The effect of different spectral LED lights on the phenotypic and physiological characteristics of lettuce (*Lactuca sativa*) at picking stage.

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Abstract

Light plays crucial roles in plant morphological and physiological processes. In this study, lettuce plants (*Lactuca sativa L.*) were exposed to different spectral LED lights for 16 hd-1 photoperiod under the following eight treatments: white light (W, the control), monochromatic red light (R), monochromatic blue light (B), monochromatic green light (G), monochromatic yellow light (Y), monochromatic purple light (P) and a combination of R and B with R/B ratios of 9/1 and 4/1. Lettuce phenotype and some quality related indices were significantly changed under different LED lights. The vitamin C content of lettuce was increased under most of different light treatments, while the anthocyanin content was significantly increased only under G LED light treatment. In addition, R/B (4/1) increased soluble sugar and protein content and R and B combinations particularly R/B (4/1) improved phenotypic characteristics including plant height, stem diameter, fresh weight above ground, Soil and Plant Analyzer Development (SPAD). These results suggested R/B (4/1) could efficiently improve the lettuce quality which will provide valuable information for optimizing the conditions of lettuce production in the plant factory setting.

Keywords: Monochromatic, Photosynthesis, Physiological characteristics, Phenotypic characteristics

Abbreviations:

LED: Light Emitting Diodes; SPAD: Soil and Plant Analyzer Development; TSP: Total Soluble Protein; GS: Glutamate Synthase; GOGAT: Glutamine Oxoglutarate Aminotransferase; GDH: Glutamate Dehydrogenase; VC: Vitamin C

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Introduction

Light is an important environmental factor and plays key roles in plant growth and morphology [1]. Different light spectrum can produce diverse morphological and physiological responses in plants [2]. The effects of light intensity on plant physiological response were conducted [3,4]. Light-emitting diodes (LEDs) have a long life, high brightness, are environment friendly and their spectrum can be manipulated [5]. Furthermore, LEDs can efficiently promote plant growth and development. Plant morphology and physiology are strongly influenced by light quality, such as colour and wavelength [6]. Red (R) and blue (B) light are both vital wavelengths for plant growth and development. In vegetable plants, including tomato, pepper, lettuce and cucumber, different R: G: B photo flux (PF) ratios have been tested [7,8].

Lettuce (Lactuca sativa) is a major fresh salad food crop with fast growth and high commercial value. Lettuce is flowering plant which is sensitive to light quality, and it is a member of the large Asteraceae family and [9,10] reported that the combination of B and R inhibited hypocotyl extension and cotyledon elongation, while other reports showed that the combination of B and R increased lettuce dry weight and leaf number [6,11]. However, R treatment alone produced higher dry weight compared with a mixture of R and B [12-14]. Cope et al. [8] found that 0.3% B produced longer stem length in lettuce than 92% B using different B:G:R ratios in lettuce. Wang et al. [2] examined different ratios of LED R light to B light on photosynthetic performance by measuring leaf morphology, photosynthetic rate, chlorophyll fluorescence, stomatal development, light response curve, and nitrogen content in lettuce. B light promoted photosynthetic performance or growth by stimulating morphological and physiological responses. Han et al. [15] optimized LED light for lettuce growth to approximately 75° for accelerating the growth of lettuce.

The aim of this study was to elucidate the effects of different LED wavelengths on plant growth and development in plant factory setting. The yield, quality characteristics, nutritional components, secondary metabolites and antioxidant activity were measured in lettuce grown under different monochromatic LED lights to gain insight into the mechanisms by which LEDs affect lettuce quality and ultimately provide valuable information for producing lettuce in a plant factory setting.

