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Effect of Different Ratios of Red and Blue Light on Maximum Stomatal Conductance and Response Rate of Cucumber Seedling Leaves

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Abstract: Light can regulate leaf stomatal development and movement, but the effects of different red-to-blue light mass ratios on leaf stomatal morphology and openness are not fully understood. In this trial, five different red-to-blue light (R:B) ratio treatments were used to study the changes in morphology, photosynthesis, and stomatal-related indexes of cucumber seedlings under fixed light intensity (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The results showed that the thickness of spongy tissue and stomatal size (SZ) of cucumber seedling leaves decreased, and the photosynthetic potential, stomatal density (SD), maximum stomatal conductance and stomatal responsiveness increased with decreasing R:B content. The experimental results showed that when R:B = is 1:9, cucumber seedlings had the greatest stomatal density and the fastest response rate, and the stomatal opening rate was accelerated with the increase in the proportion of blue light; when R:B = is 3:7, the stomatal conductance was the greatest and the net photosynthetic rate was the highest. This trial provides some implications for changing plant light quality and thus affecting stomatal development and movement.

Keywords: red light; blue light; stomatal morphology; stomatal density; stomatal dynamics

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1. Preface

A stomatal pore is a microscopic structure consisting of a pair of guard cells surrounding a central pore. Plants actively regulate the stomatal aperture by sensing environmental changes and adjusting the expansion pressure of the guard cells, thereby regulating the rate of gas exchange between the interior and exterior of the leaf [1,2]. High stomatal conductance promotes net photosynthetic rate, but at the cost of greater water dissipation, making plants more susceptible to drought stress [3–5]; low stomatal conductance, while helping plants to better adapt to adversity, will limit CO_2 diffusion and photosynthesis under well-watered conditions, thus negatively affecting biomass accumulation [4,6–8]. By controlling the expansion of guard cells, plants can precisely balance the relationship between carbon dioxide and water loss [9–11], allowing them to minimize water loss while maximizing CO_2 supply [6]. However, in the face of environmental changes, stomatal responses (closure or opening) are often an order of magnitude slower than photosynthetic responses, which leads to a mismatch between stomatal conductance (g_s) and carbon assimilation (A) [12], a lag effect that can lead to restricted carbon assimilation or increased unnecessary water dissipation [13]. Therefore, it has been suggested that enhancing the speed of stomatal response to environmental changes could significantly enhance plant carbon assimilation and water use efficiency [14].

The rate at which stomata respond to environmental changes depends on their own characteristics in addition to the environmental changes themselves, with the size and

density of the stomata influencing the rate of stomatal response and maximum stomatal conductance. It has been shown that smaller stomata respond faster than larger stomata, due to their larger defense cell membrane area to volume ratio and the faster rate of solute change within them [15,16]. Changes in stomatal density have been one of the important strategies used by plants to respond to changes in environmental signals [17], and it has been shown through genetic modification (GM) that reducing stomatal density through manipulation can improve water use efficiency without affecting plant yield [18,19] and can reduce the entry of bacteria and other pathogens [20]. On the other hand, increasing stomatal density by GM can promote CO_2 diffusion and improve photosynthetic efficiency, although this comes at the expense of water use efficiency [21].

Light is not only a source of energy for photosynthesis, but also a key signal regulating the growth and development of multifaceted plants [22]. Moreover, the development and the opening or closing of stomata are influenced by the environment in which the plant is exposed [21]. Under light conditions, the red and far-red photoreceptors (phyB and phyA) and the blue photoreceptors (CRY1/2) act synergistically to inhibit the constitutive photomorphogenic1 (*COP1*) activity as well as that of *PIF4* (one of phytochrome-interacting factors) and associated downstream networks, thereby promoting stomatal development [23]. The response of stomatal movement to light depends mainly on two signaling mechanisms, namely the red and blue light response [24–27]. Red light acts as both a signal and an energy source as it induces the opening of stomata in chloroplasts of leaf pulp and guard cells through photosynthesis, reducing the intercellular carbon dioxide concentration (Ci) [24]. The red light-driven response of stomata is similar to the carbon assimilation response to increased light intensity [28], and the red light response of stomata is mediated by a part of the low CO_2 regulatory network [29]. Further related studies have shown that the red light response is associated with anion transport [30–32], sucrose metabolism [33–35] and guard cell photosynthesis [36–40]. Blue light-induced stomatal opening differs from the red light response in that it does not require the involvement of a chloroplastic signal [41], and on a quantum basis, the stomatal blue light response drives stomatal opening 20 times more efficiently than red light [26,42]. Moreover, even when photosynthesis saturates under red light, the addition of blue light still increases stomatal opening [26]. The blue light response of stomata is important for morning stomatal opening for carbon assimilation [1]. It has been demonstrated that the sensitivity of the stomatal response to blue light depends on the background red light intensity [43], with g_s increasing when blue light is applied to red light compared to for red light alone; on the other hand, little stomatal response is observed to weak blue light when red light is not present [26].

