



# Plant Growth and Photosynthetic Characteristics of Soybean Seedlings Under Different LED Lighting Quality Conditions

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## Abstract

Light-emitting diodes (LEDs) have recently been widely used as light sources for the cultivation of plants in controlled environments. However, before LEDs can be used as the sole light source, it is imperative that soybean (*Glycine max*) response to light quality be well characterized. The objective of the present work was to investigate how soybean seedlings respond to different blue (B) and red (R) photon flux ratios (PFR) using LEDs. Light treatments were comprised of  $170 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density with B:R ratios of 0B:100R%, 10B:90R%, 25B:75R%, 50B:50R%, 80B:20R%, 90B:10R%, and 100B:0R%. Our results suggest that with the increase in B PFR from 0 to 90%, both plant height and specific leaf area inclined to decline while stem diameter gradually increased. The plants exposed to 80B:20R% showed unexpectedly greater total root length, root surface area, root volume, number of root tip, plant dry mass, leaf dry mass, root dry mass, leaf mass fraction and root mass fraction than plants exposed to all other treatments. Plants grown with 0B:100R% had the highest content of chlorophyll *b* (Chl *b*) and content of total chlorophyll [Chl (*a* + *b*)] but possessed the lowest ratio of the content of chlorophyll *a* to the content of chlorophyll *b* (Chl *a/b*). Plants grown with R alone also showed the lowest chlorophyll fluorescent parameters. The light-saturated CO<sub>2</sub> assimilation rate ( $A_{\text{sat}}$ ) and the light-saturated stomatal conductance ( $g_{\text{ssat}}$ ) for 0B:100R% were, respectively, 64.85% and 203.23% lower than those for 80B:20R%. However, plants grown with sole R accumulated higher soluble sugar and starch content during the daytime. Collectively, our results suggest that compared to R- or B-LED alone, an appropriate combination of R and B promotes plant growth and photosynthetic performance for soybean.

**Keywords** Soybean · LED · Light quality · Plant growth · Photosynthetic characteristics

## Introduction

Indoor cultivation methods are frequently applied to shorten crop breeding periods to speed up the process of crop improvement by artificially controlling the cultivation environment, thereby allowing plants to grow continuously

independent of seasonal climatic conditions. However, growth chambers or greenhouses that utilize fluorescent lamps or metal-halide lamps often have the disadvantage of generating radiant heat, low light fluence efficiency and fixed spectral distribution, thus making them inefficient (Morrow 2008; Darko et al. 2014). High temperature resulting from the radiant heat makes it difficult to position fluorescent and metal-halide lamps close to plants, consequently optimal levels of light intensity cannot be applied to the plant canopy. In addition, generating heat also results in a waste of electric energy.

The utilization of light-emitting diode (LED) technology can enhance the efficiency of indoor plant cultivation. Solid-state lighting with LEDs that has long life-expectancies, offers high luminous flux with low radiant heat, and shows the potential to optimize the light spectral distribution and intensity. At the same time, LEDs can cut down the electricity cost in the long-term due to low energy consumption (Singh et al. 2015). Despite the great potential of LEDs as a

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radiation source in the phytotrons used for plant cultivation, it is essential to further evaluate the plant morphological, biochemical and genetic responses to LED light.

Light signals (mainly light intensity, light period and spectral distribution) regulate virtually all aspects of plant growth and developmental processes throughout the life cycle (Jiao et al. 2007). Spectral distributions, especially R and B wavelengths, are involved in plant photosynthesis by providing energy required for plant growth and development and regulate seedling photomorphogenesis, stomatal opening, seed germination, chloroplast movement, shade avoidance, circadian rhythms and flower induction through the photoreceptor signal (Zeiger 1983; Wada et al. 1993; Somers et al. 1998; Mockler et al. 1999). The effects of light quality on plant growth and development have been extensively investigated in agronomic plants (Zheng and Labeke 2017; Yang et al. 2018). For example, B was associated with larger leaves than R, and the combination of both resulted in intermediate levels of total leaf area in Sweet Basil (Carvalho et al. 2016). Leaf area decreased with the increase of B photon flux when cucumber seedlings were irradiated with a combination of R and B, and seedlings grown with B alone had greater plant height than those exposed to R alone and combinations of both (Hernández and Kubota 2016). Light not only modulates changes in the aerial structures but also penetrates the plant shoot to influence growth and development of the underground root system (Lee et al. 2016). R promoted 1° lateral root number and 2° lateral density but retarded lateral root elongation in tobacco seedlings (Meng et al. 2015). It has been suggested that photoreceptors control carbon allocation and biomass production in the developing plant (Yang et al. 2016). *Mesembryanthemum crystallinum* grown with R conditions had the lowest shoot biomass, root biomass and shoot/root ratio, whereas those grown with the R-to-B ratio of 9 exhibited the highest values (He et al. 2017). R radiation alone was unsuccessful for chlorophyll biosynthesis, whereas the combination of B and R irradiation was necessary for this process (Fan et al. 2013). In wheat, R light increased the content of total chlorophyll while the ratio of chlorophyll *a* to chlorophyll *b* was augmented by the R plus B light treatment (Dong et al. 2014). In cucumber, the leaves that developed with R light alone displayed dysfunctional photosynthetic operation and lower photosynthetic capacity, whereas the combination of R and B prevented overt dysfunctional photosynthesis (Hogewoning et al. 2010). In keeping with this finding, R irradiation caused rapeseed leaves to be stressed with a low  $F_m/F_v$  (photosynthetic maximum quantum  $\Phi$ PSII) and a high content of  $O_2^-$  and  $H_2O_2$ , and induced a stress resistance response with a high activity of antioxidase (Chang et al. 2016). In lettuce, leaf net photosynthetic rates under R alone were significantly lower than those under B alone and combinations of B and R, and photosynthetic capacity and

photosynthetic rate increased as the R-to-B ratio decreased to 1 (Wang et al. 2016).

