DOI: 10.1007/s11099-015-0083-8 PHOTOSYNTHETICA 53 (2): 213-222, 2015

The importance of blue light for leaf area expansion, development of photosynthetic apparatus, and chloroplast ultrastructure of *Cucumis sativus* grown under weak light

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Abstract

The objective of this study was to evaluate the significance of blue light (B) in the growth and photosynthetic capacity of cucumber. Gas exchange, chlorophyll (Chl) fluorescence kinetics, chloroplast ultrastructure, and leaf growth were investigated to explore the influence of three different light qualities of light emitting diodes (LEDs) on plant morphogenesis and the development of photosynthetic apparatus in cucumber (*Cucumis sativus*) leaves from emergence to full expansion under weak light [50 μmol(photon) m^{-2} s^{-1}]. We found that B could significantly increase the leaf area (LA), shoot elongation, Chl *a*/*b*, net photosynthetic rate, and stomatal conductance (g_s). In addition, the comparisons of maximal quantum yield of PSII photochemistry and the photosynthetic performance index between B-, W (white light)-, and R (red light)-grown leaves suggested that B was essential for the development of photosynthetic apparatus under weak light. B-grown leaves had the lowest Chl content under weak light, however, they had well-developed chloroplasts with the highest degree of stacked lamellae and the lowest starch accumulation. This could explain to a considerable extent the highest net photosynthetic rate per Chl unit. The results demonstrated that B optimized photosynthetic performance by improving the photosynthetic rate, increasing LA, and prolonging active photosynthesis duration under low irradiance. Therefore B is necessary to ensure healthy development of chloroplasts and highly efficient photosynthetic functions in cucumbers under a weak light environment. More importantly, our study also provided theoretical and technical support for the development of light environmental control technology.

Additional key words: fluorescence transient; grana; JIP test; photosynthetic characteristics.

Introduction

Light acts on plants not only as the sole source of energy, but also as a source of external information, affecting growth and development (Kendrick and Kronenberg 1994, Smith 1995). Plant development and physiology are strongly influenced by the light spectrum of the growth environment. There are three main photoreceptors in higher plants: phytochromes, which absorb photons principally in the 600–800 nm region (Quail 1994), blue radiation photoreceptors, which absorb radiation in the 300–500 nm region (Kaufman 1993), and UV radiation photoreceptors, which absorb light between 290–300 nm (Kendrick and Kronenberg 1994). Spectral changes of illumination evoke different photosynthetic and morphogenetic responses, which may vary among different plant species. Red (R) and B are more efficiently absorbed by photosynthetic pigments than other spectral regions (Inada 1976). It is well known that B influences the stomata opening and photomorphogenesis (Senger 1982, Schuerger *et al*. 1997, Dougher and Bugbee1998, Heo *et al*. 2002). Plants grown under blue fluorescent lamps had higher Chl *a*/*b* ratios (Senger and Bauer 1987), smaller amounts of LHCII, higher Rubisco activities (Eskins *et al*. 1991), and higher photosynthetic electron transport activities (Leong and Anderson 1984) than plants grown under red fluorescent lamps. It has also been widely shown that plants grown under R LEDs exhibited significantly lower Chl *a*/*b* ratio, lower irradiance-saturated rates of photosynthetic CO_2 fixation and total plant biomass than plants grown under

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Received 14 October 2013, *accepted* 16 May 2014.

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Abbreviations: B – blue LEDs; Chl – chlorophyll; DM – dry mass; F_v/F_m – maximal quantum yield of PSII photochemistry; g_m – apparent mesophyll conductance; g_s – stomatal conductance; LA – leaf area; LED – light emitting diode; PI_{ABS} – photosynthetic performance index; P_{NA} – net photosynthetic rate per unit of leaf area; P_{NC} – net photosynthetic rate per unit of chlorophyll; R – red LEDs; W – white LEDs.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (31171998) and Natural Science Foundation of Jiangsu Province (BK2010439). We thank Dr. T. Sansotta for language correctness on the manuscript.

white (W) or B-R LEDs (Milivojević and Tyszkiewicz 1992, Brown *et al.* 1995, Goins *et al.* 1997). B can trigger photomorphogenetic processes in plants and provides sufficient energy *via* photosynthesis to sustain normal growth and development (Abidi *et al.* 2012). Costa *et al.* (2013) implicated that the photoacclimation to high light intensities required the perception of B in diatoms.

