



Influence of light spectral ratios on physiological and stomatal parameters of pak choi (*Brassica rapa* var. *chinensis*) grown in a controlled vertical hydroponic system

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ABSTRACT

Light quality, specifically the ratio of red and blue wavelength LEDs, is critical in optimising plants' photosynthetic rate and related physiological processes for higher yield and quality. The present study was carried out during 2022 and 2023 at ICAR-Indian Agricultural Research Institute, New Delhi to investigate the effects of different red (R) and blue (B) light ratios (R2B1= 2:1, R1B1= 1:1 and R1B2= 1:2) on plants stomatal characteristics and physiological parameters. Significant variations were observed for stomatal pore width, length, and guard cell dimensions across treatments, particularly with R1B1 and control (white light) conditions. Notably, R1B1 demonstrated increased stomatal pore width and guard cell dimensions over time, while control maintained higher stomatal density on the abaxial surface at 30 days after treatment (DAT). Stomatal responses varied, with R2B1 achieving the highest stomatal density and physiological efficiency as evidenced by a net photosynthetic rate of 22.26 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ and a water-use efficiency (WUE) of 72.67. The control treatment showed the lowest values across all physiological parameters, highlighting the benefits of spectral treatments. Correlation analysis revealed significant positive relationship among the physiological and stomatal parameters, particularly between net photosynthetic rate and WUE ($r = 0.97$), stomatal conductance and guard cell arc length ($r = 0.97$). The yield was observed highest (113.61 g fresh weight and 6.98 g dry weight) in R2B1 treatment. These findings underscore the critical role of light quality in optimising stomatal function and enhancing photosynthesis, transpiration and water-use efficiency in plants, suggesting potential applications in controlled agricultural environments.

Keywords: Net photosynthetic rate, Pak choi, Pigments, Stomata, Transpiration rate

Global population growth has significantly increased the demand for food, placing immense pressure on sustainable agricultural systems. However, the availability of arable land, freshwater, and other natural resources remains limited and unevenly distributed (Kumar *et al.* 2014, Cotula 2006). Rapid urbanisation and industrialisation further reduce cultivable land while intensifying the negative environmental impacts of conventional agricultural practices. Hence, there is a growing need for innovative and resource-efficient food production systems that can ensure sustainability and meet global food security goals (Sharma *et al.* 2018).

Conventional soil-based agriculture struggles to maintain productivity under these constraints, largely due to its dependence on climate, soil fertility, and water availability. Although sustainable solutions have been explored, many approaches still face challenges in achieving

high yield with minimal environmental impact. Light and water use efficiency are often overlooked in the optimisation of cultivation systems, despite being critical for plant productivity and resource conservation. Therefore, soilless cultivation techniques such as hydroponics, particularly within controlled environment agriculture (CEA), present a promising alternative. Vertical hydroponic systems can maximise yields while minimising dependence on soil and climate (Sardare and Admane 2013). Hydroponics provides plants with a balanced nutrient supply through nutrient solutions and artificial lighting (Aires 2018), enhancing growth, quality, and pest resistance. Pak choi [*Brassica rapa* subsp. *chinensis* (L.) Hanelt], a nutrient-rich leafy vegetable valued for its high vitamin C, carotenoid, flavonoid, and glucosinolate content (Cartea *et al.* 2011, Wiangsamut and Koolpluksee 2020), serves as an ideal model crop for such systems.

Light quality is a crucial environmental factor that regulates photosynthesis, stomatal behaviour, and plant water use efficiency (Sharkey and Raschke 1981). Among different spectra, red and blue light are particularly influential

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in controlling stomatal response, with blue light shown to enhance stomatal conductance more effectively than red light (Sharkey and Raschke 1981). However, previous studies have primarily focused on individual physiological responses to light and have seldom quantified overall water use efficiency under varied spectral conditions. Hence, the present study aims to advance understanding by monitoring and quantifying water use in pak choi under different red (R) and blue (B) light combinations. By measuring evapotranspiration rates, photosynthetic parameters, and stomatal characteristics, this research seeks to elucidate the effects of specific light spectra on water use efficiency, contributing to the development of sustainable hydroponic systems within controlled environments.

MATERIALS AND METHODS

Plant materials and experimental setup: Pak choi cv. Choko was grown during 2022 and 2023 at the Centre for Protected Cultivation Technology (CPCT), ICAR-Indian Agricultural Research Institute (28°37'22"–28°39'05"N, 77°08'45"–19 77°10'24"E; at an elevation of 1462 m amsl), New Delhi under controlled protected conditions. The experimental treatments included three ratios of red (R) and blue (B) light LEDs, i.e. R2B1 = 2:1 (T_1); R1B1 = 1:1 (T_2); and R1B2 = 1:2 (T_3), each featuring a specific ratio of B and R lights and control (white LED light, T_4) obtained by the measurements taken by spectroradiometer (SRI-PL-6000+, LED Grow (Plants) Light Spectrometer, OPTIMUM, Hginchu, Taiwan). The experiment was laid out in a completely randomised block design (CRBD) with four treatments and six replications. The photosynthetic photon flux density (PPFD) was kept constant at $200 \pm 10 \mu\text{mol}/\text{m}^2/\text{s}$.