Soybean is a substantially economic crop that accounts for more than half of oil seed production, and a great deal of research efforts are focused on investigating its physiological and molecular processes and stress responses (Wilson 2008). In numerous experiments, it is imperative to grow plants under controlled environmental conditions, mostly in phytotrons that emit different, yet primarily suboptimal, irradiation conditions in either intensity or spectral distribution for legumes.

Only a couple of investigations have examined the effect of LED light on legumes. Tanaka and Tanaka (2007) demonstrated that R-LED light significantly suppressed pod elongation in soybean during the light and dark period. Nevertheless, our knowledge of soybean seedling responses to light quality is fairly fragmented. In this study, soybean plants were grown in a plant growth chamber using different light qualities provided by LED light source. The objective of this work was to better understand how soybean seedlings respond to spectral distributions.

## Materials and Methods

### Plant Materials and Growth Conditions

Soybean seeds (Maodou 3, maturity group V) were sown in vermiculite and germinated with natural sunlight. Four days after sowing (DAS), eighteen plants were transferred into each of seven 38-L containers with full-strength nutrient solution in a climate chamber. The full-strength nutrient solution was composed of the following (in mM): 1.5  $KNO_3$ , 1.2  $Ca(NO_3)_2$ , 0.4  $NH_4NO_3$ , 0.3  $(NH_4)_2SO_4$ , 0.5  $KH_2PO_4$ , 0.3  $K_2SO_4$ , 0.5  $MgSO_4$ , 0.4 Fe-Na-EDTA, 0.0025  $Na_2B_3O_7$ , 0.0015  $MnSO_4$ , 0.0015  $ZnSO_4$ , 0.0005  $CuSO_4$ , 0.00016  $(NH_4)Mo_7O_{24}$ , 0.025  $MgCl_2$ , and 0.0001  $CoCl_2$ . During the experiment, the nutrient solution was continuously aerated, and the pH was adjusted regularly with 4 M hydrochloric acid and 4 M sodium hydroxide and was maintained at 5.8–6.0. The day/night temperature was set at 26 °C/21 °C, and the  $CO_2$  concentration was ambient. All plants were subjected to  $170 \pm 5 \mu mol m^{-2} s^{-1}$  irradiance (12 h/12 h day/night) provided by a mixture of B and R LEDs.

### Light Treatments

Light treatments consisted of seven different R-to-B photon flux ratios (R:B% PFRs) (Fig. 1). Each treatment was composed of six fixtures which were built with B LEDs (peak wavelength 439 nm) and R LEDs (peak wavelength

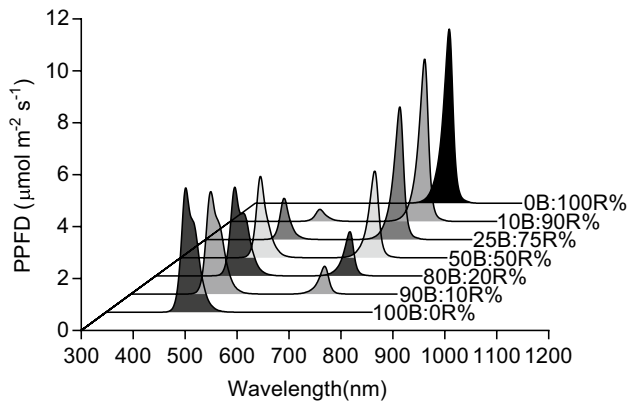


Fig. 1 Relative spectral irradiance of the light treatment

659 nm). All fixtures were 120 cm (L) × 5.3 cm (W) and were connected to a digital controller (Guangzhou Research Institute of Optics Mechanics and Electrics Technology, China) capable of controlling the output of B and R LEDs independently. Seven compartments were created inside the growth chamber using standard shelving units and nonreflective black cloth. The irradiance at the top of the canopy was regularly measured (GIZ-C, Zhejiang TOP Instrument Co., Ltd, Hangzhou, China) and the height of light was adjusted to maintain the desired photosynthetically active irradiance throughout the experiment. There were five repetitions under each treatment for the measurement, and the experiment was conducted twice.

### Measurement of Morphology

Sixteen DAS (the second fully expanded trifoliate leaf), plants were harvested and subsequently divided into leaf blades, roots and stems (including petioles). Roots and leaves were scanned; the digital images of roots were quantified using computer image analysis software (WinRhizo Pro, Régent Instrument, Québec, Canada) to measure morphological parameters, such as total root length, root surface area, root volume and the number of root tips, whereas the images of leaves were quantified using computer image analysis software (Image-Pro Plus 6.0, Media Cybernetics, USA) for calculating the leaf area. Roots were blotted dry with tissue paper, then roots, stems and leaves were individually put into paper bags, and dried at 80 °C to measure the dry mass. Biomass partitioning among the plant organs was assessed by calculating the leaf mass fraction (leaf dry mass/plant dry mass,  $\text{g g}^{-1}$ ), the stem mass fraction (stem dry mass/plant dry mass,  $\text{g g}^{-1}$ ), and the root mass fraction (root dry mass/plant dry mass,  $\text{g g}^{-1}$ ). Specific leaf area ( $\text{m}^2 \text{kg}^{-1}$ ) was determined using the ratio of leaf area/leaf dry mass.