Therefore, we measured leaf characteristics such as stomatal traits, gas exchange capacity and photosynthetic capacity of cucumber seedlings treated with different ratios of red and blue light. The aim of this study is to address the response of leaf characteristics and stomatal traits to different ratios of red and blue light, and to provide some reference value for studies that can influence relevant physiological properties of plants by altering their light environment.

2. Materials and Methods

2.1. Test Materials

This trial was conducted in October 2022–December 2022 at the Laboratory of Biological and Environmental Engineering for Facility Agriculture, College of Horticulture, Northwest Agriculture and Forestry University of Science and Technology. The test material was *cucumber* 'Xinjin You No. 1' (*Cucumis sativus* L.) and seeds were purchased from Shandong Taian Huayi Seed Co. (Taian, China). The light source was LED light board and LED control system V1.0 produced by Xi'an InChange Photoelectric Technology Co. (Xi'an, China).

Cucumber seeds were sown in 10 cm × 10 cm seedling pots in substrate culture. The seeds were sown at a depth of 1.5 cm in a nursery substrate, and covered with a thin layer of vermiculite about 1 cm thick. They were grown in an artificial climate chamber with a light

intensity of 150 μmol·m^{-2}·s^{-1}, day and night temperatures of 25 °C and 20 °C, respectively, a photoperiod of 12 h/12 h, relative humidity of 60% and the substrate was kept moderately hydrated by rehydrating once a day in the morning and once at night. Once their cotyledons had fully expanded, after around 3 d of growth, they were transplanted into a cultivator equipped with an LED light source and watered with 1/4 Hoagland Cucumber nutrient solution (pH 6.5 ± 0.1, EC 2.2–2.5 ms/cm); here, day and night temperatures of 28 °C and 25 °C, respectively, and relative humidity of 40–50% were maintained for 25 d by when about 4 leaves and 1 heart would have grown.

2.2. Experimental Design

The total light intensity of the test was set at 200 μmol·m^{-2}·s^{-1}, the photoperiod 12 h/12 h, the light intensity settings for each treatment were as shown in Table 1, and the spectra of each treatment were plotted as shown in Figure 1.

Table 1. Intensity of red light, blue light and green light under different treatments.

Treatments	Red Light (μmol·m^{-2}·s^{-1})	Blue Light (μmol·m^{-2}·s^{-1})	R:B	PAR (μmol·m^{-2}·s^{-1})
R:B = 1:9	20	180	1:9	200
R:B = 3:7	60	140	3:7	200
R:B = 1:1	100	100	1:1	200
R:B = 7:3	140	60	7:3	200
R:B = 9:1	180	20	9:1	200

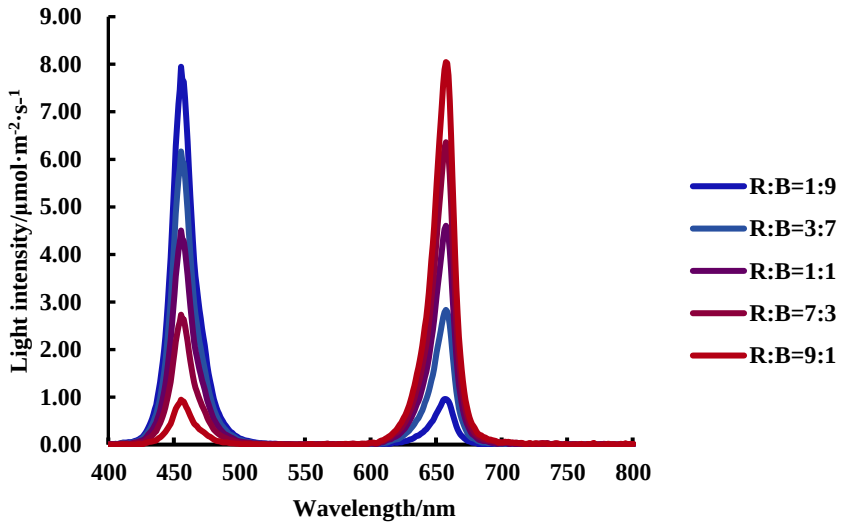


Figure 1. Spectrograms of different scales of processing.

2.3. Item Determination

2.3.1. Morphological Indicators

Six cucumber seedlings were randomly selected when their growth had reached 4 leaves and 1 heart. Their above-ground height was determined using a ruler; stem thickness was determined using a vernier caliper; leaf area was determined using a leaf area meter (AM350 portable leaf area meter, ADC Bioscientific, Hoddesdon, UK); and weight was determined using an electronic balance.

