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Blue-Light-Dependent Stomatal Density and Specific Leaf Weight Coordinate to Promote Gas Exchange of Soybean Leaves

Jiyu Chen [1,2,3,†], Jing Gao [1,2,3,†], Qi Wang [1,2,3], Xianming Tan [1,2,3], Shenglan Li [1,2,3], Ping Chen [1,2,3], Taiwen Yong [1,2,3], Xiaochun Wang [1,2,3], Yushan Wu [1,2,3], Feng Yang [1,2,3,*] and Wenyu Yang [1,2,3]

1 College of Agronomy, Sichuan Agricultural University, Chengdu 611130, China
2 Sichuan Engineering Research Center for Crop Strip Intercropping System, Chengdu 611130, China
3 Key Laboratory of Crop Ecophysiology and Farming System in Southwest, Ministry of Agriculture, Chengdu 611130, China
* Correspondence: f.yang@sicau.edu.cn; Tel.: +28-8629-0960
† These authors contributed equally to this work.

Abstract: Blue and red light are essential light signals used to regulate stomatal development and leaf structure. In the present study, stomatal and leaf traits that respond to blue and red light were studied at two light intensities (400 and 100 μmol m^{-2} s^{-1}) in soybeans. The stomatal traits and leaf characteristics were determined. Furthermore, their contribution to the operational maximum stomatal conductance (g_{opmax}) was evaluated using the rdacca.hp R package. With the light intensity significantly reduced, the stomatal size (SZ) under blue light did not change. Similarly, the decrease in light intensity did not influence the stomatal density (SD), specific leaf weight (SLW) or g_{opmax} under red light. These results implied that the regulation of SD and SLW depended on blue light and that SZ was highly sensitive to red light. In addition, SLW was strongly correlated with SD. The SLW and SD had the highest contribution rates (19.43% and 19.5%, respectively) to $g_{opmax,}$ as compared with the other parameters. In conclusion, these results suggested that in long-term exposure to blue light, the enhancements in g_{opmax} were primarily due to the synergistic promotion of SLW and SD.

Keywords: specific leaf weight; stomatal conductance; stomatal density; stomatal size

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1. Introduction

Stomata play an essential role in regulating gas exchange. Although stomata only account for 0.3 to 5% of the total leaf area, the stomata complete 95% of the gas exchange (CO_2 diffusion) [1]. Gaseous CO_2 diffuses through leaf tissue and mesophyll cells and eventually reaches the carboxylation site (rubisco enzyme in chloroplasts). The leaf structure exerts resistance to the diffusion of CO_2. Thus, the CO_2 concentration in the chloroplast (C_c) is relatively low, affecting the supply of CO_2 to the carboxylation site [2]. Some studies have shown that maximizing stomatal conductance can increase the CO_2 concentration at intercellular and chloroplast levels [3,4]. As a C3 crop, the unsaturated C_c generally limits the photosynthesis of soybean plants [2,5]. The net photosynthetic rate is closely related to stomatal conductance. The canopy-averaged stomatal conductance of rice increased with increasing photosynthetic rate [6]. In ferns, gymnosperms and angiosperms, stomatal conductance was significantly positively correlated with the net photosynthetic rate [7]. Therefore, improving stomatal conductance is beneficial for soybeans to obtain a high net photosynthetic rate.

The stomatal density (SD) and stomatal size (SZ) determine the theoretical capacity of gas exchange. In principle, an infinite combination of either of these parameters achieves the potential maximum stomatal conductance (g_{smax}). The SD and SZ can be calculated assuming all pores are open [2,7,8]. Although this principle is anatomically possible, the operational stomatal conductance (g_{op}) is not near g_{smax}. Instead, it is maintained at approximately 20% of g_{smax} because it allows guard cells to most effectively regulate

stomatal enlargement [9,10]. Previous studies proved that g_{smax} positively correlates with g_{op} [9,11,12]. Moreover, stomatal characteristics (i.e., SD and SZ) are significantly related to g_{op} in angiosperms; other unknown traits may be involved in regulating g_{op} [12,13].

Many traits work in coordination instead of alone during the growth and development of plants. The SD and SZ are also affected by regulatory factors during stomatal development. They mutually coordinate with leaf tissue development to establish an efficient and robust network that facilitates gas exchange for photosynthesis [14]. Previous studies have shown that stomatal development is coupled with the underlying mesophyll tissues. It is beneficial to achieve high leaf photosynthetic potential (V_{max}) and g_{smax} [15]. Tanaka et al. [16] reported that Arabidopsis epidermal pattern factor (EPF) mutants, with 75% more stomata than their wild-type counterparts, demonstrated significantly higher rates of the net photosynthetic rate under high light intensities. The authors attributed the net photosynthetic rate and stomatal conductance enhancement to the improvement in the C_i of the leaf and, thus, lateral diffusion of CO_2. Therefore, variations in stomatal development and leaf anatomical structure are considered adaptive strategies that help plants respond to long-term environmental fluctuations and are essential to plant evolution and adaptation to new environmental conditions [17].