### Measurement of Carbohydrates

The leaf samples for carbohydrate determination were harvested at the end of both day and night. Samples were dried at 80 °C then finely ground. Approximately 100 mg of dry mass was extracted two times with distilled water at 80 °C. The extract was used for the determination of the soluble sugar, whereas the residue was used for starch determination. Both the soluble sugar and the starch content were analyzed using the method reported by Wang et al. (2016).

### Measurement of Photosynthetic Pigments

Fresh leaf Sects. (0.1 g) were cut from the middle leaflet of the first fully expanded trifoliate leaf (from bottom to top) and were soaked in 8 mL of 80% acetone until the leaf turned white. The optical density was measured with a UV1800 spectrophotometer (Shimadzu, Japan) at 663 nm (OD663) and at 645 nm (OD645) for chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*).

### Measurement of $A_{\text{sat}}$ , $g_{\text{ssat}}$ and Chlorophyll Fluorescence

The light-saturated  $\text{CO}_2$  assimilation rate ( $A_{\text{sat}}$ ) and the light-saturated stomatal conductance ( $g_{\text{ssat}}$ ) of the first fully expanded trifoliate leaf (from bottom to top) were measured simultaneously in the growing chamber during 10:00–12:00 am with an open infrared gas analysis system LI-6400 (BioSciences, USA). Readings were taken with an LED light source, which supplied  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The spectral output of the light source had one peak centered at 470 nm and a second peak centered at 665 nm. Data were recorded when both  $A_{\text{sat}}$  and  $g_{\text{ssat}}$  were stable.

Chlorophyll fluorescence was also measured on the first fully expanded trifoliate leaves (from bottom to top) using a PAM-2500 chlorophyll fluorometer (Walz Heinz GmbH, Effeltrich, Germany). The plants were dark-adapted for 30 min prior to determination. The following chlorophyll fluorescence yields (Chl FY) were measured: minimum Chl FY in the dark-adapted state ( $F_0$ ), maximum Chl FY in the dark-adapted state ( $F_m$ ), maximum Chl FY in the light-adapted state ( $F'_m$ ), and steady-state Chl FY in the light-adapted state ( $F'$ ). Using these parameters, the following ratios were calculated: minimum Chl FY in the light-adapted state,  $F_0' = 1/(1/F_0 - 1/F_m + 1/F'_m)$ , maximum PSII photochemical efficiency,  $F_v/F_m = (F_m - F_0)/F_m$ , effective quantum yield of photochemical energy conversion in PSII,  $\Phi\text{PSII} = (F'_m - F')/F'_m$ , and coefficient of photochemical fluorescence quenching,  $qP = (F'_m - F')/(F'_m - F_0')$ .

## Statistical Analysis

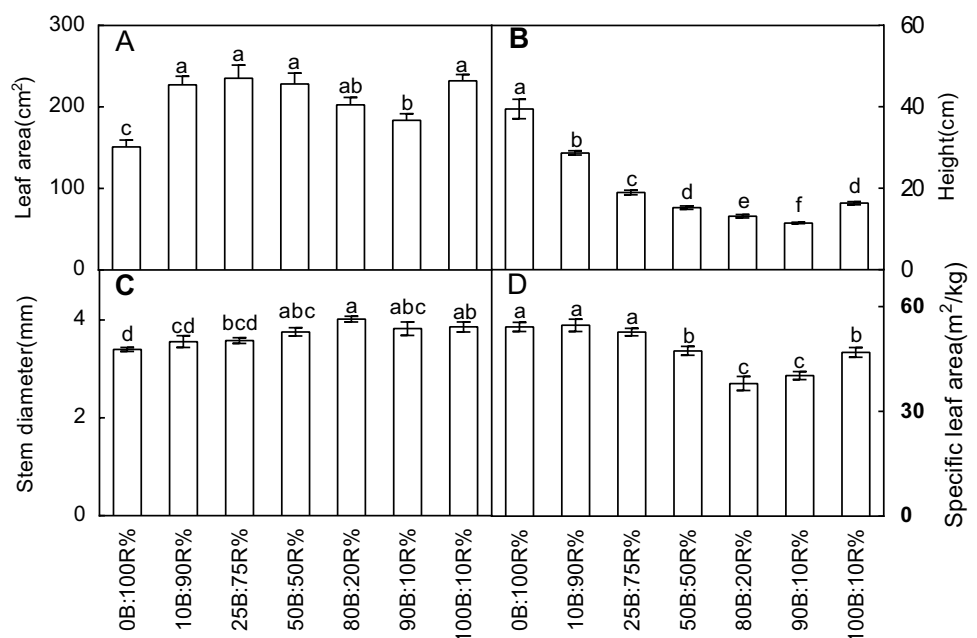
Data were expressed as the mean  $\pm$  standard error (SE) and evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test at  $p < 0.05$  level. Pairwise Pearson's correlation was calculated for main morphology and biomass against carbohydrate metabolic traits. All statistical analyses were performed using SPSS 25.0 software (SPSS, Chicago, IL, USA).

## Results

### Shoot Morphological Characteristics

We found that 0B:100R% suppressed soybean seedling leaf expansion (Fig. 2a). Seedlings grown with 10B:90R%, 25B:75R%, 50B:50R% and 100B:0R% conditions had considerably large leaf area, and the leaf area declined with the increase of B PFR, except for 100B:0R%. In contrast, plants grown with 0B:100R% possessed the tallest stem, nearly four times the height of 80B:20R%; meanwhile, stem growth was retarded with the reduction of the R PFR (Fig. 2b). Interestingly, seedlings grown with pure B had significantly higher stems than those grown with 80B:20R% and 90B:10R%. Conversely, the stem diameter was greater with increased B PFR (Fig. 2c). It appears that the specific leaf area exhibited a similar response to the light distribution as the plant height (Fig. 2b, d).