2.3.2. Determination of Blade Structure Parameters

For each treatment, three cucumber seedlings were randomly selected, the third true leaf was taken and a 10 mm × 10 mm large leaf was taken at the same leaf position avoiding the main leaf veins, placed in FAA fixative (100 mL of which contained: 50 mL 95% ethanol,

5 mL acetic acid, 10 mL 37% formaldehyde, 35 mL distilled water), then dehydrated in ethanol, rendered transparent in xylene, stained in pink-solid green and embedded in paraffin to make the final paraffin sections. These were placed under a microscope (Olympus BX63, Olympus LS, Tokyo, Japan) for observation: 10 were selected for each mount leaf thickness, upper and lower epidermal thickness, fenestrated tissue thickness and spongy tissue thickness and were all measured by ImageJ V1.8 software.

2.3.3. Measurement of Photosynthetic Properties

Three cucumber seedlings were randomly selected from each treatment, their second true leaves were taken and their transpiration rate, net photosynthetic rate, intercellular CO_2 concentration and stomatal conductance were measured using a plant photosynthesis meter 6800 (LI-6800, LI-COR Biosciences, Lincoln, NE, USA). The leaf chamber temperature was set at 24 °C, the CO_2 level at 400 μmol/mol, the relative humidity at 60%, and the light source set at R90B10 with a light intensity of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

2.3.4. Stomatal Characteristics

Three cucumber seedlings were randomly selected for each treatment, and the same leaf position of the third leaf was taken to make a clinical slide. The leaf was placed on transparent tape, lightly pressed to make a tight bond and then lightly scraped with a razor blade to remove the leaf flesh tissue. the mount was attached to a slide, and the section was observed under a microscope. Stomatal density was measured at 20× and stomatal size was measured at 40×. For each mount, ten fields of view were selected and 20 stomata were selected for each field of view. Stomatal length, stomatal width, pore length and pore width were measured by ImageJ software.

2.3.5. Stomatal Movement Measurements

Cucumber seedlings were randomly selected from each treatment, their 3rd true leaf was taken and their stomatal conductance was measured using a plant photosynthesis meter 6800 (LI-6800, USA). The leaf chamber temperature was set at 24 °C, the CO_2 level at 400 μmol/mol and the relative humidity at 60%. Before the plants were exposed to light, the leaves were acclimatized by clamping them in the LI-6800, and after the data had stabilized, the stomatal conductance, net photosynthetic rate and intercellular CO_2 concentration were recorded under dark conditions, once every 60 s. After 20 min of recording, the light intensity and light quality of the built-in light source in the leaf chamber were made consistent with the ambient light, and the stomatal-related data of seedlings in each treatment group under ambient light were recorded, once every 60 s. After 60 min, the light was switched off and stomatal data were recorded for each treatment group in the dark, once every 60 s. The measurements were completed before 12 noon to ensure that the stomatal opening and closing rhythm was maintained.

2.3.6. RNA Extraction and RT-PCR for Gene Expression Analysis

After 2 h of light treatment, 2 g of young apical tissue was taken, placed in 1.5 mL centrifuge tubes and rapidly frozen in liquid nitrogen, followed by high-frequency shaking using a high-throughput grinder. Stomatal development-related genes from the cucumber genome database (http://cucurbitgenomics.org/v2/, accessed on 23 December 2022) and SLAC1 [44] (a key gene for stomatal movement) were selected, and primers were designed by Primer Premier 5.0—the primer sequences are shown in Table 2. The extracted RNA was reverse transcribed into cDNA using the Reverse Transcription Kit (Cofitt, Chengdu, China) according to the standard reagent procedure. A volume of 2 × qPCR SmArt Mix (SYBR Green) kit (Dr. Di, China) was used for the RT-PCR reaction, using the $^{-\Delta\Delta Ct}$ analysis method.

Table 2. Primers are used for RT-PCR detection.

Gene	Forward Primer	Reverse Primer
Action	5′-CACCAAGCCCAAGAAGATC-3′	5′-TAAACCTAATCACCACCAGC-3′
CsaV3_1G006250	5′-CAGTGCCTACTGAATCAAGCGACTC-3′	5′-GTAGGTACTGCCACGATTGGATGG-3′
SLAC1	5′-CTCGGCCAACAATGACATCAGC-3′	5′-CAAACCCGTTTCCAGCGACATC-3′

2.4. Stomatal Movement Rate Analysis

The stomatal movement response curve was fitted using Matlab R2019b and its equation obtained. Curvature was calculated and plotted for each point on the equation to reflect the stomatal response rate for each treatment.

Stomatal opening rates were calculated with reference and minor modifications to the method of Lorna McAusland et al. [13].

