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Green light enhances growth, photosynthetic pigments and CO_2 assimilation efficiency of lettuce as revealed by 'knock out' of the 480–560 nm spectral waveband

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Abstract

Adding green component to growth light had a profound effect on biomass accumulation in lettuce. However, conflicting views on photosynthetic efficiency of green light, which have been reported, might occur due to nonuniform light sources used in previous studies. In an attempt to reveal plausible mechanisms underlying the differential photosynthetic and developmental responses to green light, we established a new way of light treatment modeled according to the principle of gene "knock out". Lettuce (*Lactuca sativa* L. var. *youmaicai*) was grown under two different light spectra, including a wide spectrum of light-emitting diode (LED) light (CK) and a wide spectrum LED light lacking green (480–560 nm) (LG). Total PPFD was approximately 100 μmol(photon) m^{-2} s^{-1} for each light source. As compared to lettuce grown under CK, shoot dry mass, photosynthetic pigment contents, total chlorophyll to carotenoids ratio, absorptance of PPFD, and CO_2 assimilation showed a remarkable decrease under LG, although specific leaf area did not show significant difference. Furthermore, plants grown under LG showed significantly lower stomatal conductance, intercellular CO_2 concentration, and transpiration compared with CK. The plants under CK exhibited significantly higher intrinsic quantum efficiency, respiration rate, saturation irradiance, and obviously lower compensation irradiance. Finally, we showed that the maximum ribulose-1,5-bisphosphate-saturated rate of carboxylation, the maximum rate of electron transport, and rate of triose-phosphate utilization were significantly reduced by LG. These results highlighted the influence of green light on photosynthetic responses under the conditions used in this study. Adding green component (480–560 nm) to growth light affected biomass accumulation of lettuce in controllable environments, such as plant factory and Bioregenerative Life Support System.

Additional key words: gas exchange; green light; leafy vegetable; light-response curve; pigment; P_N/C_i curve; photosynthesis.

Introduction

Light quality is thought to affect many plant physiological processes during growth and development, particularly photosynthesis (Hogewoning *et al.* 2010, 2012). Green light was always considered less efficient in driving photosynthesis comparing to blue, red or white light (Evans 1987, Wang *et al*. 2009, Johkan *et al*. 2012), because green light is absorbed only weakly by chlorophylls (Chl) and pigments extracted from green leaves (Terashima *et al*. 2009). Nevertheless. some previous works in laboratory over the last decade reported the opposite results. Green light could potentially increase plant growth and drive leaf photosynthesis more

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Abbreviations: C_i – intercellular carbon dioxide concentration; Car – carotenoids; Chl – chlorophyll; CK – wide-spectrum LED light; DM – dry mass; E – transpiration rate; g_s – stomatal conductance; I_c – compensation irradiance; I_{sat} – saturation irradiance; J_{max} – the maximum rate of electron transport; LED – light-emitting diode; LG – wide-spectrum LED light lacking green, 480–560 nm wavebands "knock out"; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; R_D – respiration rate; RuBP – ribulose-1,5-bisphosphate; SLA – specific leaf area; V_{cmax} – the maximum RuBP-saturated rate of carboxylation; V_{TPU} – rate of triose-phosphate utilization; α – intrinsic quantum efficiency; Φ_{PSII} – quantum yield of PSII electron transport.
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efficiently for its better penetration. Leaf photosynthesis could be increased to a greater extent by any additional green light absorbed by the lower chloroplasts than additional red or blue light (Sun *et al.* 1998, Kim *et al.* 2004a, Terashima *et al*. 2009, Lin *et al.* 2013).

Most of the light sources used in the experiments were colored fluorescent lamps, or halogen lamps with optical filters, or natural light with colored filters (Evans 1987, Talbott *et al.* 2002, Terashima *et al.* 2009). The wavebands of green light were not pure. LEDs can get narrow and pure green light, and have been used in previous studies (Kim *et al.* 2004a, b; Wang *et al.* 2009, Lin *et al*. 2013, Su *et al.* 2013, Dong *et al.* 2014), and plants showed significant responses to different monochromatic light. Plants were adapted to the naturally wide spectrum during the long-term evolution. There might be coupling effects among different wavebands on plants. Recent research has revealed that plant growth, CO_2 assimilation rate, and Chl contents, as well as nutritional quality, *e.g.,* nitrate and contents of antioxidants, can be significantly reduced in plants grown under red, blue, yellow, and green lights, as compared with those grown under white light (Su *et al.* 2013, Wu *et al.* 2014, Shao *et al.* 2015).

Recently, LEDs have been developed and can provide a better combination of the visible spectrum for optimizing photosynthesis and growth. In order to examine more exactly the effects of a green component (480–560 nm) on plant photosynthesis and growth, a new method was established, and special spectrum was modeled according to the principle of gene "knock out". Plant biomass, photosynthetic pigment contents, CO_2 assimilation, light responses, P_N/C_i responses, and Chl fluorescence quenching were analyzed in leaves of lettuce after cultivation for 35 d under different light spectra. This study examined photosynthetic responses of lettuce to green light (480–560 nm), but also led to the development of a method allowing the characterization of plant response to special spectral wavebands.