Measurement of stomatal and morphological parameters: For assessing stomata parameters, leaf samples at 10 and 30 DAT were taken and the impressions were made on both surfaces using transparent nail enamel. The samples were allowed to air-dry and the impressions were lifted with the help of a tweezer and mounted on a slide for microscopic observation (Olympus CX33 microscope, Magcam DC 5, India) at 100 and 400X magnifications. The stomatal density was observed at 100X magnification and other stomatal parameters like pore width, pore length, left guard cell width, guard cell width, and arc length were observed at 400X magnification and expressed in μm (Gao *et al.* 2022). Magvision software (IVMeditech, China) was used to measure the stomatal parameters, which were read on the third leaf in three biological replicates on 18 counts. The pictographic illustrations of measurements are depicted in Fig. 1 (h). The plant fresh weight (FW) was recorded using an analytical balance (± 0.001 g accuracy). Fresh biomass was expressed as grams per plant (g/plant). For the dry weight (DW) determination, the same plant samples were placed in paper envelopes and oven-dried at $70 \pm 2^\circ\text{C}$ for 48 h (or until a constant weight was achieved). After drying, the samples were cooled in a desiccator for 30 min and then weighed again using the analytical balance. The

final stable weight was recorded as the plant dry weight (g/plant).

Physiological parameters: To assess leaf gas exchange parameters, a portable photosynthesis system (LICOR, Lincoln, NE, USA) was used to measure net photosynthesis (Pn), transpiration (E) and stomatal conductance (gs). Measurements were taken on functional leaves of six plants per replicate across different LED light setups, 24°C temperature under a photon flux density of $1000 \mu\text{mol}/\text{m}^2/\text{s}$ (Chen *et al.* 2016).

Leaf photosynthetic pigments: Leaf photosynthetic pigments, including total chlorophyll (TC), chlorophyll a, chlorophyll b, and total carotenoids, were determined following the method of Hiscox and Israelstam (1979). Approximately 100 mg of leaf tissue was extracted in 10 mL of analytical grade dimethyl sulphoxide (DMSO) and incubated in a water bath at 65°C for 3 h. The pigment extract was then analysed spectrophotometrically at 663, 645, and 480 nm to quantify individual and total pigment contents.

Statistical analysis: Stomatal measurements were conducted on the third leaf, with 18 counts taken across three biological replicates. Physiological data underwent analysis of variance (ANOVA) within a completely randomised design, using a 5% significance ($p=0.05$) by Tukey's multiple comparisons test letters in R Studio (version 4.3.1). Additionally, Pearson's correlation was applied to examine the relationships between physiological parameters (Pn, E, gs), plant FW, plant DW and stomatal traits (pore W, left GW, right GW, pore L, GAL, GAW).

RESULTS AND DISCUSSION

Stomatal characteristics: With different light treatments, there was a distinct effect on photosynthesis and biomass production (Fig. 1 and Fig. 2). The role of stomata in gas exchange with the environment is critical and photosynthesis was found to be influenced by the varying R and B light ratios observed in our study. Similar results were reported by Matthews *et al.* (2020). The stomatal characteristics of the adaxial and abaxial leaf surfaces showed notable differences when subjected to different R and B light ratios. Significant differences emerge as time progresses, particularly in stomatal pore width (Fig. 1a), where R1B1 exhibits considerable variation by 30 DAT, indicating a broad range in pore sizes. In terms of stomatal pore length (Fig. 1b) and control shows significant elongation compared to the other treatments, suggesting its potential to promote enlarged stomatal structures over time. Guard cell measurements reveal that the width of the right guard cells (Fig. 1c) varied across the light treatments, with R1B1 showing significant variation at 10 DAT, thereafter, becoming uniform by 30 DAT. This pattern is mirrored in the left guard cell width (Fig. 1d), where R1B1 and control exhibit wider guard cells at 30 DAT, implying that these treatments induce larger stomatal structures. The arc length of both left (Fig. 1e) and right guard cells (Fig. 1f) displayed distinct differences; control presents shorter arc

