and 19 ml of 4 M NaOH. After 30 min, the nitrate content was measured at a wavelength of 410 nm.

2.8. Sensory analysis

A comprehensive survey of the sensory characteristics of shape, color, crispness, and sweetness of the fresh lettuce was conducted for marketable acceptability using a scale of 0–6, with 6 being the highest score, that is, 6 = like extremely, 3 = neither like nor dislike, 0 = dislike extremely. Random samples from different treatments were separately evaluated by 50 untrained consumer panelists, aged 20–64 year old, in the nutrition/sensory laboratory at the Agronomy Department, National Taiwan University. Each panelist was served 3 samples, and tested one sample at a time for every treatment of lettuce presented in three-digit coded plates. The panelists were also provided with water to cleanse the palate between samples. The sensory intensities were obtained by averaging the individual intensities for the 50 subsamples.

2.9. Statistical analysis

All measurements were evaluated for significance by an analysis of variance (ANOVA) followed by the least significant difference (LSD) test at the $p < 0.05$ level. All statistical analyses were conducted using SAS 9.1 (SAS Institute; Cary, NC, USA).

Table 1

Influence of light quality on shoot fresh weight (shoot FW), root FW, shoot dry weight (shoot DW), root DW, shoot/root DW, leaf area (LA), specific LA (SLA), chlorophyll (chl), chl a, chl b, and carotenoid (car) contents at 35 days after sowing.

Parameter	Light quality		
	RB	RBW	FL
Shoot FW (g)	136.3 b*	164.1 a	149.0 b
Root FW (g)	9.1 b	13.5 a	11.8 ab
Shoot DW (g)	7.02 a	7.97 a	7.17 a
Root DW (g)	0.43 b	0.62 a	0.58 a
Shoot/root DW	16.3 a	12.8 b	12.3 b
LA (cm^2)	6425 b	7435 a	7480 a
SLA ($m^2\,kg^{-1}$)	91.5 c	93.3 b	104.3 a
Chl a ($\mu g\,g^{-1}$ DW)	2954 a	2932 a	3053 a
Chl b ($\mu g\,g^{-1}$ DW)	971 a	925 a	988 a
Chl a + b ($\mu g\,g^{-1}$ DW)	3925 a	3857 a	4041 a
Car ($\mu g\,g^{-1}$ DW)	1789 a	1715 a	1825 a

* Values followed by the same letter within a row do not significantly differ (by the LSD test, $p = 0.05$).

3. Results

3.1. Plant growth and morphology, and pigment contents

Results of the biomass measurements of lettuce influenced by the light spectra treatments are shown in Table 1, and plants showed distinct growth responses to different light-quality treatments. Shoot FW and DW, and root FW and DW of the plants were the greatest when grown under RBW light, and lowest under RB light. The shoot FW significantly increased by 10% with the RBW treatment compared to the FL control. Plants under RB treatment (16.3) had a significantly higher S/R DW compared with those under RBW (12.8) and FL (12.3) treatments. The LA and SLA decreased in the order of plants grown under FL, RBW, and RB, and both parameters under FL were significantly higher than under RB light. In addition, a normal appearance and compact morphology with vigorous roots of the lettuce plants treated with RBW lights were observed. However, plants grown under RB light looked small or even severely dwarfed (Fig. 2).

Chl a contents of lettuce leaves in all treatments were higher than the respective chl b contents. However, no significant differences were observed in pigment contents (chl a, b, a + b, and car) regardless of the light spectra (Table 1).

3.2. Nutritional quality of lettuce plants

Fig. 3 shows the effects of light quality on the contents of soluble proteins, sugars, and nitrate in the lettuce leaves. There were no significant differences in the soluble protein content among the light

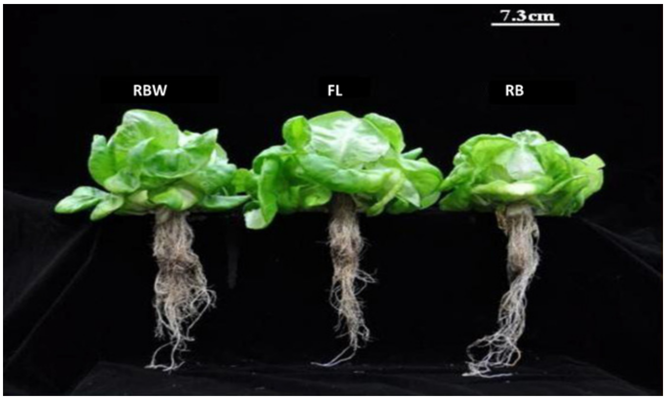

Fig. 2. Growth of lettuce plants under different light qualities for 35 days after sowing. Bar indicates 7.3 cm.

