Effects of red and blue light on leaf anatomy, CO2 assimilation, and the photosynthetic electron transport capacity of sweet pepper (*Capsicum annuum* L.) seedlings

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Abstract

Background: The red (R) and blue (B) light wavelengths are known to influence many plant physiological processes during growth and development, particularly photosynthesis. To understand how R and B light influences plant photomorphogenesis and photosynthesis, we investigated changes in leaf anatomy, chlorophyll fluorescence and photosynthetic parameters, and ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) and Calvin cycle-related enzymes expression and their activities in sweet pepper (Capsicum annuum L.) seedlings exposed to four light qualities: monochromatic white (W, control), R, B, and mixed R and B (RB) light with the same photosynthetic photon flux density (PPFD) of 300 μmol/m 2 ·s .

Results: The results revealed that seedlings grown under R light had lower biomass accumulation, CO 2 assimilation, and photosystem II (PSII) electron transportation compared to plants grown under other treatments. These changes are probably due to inactivation of the photosystem (PS). Biomass accumulation and CO 2 assimilation were significantly enriched in B- and RB-grown plants, especially the latter treatment. Their leaves were also thicker, and photosynthetic electron transport capacity, as well as the photosynthetic rate were enhanced. The up-regulation of the expression and activities of Rubisco , fructose-1, 6-bisphosphatase (FBPase) and glyceraldehyde-phosphate dehydrogenase (GAPDH), which involved in the Calvin cycle and are probably the main enzymatic factors contributing to RuBP (ribulose-1, 5-bisphosphate) synthesis, were also increased.

Conclusions: Mixed R and B light altered plant photomorphogenesis and photosynthesis, mainly through its effects on leaf anatomy, photosynthetic electron transportation, and the expression and activities of key Calvin cycle enzymes.

Background

Light is one of the most important environmental factors affecting plant growth and development [1]. Using light rather than chemicals to control plant architecture can reduce the environmental impacts [2]. Light affects the photosynthetic characteristics of seedlings by regulating chloroplast and anatomy development, and through its influence on key enzyme activities and the related expression of genes involved in the Calvin cycle, etc. [3-6].

Photosynthesis is the green engine that powers life on Earth, as it is the only biological process that allows plants, etc., to convert light energy into chemical energy [7]. Improving photosynthesis is critical to maintaining sufficient dry biomass accumulation. It is known that plants can respond to light intensity, photoperiod, and light quality [8, 9]. Light quality, which refers to the color or wavelength of the light, is a key factor in numerous adaptive responses and development transitions in plants [10-12]. Specific light qualities have precise effects on plants. For example, blue (B) and red (R) light are the most effectively utilized wavelengths during plant photosynthesis because the absorption spectra of the photosynthetic

pigments mainly focus on the B (400-500 nm) and R (600-700 nm) light spectra. Therefore, their utility and regulatory mechanisms have always been important areas of research [13, 14].

A few studies have used R and B light to examine the effects of light quality on anatomy, photosynthesis and morphology of plants. In general, R light plays an important role in controlling the functions of the chloroplast, stem and petiole growth, and the reproductive system [15, 16]. B light affects plant growth, leaf expansion, photomorphogenesis, stomatal opening, photosynthesis, and pigment accumulation [17, 18]. Furthermore, B light is shown to stimulate “sun-type” characteristics such as high photosynthetic efficiency on the chloroplast level. Studies have revealed that plants under B light had greater stomatal opening, higher chlorophyll (Chl) *a*/*b* ratios, smaller amounts of light harvesting Chl *a*/*b*-binding protein in photosystem II (PSII), higher photosynthetic electron-transport activity per unit of Chl content, and higher ribose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity levels and expressions of Calvin cycle-related genes than those plants under R light [19, 20].

The Calvin cycle, which occurs during photosynthesis, is a series of biochemical redox reactions that take place in the stroma of chloroplasts in photosynthetic organisms and plays an important role in photosynthetic carbon fixation. The regeneration rate of ribulose-1,5-bisphosphate (RuBP) affects the efficiency of carbon assimilation. Rubisco is a key enzyme in plant photosynthesis that controls both carbon dioxide and carbon fixation [21]. This set of reactions is catalyzed by Rubisco as well as other corresponding key enzymes, and finally converts carbon dioxide and water into organic sugars. Previous studies have shown that light qualities affect photosynthetic performance by regulating the expression of these related genes [22, 23].

It has also been shown that monochromatic R or B light does not satisfy normal plant growth requirements and the absence of one of the two light qualities creates photosynthetic inefficiencies [24]. Various studies have found that mixed R and B light is an effective lighting source that improves plant development, and a suitable proportion of R and B light accelerates photosynthesis and the growth of tomato, cucumber, and sweet pepper, etc. [24-26]. Leaf anatomy may directly influence light capture by its leaf thickness as well as by the differentiation of palisade and spongy mesophyll. Earlier report showed that leaf thickness increased when R light was supplemented with B light [27]. Furthermore, Klein [28] and Naznin [26] found that mixed R and B light led to higher Chl *a*, *b*, and total Chl levels, an improved electron transport rate (ETR), and an early onset of non-photochemical quenching (NPQ), all of which lead to increases in photosynthetic efficiency. Therefore, mixed R and B light is now used in research studies and commercial horticulture because of their effective photosynthetic wavelengths at the leaf level [29, 30]. Despite these achievements, the specific photosynthesis processes in plants affected by mixed R and B light remains largely unknown.

The popularity of sweet pepper (*Capsicum annuum* L.) for fresh market consumption or in ready-to-eat food has risen significantly during the past decades and these peppers are mostly produced in protected environments [31]. Mixed R and B light has an apparent influence on the growth and physiology of pepper plants [26, 32, 33]. Gaining a more complete mechanistic picture of how plants adapt and respond to R

and B light quality is important since light quality plays important roles in growth and physiology. In addition, a better understanding of the leaf anatomy, CO_2 assimilation and photosynthetic electron transport that influence responses to R and B light can improve the photosynthetic efficiency and assist in developing better methods to evaluate plant responses to light quality. Recently, light-emitting diodes (LEDs), which are light sources that have a high photosynthetic efficiency, have been successfully used in scientific research and protected horticulture [34-36]. Our previous studies have found that a suitable proportion of mixed R and B light (light intensity of R:B = 3:1, RB) accelerated pepper seedlings' photosynthesis and growth. The objective of this study was to examine how R and/or B light sources affected pepper seedling photomorphogenesis, photosynthetic characteristics, as well as the transcriptional and translation levels of key enzymes in the Calvin cycle.

Results

Plant morphology and biomass accumulation under different light treatments

A visual overview of the influence of monochromic and mixed R and B light on morphology of sweet pepper seedlings at 28 day (d) after treatment (DAT) was shown in Fig. 1 and Supplementary Fig. 2 and the differences among different treatments were significant. The plant shoot dry weight (DW) under RB was significantly increased compared with W ($P < 0.05$), and it was also higher than that under other treatments, whereas, R light produced the lowest DWs (Fig. 2a). The root DWs showed similar trends under all the treatments (Fig. 2b).

Leaf anatomy under different light treatments

Table 1 and Fig. 3 showed that R and B light had a significant effect on the anatomical structure of pepper leaves. Leaf thickness was the highest under RB, followed by B and W, while the thinnest leaves were found under R light. Furthermore, compared to W, the thickness of palisade mesophyll tissue (PT), spongy mesophyll tissue (SPT), and the upper epidermis were significantly greater under RB treatment ($P < 0.05$). These three parameters increased by 26%, 19%, and 22%, respectively, but they were significantly reduced by R light. Thinner lower epidermal thicknesses were found under R, whereas the epidermis tended to be thicker under RB although they were not significantly different from W. The effect on the PT and SPT ratio was not strong ($P > 0.05$) and the thinnest cell layers occurred under R.