Taking G_{smax} as the stomatal conductance to reach steady state in ambient light, G_{smin} as the stomatal conductance to reach steady state in darkness, k_i as the time constant for g_s to increase to G_{smax} in ambient light, S_{lmax} as the maximum rate of opening of g_s to increase photosynthetic photon flux density(PPFD) from 0 to 200 μmol·m^{-2}·s^{-1}, and r_0 as the minimum g_s for the s-type response of g_s to an increase in PPFD, then:

$$S_{l\max} = \frac{k_i(G - r_0)}{e}$$

2.5. Statistical Analysis

All statistical analyses were performed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). The significance of differences between mean values was assessed by one-way analysis of variance (ANOVA) according to Tukey's test ($p < 0.05$).

3. Results

3.1. Effect on Morphological Indicators of Cucumber Seedlings

As shown in Table 3, within a certain range, cucumber seedling height, fresh weight, total leaf area and dry weight increased with increasing the red to blue light ratio, but the growth promotion effect on the cucumber seedlings was reduced once the R:B ratio was too high. Comparing the fresh weight of R:B = 3:7 with R:B = 1:9, R:B = 3:7 and R:B = 1:1 groups, the former increased over the latter in the order of 32.55%, 24.27% and 19.97%, but the fresh weight of R:B = 9:1 treatment decreased by 14.05% compared to R:B = 7:3.

Table 3. Effects on morphological indices of cucumber seedlings.

Treatments	Plant Height (cm)	Stem Diameter (mm)	Total Leaf Area (cm^2)	Fresh Weight (g)	Dry Weight (g)
R:B = 1:9	16.020 ± 1.260 d	6.320 ± 1.005 a	492.935 ± 34.325 c	18.624 ± 2.804 c	1.644 ± 0.162 a
R:B = 3:7	17.270 ± 0.610 d	5.960 ± 1.109 a	557.733 ± 54.466 b	20.910 ± 0.595 bc	1.818 ± 0.170 a
R:B = 1:1	20.320 ± 1.850 bc	5.457 ± 1.22 a	606.001 ± 12.166 ab	22.095 ± 1.780 bc	1.954 ± 0.136 a
R:B = 7:3	32.900 ± 3.310 a	6.230 ± 1.017 a	638.663 ± 15.177 a	27.610 ± 0.690 a	2.005 ± 0.035 a
R:B = 9:1	32.300 ± 1.630 a	6.143 ± 1.289 a	629.950 ± 30.9701 a	23.731 ± 1.982 b	1.820 ± 0.099 a

Note: Within the same column, different letters represent significant differences ($p < 0.05$), while the same letters represent no significant differences ($p > 0.05$).

3.2. Effect of Different Red and Blue Light Ratios on Leaf Structure

As shown in Table 4, the inferior epidermis, palisade tissue and blade thickness of cucumber seedlings showed a significant decrease with increasing red light ratio under each treatment; the thickness of palisade tissue decreased by 44.43% in the R:B = 9:1 treatment compared to the R:B = 1:9 treatment, and blade thickness decreased by 28.01% in the R:B = 9:1 treatment compared to the R:B = 1:9 treatment. Both their upper epidermal and spongy tissues decreased to some extent with increasing red light ratio.

Table 4. Effects on structural parameters of cucumber seedling leaves.

Treatments	Upper Epidermis (μm)	Palisade Tissue (μm)	Spongy Tissue (μm)	Inferior Epidermis (μm)	Blade Thickness (μm)
R:B = 1:9	15.72 ± 0.905 a	69.001 ± 1.491 a	88.87 ± 5.443 ab	8.941 ± 0.547 a	188.964 ± 5.731 a
R:B = 3:7	13.148 ± 1.026 b	52.214 ± 1.488 bc	92.196 ± 3.804 a	8.27 ± 0.738 a	182.772 ± 13.120 a
R:B = 1:1	8.854 ± 0.570 c	48.845 ± 1.542 c	78.079 ± 2.331 c	7.773 ± 0.412 b	150.424 ± 2.823 bc
R:B = 7:3	9.895 ± 0.771 c	50.707 ± 2.333 bc	83.172 ± 2.992 ab	8.154 ± 0.515 a	155.673 ± 4.864 b
R:B = 9:1	9.357 ± 0.462 c	38.347 ± 1.946 d	75.621 ± 3.500 c	5.716 ± 0.341 c	136.028 ± 4.725 c

Note: Within the same column, different letters represent significant differences ($p < 0.05$), while the same letters represent no significant differences ($p > 0.05$).

3.3. Effect on Photosynthetic Properties

As shown in Figure 2, the transpiration rate E were significantly lower in the high R:B treatment than in the low R:B treatment under the same light quality and saturated light intensity conditions. With Pn increasing by 70.49% in the R:B = 1:9 treatment compared to R:B = 9:1. Therefore, cucumber seedlings grown under low R:B had greater photosynthetic potential.