Being photosynthetic organisms, plants are sensitive especially to their light environment. In the late autumn, spring, and winter, the shortening of illumination time and the larger irradiance fluctuation are serious problems for plant development, thus hampering crop establishment in the growth facility. What is more, this seriously affects both yield and quality of the vegetable. In general, plants grown under low light intensity are more sensitive to photoinhibition caused by exposure to higher light irradiation (Kato *et al.* 2002). This causes the bottleneck problem of vegetable production and year round forage supply since both light quality and quantity affect morphological and physiological characteristics of plants (Ballaré *et al.* 1991) and the interaction is often complex (Senger and Bauer 1987, Hogewoning *et al.* 2010, Costa *et al.* 2013). It is necessary to account for the interaction between light quality and quantity, however, previous research focused only on higher light intensity [≥ 100 μmol(photon) m^{-2} s^{-1}] investigated for light quality effects (Hogewoning *et al.* 2010, Shibuya *et al.* 2010). Little is known about how light quality influences photosynthesis and plant growth and development under weak light. The mechanism of photosynthesis acclimation to irradiance has been frequently discussed based on monitoring the changes in photosynthetic rates and the amounts of photosynthetic components (Matsuda *et al.* 2008). However, when acclimating to irradiance, plants alter not only photosynthetic rates and the amounts of photosynthetic components, but also other physiological characteristics. Chloroplast development is one that alters depending on irradiance. The chloroplast is essential for photosynthesis and the production of hormones and metabolites. As a consequence, its biogenesis and development needs to be coordinated with seedling growth to ensure optimal rates of photosynthesis upon seedling emergence. Studies on the processes of chloroplast biogenesis and development during germination have shown how chloroplast development is important for plant vitality, seed set, and growth. Indeed, even if chloroplast development is impaired only in cotyledons (the embryonic leaves of germinating seedlings) with normal chloroplast development in true leaves, plant growth and yield can be negatively impacted. In addition, some authors have suggested that acclimation responses to irradiance at the chloroplast level and at the leaf morphological level could be regulated by different mechanisms (Murchie and Horton 1998, Yano and Terashima 2001). It is possible that, even within chloroplasts, different mechanisms participate in the regulation of various acclimation responses. However, it is uncertain whether B also affects the responses of chloroplast development characteristics under weak irradiance. Thus, it is necessary to understand the regulation and mechanism of chloroplast development in leaves acclimated to various light quality at comparatively lower light intensities.

In order to understand the respective contribution of photosynthesis and photomorphogenesis on the elaboration of leaf architecture, we monitored the effect of B, R, and W on morphological and physiological acclimation of cucumber to different light qualities under relatively lower irradiance throughout the development of leaves from initial stage until the fully expanded stage. The objective of this study was to determine if B plays an important role in the photosynthesis and development of cucumber under weak light. For this purpose, Chl content, LA, CO_2 assimilation, Chl *a* fluorescence, and stomatal characteristics as well as chloroplast ultrastructure were investigated under different light qualities during leaf expansion in cucumber plants.

Materials and methods

Growth of plants: Cucumber (*Cucumis sativus* L. cv. Jinchun No. 4) seeds were germinated in four-layer, white gauze until the length of roots was 4–5 cm. The seedlings were transferred into plastic pots (5 cm in diameter and 9 cm in depth, one seedling per pot) containing a mixture of peat and vermiculite (1:1, v/v) and placed under 100 μmol(photon) m^{-2} s^{-1} fluorescent lamps (*PRX-25D*, *Safe*, China). The day/night temperature was 25/23ºC, the relative humidity was 70%, and the CO_2 concentration was about 400 μmol mol^{-1}. The seedlings were watered daily until the first true leaf was fully expanded.

LED light quality treatment: As a multi-wavelength white light control (W), the other plants were moved into incubators and given exposure to blue light (B) with dominant wavelengths of 455 nm and red light (R) with dominant wavelengths of 635 nm. W, B, and R were created by light-emitting photodiodes (*PGX-150*, *Safe*, China). All plants were subjected to 50 ± 5 μmol(photon) m^{-2} s^{-1} irradiance which was measured daily above the plant canopies and maintained by adjusting the distance of the LED lamps to the plant canopies. The light intensity and spectral distributions of the LEDs were measured with a *Quantitherm Light Meter* (*QRT 1*, *Hansatech Instruments*, Norfolk, UK) and a *STC 4000* spectrometer (*Everfine Photo-E-Info Co.*, China), respectively (Fig. 1). Two days after placing plants in the incubators, the measurements started on the second true leaf. All treatments and assays were repeated three times.

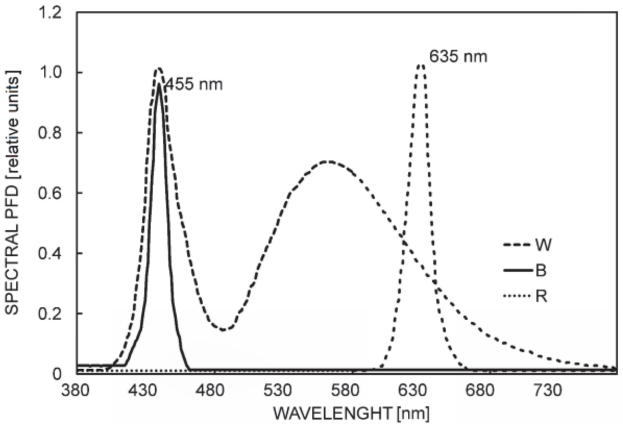

Fig. 1. Relative spectral distributions of the LEDs used in this study. W – white LEDs; B – blue LEDs; R – red LEDs; LED – light emitting diode.

LA and Chl content: LA and relative Chl content of second true leaves were measured every 2 d using a leaf area meter (*Li-3000*, *Li-Cor Inc.*, Lincoln, NE, USA) and SPAD Chl meter (*SPAD 502*, Minolta, Japan) during leaf development. A total of five plants per treatment were used for the LA and relative Chl content measurements.

Three leaf discs (1.33 cm^2) from each 12-d-old leaves were cut randomly over the LA, avoiding the leaf margins and main veins, and stored in 10 ml of 96% ethanol in the dark at –5ºC for 24 h. The absorbance of the extract was measured in the range 320–750 nm using a *UV/Vis* spectrophotometer (*Lambda 25*, *PerkinElmer*, USA), the Chl content was calculated using the equations of Lichtenthaler and Wellburn (1983).