Photosynthetic light- and CO_2-response curves under different light treatments

Both of the net photosynthetic rate (Pn) of the leaves increased rapidly along with the increment in PPFD (Fig. 4a) and CO_2 concentration (Fig. 4b) at the initial stage, after that, their increasing tendency gradually became stable. The highest Pn-PPFD response curve value was detected under RB, followed by B and W, whereas R produced the lowest value. Furthermore, different light treatments produced similar trends for Pn-CO_2. The apparent quantum efficiency (AQY), light saturation point (LSP), light-saturated maximum (Pn_{max}), carboxylation efficiency (CE), and CO_2 saturation point (CSP) levels, and the maximum RuBP

regeneration rate were significantly higher under RB ($P < 0.05$) than those under W, whereas, the light compensation point (LCP) and CO_2 compensation point (CCP) values were decreased under this treatment (Table 2 and Table 3).

Chlorophyll a fluorescence and the chlorophyll fluorescence transients under different light treatments

The effects of R and B light on the pepper seedling Chl fluorescence parameters were shown in Fig. 5. F_v/F_m, which represents the greatest light conversion efficiency or the maximum quantum yield of PS II, was significantly higher under RB and B than that under W, and there were no significant differences between RB and B treatments (Fig. 5a). Furthermore, this parameter significantly declined under R ($P < 0.05$). Φ_{PSII} represents the actual conversion efficiency of PS II or the actual quantum yield and it showed a similar reaction to the four light quality treatments (Fig. 5b). F'_v/F'_m indicates how efficiency the excitation energy is captured by open PSII reaction centers, and it was enhanced in RB-grown seedlings, followed by W and B, and there were no significant differences among these three treatments ($P > 0.05$) (Fig. 5c). However, seedlings grown under R light had significantly lower F'_v/F'_m values ($P < 0.05$), and no significant difference was found between R and B treatments.

The typical polyphasic Chl a fluorescence transient (OJIP) increased at different experimental time points were shown in Figs. 6a-d. In general, the results indicated that the W, B, and RB treatments decreased the amplitude of the OJIP curves compared with R, mainly at the J and I step, whereas they were higher under R light. There was no obvious difference in the maximal amplitude of the O and P steps among the treatments ($P > 0.05$). The JIP-test was applied to the fluorescence induction transients to further investigate the mechanisms underlying the observed changes (Figs. 7a-h). Most JIP-test parameters (e.g., the general electron carrier of the reaction center (S_m), the potential for energy conservation from photons absorbed by PSII to the reduction of the intersystem electron acceptors (PI_{ABS}), the potential for energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors (PI_{total}), the quantum yield for reduction of end electron acceptors at the PSI acceptor side (Φ_{Ro}), and the efficiency/probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side (δ_{Ro})) were significantly elevated by B and RB compared with W ($P < 0.05$), but the R light produced relatively lower values. Additionally, the fraction of PSII Chl *a* molecules that function as reaction centers (RC/ABS), the dissipated energy in the reaction center (DI_o/RC), and the maximum trapped energy exciton per active PSII reaction center (TR_o/RC) in the leaves under R were significantly greater than those under other treatments ($P < 0.05$).

Calvin cycle enzymes activity under different light treatments

Rubisco, FBPase, fructose-1, 6-bisphosphate aldolase (FBA), glyceraldehyde-phosphate dehydrogenase (GAPDH), and transketolase (TK) are key enzymes in the Calvin cycle. The results showed that the Rubisco activities increased initially and then decreased with the duration of different light quality treatments increased (Figs. 8a-e). Seedlings under B and RB had significantly higher Rubisco activities

than W-grown seedlings ($P < 0.05$) with 65% and 36% increases, respectively, at 28 DAT (Fig. 8). In contrast, R-grown plants had a significantly lower activity levels (15% less) than W-grown plants.

Sharp increases in FBPase activity were observed in pepper seedlings under the different light treatments. The FBPase activities reached their highest levels at 21 DAT and then decreased over the following days (Fig. 8b). Activities of this enzyme in plants under B light remained significantly higher than those under other treatments from 7 to 21 DAT ($P < 0.05$), but there was no significant difference between W and B at 28 DAT ($P > 0.05$). Significantly lower activities were observed under R light than those under other treatments during the experimental period. The FBA activities in plants treated with W and R light increased slowly during the experimental period (Fig. 8c), whereas, they rapidly increased in the RB and B treatments after 14 DAT, which indicated that the enzyme activity in the RB and B treatments was greater than in the W and R treatments. The GAPDH activities decreased in plants under all treatments, but the W and RB light applications alleviated the reduction (Fig. 8d). The TK activities were similar under all the treatments during the experimental period, except that the GAPDH and TK activities were significantly lower under the R-treatment than those under other treatments (Fig. 8e).

Gene expression under different light treatments

The RT-PCR method was used to analyze the relative expression levels of *FBA*, *FBPase*, *GAPDH*, and *TK* genes involved in the Calvin cycle after pepper seedling exposure to different light qualities for 28 d. Figs. 9a-d showed that the transcriptional levels of these genes varied significantly depending on the light qualities supplied and similar variation patterns were obtained for *FBA*, *FBPase*, and *GAPDH* under different treatments. Generally, compared to W, seedlings under RB showed significantly increased expression levels of these three genes, whereas exposure to R light resulted in decreased gene transcription. Additionally, the relative expression level of *TK* was up-regulated in B-treated seedlings, followed by RB and W, but R produced the lowest *TK* levels.

Discussion

During light-controlled development, it is generally assumed that the photoreceptors perceive and interpret incident light and transduce signals to modulate light-responsive nuclear genes. Amongst the light spectra, R and B wavelengths are the primary spectral wavelengths and can highly influence plant photosynthesis, physiological metabolism and morphology [37-39]. In this study, the photomorphogenesis and photosynthetic characteristics of sweet pepper seedlings were significantly influenced by the light qualities. Biomass is an important indicator of seedling quality. In this study, the seedling DW under RB was significantly greater than those under other treatments, which suggested that this spectrum was optimal because it promoted plant development and drove photosynthesis by increasing Chl *a* and total Chl contents in the seedlings [33, 40]. Previous studies also found that mixed R and B light could promote fresh weight (FW) and DW in many other plant species, such as chrysanthemum, upland cotton, and tomato [41-43]. The biomass of pepper seedling was significantly

increased under RB compared with other treatments and this was probably due to the enlarged leaf area (LA) [44] and changes to the leaf anatomy.

Light is absorbed by chloroplasts when it passes through the PT and SPT, which are both important photosynthetic tissues. In our study, RB treatment greatly increased the PT, SPT, as well as upper and lower epidermis thickness, which led to thicker leaves, and this was consistent with the results of Arena et al. [45] and Liu et al. [46]. The vertically elongated PT cells minimized light scattering, which allowed deeper penetration into the chloroplasts, while the changes to the SPT cells enhanced light capture by scattering the light [47]. This improved the photosynthetic structure, which should increase the light capture and absorbance capacities, and contribute to better photosynthetic light acclimation. In addition, leaf thickness plays a key role in determining space availability for chloroplast development [48]. The RB treatment increased leaf thickness, which enhanced the chloroplast ultrastructure [49]. The results suggested that a larger LA and increased leaf, as well as PT and SPT cells thickness improved light interception by the pepper seedlings. and this could be another important reason why RB was able to improve photosynthetic efficiency. Furthermore, the thinner leaves recorded under R light can be explained as a reaction to radiation stress on plant development and metabolic processes, as suggested by Macedo et al. [50].

The light- and CO_2-response curves reflect the plant capacity to benefit from increments in light energy and CO_2. This information offers interesting insights into the mechanisms that underlie light capture and CO_2 fixation. In this study, Pn-PPFD under the different light qualities was significantly lower than Pn-CO_2. This might be due to a CO_2 concentration limitation. The AQY and CE values showed the initial slopes of the light- and CO_2-response curves, respectively. They represent the capacity of the plant to capture low light energies and low CO_2 levels. Our results confirmed a previous study [51], which showed that mixed R and B light promoted AQY and CE, and that these increases led to a rise in Pn_{max} and maximized the RuBP regeneration rate. The RB light led to significant increases in AQY, CE, Pn_{max}, and the maximum RuBP regeneration rate. This indicated that mixed R and B light worked synergistically to increase photosynthetic capacity [52]. The LSP values, which reflect the plant ability to use the highest light intensity level, were also significantly higher under RB. This showed that RB improved the ability of the leaves to utilize mixed light qualities. Furthermore, the LCP and CCP values were significantly decreased under RB, which showed that this treatment improved photosynthetic performance and light energy utilization efficiency. These results indicated that the energy conversion of mixed R and B light into chemical energy by the leaves was very efficient, as this fraction of visible light had, by far, the highest quantum yield for CO_2 fixation compared with other light treatments [53].