Materials and methods

Plant materials and light treatments: Lettuce (*Lactuca sativa* L. var. *youmaicai*) seeds were germinated in a Petri plate (with moist filter paper) and hydroponically grown in an environmentally controlled chamber. The temperature was constant at 20°C under PPFD of approximately 80 μmol m^{-2} s^{-1} with cool white fluorescent lamps, the photoperiod was 16 h light, and relative humidity was around 65%. When the first true leaf fully expanded, uniform-sized seedlings were transplanted into three plastic containers (37 cm × 23.5 cm × 9 cm, 18 plants per container) filled with 1/2 Hoagland nutrient solution. Then they were randomly divided into two groups and treated with two different light spectra: (*1*) wide spectrum LED light (CK); (*2*) wide spectrum LED light lacking green component (LG), which was 480–560 nm waveband "knocked out" (Fig. 1S, *supplement available online*). Spectra of CK included wavebands of 480–560 nm, and the radiation energy of the 480–560 nm wavebands accounts for 15.12% of the total spectrum radiant energy (Fig. 1). All the light sources were customized by *E.shine Systems Limited*. The nutrient solution was renewed every three days and adjusted to pH 6.5 using 2 mol L^{-1} HNO_3. Photoperiod was also maintained at 16 h light (from 6:00 to 20:00 h) and 8 h (from 20:00 to next 6:00 h) dark. The total PPFD, air temperature, relative humidity, and CO_2 concentration [CO_2] for all treatments were maintained 95–105 μmol m^{-2} s^{-1}, 21 ± 2°C, 35%–50%, and 350 ± 20 ppm, respectively, throughout the whole experiment. All photosynthetic tests were performed when plants were treated for 33–35 d, and then plants were sampled for the measurements of biomass dry mass (DM) and photosynthetic pigments. All treatments were performed at the same time with three repetitions in order to avoid the possible influence of growth conditions.

Photosynthetic pigments: New mature leaves (the fourth leaf from the top) were sampled and divided into two parts at the midrib, one part for photosynthetic pigment measurements and the other part was used to determine specific leaf area (SLA) with leaf punching method. SLA was calculated as the ratio of leaf area to leaf DM. At the same time, fresh biomass per unit of leaf area was also obtained.

Three portions of the minced leaf sample (0.2 g each) were placed in a mortar with a little of quartz sand and calcium carbonate powder and 2–3 ml of 95% ethanol were added, and then homogenized. Ethanol (10 ml, 95%) was added and ground continuously until the plant tissues became white. The homogenized samples were filtered into a 25-ml volumetric flask, kept for 3–5 min, and then 100% ethanol was added and mixed. The filtrate was used to determine the absorbancies of chlorophyll (Chl) *a*, Chl *b*, and carotenoids (Car) in ethanol with a *UV–Vis* spectrophotometer (*Model SP–752*, *Shanghai Spectrum Instrument Co., Ltd.*, China), at wavelengths of 665, 649, and 470 nm, respectively. Concentrations of Chl were calculated according to Rowan (1989). In order to estimate leaf absorptance of PPFD, Chl content per unit of leaf area was calculated with the fresh biomass per unit of leaf area using molecular mass of 893.48 and 907.46 for Chl *a* and Chl *b,* (Porra *et al*. 1989), respectively; the corresponding micromolar contents were 100.73, 33.06 μmol m^{-2} in CK and 76.11, 26.45 μmol m^{-2} in LG, respectively. Leaf absorptance of PPFD was estimated from equation 1 (Evans and Poorter 2001).

$$\alpha = x/(x + 76) \quad (1)$$

where α is the leaf absorptance of PPFD and x is the sum of Chl content per unit of leaf area.

Gas exchange: Net photosynthetic rate (P_N) was measured two days before plants were sampled using a portable photosynthesis system (*LiCor–6400, LiCor Inc*., Lincoln, Nebraska, USA) with a transparent leaf chamber (*GA3128*, chamber size: 15 mm × 25 mm) at ambient CO_2 concentration under their original light sources between 0:00 and 23:59 h. Tested leaves were the new mature leaves (the fourth leaf from the top) and the tests were carried out with the original position of leaves. All the tests were made in six replicates, very similar plants per treatment, and the gas-exchange curves were plotted using their means.