Leaf light absorptance: Leaf absorptance (at 400–700 nm with a halogen light source) was measured with a spectra radio meter (*LI-1800C*, *Li-Cor Inc.*, Lincoln, NE, USA) and an external integrating sphere (*LI-1800-12S*, *Li-Cor Inc.*, Lincoln, NE, USA) ($n = 3$).

Biomass: Dry mass (DM) for each plant was obtained after 48 h at 80ºC after cleaning the roots, and then weighed using an electronic balance (*BSA124S*, *Sartorius*, Beijing, China) ($n = 3$).

Leaf gas exchange: Gas exchange was measured for 3 h, when the photoperiod of cucumbers in their growth environment started, using an open photosynthetic system (*Li-6400*, *Li-Cor Inc.*, Lincoln, NE, USA). Light intensity was the same as the growth irradiance [50 μmol(photon) m^{-2} s^{-1}], the leaf chamber temperature was kept at 25ºC, and the CO_2 reference concentration was 400 μmol mol^{-1}. Relative air humidity was controlled at 70%, the net photosynthetic rate per unit of leaf area (P_{NA}), the net photosynthetic rate per unit of chlorophyll (P_{NC}), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were determined. Apparent mesophyll conductance (g_m) was calculated as P_N/C_i (Fischer *et al.* 1998). All measurements were carried out on fully expanded 12-d-old leaves ($n = 6$).

Chl *a* fluorescence transient was measured every 2 d at room temperature by the *Plant Efficiency Analyzer* (*PEA*, *Hansatech Inc.*, Cambridge, UK) with an excitation light intensity of 3.3 mmol(photon) m^{-2} s^{-1}. All samples (five plants per treatment) were dark-adapted for 30 min prior to the measurements. Chl *a* fluorescence transients (OJIP) were quantified according to the JIP test (Strasser *et al.* 2004). Using these original data, PSII performance index on an absorption basis (PI_{ABS}) and the maximal PSII photochemistry efficiency (F_v/F_m) were calculated for quantifying PSII behavior.

Transmission electron microscopy (TEM): The same leaves used to determine gas exchange were used for observation with electron microscopy. Two leaf pieces (about 2 mm^2) were cut, from three biological replicates per treatment, and infiltrated under mild vacuum for 30 min in a 2.5% (w/v) glutaraldehyde/0.1 M cacodylate buffer (pH = 7.2). The samples were incubated for 2 h at room temperature (primary fixation), washed with a cacodylate buffer, and transferred to a 2% (w/v) osmium tetroxide solution overnight, at 4ºC (secondary fixation). After four washes of 15 min in a 0.1 M cacodylate buffer, the samples were dehydrated in graded ethanol series and sequentially transferred to propylene oxide for 1 h (once every half hour) and then to a mix of propylene oxide and Spurr embedding resin (*TAAB Laboratories*, Aldermaston, UK) (Brouwer *et al.* 2012). The following day, samples were transferred overnight to pure Spurr embedding resin, embedded on silicon frames and incubated at 60ºC for 1 d. After embedding, ultrathin sections were cut for TEM analysis. The sections were stained with 5% aqueous uranyl acetate for 30 min and then with lead citrate for 10 min before being examined under the electron microscope (*HITACHI 7650*, Tokyo, Japan).

Statistical analysis: One-way analysis of variance (*ANOVA*) was carried out using an *SPSS* computer package (*SPSS 18.0* for *Windows*, Chicago, IL, USA) for all sets of data. The values were the means of all measurements and comparisons of means were determined through *Duncan*'s new multiple range tests. Data were expressed as mean ± standard deviation (SD). A difference was considered significant at $P \leq 0.05$.

Results

Leaf growth, morphology, pigment contents, and biomass: Cucumber plants influenced by different light spectra treatments are depicted in Figs. 2 and 3. Plants showed distinct growth responses to different light quality. Shoot length, LA, and Chl *a*/*b* ratio were the greatest when grown under B, and the lowest under R (Table 1). R-grown leaves were fully expanded on the 6th d after leaf emergence (DAE). The LA under W reached peak values 9 DAE, while the LA was still increasing when grown under B (Fig. 4*A*). With the leaf development, Chl content of all treatments was increasing, and that of B-grown leaves was obviously lower compared with that of W and R, which was different from the change of LA (Fig. 4*B*, Table 1). While Chl content began to decline 9 DAE under W and R, it remained relatively stable under B. A similar pattern was seen for leaf thickness; it was the lowest under B treatment (Table 1), middle under W, and the highest under R. The B-grown leaves had a lower leaf light absorptance, however, no significant differences were observed between plants under R and W. Moreover, DM of W-grown leaves was higher compared with that of the R or B treatment. Cucumber plants under B showed a similar accumulation of DM to those under R.