Materials and methods

Plant material and treatments

The loose-headed lettuce variety "United States greatly fast-growing lettuce" was used in this study. Seeds were germinated on filter paper and placed into polyurethane cubes under dim natural sunlight for 22 d. The seedlings were transplanted into an indoor vertical plant factory system. The plants were subjected to a 16 h photoperiod of (1) white LED light (W), as control (400-800 nm); (2) P, purple LED light (peak at 430 nm); (3) B, blue LED light (peak at 460 nm); (4) R, red LED light (peak at 660 nm); (5) G, green LED light (peak at 530 nm); (6) Y, yellow LED light (peak at 590 nm); (7) 9R/1B, 90% red +10% blue LED light; (8) 4R/1B, 80% red +20% blue LED light (ZPDT802-200Zhishengpu Ltd., Zhengzhou, China), and light intensity for all treatment was 150 $\mu mol \bullet m^{-2} \bullet s^{-1}$.

The spectra parameters of the eight LED lights are shown in Figure 1. Photon fluxes were measured below the LED plate (20 cm) using a spectroradiometer (PAR-NIR, Apogee Instruments Inc, Logan, UT, USA).

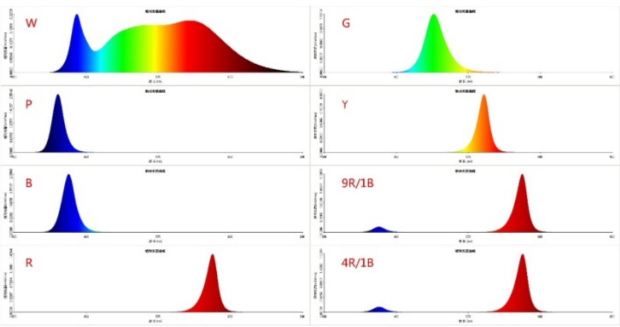

Figure 1. Absolute spectral distribution of different LED lights. Top to bottom: W LED, B LED, R LED, G LED, P LED, Y LED, R/B (9/1) and R/B (4/1). X axis indicates the wavelength and y axis indicates the absolute spectral value.

Temperature and relative humidity in the growth room were maintained at 24/18 (day/night) and 48%-52%/60%-70% (day/night), respectively. Plants were supplied with a nutritional solution for Japan Yamazaki lettuce: 236 mg/L ($Ca(NO_3)2.4(H_2O)$), 404 mg/L KNO_3, 57 mg/L $NH_4H_2PO_4$, and 123 mg/L $MgSO_4{\cdot}7H_2O$ and common microelements 20-40 mg/L EDTA ferric sodium, 2.86 mg/L boric acid, 2.13 mg/L $MnSO_4{\cdot}4H_2O$, 0.22 mg/L $ZnSO_4{\cdot}7H_2O$, 0.08 mg/L$CuSO_4{\cdot}5H_2O$ and 0.02 mg/L $(NH_4)6MO7O24{\cdot}4H_2O$. CO_2 concentration was maintained at 400 $\mu mol \bullet mol^{-1}$, electrical conductivity (EC) value and nutrient solution pH were maintained at 1.2-1.6 ms/cm and 6.0-6.9, respectively. The samples were harvested 35d after transplanting.

Phenotypic and physiological parameters measurement

After treating for 35 days under abovementioned conditions, Plant height was measured using a ruler, stem diameter and leaf thickness were measured using Vernier calipers, SPAD (Soil and Plant Analyzer Development) value was measured by chlorophyll instrument and fresh weight (FW) of above ground material was measured using an analytical balance (YajinAN110, ShangHai China). All experiments were performed with three biological replicates.

Vitamin C (VC) was measured using the 2, 6-D titration method [16]. Anthocyanin and soluble sugar was measured using spectrophotometer [17,18]. The amount of total soluble protein (TSP) and glutamate synthase (GS), glutamine synthetase (GOGAT) and glutamate dehydrogenase (GDH) were measured using a kit from Biotechnology Co., Ltd (Suzhou, China).

Statistical analysis

The data are the average of three replicates ± standard derivation (SD) for each treatment. The data was calculated by ANOVA using SPSS 20.0. Software Letters in the figures represent statistical significance at $P<0.05$ level.