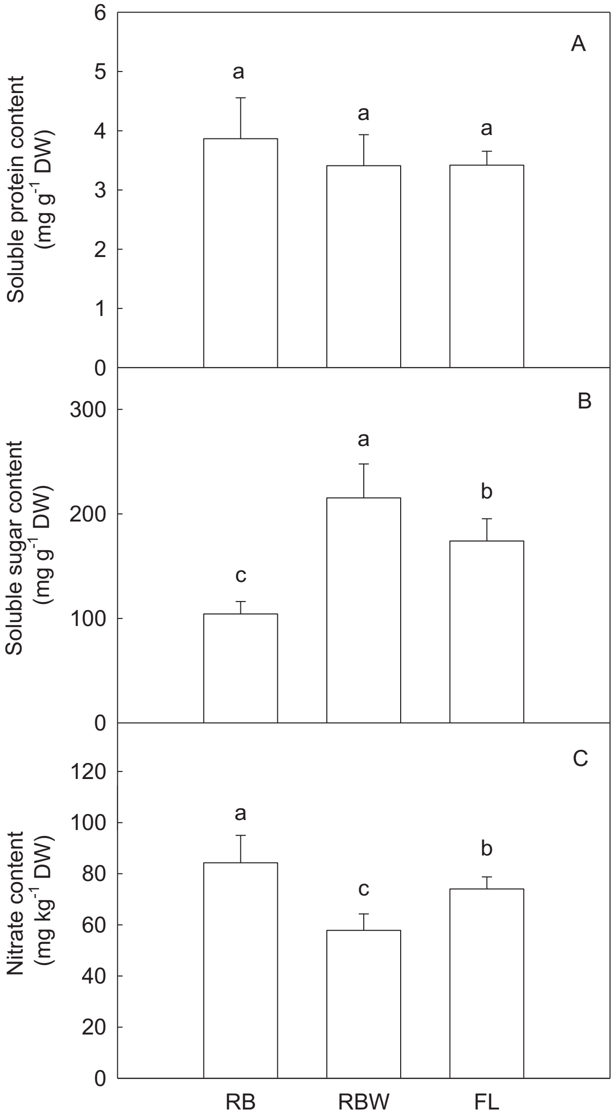

Fig. 3. Effects of light quality on the contents of (A) soluble proteins, (B) soluble sugars, and (C) nitrate in lettuce leaves. Vertical bars represent the standard deviation. Values for the same parameter with different letters significantly differed at the 5% level.

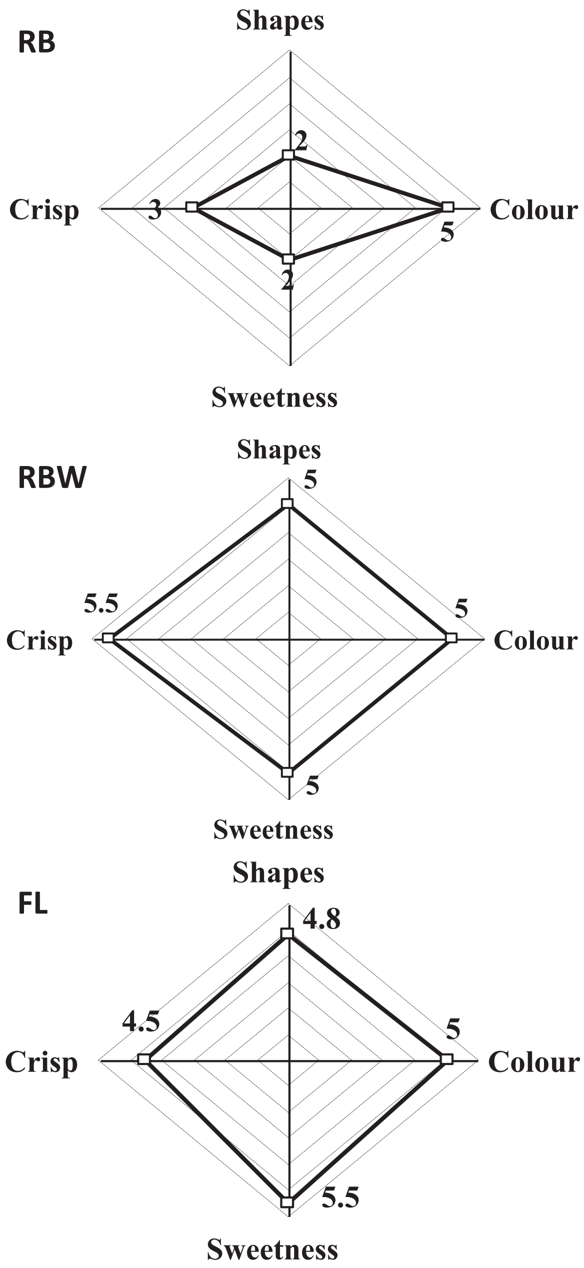

Fig. 4. Sensory analysis of the shape, color, crispness, and sweetness of fresh lettuce under red and blue (RB) LEDs, RB and white (RBW) LEDs, and fluorescent lamps (FL). The evaluation scale was 1–6, with 6 being the highest grade.