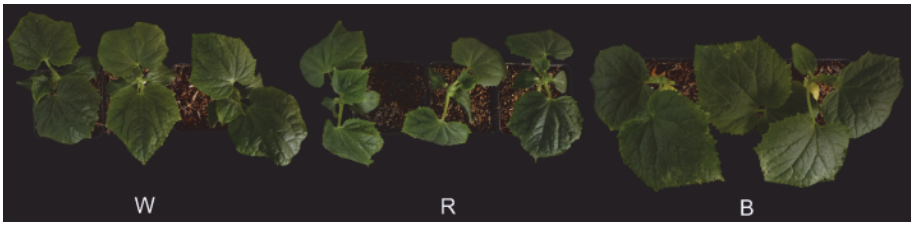

Fig. 2. Effects of light qualities on leaf area in cucumber 12 DAE. W – white LEDs; R – red LEDs; B – blue LEDs; LED – light emitting diode.

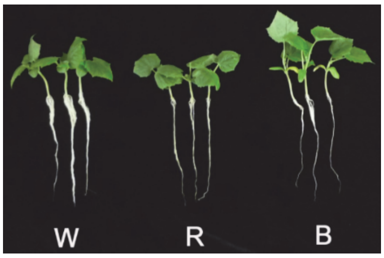

Fig. 3. Effects of light qualities on shoot length of cucumber plants 12 DAE.

Development of photosynthetic function: The W- and B-grown leaves had higher F_v/F_m compared with that of R-grown leaves (Fig. 5*A*). After nine DAE, F_v/F_m was approxi-mately 0.802 in leaves under both W and B, while it was only about 0.775 under R. A significantly lower degree of the photosynthetic apparatus development was observed under R. The above results could be manifested from PI_{ABS} (Fig. 5*B*), which reflects the photosynthetic capability of the entire PSII; it is similar to F_v/F_m, but more sensitive (Appenroth *et al.* 2001). Throughout the development process, the PI_{ABS} remained just under 20.6 under R. Furthermore, it is worth noting that the PI_{ABS} under W was significantly higher than that of B at 9 DAE. After nine DAE, the PI_{ABS} under B remained stable, but decreased under W. Finally, both F_v/F_m and PI_{ABS} of 12-d-old leaves under B were the highest among the treatments.

Leaf gas exchange: Light quality affected significantly the photosynthetic properties of leaves. Lower P_{NA} and P_{NC} were observed in R-grown leaves compared with B- or W-grown leaves (Fig. 6*A*,*B*). It was paralleled by reductions in both g_s and g_m (Fig. 6*C*,*D*). P_{NA} of B-grown leaves was similar to that of W-grown leaves (Fig. 6*A*), which exhibited higher P_{NC} than that under R and W (Fig. 6*B*). Both g_s and C_i showed a similar trend to the CO_2 assimilation rate under B conditions (Fig. 6*C*,*E*). Although

Table 1. Parameters measured or calculated on leaves (12 DAE) grown under different light qualities. *Lowercase letters* indicate the groups differentiated by *Duncan*'s new multiple range test ($P \leq 0.05$). Chl – chlorophyll; DM – dry mass. Mean ± SD ($n = 3$ or $n = 5$).

Parameter	Light quality White	Red	Blue
Shoot length [cm]	7.13 ± 0.15^{b}	6.47 ± 0.15^{c}	9.50 ± 0.35^{a}
Leaf area [cm^{2}]	21.87 ± 0.92^{b}	15.67 ± 0.69^{c}	25.29 ± 0.71^{a}
Leaf thickness [mm]	0.243 ± 0.003^{b}	1.689 ± 0.007^{a}	0.085 ± 0.016^{c}
Chl content [mg g^{-1}]	2.76 ± 0.23^{b}	3.08 ± 0.13^{a}	2.41 ± 0.06^{c}
Chl *a*/*b*	2.893 ± 0.079^{b}	2.809 ± 0.003^{b}	3.251 ± 0.184^{a}
Leaf light absorptance	0.859 ± 0.009^{a}	0.853 ± 0.175^{a}	0.830 ± 0.098^{b}
DM [g plant^{-1}]	0.235 ± 0.014^{a}	0.172 ± 0.004^{b}	0.166 ± 0.007^{b}