Results

Effect of different spectral LEDs on lettuce phenotype

The absolute spectrum curve of the eight LED light combinations is shown in Figure 1; W LED (peak wavelength 455 nm, 560 nm), B LED (peak wavelength 455 nm), R LED (peak wavelength 661 nm), G LED (peak wavelength 532 nm), P LED (peak wavelength 590 nm), Y LED (peak wavelength 560 nm), R/B (9/1) and R/B (4/1) with two peak wavelengths of 455nm and 661nm, respectively. The growth state of lettuce grown under different spectral LED lights was significantly different (Figure 2).

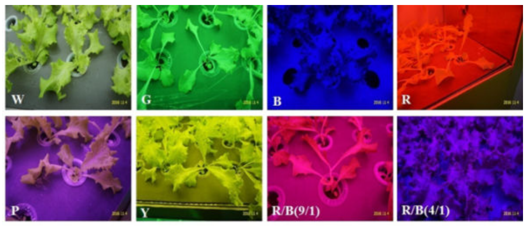

Figure 2. The leaf phenotype of lettuce grown under different spectral LED lights.

The highest length of lettuce was detected under R light compared with the W light, followed by G and R/B (9/1 and 4/1), while no difference was observed for lettuce grown under B, P and Y lights (Figure 3a). Stem diameter was lower under G and Y, while other lights had no significant effect on stem diameter compared with that of white light (Figure 3b). Leaf thickness was not significantly different under other lights except for G light (Figure 3c). Fresh weight of the above ground plant was significantly lower under G and Y lights, other lights had no differences (Figure 3d). SPAD value increased under B and R/B (4/1), but decreased under G, R, Y, R/B (9/1) compared with that under white light (Figure 3e). Number of leaves was lower under all other lights compared with that under white light (Figure 3f).

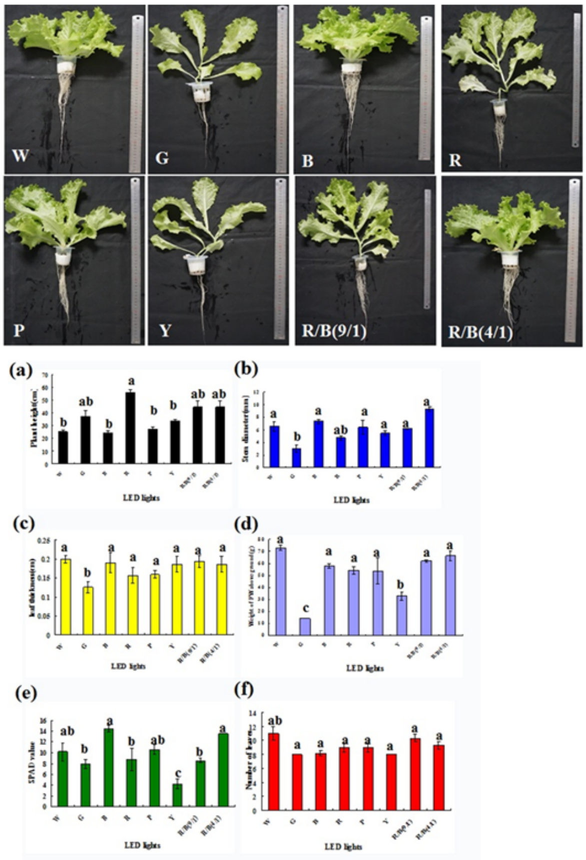

Figure 3. The leaf and root phenotype of lettuce grown under different spectral LED lights Compared with the W light, Lettuce height was highest under R light, followed by G and R/B (9/1 and 4/1), Stem diameter was lower under G and Y, while other lights had no significant effect on stem diameter (Figure 3b). Leaf thickness was not significantly different under other lights except for G light (Figure 3c). Fresh weight of the above ground plant was significantly lower under G and Y lights, other lights had no differences (Figure 3d). SPAD value increased under B and R/B (4/1), but decreased under G, R, Y, R/B (9/1) (Figure 3e). Number of leaves was lower under all other lights (Figure 3f).