Light-response curve and fittings: Measurements of CO_2 gas exchange in response to PPFD were made at eleven PPFD levels [1,200; 800, 600, 400, 350, 300, 250, 150, 50, 20, and 0 μmol(photon) m^{-2} s^{-1}] under the same conditions [C_a of 330–370 μmol(CO_2) mol^{-1}, T_{leaf} of 20 ± 3°C, and RH of 55–60%] inside the leaf chamber. Light was provided by the internal LEDs light of the leaf chamber, and the proportion of red and blue light was set according to the applied light spectra in CK and LG treatments. At each PPFD, CO_2 assimilation was monitored to ensure that they reached a steady state (3–5 min) before a reading was taken. After measuring the leaf gas exchange, the light-response curves of photosynthesis were fitted using a photosynthetic model (Eq. 2) according to a prior study (Ye and Yu 2008). A statistical program based on the Marquard algorithm was used to find the least-squares solution to obtain respective parameters. Subsequently, the saturation irradiance (I_{sat}) and light-saturated net photosynthetic rate (P_{Nmax}) were obtained using equations 3 and 4, respectively. Compensation irradiance (I_c) was estimated by Eq. 1. Fitting coefficient r^2 was also obtained between model calculations and observations of P_N.

$$P_N(I) = \alpha\frac{1-\beta I}{1+\gamma I}I - R_D \quad (2)$$

$$I_{sat} = \frac{\sqrt{(\beta+\gamma)/\beta}-1}{\gamma} \quad (3)$$

$$P_{Nmax} = \alpha\left(\frac{\sqrt{(\beta+\gamma)/\beta}-1}{\gamma}\right)^2 - R_D \quad (4)$$

where P_N is net photosynthetic rate, I is irradiance, R_D is respiration rate, α is the initial slope of irradiance-response curve of photosynthesis when irradiance approaches zero, which is also known as the intrinsic quantum efficiency, β and γ are coefficients that are independent of I, I_{sat} is the saturation irradiance, P_{Nmax} is the light-saturated net photosynthetic rate.

***P_N/C_i* response curve measurement and calculation**: Measurements of leaf P_N in response to intercellular CO_2 concentration (C_i) were made at a serie of ambient CO_2 concentrations (C_a) [400, 300, 200, 100, 50, 0, 500, 600, 800, 1,000; 1,500; and 2,000 ppm] under the same conditions (PPFD of 350 μmol m^{-2} s^{-1} predicted saturation PPFD for leaves, T_{leaf} of 20 ± 3°C, and RH of 55–60%) inside the leaf chamber. Light was also provided by the internal LED light of the leaf chamber, and the proportion of red and blue light was set according to the applied light spectra in CK and LG treatments, respectively (Habermann *et al*. 2003). At each C_a, CO_2 assimilation was monitored to ensure that it reached a steady state (3–5 min) before a reading was taken. After measuring the leaf gas exchange, P_N/C_i curves were analyzed based upon the mechanistic model of CO_2 assimilation proposed by Farquhar *et al.* (1980) and later modified by Long and Bernacchi (2003). According to this biochemical model, the P_N can be described by the equation 5.

$$P_N = \min\{w_c, w_j, w_p\}\left(1-\frac{\Gamma^*}{C_i}\right) - R_{day} \quad (5)$$

where Γ* is the CO_2-compensation point, the term (1 – Γ*/C_i) is used to take account of the proportion of recently assimilated carbon that is released in photorespiration. Day respiration is designated R_{day} and refers to the release of CO_2 in the light by processes other than photorespiration (Wullschleger 1993), w_c, w_j, and w_p are the rates of carboxylation allowed by Rubisco activity, ribulose-1,5-bisphosphate (RuBP) regeneration, and triose phosphate utilization, respectively.

$$V_c = \min\{w_c, w_j, w_p\} \quad (6)$$

where

$$w_c = \frac{V_{c,max}C_i}{C_i + K_c(1+O/K_o)} \quad (7)$$

$$w_j = \frac{JC_i}{4.5C_i + 10.5\Gamma^*} \quad (8)$$

$$w_p = \frac{3V_{tpu}}{\left(1-\frac{\Gamma^*}{C_i}\right)} \quad (9)$$

V_c is the rate of carboxylation. V_{cmax} is the maximum rate of carboxylation, competitive with respect to both CO_2 and oxygen. O is the partial pressures of O_2 in the intercellular air space. K_c and K_o are Michaeli's constants for carboxylation and oxygenation, respectively. V_{cmax} is the maximum RuBP-saturated rate of carboxylation, J_{max} is the maximum rate of electron transport. This model assumes that RuBP regeneration is limited by potential whole chain electron transport rate (J) under the given conditions of light and temperature and corrected for partitioning between oxygenation and carboxylation of RuBP. At light saturation, J is equal to J_{max}.

Nonlinear regression techniques were used to estimate photorespiration, V_{cmax}, J_{max}, and V_{TPU} for the P_N/C_i, curves compiled in this study (Long and Bernacchi 2003).

Statistical analysis: Statistical significances of means were tested with a model of one-way *ANOVA* followed by *Duncan*'s multiple range tests (*SPSS Inc.*, Chicago, IL, USA) with a *P*-level of 0.05 was used to evaluate the significance.