**Fig. 2** Effect of different light quality treatments on leaf area (a), plant height (b), stem diameter (c) and specific leaf area for soybean seedlings (d). Letters above the bars indicate significant statistical differences ( $p < 0.05$ ) according to a Duncan's test



### Root Morphology

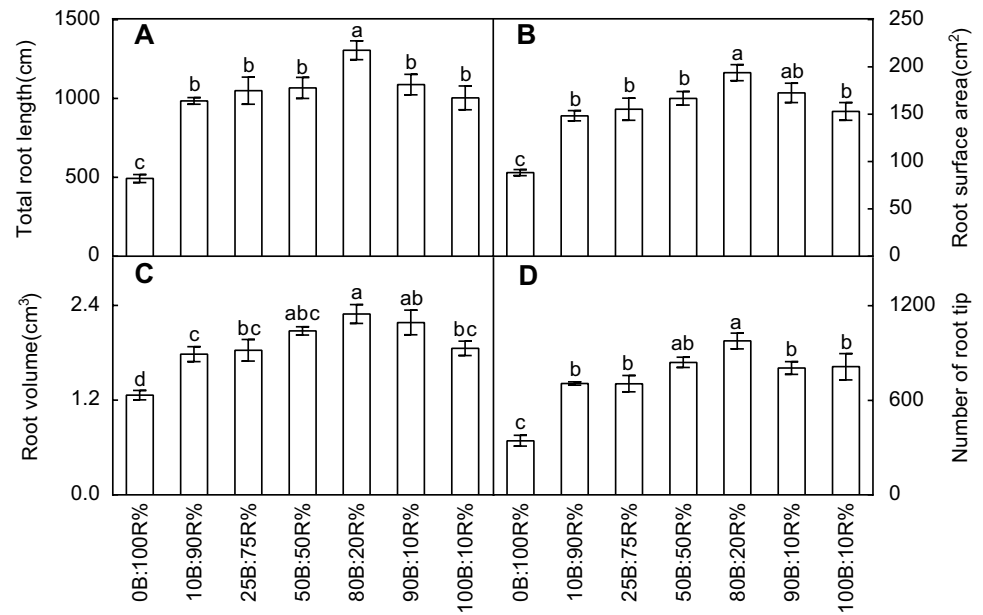
As shown in Fig. 3a, 80B:20R% stimulated the most soybean root elongation, and the total root length for plants grown with 80B:20R% was onefold greater than those for plants grown with R alone. When B PFR increased from 0 to 80%, the seedling total root length were aggrandized while it was diminished when B PFR increased from 80 to 100%. In addition, when the B PFR increased from 0 to 80%, root surface area, root volume and number of root tip gradually increased, whereas these characteristics were reduced when B PFR further increased to 100%; the root surface, root volume and number of root tip in plants grown with 80B:20R% were nearly twofold larger than plants grown with R alone (Fig. 3b–d).

### Biomass and Allocation

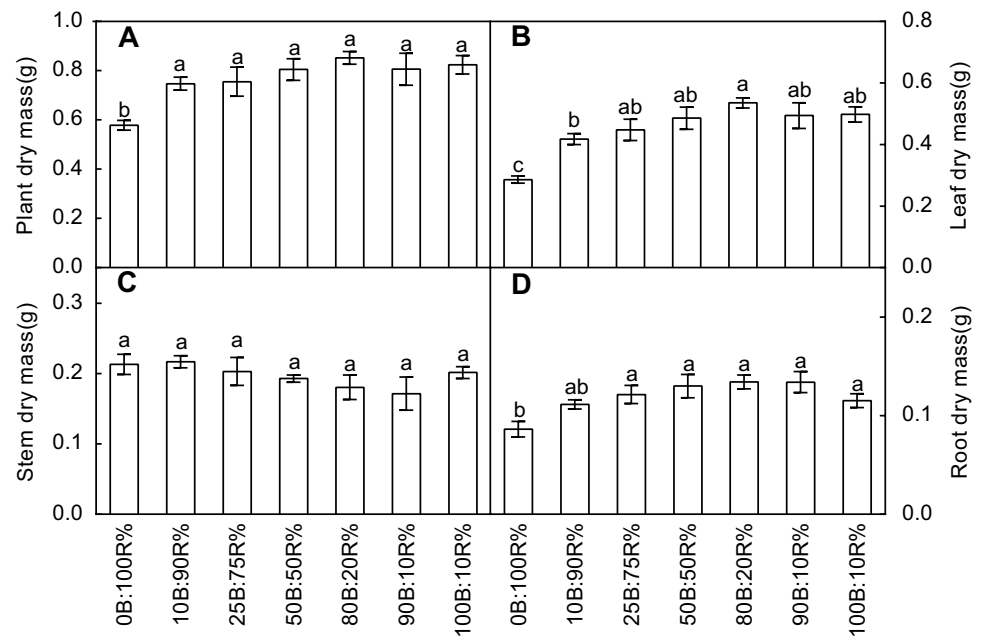
Consistent with the above morphological results, the plant dry mass in plants grown with the 100R:0B% treatment was significantly lower than that in plants grown with six other light conditions, and there was no remarkable difference between these conditions (Fig. 4a). The leaf dry mass was also lowest in plants grown with 0B:100R% (Fig. 4b). With the B PFR rising from 0 to 80%, the leaf dry mass were augmented to the maximum values. Similarly, the root dry mass increased when the B PFR was raised from 0 to 80%, but decreased with further increase in B PFR (Fig. 4d). Light quality did not have a significant effect on soybean stem biomass accumulation (Fig. 4c).