Effect of different spectral LED lights on the content of anthocyanin and vitamin C

The content of anthocyanin and vitamin C was investigated for the effect of eight different colour LED lights: white (W), green (G), blue (B), red (R), purple (P), yellow (Y), R/B (9/1) and R/B (4/1). Compared with the W treatment, the content of anthocyanin was significantly different ($p<0.05$) after G, B, P, Y and R/B=9/1 treatments; G LED light treatment significantly increased anthocyanin content (Figure 4), while, B, P, Y and R/B (9/1) treatments significantly decreased anthocyanin content. Compared with the W treatment, G, Y, P, R/B (4/1) and R/B (9/1) treatments significantly increased VC content ($p<0.05$) (Figure 4).

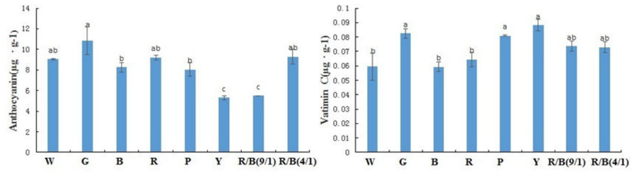

Figure 4. Anthocyanin and vitamin C content of lettuce grown under different LED lights Compared with the white control, the content of anthocyanin was significantly different ($p<0.05$) after G, B, P, Y and R/B=9/1 treatments; G treatment significantly increased anthocyanin content, while, B, P, Y and R/B (9/1) treatments significantly decreased anthocyanin content., and G, Y, P, R/B (4/1) and R/B (9/1) treatments significantly increased VC content ($p<0.05$).

Effect of different spectral LED lights on the content of soluble sugar and soluble protein

Compared with the W treatment, R, P and R/B (4/1) treatments significantly enhanced the soluble sugar content, especially for the R treatment which increased soluble sugar content by up to five-fold (Figure 5). Furthermore, B, P, R/B (9/1) and R/B (4/1) LED lights significantly affected soluble protein content; B and R/B (4/1) lights promoted the biosynthesis of soluble protein, but P and R/B (9/1) lights significantly ($p<0.05$) decreased soluble protein content compared with the W treatment (Figure 5).

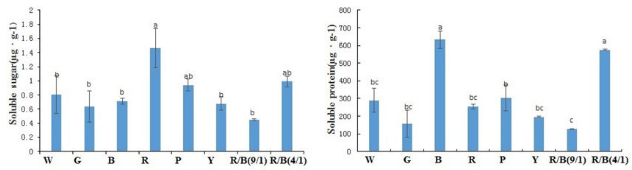

Figure 5. Soluble sugar and soluble protein content of lettuce grown under different LED lights Compared with the white control, R, P and R/B (4/1) treatments significantly enhanced the soluble sugar content, especially the R treatment, and B, P, R/B (9/1) and R/B (4/1) LED lights significantly affected soluble protein content.

Effect of different spectral LED lights on the activity of enzymes involved in ammonia assimilation

To investigate the effect of different spectral LED lights on the process of **ammonia assimilation**, three key enzymes involved in ammonia metabolism, including glutamate synthase (GS), glutamine synthetase (GOGAT) and glutamate dehydrogenase (GDH), were measured after W, G, B, R, P, Y, R/B (9/1) and R/B (4/1) treatments. The B LED light resulted in a 100-fold increase in GOGAT activity ($p<0.05$) compared with the W treatment. The G, B, P and R/B (4/1) treatments significantly altered GS activity; B and P LED lights increased the activity of GS, while G and R/B (4/1) decreased GS activity compared with the W LED. Only the R/B (4/1) treatment significantly increased the GDH activity (Figure 6).