Light is an essential environmental signal that affects leaf structure and function through light quality and intensity. According to previous studies, plant leaves' area decreases and leaf thickness becomes thinner under low light. The SD, SZ, net photosynthetic rate, transpiration, and stomatal conductance also decrease when light intensity is reduced [18–23]. Among the various light qualities, blue and red light are the two most efficient bands of photosynthesis in plants [24]. The two lights affect photosynthesis by altering the palisade and spongy mesophyll [25]. Nevertheless, the regulation of leaf structure by blue and red light is different. The blue light inhibited leaf extension, promoted leaf tissue structure and chloroplast development, and improved stomatal conductance and photosynthetic efficiency. The prolonged red light produced thinner grana lamellae and smaller starch grains, led to loosely organized leaf palisade tissues, and inhibited net photosynthesis [26,27]. In stomatal movement, the red-light-driven opening response of stomata relies on photosynthesis, and blue light induces stomatal opening by activating blue light photoreceptors [28–35]. However, whether blue and red light also has differential adjustments for stomatal characteristics, such as SD and SZ, still need to be researched.

Therefore, we measured soybean leaf characteristics, such as stomatal traits, gas exchange capacity, and photosynthetic capacity under different light treatments. This study aims to address the different responses of leaf characteristics and stomatal traits to blue and red light and reveal how these differences affect the actual gas exchange and photosynthetic capacity under blue and red light. The results will provide a theoretical basis for the rational application of different wavelength spectra in facility agriculture and intercropping systems.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Soybean (*Glycine max* L. Merr.) seeds 'Rongxian Dongdou' were used to assess growth conditions under different light environments. A light chamber was used at the Sichuan Agricultural University of China (30°71′20″ N, 103°86′87″ E). Soybean seeds were decontaminated with 75% ethanol and deionized water for 5 min and germinated on a wet sterile gauze for 48 h in the dark at 25 °C. After germination, we sowed two seeds in 400 mL pots filled with a mixed matrix of PINDSTRUP organic soil (Pindstrup Mosebrug A/S, Ryomgaard, Denmark) and vermiculite (v:v, 3:1) in a light chamber. The temperature was maintained at 25 °C during the 12 h daytime and 20 °C during 12 h of nighttime. Soybean seedlings were watered every 2 days with 0.2% Hoagland's solution [36]. When the soybean seedlings had grown to the VC stage (cotyledon leaves unrolled sufficiently and the leaf edges are not touching), the pots were transferred to different light treatments under the abovementioned environmental conditions [37]. The fully unfolded penultimate

compound leaves were used to measure morphological and physiological parameters during the V3 (three nodes on the main stem with fully developed leaves beginning with the unifoliolate nodes) [37].

The experiment used two light intensities (400 and 100 μmol m^{-2} s^{-1}) at different light qualities (blue and red). The specific light quality and intensity (Figure 1) were as follows: blue light of 400 μmol m^{-2} s^{-1} (400 B); blue light of 100 μmol m^{-2} s^{-1} (100 B); red light of 400 μmol m^{-2} s^{-1} (400 R); and red light of 100 μmol m^{-2} s^{-1} (100 R). The position of the pots was adjusted daily to maintain the same light intensity during growth under one treatment. HR-450 (Hipoint Inc., Gaoxiong, Taiwan, China) was used to measure spectral irradiance and light intensity.

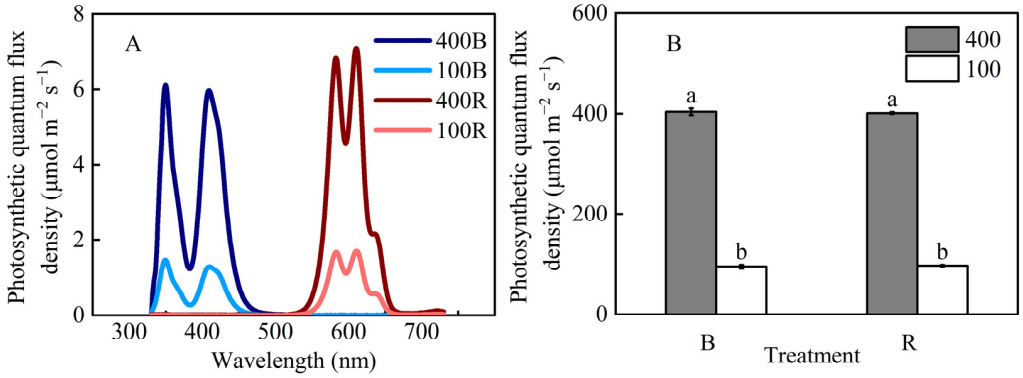


Figure 1. The light environment of different treatments. (**A**) Light quality; (**B**) Light intensity. 400 B, 100 B, 400 R, and 100 R denote 400 μmol m^{-2} s^{-1} under blue light, 100 μmol m^{-2} s^{-1} under blue light, 400 μmol m^{-2} s^{-1} under red light, and 100 μmol m^{-2} s^{-1} under red light, respectively. The mean ± *SE* is expressed by each value. Different lowercase letters indicate significant differences between treatments ($p \leq 0.05$).

2.2. Measurement of Morphological Characteristics

Four soybean seedlings of penultimate compound leaves (fully expanded) were used. Samples were overdried at 105 °C for 0.5 h and then dried at 80 °C for 72 h to constant weight. The dry mass per unit leaf area of the penultimate compound leaf indicates the specific leaf weight (SLW, mg/cm^2). The average value is considered the final result.