Leaf mass fraction gradually increased with increasing B PFR, with the greatest value under 80B:20R%, and

**Fig. 3** Effect of different light quality treatments on total root length (a), root surface area (b), root volume (c) and number of root tip for soybean. Letters indicate significant differences ( $p < 0.05$ ) according to a Duncan's test



**Fig. 4** Effect of different light quality treatments on plant dry mass (a), leaf dry mass (b), stem dry mass (c), root dry mass (d). Letters indicate significant differences ( $p < 0.05$ ) according to a Duncan's test

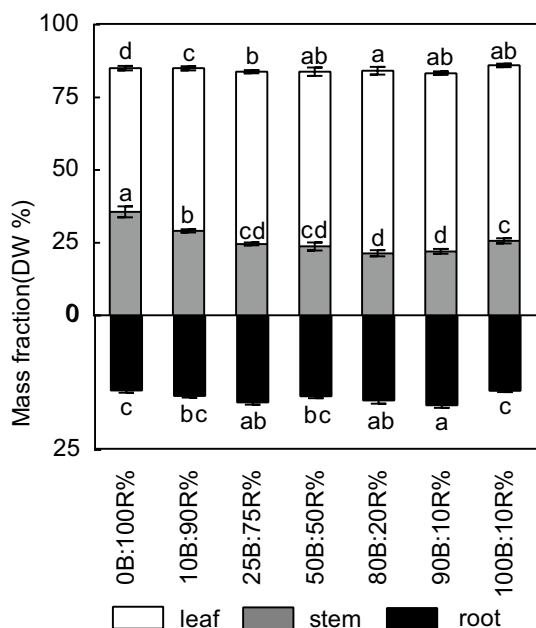


then declined (Fig. 5). We observed a similar effect on root mass fraction. Interestingly, the root mass fraction for plants grown with B alone was the lowest, whereas plants grown with 80B:20R% had the highest value. In contrast, plants grown with R alone possessed the highest stem mass fraction. The increase in the B PFR led to a decrease in stem mass fraction; the lowest value was observed under 80B:20R%, and no significant difference was observed between 80B:20R% and 90B:10R%. Besides, stem mass fraction for plants grown with 100B:0R% was significantly higher when compared with those grown with 80B:20R%.

### Chlorophyll Content and Chlorophyll Fluorescence

Light quality may have a significant effect on the chlorophyll biosynthesis process. Our work suggests that soybeans grown with 100B:0R% and 10B:90R% both had remarkably lower Chl *a* content when compared with those grown with other treatments (Table 1). Interestingly, plants grown with R alone showed the highest Chl *b* content, whereas plants grown with B alone showed the lowest. Our work also suggests that 0B:100R% induced greatly the accumulation of chlorophyll *a* long with 25B:75R%, 50B:50R%, 80B:20R% and 90B:10R% treatments. However, 100B:0R%



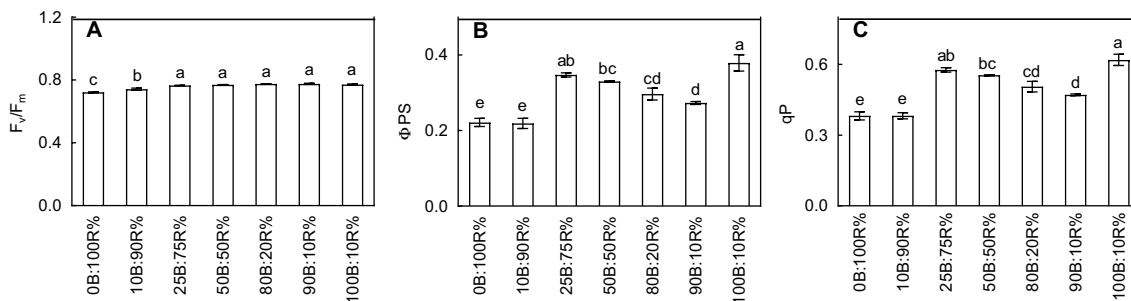


**Fig. 5** Effect of different light quality treatments on the leaf mass fraction, stem mass fraction, and root mass fraction for soybean. Letters indicate significant differences ( $p < 0.05$ ) according to a Duncan’s test

**Table 1** Effects of light qualities on photosynthetic pigments of soybean leaves

Treatment	Chl <i>a</i> (mg g <sup>-1</sup> FW)	Chl <i>b</i> (mg g <sup>-1</sup> FW)	Chl <i>a</i> + <i>b</i> (mg g <sup>-1</sup> FW)	Chl <i>a/b</i>
0B:100R%	1.60 ± 0.03ab	0.63 ± 0.02a	2.20 ± 0.04a	2.68 ± 0.03f
10B:90R%	1.50 ± 0.02b	0.49 ± 0.00bc	1.99 ± 0.02bc	3.09 ± 0.04e
25B:75R%	1.65 ± 0.02a	0.52 ± 0.00bc	2.17 ± 0.02a	3.19 ± 0.03d
50B:50R%	1.72 ± 0.07a	0.53 ± 0.02b	2.25 ± 0.09a	3.27 ± 0.02cd
80B:20R%	1.60 ± 0.03ab	0.48 ± 0.01c	2.10 ± 0.04a	3.37 ± 0.03b
90B:10R%	1.66 ± 0.06a	0.52 ± 0.02bc	2.22 ± 0.06a	3.33 ± 0.03bc
100B:0R%	1.47 ± 0.05b	0.42 ± 0.01d	1.89 ± 0.07c	3.47 ± 0.03a