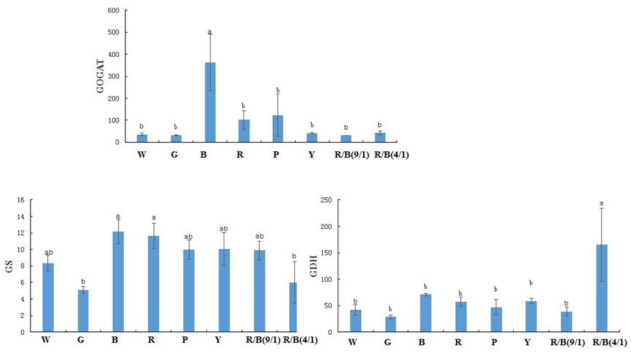

Figure 6. The activity of enzymes involved in ammonia assimilation under different LED lights Compared with the white control, the B LED light resulted in a 100-fold increase in GOGAT activity ($p<0.05$). The G, B, P and R/B (4/1) treatments significantly altered GS activity; B and P LED lights increased the activity of GS, while G and R/B (4/1) decreased GS activity. Only the R/B (4/1) treatment significantly increased the GDH activity.

Discussion

Light influences plant growth and development mainly via photo morphogenetic pigments. These include the red/far-red light absorbing phytochromes and blue/UV light absorbing pigments, which were decided by quality, quantity, direction and duration of light [19]. Plants grown under optimal R and B combination tend to have the highest Pn and Chl concentrations, although the optimal R and B ratio varies among different plant species [20-24]. In tomato, higher B:R ratios decreased tomato plant height under single source electrical light [7,14,25]. In this study, lettuce height was highest when grown under R light, followed by G and R/B (9/1 and 4/1) combinations; no difference was observed when grown under B, P and Y LED lights compared with the W LED.

Oh-Hama and Hase [26] showed that B light is critical to initiate chlorophyll biosynthesis and increasing B photo flux could improve chlorophyll concentration in green algae [27-29]. Additionally, many studies have shown that only B light affects leaf photosynthetic performance including chlorophyll content, chlorophyll a/b ratio, chlorophyll a/b-binding protein of the PSII system and the electron transport chain [30-32]. To our knowledge, SPAD value was positively related with chlorophyll concentration. In our study, SPAD significantly increased under B and R/B (4/1) lights, however, B and Y light significantly repressed chlorophyll content in lettuce. Interestingly, the SPAD value decreased under monochromatic R and G, but increased when R and B were combined. This is consistent with Tripathy and Brown [33] who showed that B and R combination restored chlorophyll biosynthesis of wheat seedlings initially grown under only R light.

However, earlier studies showed that G light could inhibit the growth of algae, fungi, plant cell cultures and some higher plants [32-35]. Herein, we also observed that G light inhibited the growth of lettuce. Plant height; stem diameter, leaf thickness, and fresh weight above ground. SPAD value and number of leaves significantly decreased under G LED light compare with the other LED light treatments. Stem diameter and fresh weight above ground were particularly affected by G LED light, characteristics which dictate lettuce yield. However, anthocyanin content was significantly increased under G LED lights compared with the other light treatments.

Kim et al. [36] found that plants grown under 24% G LED light had greater fresh mass, dry mass and leaf area than plants grown under combinations of B:R and B:G:R in lettuce. Furthermore they found that 24% G increased lettuce growth, and more than 50% G inhibited plant growth [37]. Here, we found that lettuce quality characteristics were better under R:B combination than under monochromatic G, R, B, P and Y; in particular, the quality characteristics of lettuce grown under R/B (4/1) was better that under other lights.

Furthermore, many studies have reported that the combination of R and B lights plays important role in leaf expansion and biomass accumulation [38-41]. Wang et al. [2] studied leaf morphology and shoot dry weight grown under different combinations of R/B light ratios, including 12, 8, 4, and 1. The authors found that shoot dry weight increased with increasing R/B light ratio and was mainly due to an increase in leaf number and leaf area.

Herein, fresh weight above ground of lettuce grown under R/B combinations significantly increased compared with that of G and Y; while other LED light treatments resulted in no difference. Moreover, the vitamin C, soluble sugar, soluble protein and GDH significantly increased under R/B (9/1) and R/B (4/1) combinations compared with lettuce grown under W light [42]. These parameters were especially high when grown under R/B (4/1), indicating this combination can efficiently contribute to increased quality characteristics in lettuce.

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Conflict of Interest

All authors contributed equally and declare that there are no conflicts of interest.

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