2.3. Leaf Anatomical Feature Measurements

Leaf pieces (4 mm^2), from approximately four replications for each treatment without midribs, were fixed in formalin–acetic acid–alcohol solution (FAA; 90% ethanol; 5% formaldehyde; 5% glacial acetic acid, *v*/*v*/*v*) at 4 °C for 3 days. The leaf samples were dehydrated in a gradient series of ethanol solutions (95%, 75%, 50%, 25% and 10% ethanol) and embedded in paraffin. Tissue sections of 4 μm thickness were cut with a rotary microtome (RM2235, Leica Microsystems Ltd., Karlsruhe, Germany). The tissue sections were stained with toluidine blue, observed with a light microscope (ECLIPSE Ts2, Nikon Instruments Inc., Tokyo, Japan), and captured by a digital camera (Digital Sight DS-U3, Nikon Instruments Inc., Tokyo, Japan). ImageJ was used to measure the thickness of the leaf, spongy tissue, palisade tissue, and epidermal cells on the upper and lower sides. The parameters of the leaf tissue compactness (LC), leaf tissue looseness (LL), and the ratio of palisade tissue to spongy tissue (P/C) were calculated based on the following formula [38]:

$$LC = PT/LT, \quad (1)$$

$$LL = ST/LT, \quad (2)$$

$$P/C = PT/ST, \quad (3)$$

where *LC* is leaf tissue compactness, *PT* and *LT* are the thickness of the palisade tissue and leaf, respectively, *LL* is the leaf tissue looseness, *ST* is the thickness of spongy tissue, and *P/C* is the ratio of palisade tissue to spongy tissue.

2.4. Determination of Stomatal Structure Characteristics

From the base 1/3 of the fully unfolded penultimate compound leaf near the central vein, a small piece of 1 cm × 1 cm was cut. The samples were immediately placed into FAA fixative solution and fixed in a refrigerator at 4 °C for more than 48 h. Ethanol was used to perform step-by-step dehydration as follows: 50% ethanol soaked for 1 h, 70% ethanol soaked for 1 h, 85% ethanol soaked for 1 h, 95% ethanol soaked for 40 min twice, and 100% ethanol soaked for 40 min twice. The dehydrated samples were soaked in 5% sodium hydroxide solution for 42 h or more until the leaves turned white. The deionized water was used to clean the samples twice. Then, the samples were transferred to a saturated trichloroacetaldehyde solution. After completely transparent samples were obtained, deionized water was utilized to clean the trichloroacetaldehyde. Finally, each sample was stained with 1% toluidine blue aqueous solution and observed with an optical microscope (Nikon Eclipse 50I, Tokyo, Japan). A digital camera (Sight DS-U3, Nikon Instruments Inc., Tokyo, Japan) was used [39]. The SZ (stomatal long axis length) and SD (mm^{-2}) on the upper and lower epidermis were measured using ImageJ in five visual fields for four replicates of each treatment.

2.5. Photosynthesis Measurements

Under each treatment, the fully unfolded penultimate compound leaves of five plants were used. The acquisition of photosynthetic parameters was conducted in a 6 cm^2 leaf chamber with a CO_2 concentration of 400 μmol mol^{-1} using a portable infrared gas analyzer (Li-6400, LI-COR Inc., Lincoln, USA). The spectral composition of LICOR 6400 was a combination of red and blue light with a ratio of 3:2, which was different from the growth light environment of the plants in the experiment. Fourteen light intensity levels (0, 20, 50, 100, 150, 200, 400, 600, 800, 1000, 1200, 1500, 2000 and 2300 μmol m^{-2} s^{-1}) were imposed at increments of 2 min. The net photosynthetic rate and operational stomatal conductance (g_{op}) response curve to light under different treatments were fitted with a smooth curve.

2.6. Statistical Analysis

In this experiment, SPSS statistics 25 was used to compare the differences between treatments via a one-way analysis of variance. Mean ± standard error values are presented for all statistical analyses, and at least three biological replicates were analyzed for significant differences at the 5% level in all parameters. Microsoft Excel 2016 was utilized for data wrangling, and the figures were drawn using Origin 2021b.

3. Results

3.1. Leaf Structure Response to Blue and Red Light

The response of soybean leaf structures to blue and red light at different light intensities is shown in Table 1. The SLW, leaf thickness, palisade tissue thickness, and spongy tissue thickness under 100 B treatment was significantly decreased compared with those under 400 B treatment. The LL and P/C of spongy tissue under 400 B were not significantly different from that under 100 B. However, the LC significantly increased by 9% under 100 B, as compared with 400 B. The thicknesses of the leaf, palisade tissue, spongy tissue, upper epidermal cells, and lower epidermal cells of soybean leaves under the 100 R treatment were significantly lower than those under 400 R. Nevertheless, the LC and SLW under 400 R were not significantly different from those under 100 R. Regardless of blue or red light, low light intensity inhibited the development of leaf structure, but the regulation of SLW was highly dependent on blue light.

Table 1. Soybean leaf structure under different light intensities of blue and red light: 400 B and 100 B denote the blue light with 400 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}, respectively. 400 R and 100 R denote the red light with 400 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}, respectively.