Results

Spectral analysis: Light spectra of CK and LG were both analyzed at the shoot top with an *Avaspec–2048–USB2–UA* spectroradiometer (*Avantes B.V.*, Netherlands). Results showed the total PPFD was maintained at 95–105 μmol m^{-2} s^{-1}. The spectrum was divided into six wavebands, including 360–420, 420–480, 480–560, 560–610, 610–710, and 710–900 nm. When the waveband at 480–560 nm was cut off, the total PPFD was adjusted to the original PPFD by adjusting the distance from the LED light plate to the growth surface. Specifically, the radiation at 480–560 nm was reduced to almost zero [0.57 μmol(photon) m^{-2} s^{-1}], while the proportions of other wavebands were not changed obviously (Fig. 1). Hence this new spectrum was considered as the 480–560 nm "knocked out" spectrum on basis of the original wide spectrum.

Biomass and SLA: DM of lettuce roots and shoots was measured at the harvest time (35 d after sowing) and the results are shown in Fig. 2*A*. The elimination of the waveband at 480–560 nm not only resulted in a decrease in root biomass but also in shoot biomass. Shoot DM decreased by 37.3%, while root DM increased by 18.9% on average in LG compared with CK. CK had higher total biomass, which was the sum of shoot biomass and root biomass (Fig. 2*A*). According to the compositions of two light sources (Fig. 1), the largest difference between these spectra was the proportion of green light (480–560 nm), which accounted for 22.09% and 0.58% of the spectra of CK and LG, respectively. Therefore, the biomass was improved by increasing the proportion of green light from 0.58% to 22.09% (Fig. 2*A*). SLA was slightly but not significantly lower in LG *vs*. CK (Fig. 2*B*).

Photosynthetic pigments and absorptance of PPFD: The analysis of photosynthetic pigments in plant leaves treated by different spectra showed the Chl patterns varied considerably. Spectral lack of green component (480–560 nm) significantly affected the photosynthetic pigments of lettuce leaves (Fig. 2*C*). The contents of Chl *a*, Chl *b*, and Car after the LG treatment were all significantly reduced compared with CK (Fig. 2*C*). The absorptance of PPFD and the ratio of total Chl/Car were also estimated based on the data of Chl contents, and the results in Fig. 2*D*,*E* showed the similar trend as the Chl contents. But the Chl *a*/*b* ratio did not show any significant difference between light treatments (Fig. 2*F*).

Gas exchange: The P_N during a diurnal cycle was analyzed under their growth conditions after 34 d from sowing and thereby their carbon assimilation efficiencies were compared. The leaves stopped assimilating CO_2 and began releasing CO_2 to the air when lights were shut off. Thus, P_N was negative in all cases and its absolute value should represent the respiration activity of plants. As shown in Fig. 3, P_N in LG-treated leaves was only half that of CK during the light hours, while the dark-period respiration under LG was about 1/3 of that in CK. Hence, the "knock out" of green light weakened the CO_2 fixation and reduced the foliar respiration compared with CK, since the green light could enhance photosynthesis and dark respiration in lettuce.

Light response: The responses of P_N, stomatal conductance (g_s), intercellular carbon dioxide concentration (C_i), transpiration (E), leaf temperature, and vapour pressure deficit to PPFD were also analyzed by a photosynthetic model before plant sampling (Fig. 4). The identified model parameters, including saturation irradiance (I_{sat}), compensation irradiance (I_c), maximum photosynthetic rate (P_{Nmax}), intrinsic quantum efficiency (α) and dark respiration (R_D), were calculated and listed in Table 1. The simulated results agreed well with the observed results

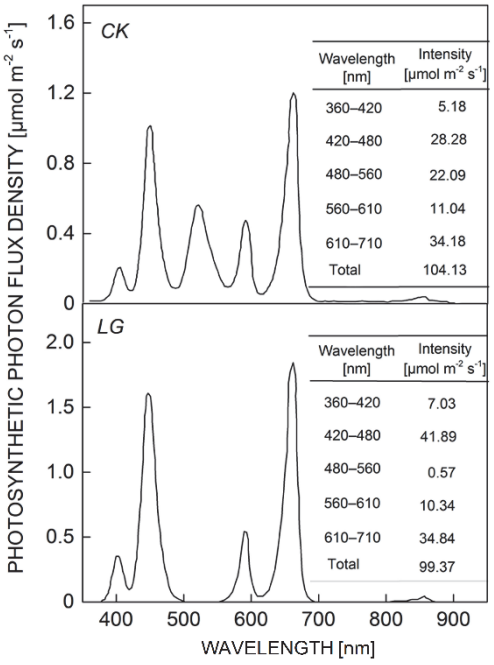

Fig. 1. Spectral distribution of the similar energy in CK and LG light sources. Spectral scans were recorded by a spectroradiometer (*Avaspec-2048–USB2–UA, Avantes B.V.*, Netherlands) at the top of plant shoots. CK – wide spectrum LED light; LG – wide spectrum LED light without the waveband of 480–560 nm.