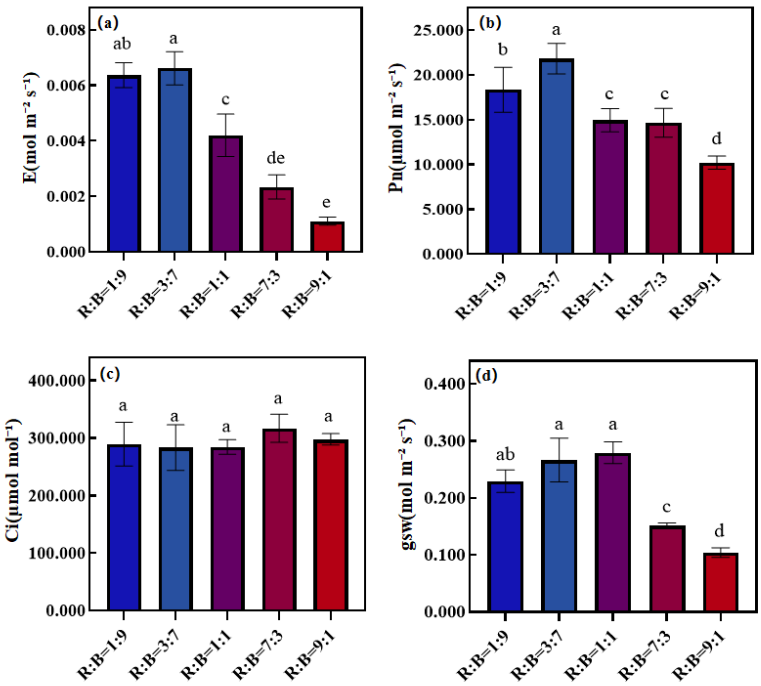


Figure 2. Effect on photosynthetic properties: (**a**) effect of transpiration rate (E) between treatments; (**b**) effect of net photosynthetic rate (Pn) between treatments; (**c**) effect of intercellular CO_2 (Ci) between treatments; (**d**) effect of stomatal conductance (gsw) between treatments. Note: In the same figure, different letters represent significant differences ($p < 0.05$) and the same letters represent insignificant differences ($p > 0.05$).

3.4. Effect on Stomatal Characteristics

As shown in Figure 3a–d, the stomatal length, stomatal width, pore length and pore width of leaf stomata of cucumber seedlings grown under low R:B were lower than those of high R:B. Therefore, their stomatal pore area was also significantly lower than that of the high R:B treatment, where the stomatal area of the R:B = 1:9 treatment was significantly lower than that of the R:B = 7:3 treatment by 38.43%, with smaller stomatal size and pore openings (e.g., Figure 3e). However, as the red light ratio increased, their leaf stomatal density showed a significant decrease, with the R:B = 9:1 treatment showing a significant 45.75% decrease in stomatal density compared to the R:B = 1:9 treatment, as shown in Figure 3f.