Light qualities can regulate photosynthesis by affecting the formation of different types of chloroplast proteins and electron transport between light systems [54]. Chl fluorescence can partly reflect the photosynthetic ability of plants [55] and the efficiency of PSII photochemistry (Φ_{PSII}) can be used to reveal the physiological state of plants [56]. Our results showed that there was a reduction in Φ_{PSII} in pepper seedlings after exposure to the RB treatment. F_v/F_m represents the maximal efficiency of the excitation

energy captured by the PSII reaction centers and the significantly higher value observed in RB-treated seedlings indicated that resistance to photoinhibition was up-regulated under this treatment [57]. Additionally, the higher F_v/F_m and Φ_{PSII} levels under RB treatment showed that mixed R and B light increased the openness and electron transport efficiency of PSII, which meant that more electrons could be absorbed, captured, and transported.

The J-step, I-step, and IP phases of Chl fluorescence transients are correlated with the redox state of quinone electron acceptor (Q_A), the redox state of plastoquinone, and the redox state of the end acceptors at the PSI electron acceptor side, respectively [58, 59]. The finding that R-treated leaves increased the J- and I-step suggested that electron transport at both the donor and acceptor sides of PSII was inhibited. Thus, the imbalance of excitation energy distribution between PSI and PSII was induced, which would reduce CO_2 assimilation. Monochromatic B and mixed R and B light induced a decrease in all the OJIP steps during the experimental period compared with other treatments, which altered both the donor and acceptor sides of PSII and affected electron transport [60]. These changes maintained electron transportation on both the donor and acceptor sides. Furthermore, we found that RB increased S_m, PI_{ABS}, PI_{total}, Φ_{Ro} and δ_{Ro}, but decreased RC/ABS, DI_o/RC, and TR_o/RC (Fig. 7). This indicated that there was less damage to the photochemical and non-photochemical redox reactions and that there was an enhanced capacity for electron transport, which would accelerate ATP synthesis and RuBP regeneration [61].

In C3 plants, the Calvin cycle is the predominant pathway for CO_2 assimilation [62]. Rubisco is a representative and unique enzyme in the Calvin cycle and other Calvin cycle enzymes, including FBPase, FBA, GADPH, and TK, play an important part in modulating this pathway [63, 64]. Light is an important environmental signal that triggers gene expression and regulates corresponding enzyme activities in plants during development. A few studies have examined how light regulates the expression and activities of enzymes which involved in photosynthesis [52, 65]. These previous studies were verified by the present study. The Rubisco activity in B- and RB-treated plants was significantly higher than those in the plants treated with other light wavelengths. This finding suggested that the application of B or RB could increase carbon assimilation and RuBP regeneration in the Calvin cycle. We also found that under R light, the decreased photosynthetic rate was accompanied by the reductions in Rubisco activity, and the transcriptional levels of most genes involved in the Calvin cycle. This result was consistent with an earlier observation and implied that the inhibition of CO_2 carboxylation in the Calvin cycle and PSII slow down as a result of the impaired activity of Rubisco activase, which removes inhibitors bound to Rubisco, are probably responsible for the decreased CO_2 assimilation rate in R-grown seedlings compared with other light treatments [66, 36]. Furthermore, a previous study found that the stomatal factor, which differentially regulates the availability of RuBP and CO_2, might also be involved in the regulation of gene expression because the expression levels of the genes examined were highly correlated with the changes in stomatal conductance [36].

The FBA and FBPase activities directly affect photosynthetic efficiency and carbon accumulation [67]. Furthermore, a previous study showed that a significantly decrease in TK activity led to a significant

reduction in RuBP regeneration and significantly inhibited the plant photosynthetic rate [68]. In our study, the activities of these enzymes under B and RB and the relative expression of their associated genes, except for *FBA* and *TK*, were significantly elevated, which promoted RuBP regeneration and increased Pn [67, 68]. Chloroplast GAPDH is a key enzyme involved in the carbon reduction process during photosynthesis [69] and the greater *GAPDH* expression level under RB light in the present study may be due to the increased demand for carbon flux [70], suggesting that maintenance of active *GAPDH* expression in the carbon reduction process could be an important factor contributing to superior photosynthesis under RB light [71]. Changes in activities of FBA and TK as well as their expression under all treatments were not positively correlated, suggesting that transcript abundance is poorly linked to de novo protein synthesis due to profound regulation at the level of translation Oelze et al. [72]. Moreover, the different patterns of gene expression and activity are probably correlated with regulatory factors other than light quality, but this needs further investigation.

Conclusions

Light quality is an important environmental factor that regulates the plant photomorphogenesis and photosynthetic characteristics. In conclusion, sweet pepper growth, development, and photosynthesis are precisely controlled and genetically regulated by light quality. The results indicated that photosynthesis in seedlings under R light was inhibited by the decreased photosynthetic electron transport capacity, which caused a reduction in CO_2 assimilation. This led to down-regulation of Calvin cycle associated gene expressions and their related enzymatic activities. However, the use of monochromatic B and mixed R and B light, especially the latter, could enhance the activity of the PSII reaction center, and improve photosynthesis and the expression and activities of Calvin cycle-related enzymes, including Rubisco, FBPase and GAPDH, which are probably the main enzymatic factors contributing to RuBP synthesis. Therefore, mixed R and B light may provide more suitable light conditions for the growth of sweet pepper seedlings.

Methods

Plant material and climate conditions

The experiment was performed from June to October, 2016 in a Chinese solar greenhouse (CSG) and an artificial climate chamber (ACC, Zhejiang Qiushi Environment Co., Zhejiang, China) at the Horticultural Research Center, Shandong Agricultural University, P. R. China. Sweet pepper (*Capsicum annuum* L. cv. Hongqijian) seeds (Jinan Weili Seeds Co., Ltd., Shandong, China) were immersed in water for 15 min at 55 ^{o}C and then soaked in cold water (4 ^{o}C) for 24 h. The seeds were sown into 50-cell plug trays (54.0 × 30.0 × 4.4 cm) filled with a mixture of peat (Floragard Seed 2, Floragard Co., Oldenburg, Germany) and vermiculite (2:1, v/v) in the CSG. All seedlings were watered daily with half-strength Yamazaki's pepper nutrient solution. Three weeks later, when their second true leaf had fully expanded, the seedlings were transplanted into plastic pots (8 cm long, 8 cm wide and 10 cm deep, one seedling per pot) containing the same substrate and watered with full-strength nutrient solution. Then, a total of 480 seedlings were

selected, moved into the ACC, and cultured under four light quality treatments for 28 d. Each light treatment was repeated three times in the same ACC and there were 40 plants for per replication per treatment. Five plants were randomly sampled at 7, 14, 21, and 28 DAT from each replication each treatment and were subjected to morphological and biochemical analyses. There was ventilation in the controlled environment, so the CO_2 level was the same as the CO_2 level of atmosphere outside. The relative humidity (RH) was maintained at 70 ± 10 %, with a 12 h photoperiod and a temperature of 26 ± 1 ^{o}C during the daytime and 18 ± 1 ^{o}C at night.

Light treatments

All the mixed LEDs had a uniform spectrum for R and B light and were designed by Chunying Optoelectronics Technology Co., Ltd., Guangdong, China. The cultivation rack in the ACC was a steel frame structure with an LED light source placed at the top. The different treatments were insulated from one another by silver shading material. The plants were grown under the following light conditions: monochromatic B light with a maximum intensity at 457 nm, R light with a maximum intensity at 657 nm or mixed R and B light (3:1, RB: 75% R light at a wavelength of 657 nm and 25% B light at a wavelength of 457 nm). There was a multi-wavelength W light treatment as control (Supplementary Fig. 1). The light intensity, expressed as PPFD at the canopy level, was set at 300 μmol/m^2·s, which was measured using a quantum sensor (LI-250, LI-COR Inc., Lincoln, NE, USA) and maintained by adjusting the distance of the LEDs from the canopies. The distance between the LEDs and the canopy was approximately 10 cm. The spectral photon flux density distributions (SPDs) of the LEDs were measured using a spectroradiometer (Unispec-SC Spectral Analysis System, PP Systems Inc., Haverhill, MA, USA).