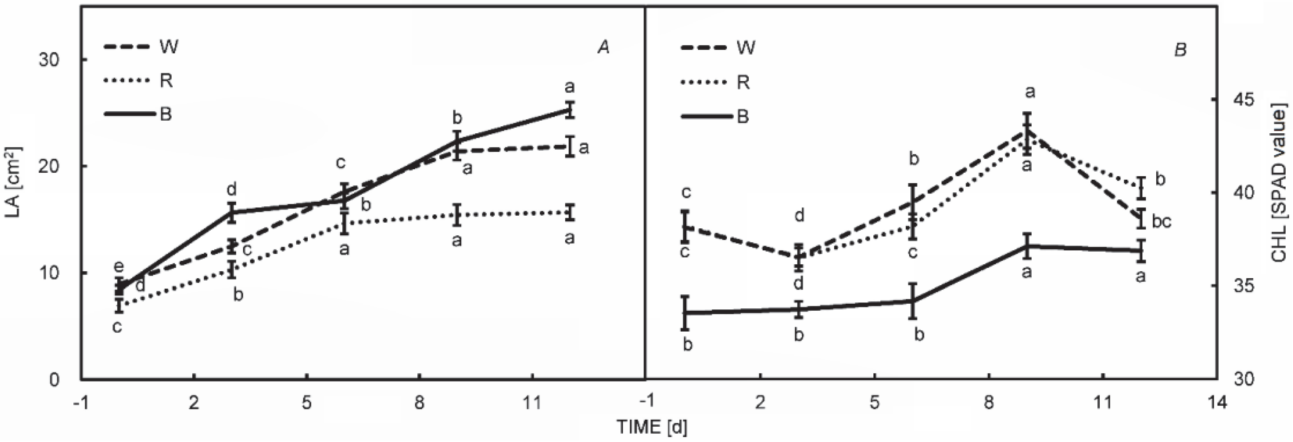

Fig. 4. Changes of leaf area (LA) (*A*) and chlorophyll (Chl) content (*B*) in the developmental process of cucumber leaves. W – white light; R – red light; B – blue light. Different *lowercase letters* indicate significant differences using *Duncan*'s new multiple range test ($P \le 0.05$). Error bars indicate the standard deviation ($n = 5$).

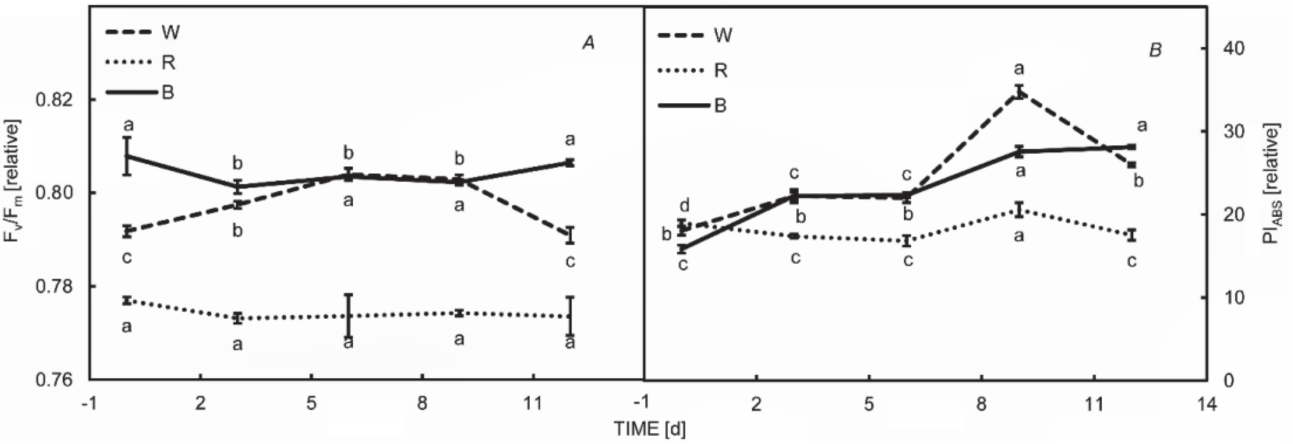

Fig. 5. Changes of photosynthetic function in the developmental process of cucumber leaves: the maximal quantum yield of PSII photochemistry (F_v/F_m) (*A*), the photosynthetic performance index (PI_{ABS}) (*B*). W, R, or B – white, red, or blue LEDs, respectively. Different *lowercase letters* indicate significant differences using *Duncan*'s new multiple range test ($P \le 0.05$). Error bars indicate SD ($n = 5$).

g_s and C_i significantly increased under B conditions, the g_m was smaller in the B-treated than that in the W-treated leaves (Fig. 6*D*).

Chloroplast ultrastructure: Twelve days after exposure to different light quality, B-grown leaves showed healthy chloroplasts with well-developed grana and stroma lamellae, which were oriented parallel (from cross-sectional view) to the long axis of the organelle and thus, maintained their standard configuration (Fig. 7*A*,*B*). The number of grana lamellae under B, W, and R was about 27–33, 18–21, and 6–11, respectively (Fig. 7*B,D,F*). Compared with that of W and R, the number of grana lamellae was the highest under B with the most stacked lamellae and the minimum starch grains in the chloroplast. However, the starch grains became larger under W. It led to the chloroplast swelling, thus the grana and stroma lamellae were slightly compacted and the stroma lamellae were irregular. Furthermore, more and large starch granules filling the whole chloroplast profile were observed in the R-grown leaves (Fig. 7*E,F*). Starch build-up altered the normal structure of the chloroplast and perturbed the configuration of granal stacks. Chloroplasts appeared swollen and distended with starch, showed limited presence of stroma, and visible internal membranes were distorted and forced to the periphery of the organelle under R.