Letters indicate significant differences ( $p < 0.05$ ) according to a Duncan’s test, whereas the same letter indicates no significant difference between treatments



**Fig. 6** Effect of different light quality treatments on  $F_v/F_m$  (a),  $\Phi$ PSII (b) and qP (c) for soybean. Letters indicate significant differences ( $p < 0.05$ ) according to a Duncan’s test

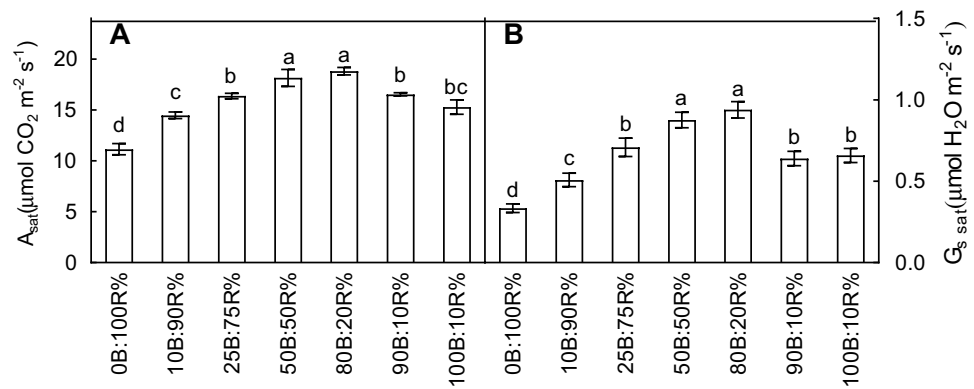
did not favor chlorophyll biosynthesis. In contrast, plants grown with R alone had the lowest ratio of Chl *a/b*, whereas plants grown with B alone had the highest value. Moreover, the ratio increased gradually with the increase of B PFR (Table 1).

Fluorescence is a property of chlorophyll that provides an abundant source of information about the light reaction of photosynthesis. As shown in Fig. 6a, plants grown with R alone exhibited the lowest  $F_m/F_v$ , and  $F_m/F_v$  increased with the increase of B PFR. Furthermore, plants grown with B alone had the highest  $\Phi$ PSII, whereas plants grown with 100B:0R% and 90R:10B% had comparably lower  $\Phi$ PSII (Fig. 6b). When the B PFR increased from 25 to 90%,  $\Phi$ PSII in plants tended to decline. Light quality had a similar effect on qP as  $\Phi$ PSII (Fig. 6b, c).

**Photosynthetic Parameters**

Light provides energy for plant photosynthesis, and its energy distribution depends on the light wavelength. Our data showed that sole R was the most inefficient in driving photosynthesis (Fig. 7a). The  $A_{sat}$  was gradually aggrandized with the increase of B PFR from 0 to 80%. Plants grown with 90B:10R% and 100B:0R% had a significantly lower  $A_{sat}$  than plants grown with 80B:20R% had. In addition, the  $g_{ssat}$  and the  $A_{ssat}$  exhibited a similar spectral response (Fig. 7b).

**Fig. 7** Effect of different light quality treatments on  $A_{\text{sat}}$  (a) and  $g_{\text{ssat}}$  (b) for soybean. Letters indicate significant differences ( $p < 0.05$ ) according to a Duncan's test



## Carbohydrate Content and Correlations

Photosynthetic carbon fixation generates organic sugars, including sucrose, which is the most abundant sugar for translocation and energy supply. A large percentage of the photosynthate generated during the daytime is stored as starch reserves, which are degraded after photosynthesis ceases to sustain plant respiration and growth during the night. The low photosynthetic rate in plants grown with R alone suggests that the sugar levels may be lower in these plants. However, our data showed that plants grown with 0B:100R% accumulated unexpectedly higher levels of soluble sugar and starch at the end of the day when compared with plants grown with other irradianations (Fig. 8a, b). Plants grown with 25B:75R%, 50B:50R% and 80B:20R% also showed higher soluble sugar and starch levels when compared with those grown with 10B:90R%, 90B:10R% and 100B:0R%. Even though plants grown with R alone had more sugar at dusk, both the soluble sugar and starch levels were depleted to levels comparable with those in plants grown with other treatments.

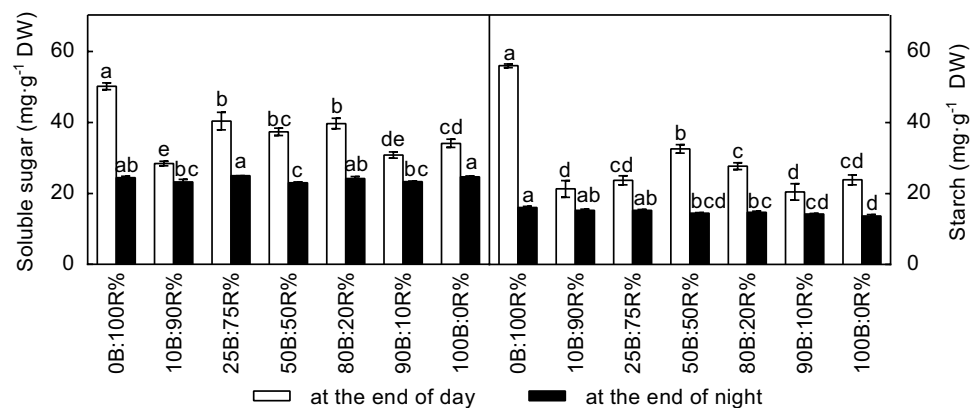
Starch showed highly significant negative correlations with leaf area, total root length, root surface area, root volume, number of root tip and leaf mass fraction, and a lower but significant negative correlation with plant dry