Biomass analysis

Five seedlings, including leaves and roots, were removed from each replication each treatment at 28 DAT and dried in an oven at 105 °C for 30 min. The oven temperature was changed to 75 °C and the plants were dried to a constant weight. Then, the DWs of leaves and roots were measured using an electronic balance (precision: ± 0.1 g, Model LA16001S, Sartorius Co., Hamburg, Germany).

Leaf anatomy

Leaf anatomy was measured on the fully expanded second leaves from five pepper seedlings at a similar position for each replication each treatment [46] on 28 DAT. Leaf segments of 5 mm × 5 mm were taken from the central leaf blade next to the main vein, fixed with formalin-acetic acid-alcohol (FAA) fixative, dehydrated in an alcohol and xylene series, embedded in paraffin, cross-sectioned to a thickness of 10 μm, and stained with red-solid green. The total thickness of the whole leaf and the thickness of the upper epidermis, lower epidermis, PT, and SPT were measured under a transmission light microscope (DP71, Olympus Inc., Tokyo, Japan). Images were collected using a digital camera (Camedia C4040, Olympus Inc., Tokyo, Japan) and analyzed by AnalySIS 5.0 (Olympus Inc., Tokyo, Japan).

Photosynthetic light- and CO_2-response curves

The photosynthetic light-response curves and CO_2-response curves were measured on the second fully expanded leaf between 09:00 am and 14:00 pm using a portable photosynthesis systems machine (LI-6400XT, Li-COR, Lincoln, NE, USA) at 28 DAT. The measurement technique was based on a modified method described by Pan et al. [52]. The leaf chambers were set to temperature 26 ± 1 ∘C, air relative humidity 65 ± 5 %, and flow rate 300 μmol/s. The light-response curves were measured under a graded PPFD series of 1800, 1500, 1200, 1000, 800, 600, 400, 300, 200, 150, 100, 50, 20, and 0 μmol/m^2·s. When the CO_2-response curve measurements were taken, the light intensity and CO_2 concentration of the leaf cuvette were set to 1000 μmol/m^2·s and 400 μmol/mol, respectively, for 30 min. After it reached a steady state, a CO_2 mixer was used to measure the CO_2-response curves under a graded Ci value series of 400, 300, 200, 100, 50, 100, 200, 300, 400, 600, 800, 1000, 1200, 1500, and 1800 μmol·CO^2/mol. It took about 120 to 180 s for the leaf chamber to adjust to its new microclimate for each measurement with one match. Each curve was measured three times and fitted with a non-linear regression equation, as previously reported [73, 74], so that the LCP, LSP, Pn_{max}, CCP, CSP, and the maximum RuBP regeneration rate. The AQY was the initial slope of the light-response curve and the CE was the initial slope of the CO_2-response curve.

Chlorophyll fluorescence and chlorophyll fluorescence transients

The Chl fluorescence measurements were performed using a portable pulse modulation fluorometer (FMS-II, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). The second fully expanded leaves of five seedlings from each replication each treatment were dark adapted for 20 min, and the F_o (original fluorescence yield) and F_m (maximum fluorescence yield) were determined. The leaves were then illuminated by natural light for 1 h, and the F'_o, F'_m, and F_s values were measured under 800 μmol/m^2·s activating light. The saturation pulse intensity and duration were 3000 μmol/m^2·s and 0.8 s, respectively. F'_o and F'_m represent the minimum and maximum fluorescence yields of an illuminated leaf, respectively, and were measured using the saturation pulse method. F_s represents the steady state fluorescence yield. The maximum photochemical efficiency of PSII was calculated using $F_v/F_m = (F_m - F_o) / F_m$, actual PSII photochemical efficiency was calculated using $(\Phi_{PSII}) = (F'_m - F_s) / F'_m$, and maximum photochemical efficiency of PSII under light adaptation was calculated using $(F'_v/F'_m) = (F'_m - F'_o) / F'_m$.

The OJIP was measured on the second leaves by a plant efficiency analyzer (Handy PEA, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). The JIP-test formulae and glossary of terms were calculated according to Strasser [75, 76]. The following derivative parameters were determined according to Lin et al. [61] and Miao et al. [30]: RC/ABS, S_m, DI_o/RC, TR_o/RC, PI_{ABS}, PI_{total}, Φ_{Ro}, and δ_{Ro}.

Calvin cycle enzymes activity

The second leaves from the top of 15 plants per treatment were sampled at 7, 14, 21, and 28 DAT to determine the enzyme activities. Leaf tissue (0.5 g) was homogenized in 4 mL of ice-cold extraction buffer: (25 mM Hepes (K^+), pH 7.5, 10 mM $MgSO_4$, 5 mM dithiothreitol (DTT), 1 mM Na_2EDTA, 1 mM

phenylmethanesulfonyl fluoride (PMSF), 5% (w/v) insoluble polyvinylpyrrolidone (PVP), and 0.05% (v/v) Triton X-100). The homogenate was filtered through muslin cloth and centrifuged at 14,000 × g for 5 min at 4 °C. The supernatant was used as the enzyme extract for the enzyme activity assays [77].

The Rubisco (EC 4.1.1.39), FBPase (EC 3.13.11), FBA (EC 4.1.2.13), GAPDH (EC 1.2.1.12), and TK (EC 2.2.1.1) activities were determined using an ELISA kit (Shanghai Yanji Biological Technology Ltd., Shanghai, China) and the extraction methods used for these enzymes were according to Rao and Terry [78] and Wang et al. [36] with some modification. The frozen leaf samples (0.5 g) were ground to a fine powder in liquid nitrogen with a mortar and pestle, transferred to a centrifuge tube, and then extracted in pre-chilled extraction buffer (5 mL). The enzyme extraction solution was centrifuged at 12,000 × g for 15 min at 4 ^{o}C. The supernatant was used for the Calvin cycle enzymes activity assay. Subsequently, the activities of the Calvin cycle enzymes were determined using a microplate absorbance reader (Bio-Tek ELX800, Bio-Tek Instruments, Winooski, VT, USA) at an absorbance of 450 nm according to the manufacturer's instruction.

The protein concentration of each enzyme extraction solution was measured according to Bradford [79]. The results were expressed as U/g of protein.

Gene expression

Total RNA was extracted using Quick RNA Isolation Kit according to the supplier's instructions (Huayueyang Biotech Co., Ltd., Beijing, China). Reverse transcription was conducted using a ReverTra Ace qPCR RT-Kit (Toyobo Bio-Technology, Co., Ltd., Osaka, Japan). The gene expression analysis was conducted using real-time PCR, with 18S rRNA as an internal control. The thermal cycler program was one initial cycle of 94 °C for 2 min, followed by 40 cycles of 94 °C for 10 s, 60 °C for 20 s, and 72 °C for 30 s. Relative gene expressions were analyzed using the method described in Livak and Schmittgen [80]. The specific gene primers used for real-time PCR analysis of the genes involved in the PS complexes are shown in Supplementary Table 1.

Data analysis

The experiment had a completely randomized design. Values presented are the mean ± standard deviation (SD) of three replicates. The data were analyzed by one-way analysis of variance (ANOVA) and the differences between the means were tested using Duncan's multiple range test ($P < 0.05$). The charts were created using Origin (version 8.5, Microcal Software Inc., Northampton, MA, USA).