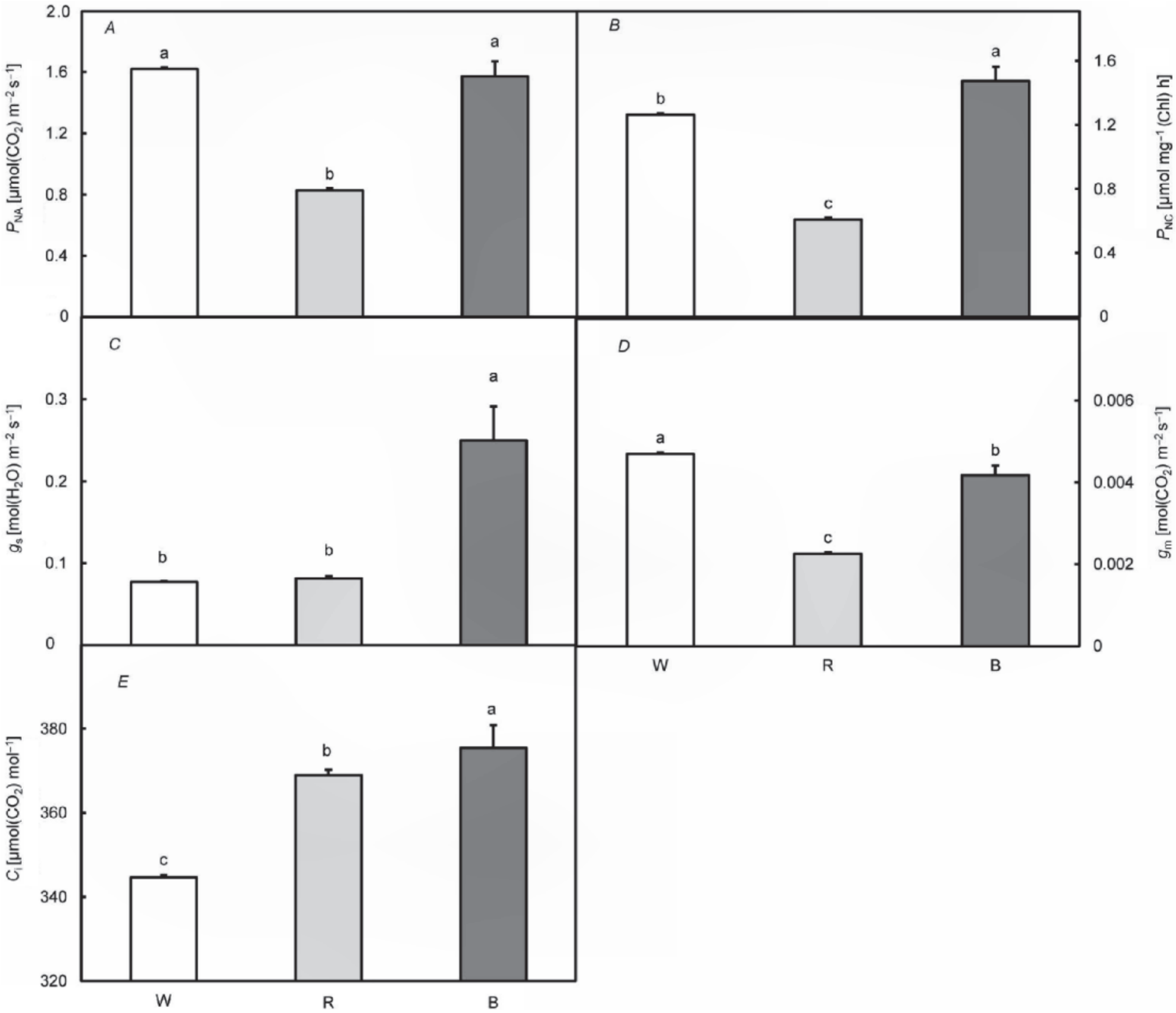

Fig. 6. Effects of light qualities on the gas exchange of 12-d-old cucumber leaves: net photosynthetic rate per unit of leaf area (P_{NA}) (*A*), net photosynthetic rate per unit of chlorophyll (P_{NC}) (*B*), the stomatal conductance (g_s) (*C*), the apparent mesophyll conductance (g_m) (*D*), and the intercellular CO$_2$ concentration (C_i) (*E*). Different *lowercase letters* indicate significant differences using *Duncan*'s new multiple range test ($P \leq 0.05$). Error bars indicate the standard deviation (n = 6). W – white light; R – red light; B – blue light.

Discussion

Leaf growth and morphogenesis under weak light: Light quality and quantity affect plant adaptation to changing light conditions. Certain wavelengths in the visible and near-visible spectrum are known to have discrete effects on plant growth and development. Plant response to B is less constant than that to R and depends on the species (Rajapakse and Kelly 1995, Khattak *et al.* 2004). Shoot elongation occurs under B in pepper (Brown *et al.* 1995), cucumber (Piszczek and Glowacka 2008), and *Petunia* (Fukuda *et al.* 2011), whereas it is repressed in *Pinus* (Sarala *et al.* 2007) and in *S. tuberosum* (Wilson *et al.* 1993). Dougher and Bugbee (2001) defined long-term B dose-response curves for LA and stem length in soybean and lettuce. They showed that both parameters in soybean decreased with the increment of the B proportion, while LA of lettuce increased with an increasing B fraction. However, R can greatly improve Chl content compared with B as reported by Xu *et al.* (2005). Under our experimental conditions, B-grown cucumber leaves invested relatively less in Chl content but exhibited significantly higher LA and shoot elongation than those under R (Fig. 4*B*, Table 1).