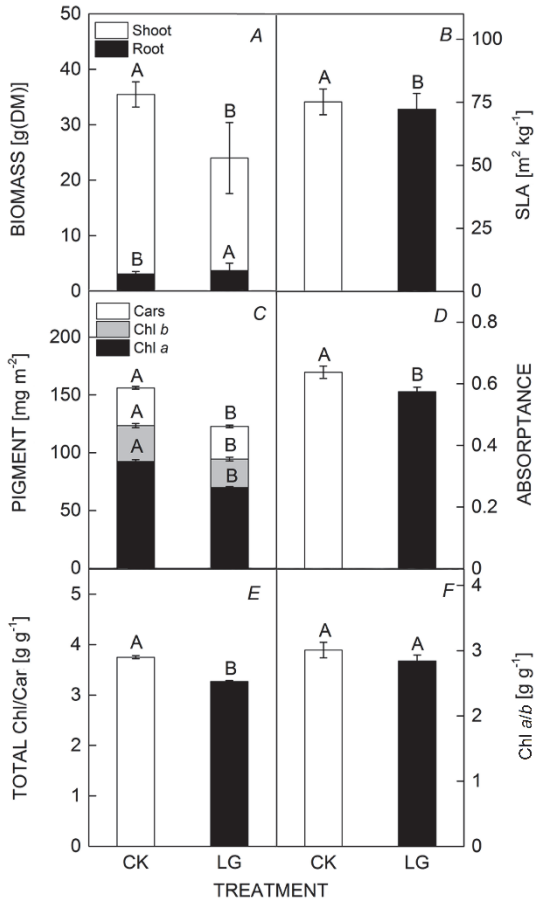

Fig. 2. Biomass (*A*), specific leaf area (SLA) (*B*), photosynthetic pigment contents (*C*), absorptance of photosynthetic photon flux density (PPFD) (*D*), total Chl/Car ratio (*E*), and Chl *a*/*b* ratio (*F*) of lettuce leaves grown under CK and LG spectra. Car – carotenoids; Chl – chlorophyll; CK – wide spectrum LED light; LG – wide spectrum LED light without the waveband of 480–560 nm.

(r^2> 0.99). Among the parameters analyzed, P_N responded strongly to the light supply, and was significantly higher in CK than that in LG (Fig. 4*A*). In LG, the 480–560 nm "knock out" decreased P_N (40.7% on average), g_s (14.9%), C_i (5.8%), E (28.6%), α (55.7%), P_{Nmax} (57.5%), I_{sat}

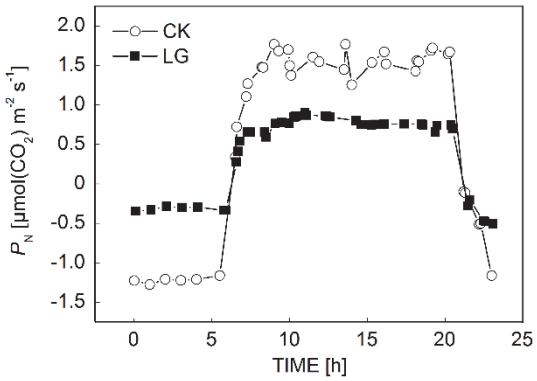

Fig. 3. Net photosynthetic rate (P_N) during a diurnal cycle of lettuce leaves grown under CK and LG spectra. The temperature varied between 20°C at night and 25°C in early afternoon. CK – wide spectrum LED light; LG – wide spectrum LED light without the waveband of 480–560 nm.

(18.8%), R_D (34.2%), but increased in I_c (46.5%) compared with CK, which had higher values of P_N, g_s, C_i, E, α, P_{Nmax}, and I_{sat} (Table 1). Here, R_D was slightly lower than the data showed in Fig. 3. This might be due to the values of R_D in Fig. 4 and Table 1 were calculated from the test data using photosynthetic model, and the instantaneous state of the leaf was different from the original state after the gradient PPFD treatment. Leaf temperature and vapour pressure deficit did not show obvious difference between the two groups, while the vapour pressure deficit showed the opposite trend to g_s (Fig. 2*E*,*F*).

***P*N/*C*i curves**: P_N increased more slowly and at lower concentrations in LG than that in CK (Fig. 5*A*). With nonlinear regression analysis based on the equations described above, the maximum rate of electron transport (J_{max}), maximum RuBP-saturated rate of carboxylation (V_{cmax}), photorespiration rate, and rate of triose-phosphate utilization (V_{TPU}) were estimated. First, J_{max}, V_{cmax}, and V_{TPU} in LG *vs*. CK decreased by about 40.0, 35.1, and 56.3%, respectively, but photorespiration rates were not significantly different (Fig. 5*B*–*E*). Second, significant linearity was found between V_{cmax} and J_{max} in both treatments (R^2 > 0.995) (Fig.5*F*).