Treatments	400 B	100 B	400 R	100 R
SLW (mg/cm^2)	0.223 ± 0.04 a	0.185 ± 0.0 b	0.14 ± 0.01 c	0.125 ± 0.01 c
LT (μm)	115.678 ± 4.4 a	63.252 ± 4.5 c	80.334 ± 6.2 b	55.401 ± 2.9 d
UET (μm)	12.346 ± 1.7 a	5.813 ± 0.9 b	12.976 ± 2.1 a	6.581 ± 1.1 b
LET (μm)	7.161 ± 1.2 a	4.081 ± 1 b	6.979 ± 1.2 a	4.453 ± 0.7 b
PT (μm)	68.541 ± 4.4 a	40.826 ± 3.3 b	49.209 ± 5.3 c	24.386 ± 2.2 d
ST (μm)	33.485 ± 3.8 a	19.014 ± 2.8 b	17.873 ± 2.1 b	14.923 ± 1.5 c
LC	0.593 ± 0.04 b	0.649 ± 0.08 a	0.614 ± 0.07 ab	0.622 ± 0.04 ab
LL	0.289 ± 0.03 ab	0.298 ± 0.04 a	0.221 ± 0.02 c	0.268 ± 0.03 b
P/C	2.07 ± 0.25 c	2.19 b ± 0.35 bc	2.805 ± 0.3 a	2.35 ± 0.25 b

Notes: SLW—specific leaf weight; LT—leaf thickness; UET—upper epidermal cell thickness; LET—lower epidermal cell thickness; PT—palisade tissue thickness; ST—spongy tissue thickness; LC—leaf tissue compactness; LL—leaf tissue looseness; P/C—ratio of palisade tissue to spongy tissue. The mean ± SE is expressed by each value. Different lowercase letters indicate significant differences between treatments ($p \leq 0.05$).

3.2. Stomatal Size of the Two Sides

Figure 2 shows the SZ of the upper and lower epidermises response to different light environments. The SZ on the upper and lower epidermis under 100 B was significantly increased compared to those under 100 R. The SZ of the upper epidermis under 400 R was considerably higher than that under 400 B and no significant difference was observed in the lower epidermis (Figure 2B,D). As the light intensity decreased from 400 to 100 μmol m^{-2} s^{-1}, it significantly reduced the SZ on the upper and lower epidermis by 17.5% and 11.7%, respectively, under red light. In contrast, the significant reduction in light intensity under blue light (400 and 100 B) did not affect the SZ of the upper and lower epidermis (Figure 2B,D). These results showed that SZ was susceptible to red light.

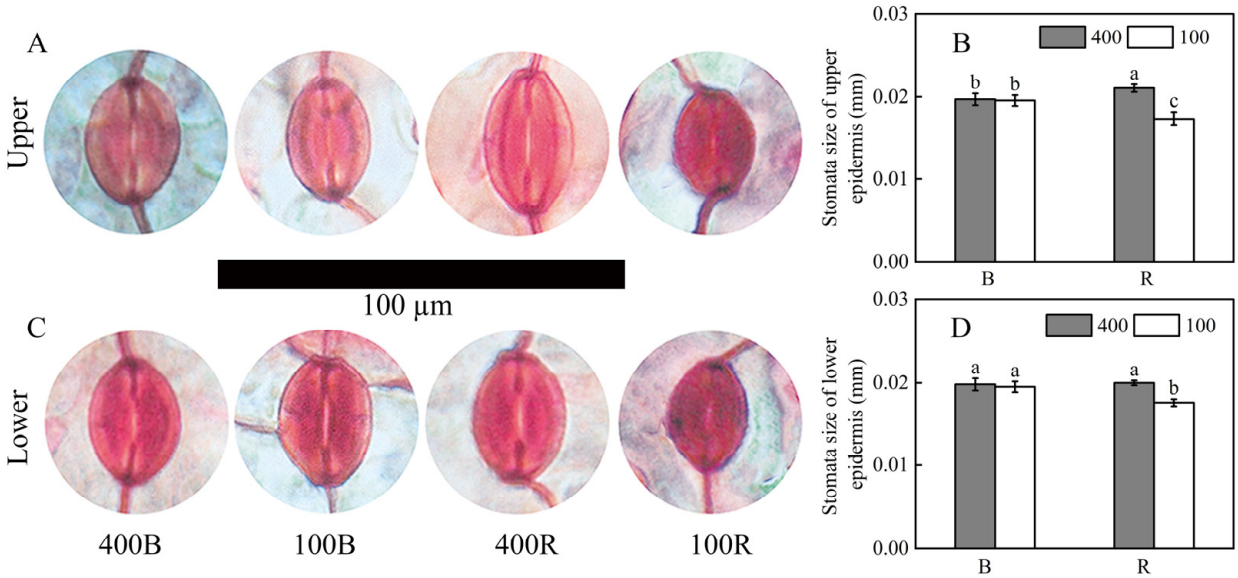


Figure 2. The response of stomatal size to various light treatments. (**A**,**B**) Stomatal size on the upper epidermis. (**C**,**D**) Stomatal size on the lower epidermis. 400 B and 100 B denote the blue light with 400 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}, respectively. 400 R and 100 R denote the red light with 400 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}, respectively. The mean ± *SE* is expressed by each value. Different lowercase letters indicate significant differences between treatments ($p \leq 0.05$).

3.3. Stomatal Density of the Two Sides

The SD of the two sides in soybean leaves is shown in Figure 3. There was no significant difference between the SD under 400 R and 100 R on the upper and lower epidermis. The SDs of the upper and lower epidermises at 400 B were significantly higher than those at 400 R. Compared to 100 R, the 100 B treatment significantly improved the SD on the upper epidermis but had no significant impact on the SD of the lower epidermis. In addition, the 100 B treatment significantly reduced the SD of the upper and the lower epidermis by 33.2% and 29.5% compared with those under 400 B, respectively (Figure 3B,C). Although light intensity could significantly regulate the SD of the upper and lower epidermis, blue light was indispensable.