In general, plants grown under R demonstrated a shade-tolerant response and increased both the relative amount of photons captured and the efficiency of photon capture under low irradiance, and invested a greater part of their biomass into photosynthetic structures but not into stem

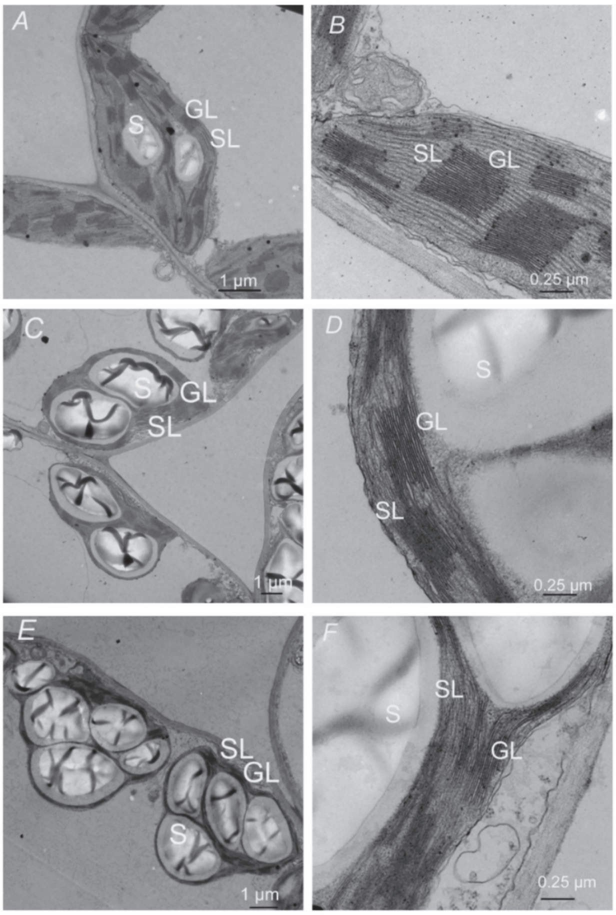

Fig. 7. Chloroplast ultrastructure in the second fully expanded cucumber leaves, which were grown at 50 µmol(photon) m^{-2} s^{-1}. Blue light (*A*,*B*); White light (*C*,*D*); Red light (*E*,*F*). Representative starch granules (S), granna lamellae (GL), and stroma lamellae (SL) were indicated.

growth (Smith 1982). In this study, the photosynthesis under R was worst despite the highest Chl content and leaf light absorptance (Table 1). It might be related to a too low PAR. On the other hand, although Chl contents of B-grown leaves were relatively lower, both LA and shoot length were largely enhanced during leaf development (Figs. 2,3, Fig 4*A*, Table 1). It could indicate that B-grown cucumber seedlings might increase light intensity by being close to the light source and harvest more light by expanding the LA to adapt to weak light stress. Surprisingly, at 9 DAE, Chl content under W and R was declining while it was kept stable under B (Fig. 4*B*). Many studies showed a close relationship between photosynthetic activity and Chl content. The higher Chl content could further increase light absorption and, therefore, photosynthetic rate. Furthermore, leaf senescence is characterized by loss of Chl and proteins (Selivankina *et al.* 2001). In our experiment, a decrease in Chl content in W- and R-grown plants suggested that leaf senescence appeared in later stages of leaf development due to weak light, but B delayed the leaf senescence. In fact, a number of studies have demonstrated that B is beneficial in delaying leaf senescence. It is related to maintaining high catalase activity and delaying Chl degradation which depends on the presence of cytokinins under B treatment (Causin *et al.* 2006). However, cucumbers under W appeared to accumulate more DM (Table 1) as it had a wider spectrum in comparison to that of B and R treatments. Cucumber under B showed a similar accumulation of DM to those under R, but we noted that the quantum yield of R was considerably greater than that of B, because some fraction of B was absorbed by flavonoids in vacuoles and/or carotenoids in chloroplasts. This illustrated the importance of the B under weak light. Our results showed that B exerted specific morphogenetic effects on cucumber seedlings, such as increasing LA and the shoot length along with delaying leaf senescence compared with those under W and R. Although light interception under B was the lowest (Table 1), LA and the stem length increased to harvest more light and thus compensate for the negative influence caused by lower Chl content per unit of leaf area. Such modification was also manifested by a greater photosynthetic rate under B than that under W and R.

Development of photosynthetic apparatus and senescence: The effects of light quality on PSII behavior was quantified by PI_{ABS} (Appenroth *et al.* 2001). Values of F_v/F_m and PI_{ABS} showed no significant difference under W and B, but they were higher than those under R during 9 d. However, the development of photosynthetic apparatus under W was superior to B if represented by PI_{ABS}. While, the PI_{ABS} of W-grown leaves were declining after 9 d, it was kept stable under B. (Fig. 5*B*). At 12 DAE, F_v/F_m and PI_{ABS} of B-grown leaves were the highest. The development of photosynthetic apparatus was ensured best under W, but its active photosynthesis duration was not long, and declined rapidly during the later developmental stage. We concluded that 100% of B played a crucial role in maintaining the stability of the photosynthetic function. Moreover, the developmental process of photosynthetic apparatus was consistent with that of Chl synthesis (Fig. 4*B*), indicating that B could maintain a long duration of active photosynthesis under low light stress, which was closely related to the delay of the degradation of photosynthetic pigments under B. In *Lolium temulentum*, it was calculated that delaying the onset of senescence by only 2 d could result in an increase in carbon fixation of about 11% (Thomas and Howarth 2000). A similar phenomenon was also observed in tobacco (Gan and Amasino 1995) and sorghum (Borrell *et al.* 2000). Our results demonstrated that although B-grown leaves maintained lower Chl content, this was an advantage in maintaining photosynthetic activity during senescence. This characteristic of B-grown leaves might be an acclimation strategy to weak light.