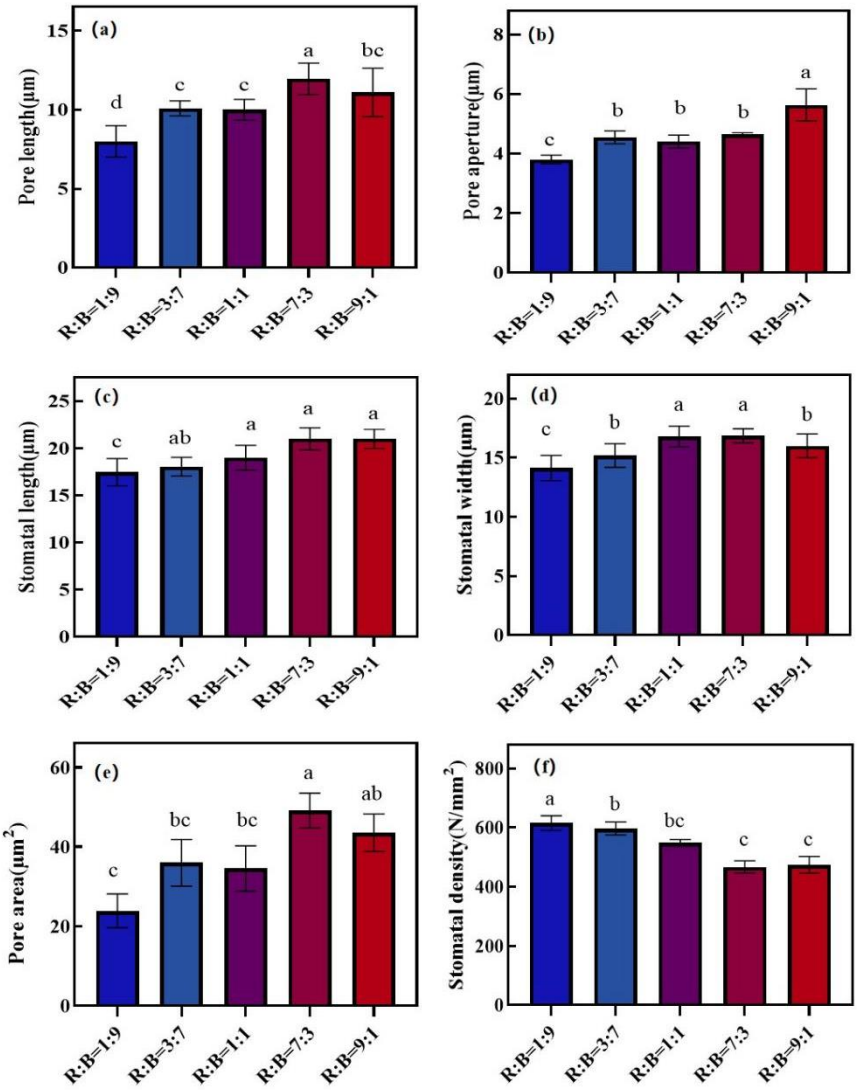
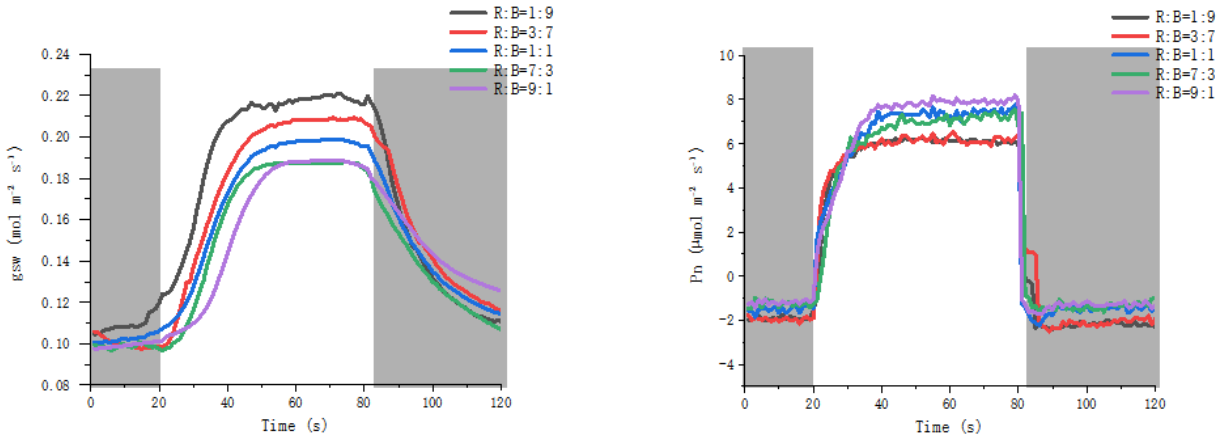


Figure 3. Effect on stomatal characteristics: (**a**) effect of different treatments on stomatal pore length; (**b**) effect of different treatments on stomatal pore width; (**c**) effect of different treatments on stomatal pore length; (**d**) effect of different treatments on stomatal pore width; (**e**) effect of different treatments on stomatal pore area; (**f**) effect of different treatments on stomatal pore density. Note: In the same figure, different letters represent significant differences ($p < 0.05$) and the same letters represent insignificant differences ($p > 0.05$).

3.5. Effect on Stomatal Dynamics

As shown in Figure 4, cucumber seedlings under the low R:B treatment had greater stomatal conductance and were more sensitive to ambient light than the high R:B treatment, entering a steady state of stomatal conductance more quickly, with the maximum stomatal conductance of the R:B = 1:9 treatment being 17.01% higher than that of the R:B = 9:1 treatment, but the net photosynthetic rate of the R:B = 1:9 treatment was lower than that of the R:B = 9:1 treatment.

For the stomatal response curve, the stomatal opening section was curve fitted to obtain the values shown in Figure 5a, and the curvature was calculated for each point on it to obtain the values shown in Figure 5b. The response rate for the low R/B treatment reached the maximum faster and gradually leveled off in the region, while the response rate for the high R/B treatment lagged behind the low R/B treatment, with the R:B = 9:1 treatment opening the slowest and reaching the maximum curvature the latest.



(**a**) (**b**)

Figure 4. Effect on stomatal dynamics: (**a**) effect of different treatments on stomatal conductance; (**b**) effect of different treatments on net photosynthetic rate (each curve is the mean of the curves for 5−7 plants). Note: grey boxes represent plants in darkness, white boxes represent light conditions.