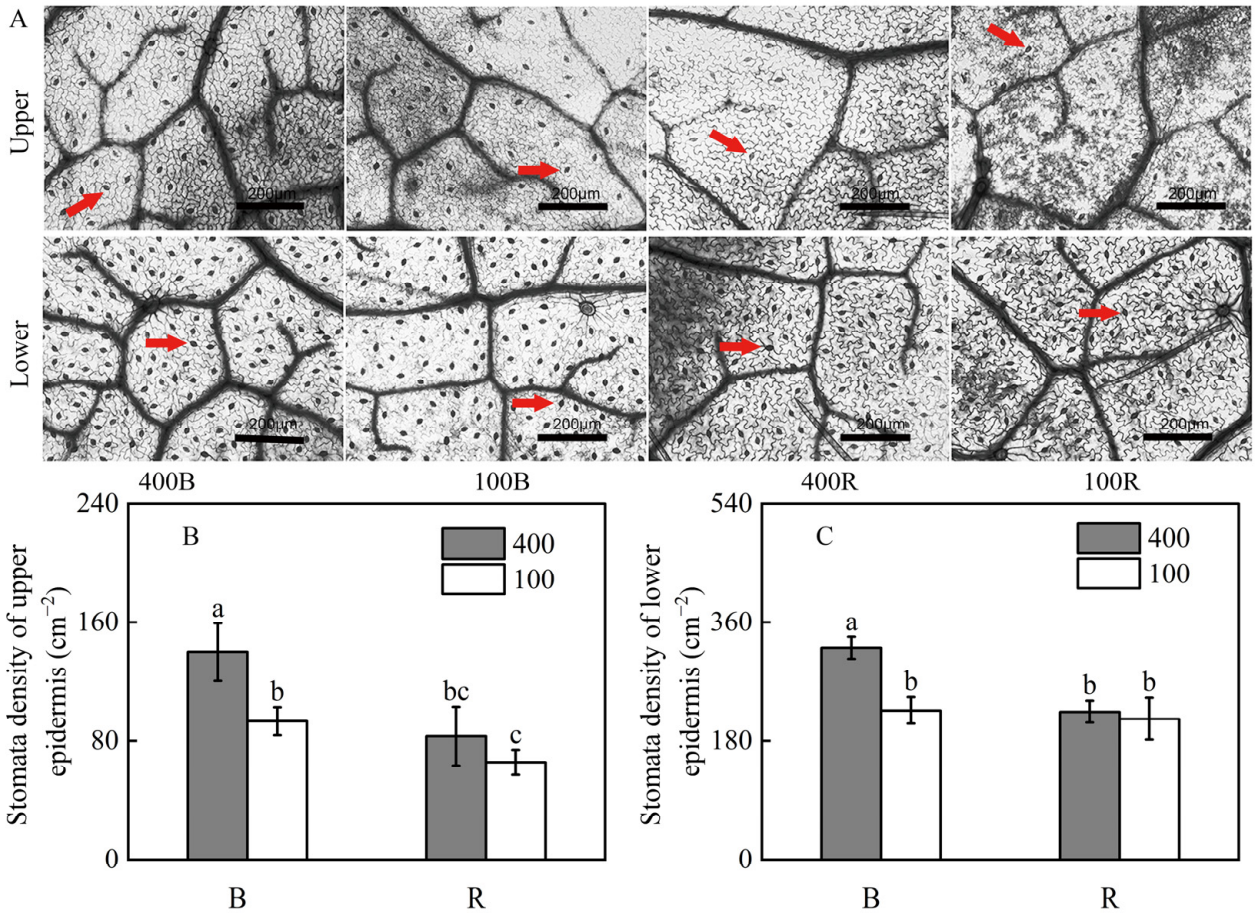


Figure 3. Stomatal density under different light intensities of blue and red light. (**A**) Photos; (**B**) the stomatal density of the upper epidermis; (**C**) the stomatal density of the lower epidermis. 400 B and 100 B denote the blue light with 400 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}, respectively. 400 R and 100 R denote the red light with 400 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}, respectively. Black spots indicate stomata. The stomatal density is higher when the number of black spots is higher. The mean ± SE is expressed in each value. Different lowercase letters indicate significant differences between treatments ($p \leq 0.05$).

3.4. The Response Curves of Light Intensities

With the light intensity increasing, the net photosynthetic rate of soybean leaves under various treatments gradually rose and remained stable after reaching the maximum (Figure 4A). The statistical analysis for the maximum net photosynthetic rate indicated that low light and red light significantly decreased the maximum net photosynthetic rate (Figure 4B) compared with high light and blue light, respectively. Under the same light intensity, the g_{op} of soybean leaves in the red light treatment was significantly lower than

that under blue light. In addition, the high light intensity increased the g_{op} compared with the low light intensity under the same light quality (Figure 4C). In terms of the operational maximum stomatal conductance (g_{opmax}), the treatments were ordered from highest to lowest as follows: 400 B > 100 B> 400 R > 100 R treatments (Figure 4D). The g_{opmax} under 100 B was significantly lower than that under 400 B. However, the g_{opmax} between 400 R and 100 R showed no significant effects. The blue light was more critical in regulating the gas exchange capacity than red light.