List Of Abbreviations

R: Red; B: Blue; W: White; RB: Mixed red and blue light; PPFD: Photosynthetic photon flux density; Chl: Chlorophyll; ETR: Electron transport rate; NPQ: Non-photochemical quenching; LED: Light-emitting diode; LA: Leaf area; CSG: Chinese solar greenhouse; ACC: Artificial climate chamber; D: Day; DAT: Day after treatment; RH: Relative humidity; SPD: Spectral photon flux density distribution; DW: Dry weight; FAA:

Formalin-acetic acid-alcohol; EP: Epidermis cell; PT: Palisade mesophyll tissue; SPT: Spongy mesophyll tissue; Pn: Net photosynthetic rate; LCP: Light compensation point; LSP: Light saturation point; Pn_{max}: Light-saturated maximum; CCP: CO_2 compensation point; CSP: CO_2 saturation point; AQY: Apparent quantum efficiency; CE: Carboxylation efficiency; F_v/F_m: Maximum photochemical efficiency of PSII; Φ_{PSII}: Actual PSII photochemical efficiency; F'_v/F'_m: Maximum photochemical efficiency of PSII under light adaptation; OJIP: Chl *a* fluorescence transient; RC/ABS: Fraction of PSII Chl *a* molecules that function as reaction centers; S_m: General electronic carrier of the reaction center; DI_o/RC: Dissipated energy in the reaction center; TR_o/RC: Maximum trapped energy exciton per active PSII reaction center; PI_{ABS}: Potential for energy conservation from photons absorbed by PSII to the reduction of the intersystem electron acceptors; PI_{total}: Potential for energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors; Φ_{Ro}: Quantum yield for reduction of end electron acceptors at the PSI acceptor side; δ_{Ro}: Efficiency/probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side; RuBP: Ribulose-1, 5-bisphosphate; Rubisco: Ribulose-1, 5-bisphosphate carboxylase/oxygenase; FBPase: Fructose-1, 6-bisphosphatase; FBA: Fructose-1, 6-bisphosphate aldolase; GAPDH: Glyceraldehyde-phosphate dehydrogenase; TK: Transketolase; DTT: Dithiothreitol; PMSF: Phenylmethanesulfonyl fluoride; PVP: Polyvinylpyrrolidone.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

WM conceived and designed research. XGF conducted experiments and analyzed data. LY wrote the manuscript. LC modified the paper. All authors have read and approved the manuscript.

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Tables

Table 1 Effects of different light treatments on leaf anatomy of sweet pepper seedlings at 28 DAT.

Treatments	Leaf thickness (μm)	Palisade mesophyll tissue thickness (μm)	Spongy mesophyll tissue thickness (μm)	Upper epidermis thickness (μm)	Lower epidermis thickness (μm)	Palisade mesophyll tissue/ spongy mesophyll tissue ratio
W	122.54 ± 4.92 b	39.73 ± 2.11 b	67.92 ± 3.02 b	8.35 ± 0.39 b	6.21 ± 0.11 ab	0.58 ± 0.02 ab
R	103.25 ± 3.78 c	30.21 ± 1.32 c	59.03 ± 2.82 c	6.23 ± 0.15 c	5.88 ± 0.19 b	0.51 ± 0.04 b
B	130.22 ± 3.15 b	43.33 ± 1.87 b	73.24 ± 1.45 b	7.96 ± 0.27 b	6.07 ± 0.14 b	0.59 ± 0.02 ab
RB	146.90 ± 5.21 a	50.07 ± 2.56 a	81.02 ± 2.56 a	10.18 ± 0.11 a	6.42 ± 0.12 a	0.62 ± 0.02 a

Note: Data are presented as means ± SE, n = 3. Different letters indicate significant differences between values ($p < 0.05$). W, white light; R, monochromatic R light; B, monochromatic B light; RB, mixed R and B light of 3:1. The same as below.

Table 2 Effects of different light treatments on photosynthetic light-response curve parameters of sweet pepper seedlings at 28 DAT.

Treatments	AQY (μmol/m^2·s)	LCP (μmol/m^2·s)	LSP (μmol/m^2·s)	Pn_{max} (μmol/m^2·s)
W	0.051 ± 0.003 b	26.6 ± 2.34 a	729 ± 38.34 c	13.0 ± 0.55 b
R	0.030 ± 0.002 c	27.2 ± 2.13 a	520 ± 29.56 d	6.1 ± 0.42 c
B	0.050 ± 0.002 b	23.7 ± 1.82 b	924 ± 27.45 b	15.2 ± 0.72 a
RB	0.056 ± 0.001 a	22.8 ± 2.91 b	968 ± 28.17 a	16.3 ± 0.68 a

Note: AQY, apparent quantum efficiency; LCP, light compensation point; LSP, light saturation point; Pn_{max}, light-saturated maximum.

Table 3 Effects of different light treatments on photosynthetic CO_2-response curve parameters of sweet pepper seedlings at 28 DAT.

Treatments	CE	CCP	CSP	Maximum RuBP regeneration rate
	□mol/m^2·s^1□	□μmol/m^2·s□	□μmol/m^2·s□	□μmol/m^2·s□
W	0.047 ± 0.006 b	81 ± 5.64 b	1087 ± 25.12 c	23.1 ± 3.92 b
R	0.032 ± 0.004 c	92 ± 3.12 a	1213 ± 12.39 b	11.0 ± 1.87 c
B	0.057 ± 0.009 b	57 ± 3.28 c	1040 ± 17.28 d	21.4 ± 1.93 b
RB	0.066 ± 0.003 a	61 ± 6.77 c	1443 ± 21.32 a	39.5 ± 2.67 a

Note: CE, carboxylation efficiency; CCP, CO_2 compensation point; CSP, CO_2 saturation point.

Figures

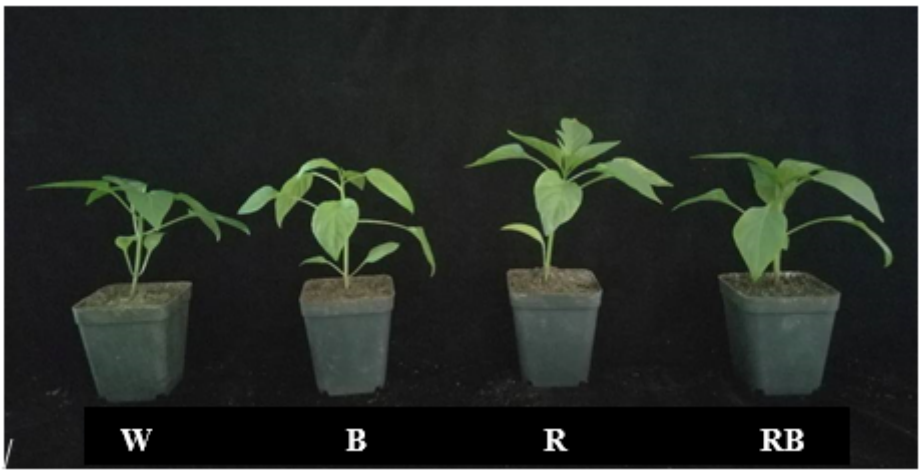

Figure 1

Effects of different light treatments on plant morphology of sweet pepper seedlings at 28 DAT. W, white light; R, monochromatic R light; B, monochromatic B light; RB, mixed R and B light of 3:1.

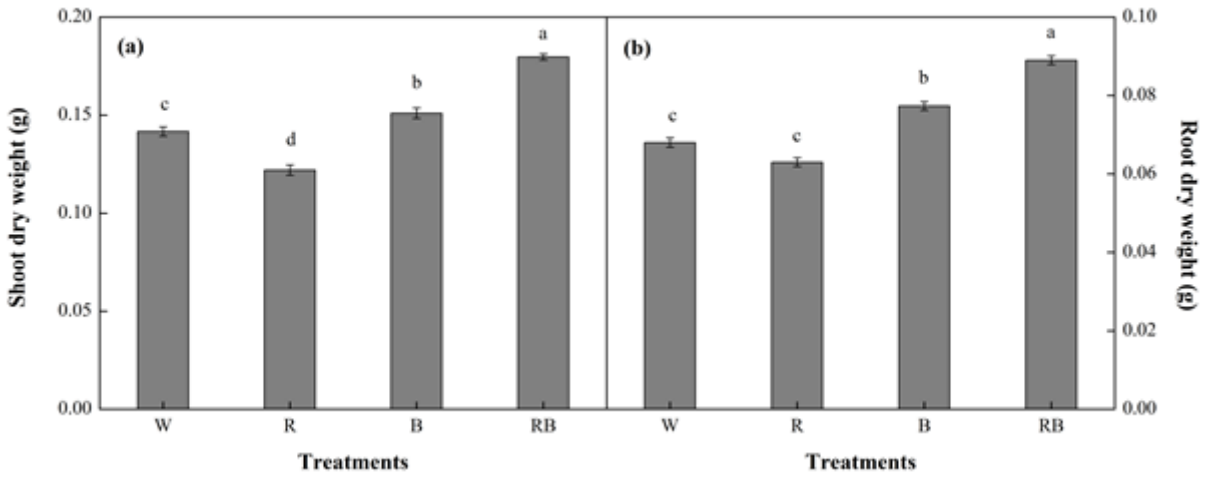

Figure 2

Effects of different light treatments on (a) shoot dry weight and (b) root dry weight of sweet pepper seedlings at 28 DAT. Different letters indicate significant differences between values ($p < 0.05$), n = 3. W,

white light; R, monochromatic R light; B, monochromatic B light; RB, mixed R and B light of 3:1.