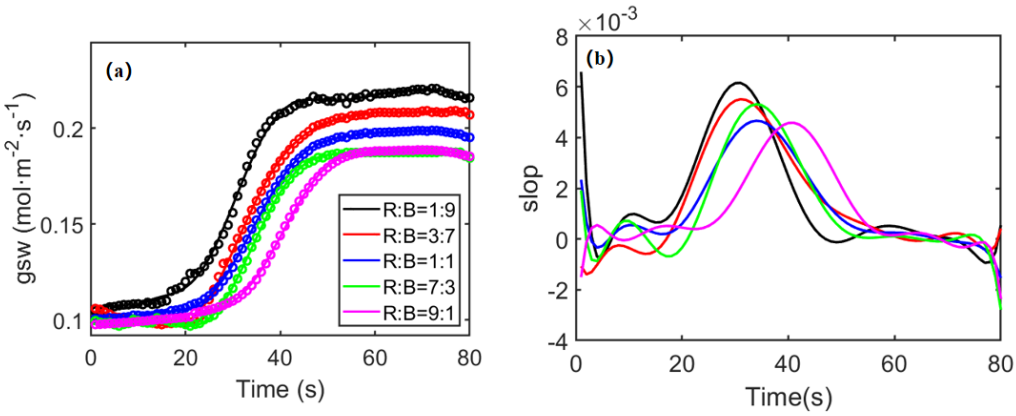


Figure 5. Stomatal movement response curve (stomatal opening) fitted: (**a**) plot of fitted curve of stomatal opening section; (**b**) plot of curvature of stomatal opening curve.

Calculations for the open portion of the stomatal response curve in Figure 6a yielded Table 5. The stomatal opening rate at the low R/B ratio shown in Table 5 is faster, with smaller ki values; the response rate is faster, with smaller Slmax; and the maximum stomatal conductance is greater, with larger Gsmax at steady state.

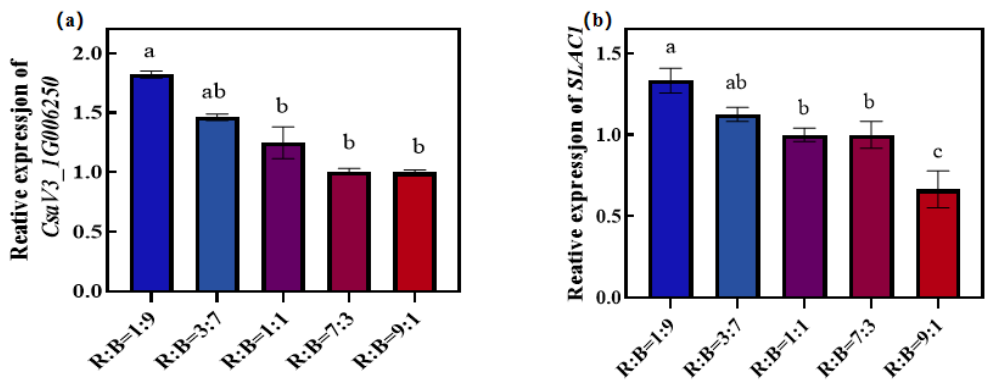


Figure 6. Different red and blue light ratios on stomatal development and motility genes: (**a**) effect of different treatments on the relative expression of *CsaV3_1G006250*; (**b**) effect of different treatments on the relative expression of *SLAC1*.

Table 5. Stomatal ringing curve parameters related to the stomatal opening section.

Treatments	k_i	S_{lmax}	G_{smax}	r_0
R:B = 1:9	22	5.781	0.221	0.124
R:B = 3:7	20	6.004	0.209	0.099
R:B = 1:1	26	6.461	0.199	0.108
R:B = 7:3	25	6.185	0.188	0.097
R:B = 9:1	30	7.111	0.189	0.102

3.6. Effect on Stomatal Development and Motility Genes

The expression of stomatal development-related genes was significantly higher in the R:B = 1:9 treatment than in the R:B = 1:1, R:B = 7:3 and R:B = 9:1 treatments. Moreover, there was a trend that the relative expression of *CsaV3_1G006250* decreased with increasing red light ratios.

The key gene for stomatal movement, *SLAC1, was* also closely related to its stomatal number, with the R:B = 1:9 treatment significantly higher than the R:B = 1:1, R:B = 7:3 and R:B = 9:1 treatments. Indeed, the relative expression of *SLAC1 was* significantly increased by 100.75% under the R:B = 1:9 treatment compared to the R:B = 9:1 treatment.

4. Discussion

LEDs offer a variety of spectral possibilities for plant growth, with red and blue light often used in combination for plant supplementation, and it has been shown that plant growth under single red or blue light were worse than under mixed red and blue light [45]. However, the optimal R/B ratio for plants depends on plant species differences or genotypic differences between plant species. In lettuce, for example, leaf photosynthetic capacity and photosynthetic rate increase as the R/B ratio decreases, which correlates with changes in its stomatal conductance g_s [46]. It has also been shown that lettuce growth rate decreases with increasing blue light intensity [47]. The results of this experiment showed that the height, fresh weight, total leaf area and dry weight of cucumber seedlings increased with increasing red light ratios, with optimum performance in the R:B = 7:3 treatment (Table 3). This is consistent with the results of Di Q et al. in aubergine seedlings, where photosynthetic efficiency was highest when PSI and PSII efficiencies were in equilibrium [48]. Wollaeger and Runkle [49] suggested that light quality is an important factor affecting photosynthetic pigments, with red light having a greater influence than other light qualities [50].