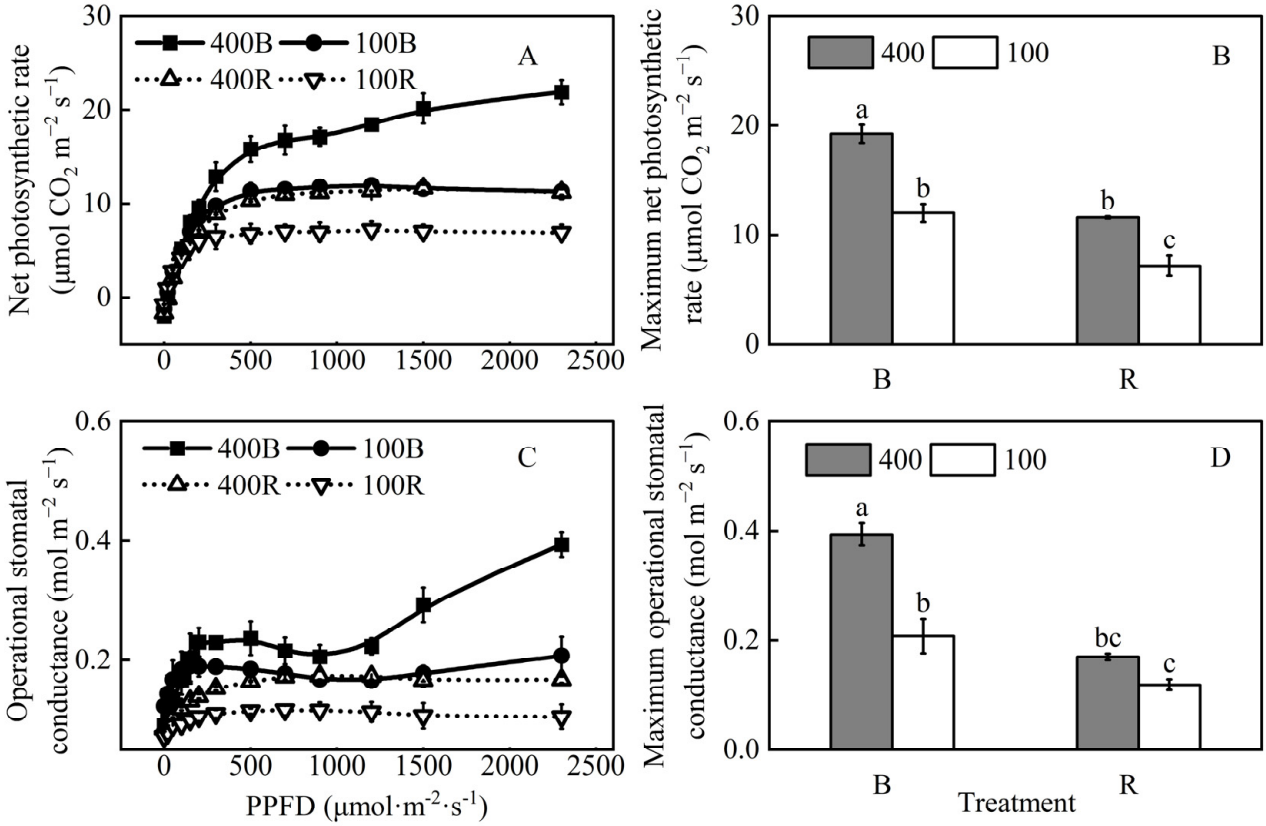


Figure 4. Effects of different light treatments on photosynthetic parameters. (**A**,**C**) The response curves of net photosynthetic rate and operational stomatal conductance (g_{op}). (**B**,**D**) The maximum net photosynthetic rate and operational maximum stomatal conductance (g_{opmax}). 400 B and 100 B denote blue light with 400 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}, respectively. 400 R and 100 R denote the red light with 400 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}, respectively. The mean ± SE is expressed in each value. Different lowercase letters indicate significant differences between treatments ($p \leq 0.05$).

3.5. Relationship between Net Photosynthetic Rate and Operational Stomatal Conductance

The dynamic g_{op} positively correlated with the net photosynthetic rate under 400 B, 100 B, 400 R, and 100 R. The correlation coefficients were as high as 0.841, 0.992, 0.876 and 0.983, respectively (Figure 5). These results showed that net photosynthetic rate was highly correlated with g_{op} under different light quality and intensity environments.

3.6. Contribution of Leaf Characteristics and Stomatal Traits to Maximum Operational Stomatal Conductance

Leaf characteristics and stomatal traits greatly affected the g_{opmax} of soybean leaves. The contribution rates of leaf characteristics to g_{opmax} reached 60.48%. The main leaf traits were SLW, leaf thickness, palisade tissue thickness, and spongy tissue thickness, whose contribution rates to g_{opmax} were 19.43%, 15.65%, 13.39% and 12.01%, respectively (Figure 6A). Stomatal traits contributed to g_{opmax} by 24.22%, mainly including SD on the

upper (9.75%) and lower epidermis (9.7%). By contrast, g_{opmax} was nearly unaffected by SZ. The correlation analysis also proved that leaf characteristics and stomatal traits had a highly significant positive correlation with g_{opmax} (Figure 6B). The SD was positively correlated with leaf characteristics but not with the SZ of the upper and lower epidermis.