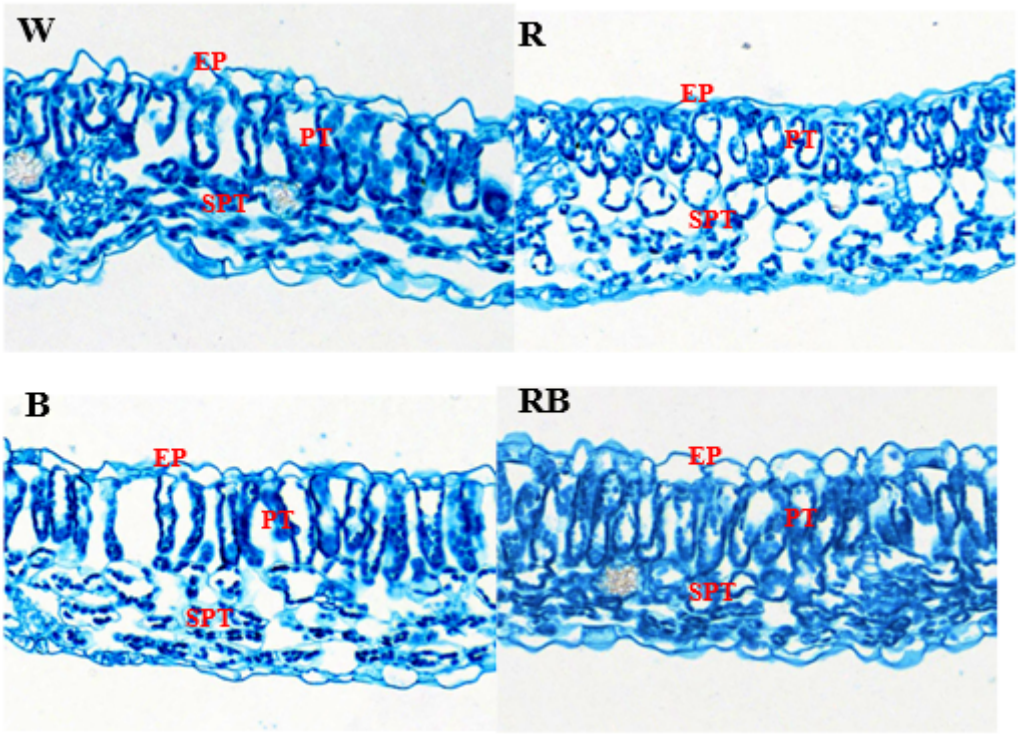

Figure 3

Effects of different light treatments on leaf sectioning anatomy of sweet pepper seedlings at 28 DAT. Note: EP, epidermis cell; PT, palisade mesophyll tissue; SPT, spongy mesophyll tissue. W, white light; R, monochromatic R light; B, monochromatic B light; RB, mixed R and B light of 3:1.

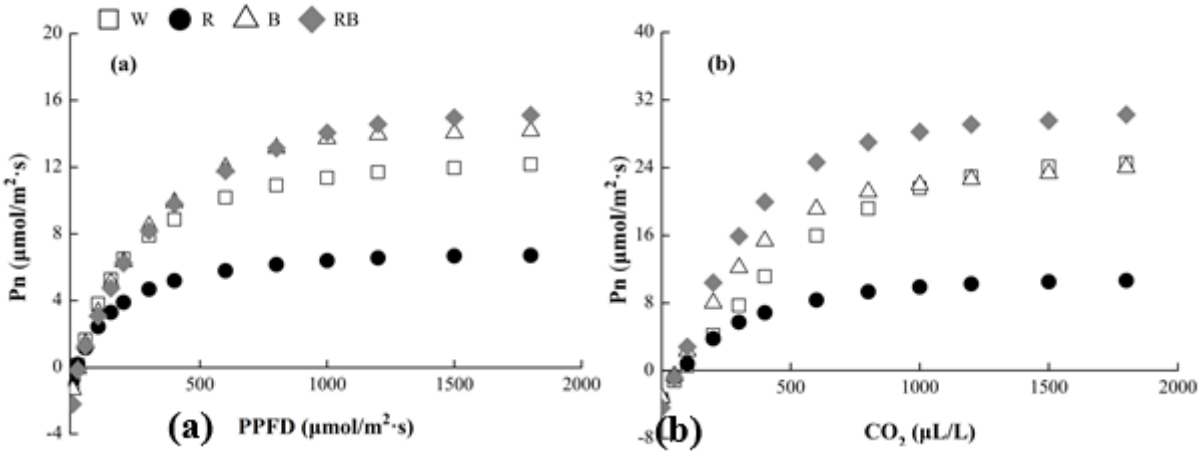

Figure 4

Effects of different light treatments on (a) photosynthetic light- and (b) CO2-response curves of sweet pepper seedlings at 28 DAT. Pn: net photosynthetic rate; PPFD: photosynthetic photon flux density; W, white light; R, monochromatic R light; B, monochromatic B light; RB, mixed R and B light of 3:1. □ W; ● R; △ B; ▯ RB.

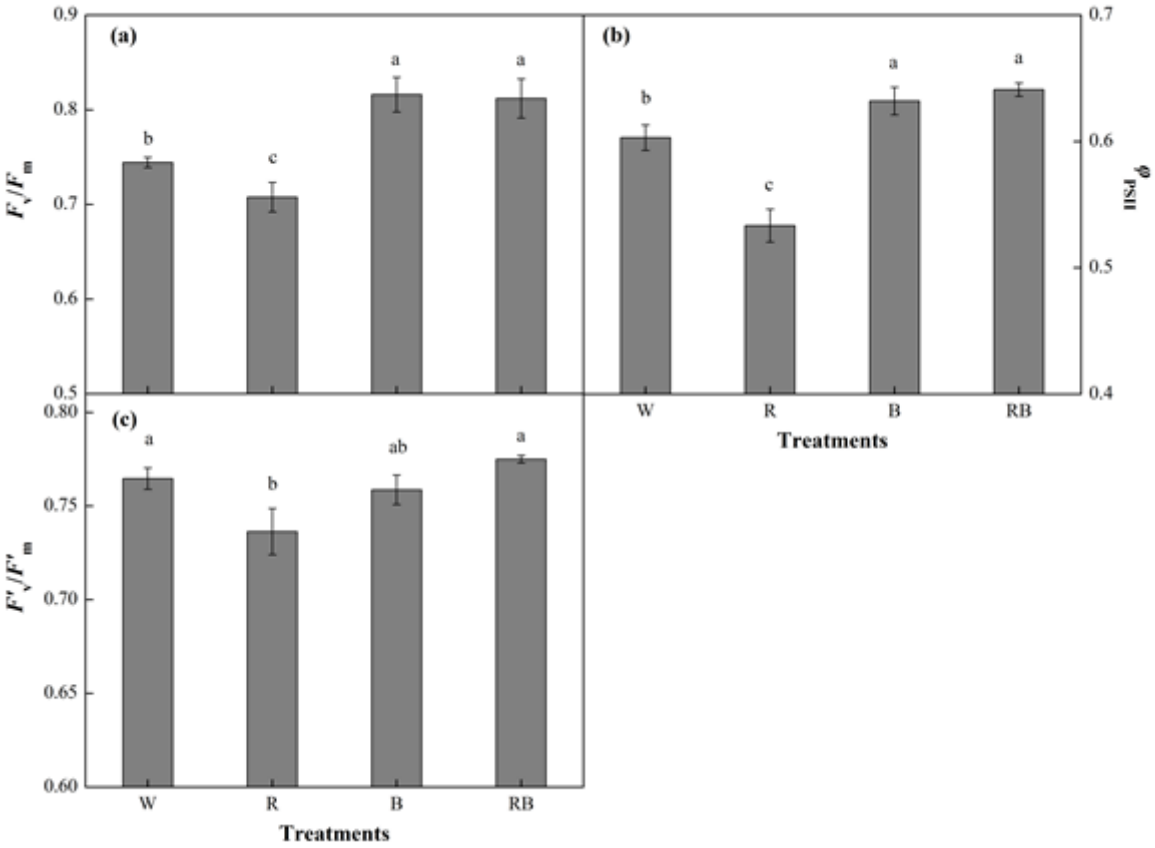

Figure 5

Effects of different light treatments on chlorophyll fluorescence parameters of sweet pepper seedlings at 28 DAT: (a) Fv/Fm: maximum photochemical efficiency of PSII; (b) ΦPSII: actual PSII photochemical efficiency; (c) F'v/F'm: maximum photochemical efficiency of PSII under light adaptation. Different letters indicate significant differences between values ($p < 0.05$), n = 3. W, white light; R, monochromatic R light; B, monochromatic B light; RB, mixed R and B light of 3:1.