Prolonged exposure to a specific light environment alters plant leaf anatomy and thus affects plant photosynthesis [51,52], with increased leaf thickness and fenestra tissue cell length under blue light treatment compared to plants grown under red or green light [22,53]. Our experimental results showed that leaf fenestra tissue thickness and leaf upper epidermal thickness were significantly higher after low R/B treatment than high R/B, and that R:B = 9:1 treatment reduced fenestra tissue thickness by 44.43% compared to R:B = 1:9 treatment (Table 4). Jiao et al. suggested that red light could stimulate the conversion of photosensitive pigments to the Pfr form and was associated with phytochrome interacting factor 3 (PIF3) [22]. Excess PIF3 stimulates the expression of skotomorphogenesis genes, which act as negative regulators of photomorphogenesis and affect leaf development [54,55]. Therefore, as the R/B ratio decreases, the leaf thickness of cucumber seedlings shows a decreasing trend.

When performing plant photosynthetic measurements, a higher R/B ratio will result in a greater net photosynthetic rate when the trials light source is consistent with the ambient light source, as the quantum yield of red light is higher than that of blue light [56,57]. However, when the light source measured was either saturated or was of high light intensity and the light quality was consistent, the results would be different, with blue light stimulating the photoprotective ability of the plant. McCree [56] and Inada [57] showed that plants grown under monochromatic blue light compared to white light treatments had higher instantaneous photosynthetic rates when using mixed red and blue light sources as the measured light. The significant effect of blue and red light on leaf morphological

anatomy and single leaf gas exchange may be due to differences in chloroplast structure and chloroplast accumulation within the cell chloroplasts [58]. Our results showed an increase in instantaneous photosynthetic efficiency under low R/B treatments, with a 70.49% increase in Pn for the R:B = 1:9 treatment compared to R:B = 9:1.

Light is not only a source of energy for photosynthesis but is also a key signal regulating the growth and development of multifaceted plants [59], and the development and opening and closing of stomata are influenced by the environment to which the plant is exposed [60]. The extent to which light quality affects stomatal development varies across species. Gitz et al. found that soybean leaves had a reduced number of stomata after UV-B irradiation, which was thought to improve drought tolerance and photosynthetic performance [59]. Barillot et al. found that blue light irradiation increased the stomatal density of tall fescue leaves and greatly increased their stomatal conductance [60]. Sander W. et al. showed that as the proportion of blue light increased, gsw increased with it, and that the larger gsw was due to more proximal stomata [61]. The results showed that leaf stomatal density was significantly reduced with an increasing percentage of red light, and it was significantly reduced by 45.75% in R:B = 9:1 treatment compared to that in the R:B = 1:9 treatment. This was also confirmed by RT-PCR validation of key genes for stomatal development. Plants with lower stomatal densities have larger leaf stomatal sizes; they also have lower transpiration, faster growth rates and higher biomass [19]. Our results are consistent with this trend, with greater cucumber biomass at high R/B and lower stomatal length and stomatal width at low R/B (Figure 3c,d). In contrast, plants with higher stomatal densities had no increase in biomass, but if these plants were in a state of rapidly changing environmental conditions, such as increased light, increased CO_2 supply, and fluctuations in light, they would perform better [19]. Therefore, increasing the proportion of blue light added at the right time (e.g., in the morning) will result in greater photosynthetic products from cucumber seedlings.

The response of stomatal movement to light depends mainly on two signaling mechanisms, namely the red and blue light responses [24–27]. The red light-driven response of stomata is similar to the carbon assimilation response to increasing light intensity [28]. On the other hand, blue light-induced stomatal opening differs from the red light response in that it does not require the involvement of a chloroplast signal [41], and on a quantitative basis, the stomatal blue light response drives stomatal opening 20 times more efficiently than does red light [26,42]. We observed that the low R/B treatment had greater gsw under the same measured light as the ambient light and that it reached stability before the high R/B treatment.

5. Conclusions

In this work, it is the first time which is shown the rate of stomatal movement under mixed red and blue light. The effect of light quality on stomatal development was significant. Cucumber seedlings under low R/B had lower biomass than those treated with high R/B, but they had higher stomatal density, smaller stomatal size and responded faster than under high R/B. When R:B = 1:9, cucumber seedlings had the highest stomatal density and the fastest response rate. This will provide some reference for changing the light environment of plant seedlings to improve plant adaptability and photosynthetic potential.

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