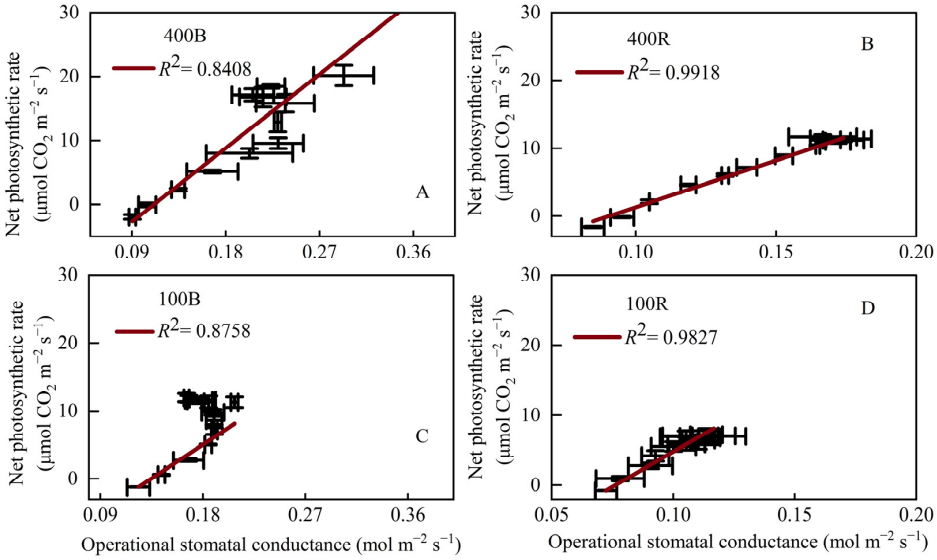


Figure 5. The correlation of net photosynthetic rate and operational stomatal conductance (g_{op}). (**A**) 400 B; (**B**) 400 R; (**C**) 100 B; (**D**) 100 R. 400 B and 100 B denote the blue light with 400 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}, respectively. 400 R and 100 R denote the red light with 400 μmol m^{-2} s^{-1} and 100 μmol m^{-2} s^{-1}, respectively. Values represent the mean of three replicates. The bars represent the standard error.

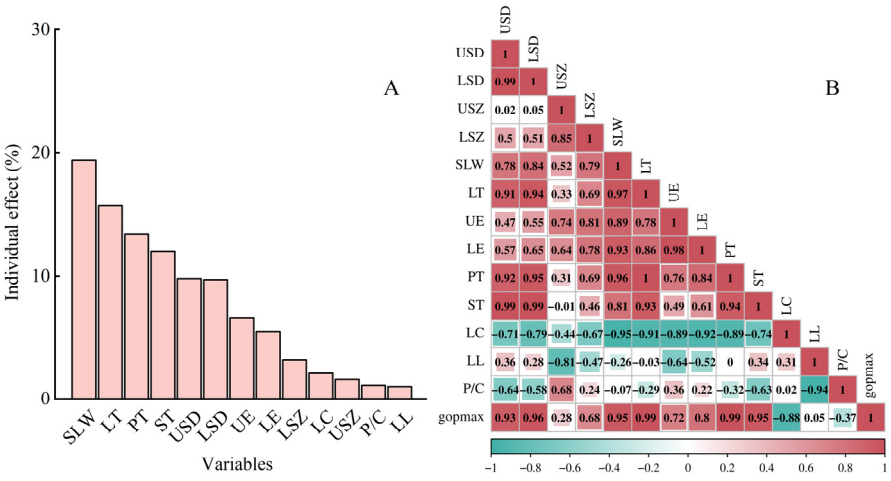


Figure 6. The relative importance of individual variables on g_{opmax} and the correlation between g_{opmax} and influence variables. (**A**) The contribution value analysis; (**B**) the correlation analysis. Blue indicates a passive correlation, and red shows a positive correlation. The darker color indicates a more robust correlation. Note: g_{opmax}—maximum operational stomatal conductance; LC—leaf tissue compactness; LET—lower epidermal cell thickness; LT—leaf thickness; LL—leaf tissue looseness; LSD—stomatal density of the lower epidermis; LSZ—stomatal size of the lower epidermis; P/C—ratio of palisade tissue to the spongy tissue; PT—palisade tissue thickness; SLW—specific leaf weight; ST—spongy tissue thickness; UET—upper epidermal cell thickness; USD—stomatal density of the upper epidermis; USZ—stomatal size of the upper epidermis.

4. Discussion

Light quality can affect the arrangement of palisade and spongy cells and, thus, affect photosynthesis [25,40]. However, the response of leaves to various light qualities differed (Table 1). In many plant species, red and blue combined light and blue light affect the growth of palisade tissue when compared with red light and white light [25]. In the present study, when the light intensity was significantly reduced, the LC was unchanged under red light but increased under blue light. On the contrary, the LL did not significantly change under blue light and improved under red light. The results suggested that the blue light partially alleviated the reduced thickness of palisade tissue caused by the decreased light intensity but had no effect on the proportion of spongy tissue (Table 1), thereby increasing the proportion of palisade tissue in leaves. This may be because palisade histiocyte development depends on the activation of phototropin under blue light [41–44]. In addition, our results also showed that the decreased light intensity under red light increased the proportion of spongy tissue in the leaf. This may be because red light did not change the proportion of palisade tissue (Table 1) and simultaneously alleviated the reduction in spongy tissue thickness caused by declined light intensity.