mass, whereas highly significant positive correlations were observed with plant height and stem mass fraction (Table 2). Stem diameter and root mass fraction had no marked correlation with starch. In contrast, leaf area, together with total root length, root surface area, number of root tip and leaf mass fraction, exhibited lower but significant negative correlations with soluble sugar than starch, whereas both plant height and stem mass fraction exhibited relatively lower significant positive correlations. However, soluble sugar did not correlate markedly with stem diameter, root volume, plant dry mass and root mass fraction. Overall, starch showed more correlations with morphological traits and biomass than soluble sugar.

## Discussion

Plant photomorphogenesis is controlled by light quality through multiple photoreceptors including B photoreceptors, the cryptochromes (CRYs, CRY1 and CRY2), and red/far-red light photoreceptors, the phytochromes (PHYs, phy A to phy E) (Cashmore et al. 1999; Quail 2002). The synergism between R and B requires the coaction of phytochrome B (phyB) and cryptochromes (Sellaro et al. 2009). In this study, soybean plants grown with R alone appeared

**Fig. 8** Effect of different light quality treatments on the soluble sugar (a) and starch in leaves (b) for soybean. Letters indicate significant differences among different light treatments ( $p < 0.05$ ) according to a Duncan's test



**Table 2** Correlation between carbohydrates and morphological traits and biomass

	Starch	Soluble sugar
Leaf area	− 0.567**	− 0.471*
Plant height	0.741**	0.526*
Stem diameter	− 0.39	− 0.292
Total root length	− 0.710**	− 0.456*
Root surface area	− 0.665**	− 0.454*
Root volume	− 0.569**	− 0.42
Number of root tip	− 0.661**	− 0.473*
Plant dry mass	− 0.536*	− 0.363
Leaf mass fraction	− 0.638**	− 0.434*
Stem mass fraction	0.756**	0.535*
Root mass fraction	− 0.389	− 0.227

Correlations were calculated from three individual values. Significant correlations at  $p < 0.01$  and  $p < 0.05$  are indicated by \*\* and \*, + and − distinguish positive and negative correlations, respectively

to exhibit an abnormal morphological response, characterized by exaggerated stem elongation, and plant height decreased with the increase of B PFR up to the treatment of 80B:20R%. Treatments of 25B:75R%, 50B:50R% and 80B:20R% induced relatively appropriate plant heights. It has been suggested that the response of stem length to the phytochrome photostationary state ( $P_{fr}/P_{total}$ ) is linear; that is, with an increase in  $P_{fr}/P_{total}$ , the stem extension rate decreases (Smith 1982). Hernández and Kubota (2016) found that the  $P_{fr}/P_{total}$  decreased with the increase of B PFR. However, soybean stem elongation showed the unexpected response, with the exception of 100B:0R%, which suggests that the change in stem extension cannot be attributed to a phytochrome-mediated response. Cryptochromes are suggested to have an inhibitory effect on hypocotyl elongation (Ahmad and Cashmore 1993). Therefore, the reduction of stem length by the increase of B PFR may be illuminated by the stimulated cryptochrome. Similar responses in stem elongation were observed for other plant species. For example, Liu et al. (2010) showed greater stem length in cherry tomato seedlings grown with R alone than that in seedlings grown with B alone and their combination treatments. Chen et al. (2014) showed that rice seedlings cultivated with R had 50% greater plant height than plants grown with B. However, we also found that B irradiation promoted stem elongation when compared with treatments of 80B:20R% and 90B:10R%. Thus, cryptochrome activation could not totally account for soybean stem growth. In plants, investment in height improves access to light. It has been suggested that plants adapt phenotypically to different conditions of light and nutrient supply, supposedly to achieve colimitation of these resources; the change of the special leaf area is critical for plant morphological response to light availability (Freschet et al. 2015). In this study, we observed a clear increase

for special leaf area in plants grown with B alone when compared with plants grown with 80B:20R% and 90B:10R%. Meanwhile, plants grown with 80B:20R% and 90B:10R% both showed greater  $A_{sat}$  than plants grown with B alone. These data suggest that B may provide lower light availability for soybean growth and development than the mixture of R and B, resulting in the promotion of stem elongation. On the other hand, R induced exaggerated height, spontaneously resulting in the severe inhibition of other organs' growth, because investment in height prompted by R incurred substantial costs in construction and maintenance of the stem.

Our results of total plant, leaf and root mass (DM) indicated that 20R:80B% was the best combination of R and B for soybean growth and development, followed by 50B:50R% and 25B:75R%. These data are consistent with numerous studies suggesting that the combination of R and B is significant for plant productivity (Hogewoning et al. 2010; Johkan et al. 2010; Lin et al. 2011). For example, a mixture of R and B augmented the plant biomass for plant species including rice (Ohashi-Kaneko et al. 2006), wheat (Dong et al. 2014), lettuce (Wang et al. 2016), cucumber (Hernández and Kubota 2016), and dendrobium candidium (Lin et al. 2011). It has been demonstrated that phytochrome is the major regulator of plant biomass and its deficiency results in sizeable reduction in plant biomass (Yang et al. 2016). Our work suggests that a slight reduction in plant biomass for plants grown with B was observed when compared with plants grown with 80B:20R%. Furthermore, when compared with plants grown with other conditions, we observed a clear reduction of plant dry mass for plants irradiated with R alone. Thus, the photoreceptor cryptochrome activation by B may play a key role in biomass accumulation for soybean plants.