Discussion

Here, a new method of specific waveband "knock out" was established in order to investigate the effects of different light qualities on plant development and metabolism. This method could eliminate the radiation of a specific waveband while keeping the proportions of other bands in the wide spectrum unchanged (Fig. 1). Hence, we tentatively used this method to clarify the effects of specific bands on plant growth and development.

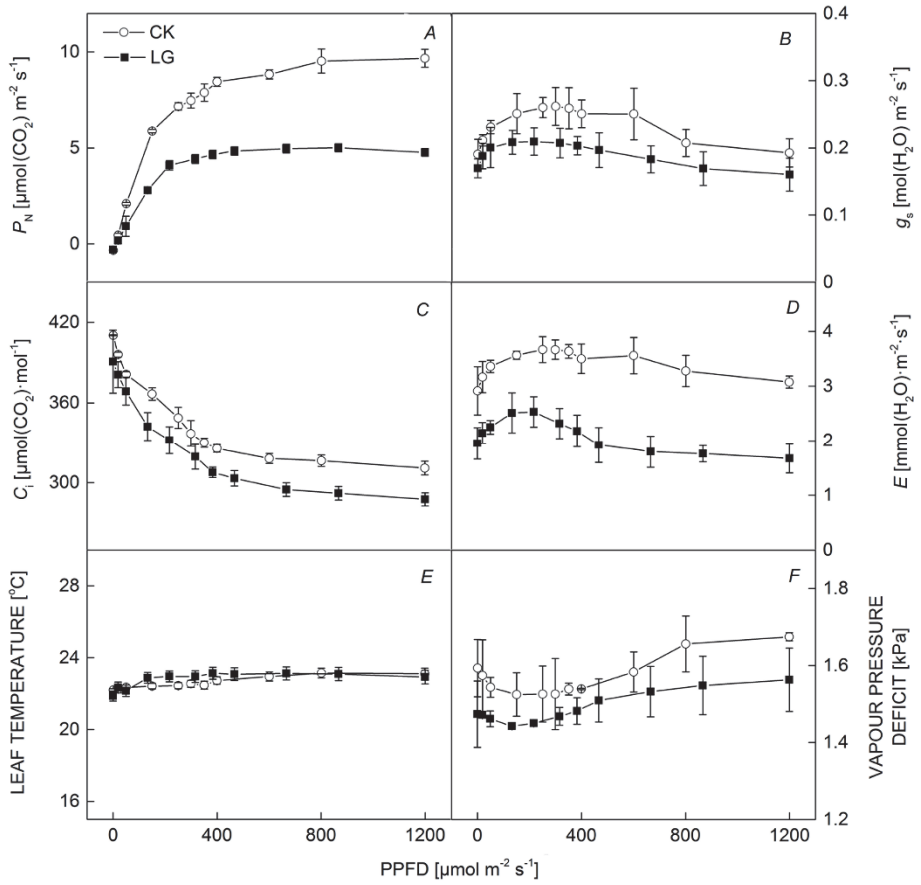

Fig. 4. The influences of different spectral composition on responses of net photosynthetic rate, P_N (*A*), stomatal conductance, g_s (*B*), intercellular carbon dioxide concentration, C_i (*C*), transpiration, *E* (*D*), leaf temperature (*E*), and vapour pressure deficit (*F*) to photosynthetic photon flux density (PPFD). CK – wide spectrum LED light; LG – wide spectrum LED light without the waveband of 480–560 nm. Vertical bars are means ± SD.

Table 1. The influences of different spectral composition on the saturation irradiance (I_{sat}), compensation irradiance (I_c), maximum photosynthetic rate (P_{Nmax}), intrinsic quantum efficiency (α), and respiration rate (R_D). These values were determined from the light-response curves using the model described by Ye and Yu (2008). Mean ± SD (n = 6). Means in the same row with *different letters* are significantly different ($P \leq 0.05$). CK – wide spectrum LED light; LG – wide spectrum LED light without the waveband of 480–560 nm.