In addition, the SZ under blue light was not significantly different from high to low light intensity but was decreased considerably under red light (Figure 2). These results suggested that SZ was highly dependent on the red light. Seif et al. [45] also found that red light reduces stomatal size but promotes gas exchange in the chrysanthemum. With light intensity significantly reduced in the current study, the SD and SLW under red light were unaffected but decreased observably under blue light. These results proved that SD and SLW were highly sensitive to blue light (Figure 3). This is consistent with the results of previous studies, where SLW is much less affected than the maximum net photosynthetic rate in the spectrum containing relatively little blue [24,46]. Increasing blue light under red light could increase SD [47,48]. Our results showed that blue and red lights specifically regulated SD and SZ. However, different light qualities usually regulate plant development in different ways. In stomatal movement, the stomatal blue light response is mediated by blue light photoreceptor protein kinases [30,33,35]. The red light required the involvement of a mesophyll signal to regulate stomatal opening [49]. Therefore, SD and SZ responding specifically to blue and red light may also be regulated by corresponding photoreceptors, but further study is needed.

A high correlation between leaf characteristics and stomatal traits (Figure 6B) indicated that they could influence and coordinate with each other. Some previous studies found that stomatal development of stomata-specific regulators could alter leaf structural changes to obtain a high g_{smax} [14,15]. In the present study, the SD was highly correlated with functional leaf characteristics, such as SLW and leaf thickness, among which the SD correlated most closely with leaf thickness. In contrast, no correlation was observed between SZ and leaf anatomical characteristics, regardless of the upper or lower epidermis. Previous studies have shown that the epidermal pattern factor increases the density of palisade mesophyll cells below the paraxial epidermis [15].

Manipulating transcription factor SPEECHLESS's activity can change the degree of stomatal lineage proliferation, which increases leaf area and thickness [50,51]. Our results suggested that specific regulators of SD were more pivotal in influencing leaf development, especially for leaf tissue development, than SZ for the high gas exchange capacity obtained. For SZ, many studies have reported that small stomata usually accelerate the stomatal response because the membrane surface area is larger, relative to the volume ratio of guard cells. Thus, increasing the rate of ion flux is conducive to a rapid response to fluctuating light environments, which improves the photosynthetic rate [52–54]. However, the association of stomatal response speed with SZ varies across many species [55,56], and the molecular genetic mechanisms that control SZ remain unclear. Thus, further investigations are needed.

Previous studies have widely proven the intense influence of gas exchange capacity on photosynthesis [7,57–59]. We also demonstrated that the net photosynthetic rate was

positively associated with stomatal conductance under blue and red light (Figure 4). Stomatal conductance is related to SZ and SD [53,60,61]. A study discovered that the structural differences between the leaves of angiosperms and ferns partly explain the decline in gas exchange capacity in ferns, according to the comparison of the leaf anatomical characteristics between ferns and angiosperms [62]. In particular, leaf thickness affects the transport of water and CO_2, which influences stomatal conductance [63–65].

Moreover, SD and SZ have a significantly negative correlation, determining g_{smax} [53,64]. Nevertheless, there was nearly no connection between SZ and SD in the present study (Figure 6B). This finding is the same as previous research, where SD and SZ showed no coincident trend during the breeding process of japonica rice varieties [66]. Although increasing g_{op} within species is possible, by decreasing SZ and increasing SD, this trend may only apply to some species due to variations in the natural habit of species [52,53]. In this study, we quantified the relative importance of individual structural variables in g_{opmax} using rdacca.hp [67], which indicated that the leaf characteristics SLW and SD strongly affected g_{opmax} (Figure 6). These results are consistent with previous studies, which proposed that SZ and g_{opmax} had no significant correlation. High SD significantly promoted g_{opmax} [12]. By practical consequence, leaf characteristics had a more substantial ability to regulate g_{opmax} than SD. Especially in a red-light background, we found that g_{opmax} was concordant with SD and SLW and unaffected by the light intensity change (Figure 6). This result indicated that SD and SLW regulation depended on blue light. Beyond that, the SLW and SD harmoniously improved gas exchange and photosynthetic capacity under blue light. Among them, the SLW appeared to be more critical in influencing g_{op}. As a result, the amount of SD in plants is reduced by approximately half and has no adverse effects on plant growth and development [8,68,69]. However, broader studies and more profound research are needed to elucidate the specific mechanisms.

5. Conclusions

The stomatal traits and leaf characteristics of soybean leaves had different responses to blue and red light during their growth and development. Nevertheless, stomatal traits were usually coordinated with leaf traits to better adapt to environmental changes. Our results were as follows: (1) Light intensity and quality could significantly affect the development of leaf characteristics and stomatal traits. (2) The stomatal density and specific leaf weight were blue light-dependent, while the stomatal size was highly sensitive to red light. (3) Although the specific leaf weight had a more significant effect on stomatal conductance than stomatal density, the improvement in stomatal conductance under blue light was more due to the long-term synergistic promotion of stomatal density and specific leaf weight. These results emphasized that coordinated optimization among plant leaf traits may be a new direction for improving photosynthetic capacity.

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