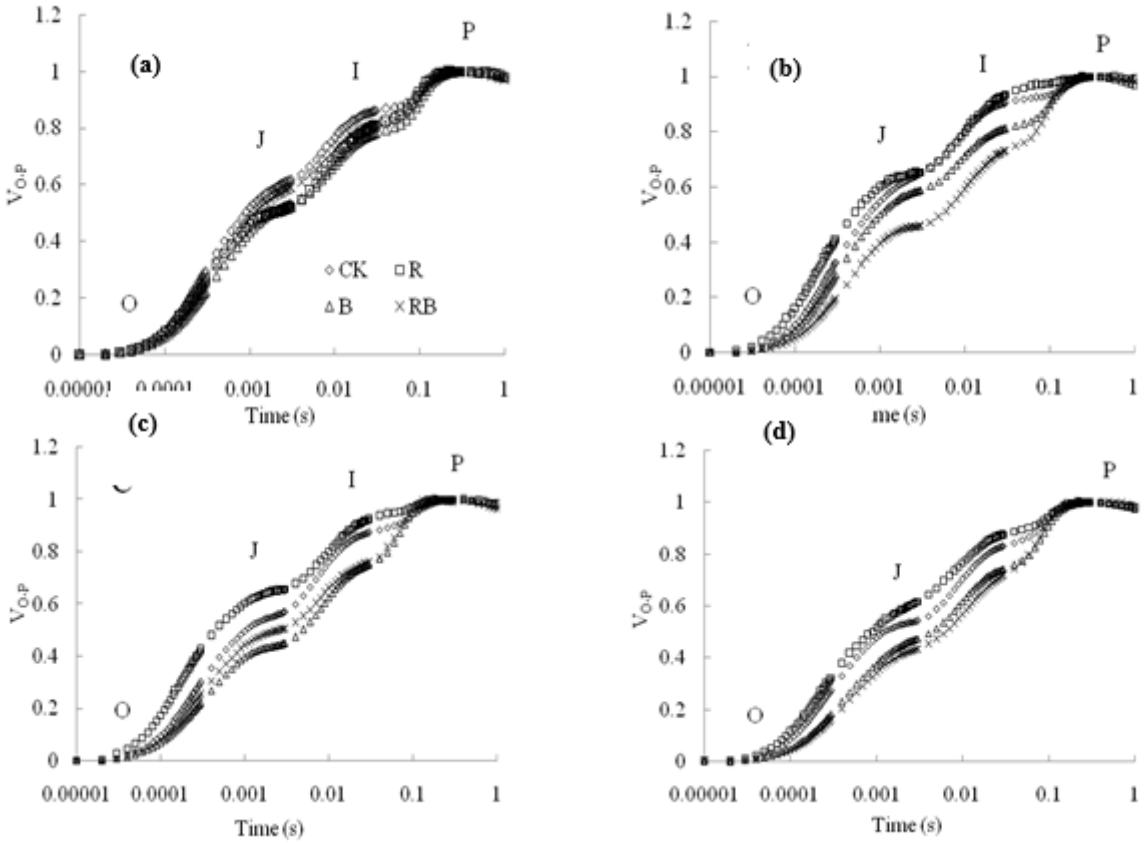

Figure 6

Effects of different light treatments on chlorophyll a fluorescence transient (OJIP) of sweet pepper seedlings at different experimental periods. (a), (b), (c), and (d) were at 7, 14, 21, and 28 DAT, respectively. W, white light; R, monochromatic R light; B, monochromatic B light; RB, mixed R and B light of 3:1.

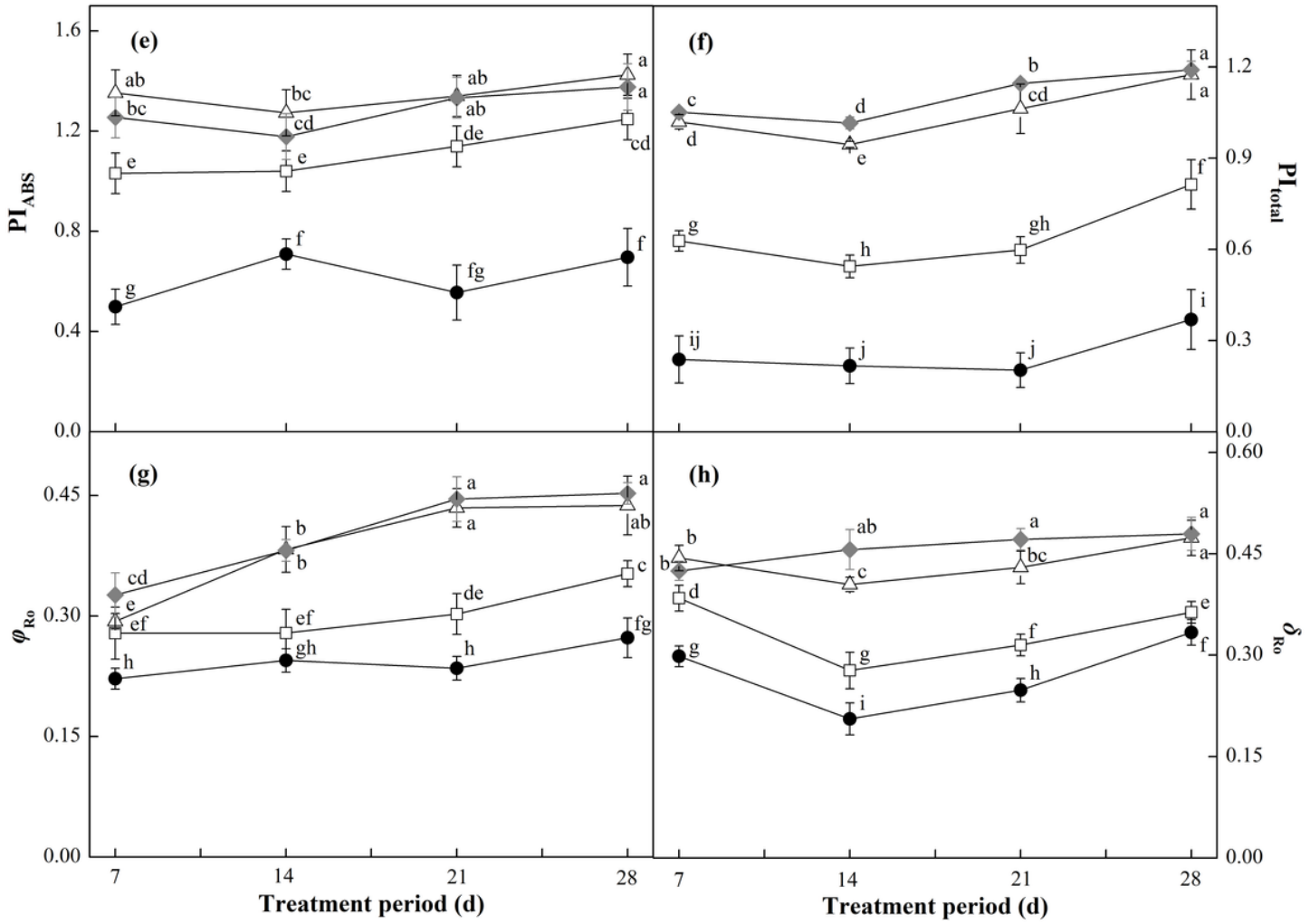

Figure 7

Effects of different light treatments on JIP-test parameters: including (a) RC/ABS: fraction of PSII Chl a molecules that function as reaction centers, (b) Sm: general electronic carrier of the reaction center, (c) DIo/RC: dissipated energy in the reaction center, (d) TRo/RC: maximum trapped energy exciton per active PSII reaction center, (e) PIABS: potential for energy conservation from photons absorbed by PSII to the reduction of the intersystem electron acceptors, (f) PItotal: potential for energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors, (g) ΦRo: quantum yield for reduction of end electron acceptors at the PSI acceptor side, (h) δRo: efficiency/probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side of sweet pepper seedlings at different experimental periods. Different letters indicate significant differences between values ($p < 0.05$), n = 3. W, white light; R, monochromatic R light; B, monochromatic B light; RB, mixed R and B light of 3:1. □ W; ● R; △ B; ▯ RB.