Parameter	Treatment	
	CK	LG
α	0.0793 ± 0.0028^a	0.0351 ± 0.0064^b
P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}]	9.766 ± 0.55^a	4.152 ± 0.88^b
I_{sat} [μmol m^{-2} s^{-1}]	795.5 ± 100.40^a	646.3 ± 55.28^b
I_c [μmol m^{-2} s^{-1}]	8.24 ± 0.17^b	12.07 ± 0.64^a
R_D [μmol(CO_2) m^{-2} s^{-1}]	0.6031 ± 0.019^a	0.3968 ± 0.051^b
r^2	0.9951 ± 0.0019	0.9907 ± 0.0030

Results showed that biomass accumulation was significantly repressed by the LG treatment (Fig. 2*A*). The mechanism underlying the effect of green light on plant biomass accumulation might be complex, so here it was tentatively investigated from the perspective of photosynthesis. SLA is a leading parameter for plant leaf features and much influenced by leaf thickness (Wilson *et al*. 2002). However, we found the green component (480-560 nm) in plant growth light did not exert significant effect on SLA in lettuce (Fig. 2*B*), but remarkably reduced photosynthetic pigment contents compared with CK (Fig. 2*C*). Pigments are integrally related to the physiological functions of leaves. As the main type of pigments, Chls absorb and transfer light energy into the photosynthetic apparatus (Gates *et al*. 1965, Sims and Gamon 2002, Wang *et al.* 2015), and especially, are the most

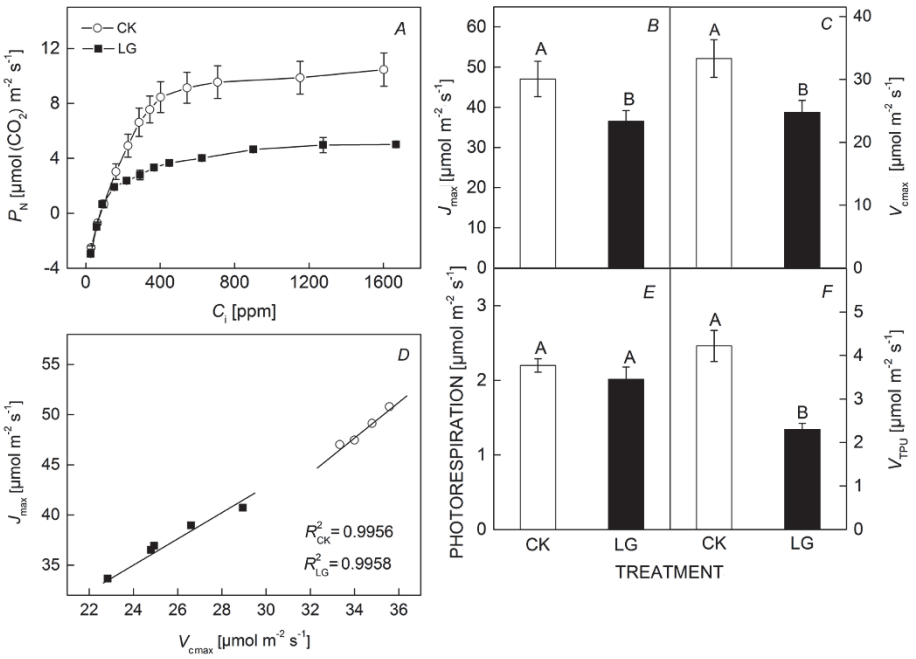

Fig. 5. The influences of different spectral composition on responses of net photosynthetic rate, P_N (*A*), the maximum rate of electron transport, J_{max} (*B*), the maximum RuBP-saturated rate of carboxylation, V_{cmax} (*C*), the relationship between V_{cmax} and J_{max} (*D*), photorespiration (*E*), and rate of triose-phosphate utilization, VTPU (*F*), J_{max}, V_{cmax}, photorespiration, and VTPU were calculated from the P_N/C_i curves of lettuce plants. Vertical bars are means ± SD.

important light-absorbing leaf pigments in the green–red spectrum (Paradiso *et al*. 2011). Hence, the calculated absorptance of PPFD was also significantly reduced in LG compared with CK (Fig. 2*D*). The accurate light absorption in leaves represents absorption in all pigments, including nonphotosynthetic pigments. Green light is absorbed more by intact leaves than by Chl and Car in solution (Ouzounisv *et al*. 2015). Our results suggested the green component (480–560 nm) in growth light spectrum affected the light absorption utilized for photosynthesis under the conditions used in this study.

Photosynthetic rate of LG was significantly lower during the light period compared with CK (Fig. 3). At low irradiances, the photosynthetic rate depends on the proportion of leaf-absorbed incident light (Evans 1996). Previous studies have reported that blue and red light are efficiently absorbed close to the surface, whereas green light contributes more to photosynthesis in deeper leaf layers (Brodersen and Vogelmann 2010, Sun *et al*. 1998), decreasing the potentially negative effect of the internal light gradient within the leaf (Ouzounisv *et al*. 2015). Although the light intensity of plant cultivation in this study was in the initial slope region for the flat leaves, the penetration advantage of green light can also be obtained (Ichiro et al. 2009) for the detour effect. Johkan *et al.* (2012) also showed the similar results with the similar PPFD (100 μmol m^{-2} s^{-1}) compared with white fluorescent light. Thus, photosynthesis ultimately affects plant growth and yield (Taiz and Zieger 2006). Thus, we hypothesize that the green light "knocked out" might induce the reduction of photosynthetic pigments in lettuce and the consequent reduction in Chl contents led to the inhibited CO_2 assimilation (Fig. 3) and ultimate yield decline (Fig. 2*A*) under LG spectrum at the conditions used in this study.