treatments, but soluble sugar and nitrate contents were greatly affected by the light quality. The soluble sugar content in plants was highest under RBW treatment (220 mg g^{-1} DW), followed by FL treatment (176 mg g^{-1} DW), and then RB treatment (104 mg g^{-1} DW). On the contrary, in the RBW treatment (58 mg kg^{-1} DW), the nitrate content of lettuce plants was significant lower than those of plants under RB (86 mg kg^{-1} DW) and FL (73 mg kg^{-1} DW) treatments.

3.3. Sensory evaluation

Shape, color, crispness, and sweetness are the four major marketable properties of Boston "butter head" lettuce, and therefore were selected for the sensory analysis. To show trends in the evaluation of attributes by the panelists, results for mouthfeel and visual characteristics were depicted for each treatment as spider-web-graphics (Auerswald et al., 1999). Plants under RBW treatment had high grades of 5–5.5 for all parameters. FL-treated plants (4.5) had a lower level for crispness than RBW-treated plants (5.5). The ranges of shape, crispness, and sweetness observed in RB plants were 2–3 (Fig. 4). The larger the rhombus was, the higher edible quality it represented. All of the sensory characteristics from RBW- and FL-treated plants had above-average scores. Nevertheless, the shape, crispness, and sweetness of RB-treated plants were not acceptable for the market.

4. Discussion

Lettuce is widely grown in Taiwan, and its production is very important, both economically and commercially. Lettuce is also a major crop grown in greenhouses worldwide and seems to be a model crop due to its fast growth and sensitivity to different light qualities (Dougher and Bugbee, 2001). The spectral quality of lights is the relative intensity and quantity of different wavelengths emitted by a light source and perceived by photoreceptors within a plant. Plant yields and quality are the result of interactions of various environmental factors under which plants are grown. The present study examined the effects of different light spectral conditions on the yield and quality of lettuce plants grown under the same environmental conditions. The biomass of lettuce leaves and roots was comparatively greater in plants grown under RBW and FL treatments than under RB treatment. However, FL-treated plants had a greater SLA than RBW- and RB-treated plants (Table 1). These results indicate that FL-treated plants exhibited puffiness with a loose shoot structure. The shoot structure of RBW- and RB-treated plants had a tight appearance, but observations of the growth and morphological features indicated that RB treatments were deleterious or adversely affected plant performance (Fig. 2). The addition of W LED light may have further increased plant growth, since W LED light might better penetrate the plant canopy than RB LED light for photosynthesis. Perhaps, RBW treatment achieved a balanced spectral environment by supplementing a favorable amount of W light to plants at 210 μmol m^{-2} s^{-1} PFD. Reductions in the total lettuce biomass suggest that light quality can alter growth, decrease the mean weight of lettuce, and lower its market value. Plant appearances under RBW light treatment were similar to those grown in greenhouses with high market value. Both FL-treated plants with a loose shoot structure and RB-treated plants with a smaller size could represent a lower value in the market. A growth-retarding effect might have been caused by an insufficient quality of light.

Exposure to only R LED light resulted in both plant elongation and reduced biomass of lettuce (Hoenecke et al., 1992). B LED light is important for leaf expansion and enhances the LA and biomass production (Li et al., 2010; Hogewoning et al., 2010; Johkan et al., 2012). Yorio et al. (2001) also reported that there was higher dry matter weight accumulation in lettuce grown under R light supplemented with B light than in lettuce grown under R light alone. However, shoot dry matter weight of leaf lettuce plants irradiated with B light decreased compared with that of W light (Ohashi-Kaneko et al., 2007). In the present experiments, the RB treatment was shown to be an inferior irradiation source compared to FL treatment for the growth and development of lettuce. Kim et al. (2004a) reported similar results for *in vitro* multiplication of potato. These results indicate that plant responses to light quality are species or cultivar dependent.

The biomass of lettuce shoots significantly increased with RBW treatment compared to RW treatment probably due to the enlarged LA (Table 1). The larger leaf allowed greater light interception, which may have led to the significant increase in biomass. The higher SLA under RBW light is a good indicator of higher photosynthetic surface area per unit investment in leaf tissue (Kim et al., 2004b). Vigorous roots support shoot growth by fully supplying the plant with water and mineral nutrition. In contrast, sprouting seedlings in which stems rapidly elongate under low irradiation or excess water have small roots that do not take up sufficient water or mineral nutrients, which decreases plant growth. Poor roots cannot supply sufficient water for large shoots, so plants with high S/R ratios are unsuitable for active growth (Johkan et al., 2010). In our study, the S/R DW ratio was suboptimal under RB light (16.3) compared to RBW light (12.8) and FL (12.3). This observation is indicative of the poor growth of roots under RB light and also indicates that root induction is probably also dependent on the spectral quality of light.