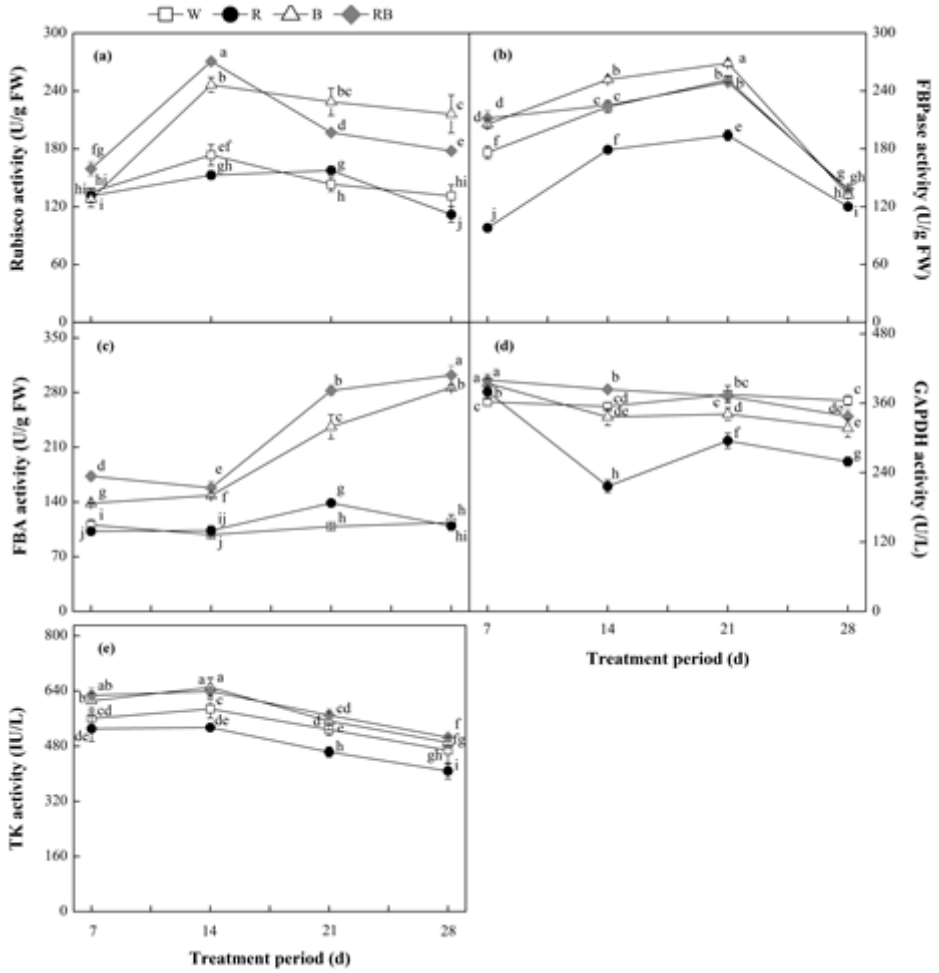

Figure 8

Effects of different light treatments on activities of Calvin cycle-related enzymes: including (a) Rubisco: Ribulose-1, 5-bisphosphate carboxylase/oxygenase, (b) FBPase: Fructose-1, 6-bisphosphatase, (c) FBA: Fructose-1, 6-bisphosphate aldolase, (d) GAPDH: Glyceraldehyde-phosphate dehydrogenase, (e) TK: Transketolase from sweet pepper seedlings at different experimental periods. Different letters indicate significant differences between values ($p < 0.05$), n = 3. FW: fresh weight; W, white light; R, monochromatic R light; B, monochromatic B light; RB, mixed R and B light of 3:1. □ W; ● R; △ B; ▯ RB.

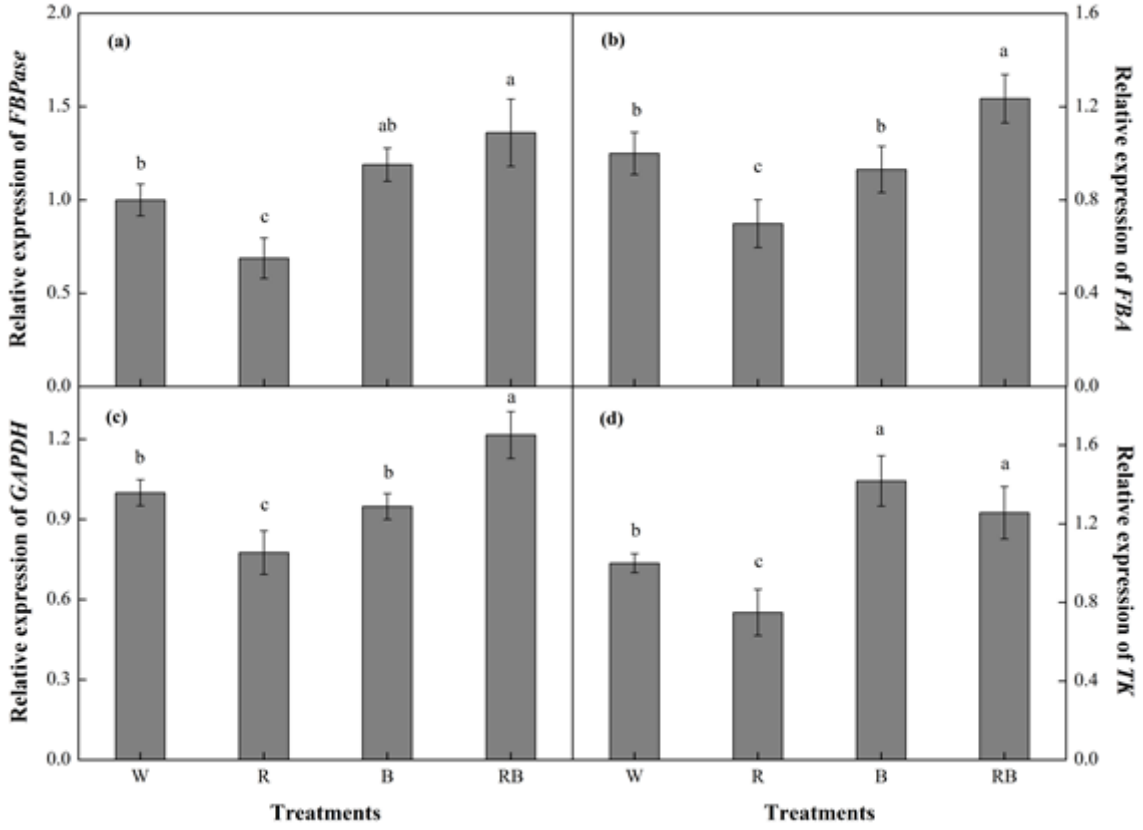

Figure 9

Effects of different light treatments on expression of (a) FBA, (b) FBPase, (c) GAPDH, (d)TK from sweet pepper seedlings at 28 DAT. Different letters indicate significant differences between values ($p < 0.05$), n = 3. W, white light; R, monochromatic R light; B, monochromatic B light; RB, mixed R and B light of 3:1.

Supplementary Files

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- Supplementarydata.docx