Previous studies have suggested that phytochrome deficiency slows the pace of adult plant growth (Karen et al. 2003; Strasser et al. 2010). Yang et al. (2016) further speculated that this situation could arise from reduction in carbon supply. For example, seedling studies have shown that R stimulates photosynthetic pigment production, whereas phytochrome mutant seedlings grown with R light have lower chlorophyll levels (Strasser et al. 2010). Michael et al. (2008) also implied that there is a significant reduction in the expression of photosynthesis and chlorophyll biosynthetic genes in phy-9 seedlings when compared with wild-type seedlings. Similar with these results, soybean plants grown with B showed a remarkable decrease in  $A_{sat}$  and Chl  $a + b$  when compared with plants grown with combination treatments, except 10B:90R%, in this study. On the other hand, we also observed a clear photosynthetic capacity depletion in soybeans grown with R when compared with plants grown with other treatments. In *Arabidopsis*, Ohgishi et al. (2004) demonstrated that cryptochromes control both photosynthetic reactions.



Therefore, we speculated that B irradiation was imperative for efficient photosynthetic reactions for soybean. Furthermore, it has been observed that stomatal conductance changes in nearly the same proportion as the rate of CO<sub>2</sub> assimilation with the capacity to fix carbon changed (Wong et al. 1979), which was consistent with our data.

Chlorophyll is the central part of the photosynthetic system for virtually every green plant. Chl *a* is a component of both photosynthetic reaction centers and the light-harvesting antennae, whereas Chl *b*, which is synthesized from Chl *a*, is only located in the antenna complexes. Our data showed that soybeans grown with R had the highest Chl *b* level, relatively high Chl *a* content, highest Chl *a* + *b* and lowest Chl *a/b*. Thus, monochromatic R may promote the conversion from Chl *a* to Chl *b* to a large extent, consistent with results for tomato (Yang et al. 2018), lettuce (Wang et al. 2016), and rapeseed (Chang et al. 2016). The highly uncoordinated Chl *a/b* ratio induced by R irradiation may contribute to low efficiency in the photosynthetic reaction center, leading to the reduction of photosynthetic system capacity.

The reaction center core of photosystem II is composed of two chlorophyll-binding proteins, D1 and D2, which are encoded by the chloroplast genes *psbA* and *psbD*, respectively. These chlorophyll-binding proteins are damaged during the photoreaction. B light-activated transcription of *psbD* via a special light-responsive promoter is part of the repair system (Thum et al. 2001). In line with these findings, Hogewoning et al. (2010) proposed that the B deficiency condition is adverse for cucumber growth. Similarly, Chang et al. (2016) indicated that plants grown with R alone exhibited stress resistance responses, characterized by low  $F_v/F_m$  and a high content of O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>. In the present study, R induced a large depletion of  $F_v/F_m$ ,  $\Phi$ PSII and qP. Thus, we speculate that R is also an adverse spectrum for soybean plant growth.

It is demonstrated that both phytochrome and cryptochrome play a role in the circadian entrainment for *Arabidopsis* (Somers et al. 1998). It has been further suggested that the photoreceptor phytochrome performs the function of coupling carbon resource availability to plant diurnal growth (Yang et al. 2016). We have showed that even though B deficiency reduces the photosynthetic capacity, soybean grown with R light accumulated more daytime soluble sugar and starch, which is contrary to the expected response. Therefore, we speculate that soybeans grown with R may be less efficient at exploiting sugar for growth during the day. In contrast, the nearly complete nocturnal depletion of the abundant soluble sugar and starch pools implied that soybeans grown with R may grow more at night. Therefore, photoactivation of cryptochrome by B may be essential for a normal soybean diurnal growth pattern. On the other hand, plants grown with B exhibited normal daily growth, potentially resulting from the fact that an important absorption

peak of the phytochrome occurs in the B region (Neff and Chory 1998).

Sugars not only fuel cellular carbon and energy metabolism but also play crucial roles as signaling molecules, which can modulate plant growth, development, and stress responses (Rolland et al. 2006). For example, modulation of endogenous sugar levels can manipulate root architecture for optimized development by regulating lateral root emergence as well as lateral root development (Gupta et al. 2015); the biosynthesis and degradation of auxin are regulated by sugars via phytochrome interaction factors (Sairanen et al. 2013). In the present study, numerous morphological traits, together with biomass parameters, were closely negatively related to the leaf starch level at the end of day in agreement with an earlier report showing that starch acts as a major integrator for the regulation of plant growth (Sulpice et al. 2009), suggesting that starch may also integrate the change by light quality for soybean.

## Conclusion

The results of this study suggest that the proper combination of R and B facilitates soybean plant growth and photosynthesis when compared with R- or B-LED alone. The combination of R and B was imperative for chlorophyll biosynthesis and function. R alone could be adverse for soybean growth, stimulated excessive carbohydrate accumulation in leaves during the daytime and disturbed nocturnal growth for soybean. B alone may be insufficient for soybean growth and development. In conclusion, soybean plants grown with combinations of 80B:20R% and 50B:50R% exhibited suitable plant architecture for the controlled environment, possessed higher  $A_{\text{sat}}$  and  $g_{\text{ssat}}$ , and accumulated higher biomass.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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