Plant pigments have specific wavelength absorption patterns known as absorption spectra. Biosynthetic wavelengths for the production of plant pigments are referred to as action spectra (Wang et al., 2009). Chl and car have high light absorption at 400–500 and at 630–680 nm, respectively, and low light absorption at 530–610 nm. Although different quality lights for all treatments were applied at the same PFD level, plants showed similar absorption spectra of photosynthetic pigments, chl a, b, a + b, and car (Table 1). Perhaps, the applied PFD level (210 μmol m^{-2} s^{-1}) had reached a certain minimal PFD, which is essential for sufficient synthesis and activity of photosynthetic pigments and electron carriers. Saebo et al. (1995) reported that plants with smaller chl contents seemed to use the chl more efficiently than plants with excessive chl. In our study, although the chl a, chl b, chl (a + b), and car contents in the leaves did not statistically differ among treatments, the chl and car contents under RBW lights were the lowest. This indicates that the lettuce plants might be using the chl more efficiently under RBW LED lights than under RB LED lights. Although the mechanisms of changes in photochemicals under different supplemental light qualities are not well known, the lower values of chl and car in the RBW treatment might have contributed to “dilution” due to the enhancement of shoot DW under RBW treatment (Li and Kubota, 2009). Plants grown under all treatments appeared to synthesize more chl a as it has a wider spectrum compared to that of chl b (Table 1), and chl a is the molecule that makes photosynthesis possible (Calatayud and Barreno, 2004).

A specific light quality can be used to improve the nutritional quality of vegetables and yields in commercial production. The selected LED lights differentially affected the metabolic system of the investigated vegetables. The most sensitive response was in sugars, the main photosynthesis product, and their accumulation in leaves (Lefsrud et al., 2008). Therefore, changes in lights not only affect sugars, as an index of nutritional quality and content, but they also participate as signaling molecules in regulating important vital processes. A high content of soluble sugars may be a desirable parameter in terms of food quality. Our results showed that lettuce plants had the highest soluble sugar content under RBW LEDs, and this light source might be beneficial for the accumulation of soluble sugars in lettuce plants (Fig. 3). However, the amounts of soluble proteins in the plant leaves showed no significant differences among all treatments. This suggests that the light spectrum might not be advantageous for protein synthesis, and the soluble protein content might not be a suitable parameter to assess the nutritional quality of lettuce plants. Moreover, a reduction in the nitrate content is definitely important for improving the nutritional quality of vegetables for human consumption. Results from Fig. 3 also show that the accumulation of nitrate in lettuce plants significantly differed among the light quality treatments. The RB LED possibly stimulated vital activities of plants and nitrate uptake, such that its concentration increased in leaves. Lillo (1994) reported that lights stimulated the *de novo* synthesis and activation of higher plant nitrate reductase, and sugar can replace lights in eliciting an increase in nitrate reductase messenger RNA accumulation. Therefore, the addition of broad spectral energy (500–600 nm) to R and B irradiations enhanced the accumulation of sugars and degraded the nitrate level in RBW-treated plants. The higher sugar level might also result in a sweeter taste (Fig. 4), and the lower nitrate level can be beneficial to human health.

Sensory properties are very important for the assessment of vegetable quality by consumers and for their purchase behavior. Boston lettuce is an annual, is a vegetable that heads in the cool season, and is commonly used for salads in restaurants. In Taiwan, an acceptable weight for lettuce for the market is around 150 g. In addition to yields, the shape, crispness, color, and sweetness of the lettuce are also important for market acceptance. In a comprehensive sensory evaluation, plants grown under RBW and FL treatments had significant higher overall acceptability than those under RB treatment based on the sensory analysis (Fig. 4). Among plants treated with three light qualities, consumer panelists selected the RBW-treated plants as the most preferable item; in particular, the crispness was the most popular parameter.

5. Conclusions

In agricultural production, yields and costs are the two most important criteria by which optimization of environmental factors is conducted. The final goal of our project is to develop a new light apparatus with LEDs optimized for vegetable production in plant factories. In the present study, we investigated the effective light quality with sufficient intensity for growing healthier lettuce plants more rapidly. Based on this study, it appears that the combined RBW LEDs resulted in many positive effects on growth, development, nutrition, appearance, and the edible quality of lettuce plants. The bioregenerative and hydroponic culture systems may comply with commercial requirements for rapid, large-scale, and precise management of plant production.

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