Starch accumulation and chloroplast ultrastructure: Both light quality and quantity greatly influenced the development of chloroplast. In this study, at 12 DAE, the number of grana lamellae was the largest in plants grown under B (Fig. 7). In addition, thylakoid membranes of B-grown leaves were stacked the most, which implied a higher ability for light harvesting and, thereafter, increased the energy conversion efficiency of the photosynthetic membranes (Bondada and Syvertsen 2003). Our results showed a strong adaptability to a low light environment in B-grown leaves. Starch accumulates in the chloroplast during the day and is depleted in the dark. Persistence of accumulated starch in the chloroplast, a reflection of depressed translocation, causes severe impairment to chloroplast structure and function (Paul and Foyer 2001). Under R, conspicuous starch granules, which filled the whole chloroplast, squeezed severely the distribution of stroma and grana lamellae, resulting in the blurry and irregularity of stroma and grana lamellae. The disorientation of the grana and stroma lamellae occurred due to displacement of the photosynthetic membrane system towards the periphery of the organelle by the starch granules. It resulted in a loss of structural integrity, eventually leading to its reduced photosynthetic function (Figs. 6,7). In contrast, starch grains hardly squeezed the distribution of stroma and grana lamellae under B. Starch grains of W-grown leaves became larger, and they only slightly squeezed the thylakoid membranes. Our data presented above demonstrated that the B region of the light spectra was a key signal for the development of chloroplast in shaded cucumber leaves. However, the leaves developed under R alone displayed dysfunctional photosynthetic apparatus. This was also confirmed by Hogewoning *et al*. (2010) using irradiance of 100 μmol (photon) m^{-2} s^{-1}. These results indicated that B light was important for the development of chloroplast and photosynthetic apparatus, even under low light [50 μmol (photon) m^{-2} s^{-1}].

Net gas-exchange rates and carbohydrate export: A lower photosynthetic rate in plants grown under R alone has been shown in several crop plants (Matsuda *et al.* 2004, Hogewoning *et al*. 2010). Our data were consistent with previous results. The lower photosynthetic rate under R was not only related to the lower g_s (Fig. 6*C*), but also to the lowest g_m (Fig. 6*D*). Many studies have shown that R light promotes significantly less stomatal opening than does B light. The lowest g_m under R might be associated with the reductions in Rubisco activity and transcriptional levels of most genes which are involved in the Calvin cycle (Wang *et al*. 2009).

B generally promotes stomatal opening more than other wavelengths and this has been shown to contribute to an increase in the photosynthetic rate along with the enhancement of DM production in many plant species (Goins *et al.* 1997, Zeiger *et al.* 2002). In our experiments, no significant differences in P_{NA} were observed in B- and W-grown plants (Fig. 6*A*). This might be related to the significantly lower g_m under B than that of W, because of its higher C_i compared with that of W-grown leaves (Fig. 6*D,E*). Terfa *et al.* (2012) reported that rose plants grown under LED irradiation showed higher photosynthesis. It was a result of thicker leaves with more photosynthetic units per unit of LA. In this study, B-grown leaves had the largest LA and thinnest leaves (Table 1), thus, less photosynthetic units per unit of leaf area was probably the most important reason for not having the highest P_{NA} under B, despite having the largest g_s (Fig. 6*C*). In fact, B-grown cucumber seedlings exhibited higher P_{NC} than that under R and W (Fig. 6*B*). In addition, the highest Chl *a*/*b* ratio was found in B-grown leaves (Table 1). A higher Chl *a*/*b* ratio indicated a high light-adapted photosynthetic apparatus with less Chl *b* containing light-harvesting antennae, and therefore a higher capacity for electron transport and more Calvin cycle enzymes on a Chl basis (Evans 1988). B-grown cucumbers had the highest photosynthetic activity on the Chl basis.

The highest photosynthesis under B was also associated with carbohydrate export from the leaf. The export of carbohydrate from photosynthesizing leaves (sources) provides the substrate for the growth and maintenance of nonphotosynthetic plant tissues (sinks). Generally, when sink activity is decreased by carbohydrates that accumulate in leaves, photosynthesis is inhibited (Paul and Pellny 2003). Also accumulation of starch excess in source leaves is involved in suppressing photosynthetic gene expression and subsequent growth (Sheen 1990, Stitt *et al.* 2010). In our experimental conditions, starch grains accumulated least in B-grown leaves among the three light quality treatments. The accumulation of starch within the chloroplasts is accompanied by damage and disorientation of grana and thylakoids (Carmi and Shomer 1979, Bondada and Oosterhuis 2003), which may result in reduced photosynthetic rates (Paul and Foyer 2001, de Groot *et al.* 2003). As a consequence, cucumber seedlings grown under B had greater P_{NC} than that from R or W.

Conclusion: In summary, we concluded that B light promotes plant growth by controlling and integrating a variety of responses that optimize photosynthetic performance under low radiation. This was mainly manifested by an improvement in the photosynthetic rate, increasing LA, and prolonging the duration of active photosynthesis. We found that this might ensure healthy chloroplast development under low light intensities to achieve a higher photosynthetic rate and efficiency. B light can increase light harvesting through the expansion of LA and stem elongation, thereby improving single leaf assimilation. B might delay the process of leaf senescence caused by low light and prolong the duration of active photosynthesis, thus enabling more carbon assimilation.

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