In order to further estimate the effect of green light component on photosynthesis in lettuce leaves, we analyzed the light responses and P_N/C_i responses. Plants grown under LG light showed significantly lower P_N, g_s, C_i, and E compared with CK (Fig. 4), suggesting that long-time treatment by green light "knocked out" (480–560 nm) weakened the foliar activity for gas exchange. Moreover, the light energy during photosynthesis is first absorbed and transferred into the photosynthetic apparatus by Chls, and can also be supplied by Car to the photosynthetic system (Sims and Gamon 2002, Wang *et al*. 2015). The efficiency of PPFD-driven photosynthesis can be assessed as quantum efficiency (Foyer *et al*. 2012). As showed in Table 1, LG showed significantly lower intrinsic quantum efficiency compared with CK (α = 0.0351 *vs*. 0.0793). It is known that the energy conversion efficiency from incident photons to chemical energy by leaves is wavelength-dependent (Hoover 1937). Even on basis of absorbed light, the quantum yields for CO_2 fixation or O_2 evolution vary in a wavelength-dependent way (Hogewoning *et al.* 2012). Upon the absorption by leaves, the green light drives photosynthesis very efficiently (Björkman 1968, Balegh and Biddulph 1970, Inada 1976). The α at waveband of 480–560 nm ranges approximately from 0.063 to 0.082 (Hogewoning *et al*. 2012).

According to analysis of P_N/C_i curves, the plants

treated by different spectra differed considerably in their biochemical capacities to assimilate atmospheric CO_2 (Fig. 5*A*). The value of V_{cmax} and J_{max} in the LG group was obviously repressed compared with CK (Fig. 5*B*,*C*), indicating that LG inhibited the efficiency of CO_2 assimilation in lettuce leaves. Furthermore, V_{TPU} was also reduced by LG compared with CK, although there was no significant difference in photorespiration (Fig. 5*D*,*E*). Rubisco activity, electron transport used in the RuBP regeneration, and triose-phosphate utilization are important processes in the Calvin cycle, which is the major route through which carbon enters metabolism in many plants (Martin and Schnarrenberger 1997). The Calvin cycle is still the only known pathway of CO_2 fixation in eukaryotes (Calvin 1956). Therefore, the radiation of waveband 480–560 nm was probably beneficial for the CO_2 fixation in the Calvin cycle, and hence promoted the photosynthesis in lettuce for the conditions used in this study.

An important property of the reaction centers of both photosystems (PSI and PSII) is their ability to use the fugitive high-energy Chls to drive an otherwise endothermic charge separation, a high-efficiency stable chemical transformation, implying very rapid chemistry (Lavergne and Joliot 2000, Mauzerall 2013). In addition, the balance of excitation between PSI and PSII is also wavelength-dependent (Evans 1987, Chow 1990, Melis 1991, Walters and Horton 1995), while an imbalance results in quantum yield loss (Pfannschmidt 2005). However, the significant linearity between V_{cmax} and J_{max} showed that lettuce grown under different spectra preserved a close functional balance of the two component processes *in vivo* (Fig. 5*F*): apparent Rubisco activity and the electron transport used in RuBP regeneration (Thompson and Kriedemann 1992, Long and Bernacchi 2003). These results suggest that across a series of environmental conditions, plants have the ability to optimize the resource allocation, so as to preserve a balance between enzymatic (*i.e.*, Rubisco) and light-harvesting (*i.e.*, Chl) capabilities (Wullschleger 1993).

Conclusion: In this study, a new method using LEDs for specific waveband "knock out" was established for investigating the effect of light quality on the development and physiological metabolism of plants. The biomass was improved by increasing the proportion of green light from 0.58% to 22.09%. SLA was slightly but not significantly lower in LG compared with CK. Chlorophyll contents and the absorbance of PPFD in LG also showed the similar trend. Through photosynthetic analysis, we found that "knock out" of green light weakened the CO_2 fixation and repressed the foliar respiration compared with CK. Green light "knock out" might induce the reduction of photosynthetic pigments in lettuce and the consequent reduction in chlorophyll contents led to the inhibited CO_2 assimilation and ultimate yield decline under LG spectrum at the conditions used in this study. But plants grown under different light spectra had the ability to optimize the resource allocation, so as to preserve a balance between enzymatic (*i.e.*, Rubisco) and light-harvesting (*i.e.*, chlorophyll) capabilities. Accordingly, this study provides valuable insights for growing lettuce with wide spectrum containing moderate amounts of green light which can obtain a higher photosynthetic capacity and thus to increase biomass yield in plant factory, and is a useful practical consequence for research and agriculture.

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