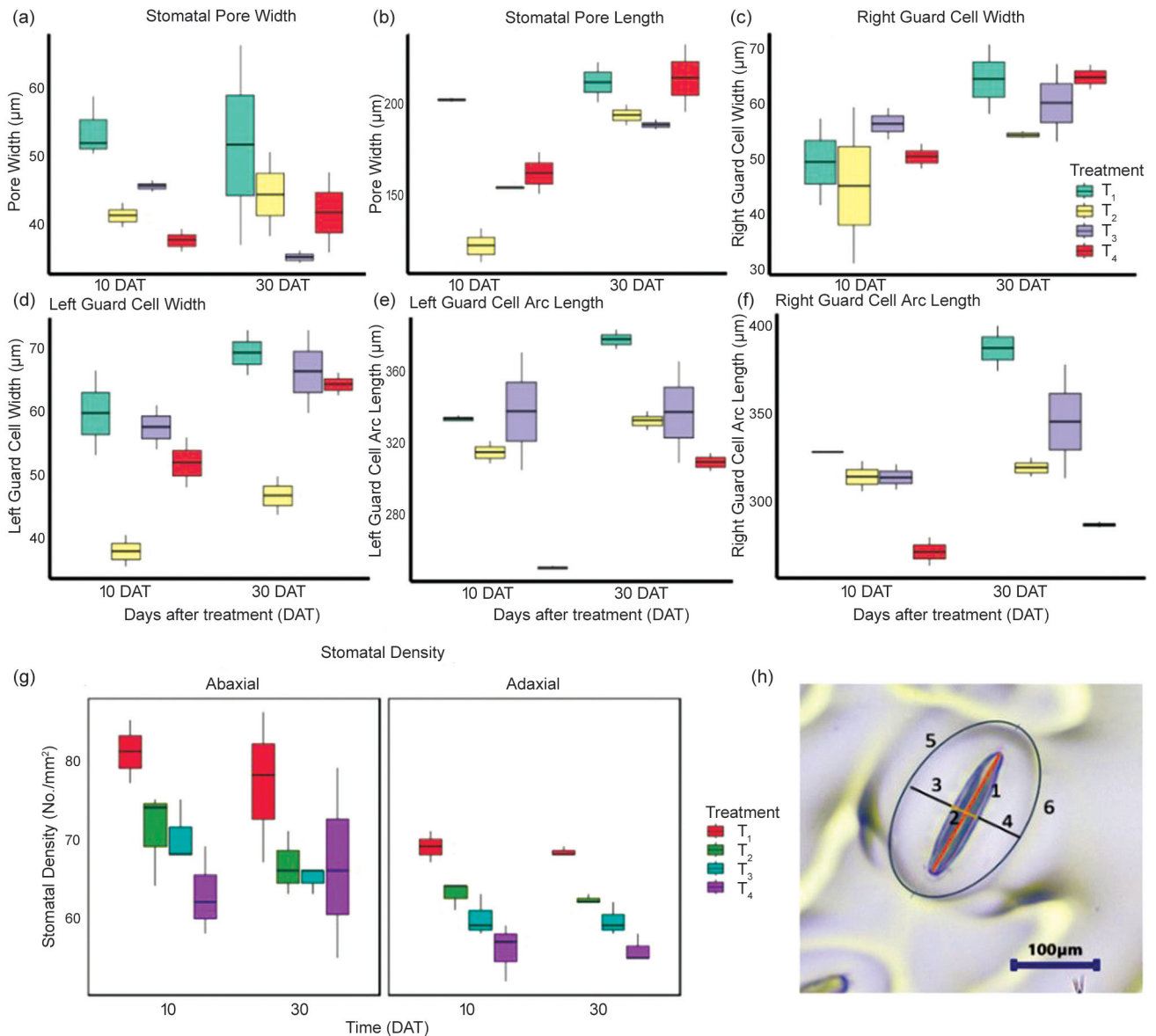


Fig. 1 Acclimation of stomata of pak choi plants exposed to different ratios of red: blue lights and control. (a) Stomatal pore width; (b) stomatal pore length; right (c) and left (d) guard cell width; left (e) and right (f) guard cell arc length; (g) Stomatal density in the adaxial and abaxial sides of pak choi leaves; (h) illustrations of measurements.

Treatment details are given under Materials and Methods.

lengths at 30 DAT, while R2B1 and R1B1 maintain longer arcs, indicating that the treatments differentially affect the shape and curvature of guard cells. Stomatal density (Fig. 1g) shows a marked decline over time, especially on the adaxial surface, suggesting reduced stomatal development or retention under experimental conditions. Notably, at 30 DAT, control plants exhibited higher stomatal density on the abaxial surface compared to the other treatments indicating it may enhance the stomatal retention and potentially improve the gas exchange and transpiration efficiency. Furthermore, Fig. 1h depicts a microscopic image of the stomatal structure, highlighting key measurements such as pore width, guard cell arc length, and guard cell dimensions. Overall, these findings underscore the significant impact of the treatments

on stomatal development, with control promoting larger stomatal dimensions and higher density, particularly on the abaxial surface, thereby optimising stomatal function and potentially enhancing physiological responses to environmental conditions. Illustrations of measurements of stomatal parameters are shown in Fig. 1h [stomatal pore length (1), stomatal pore width, left (3) and right (4) guard cell width, left (5) and right (6) guard cell arc length].

Physiological and leaf photosynthetic parameters: Light influences plant growth via photosynthesis, and stomatal dynamics play a crucial role in facilitating this process by controlling gas exchange parameters and water regulation. The results align with findings that blue light triggers rapid stomatal opening, enabling increased gas exchange,

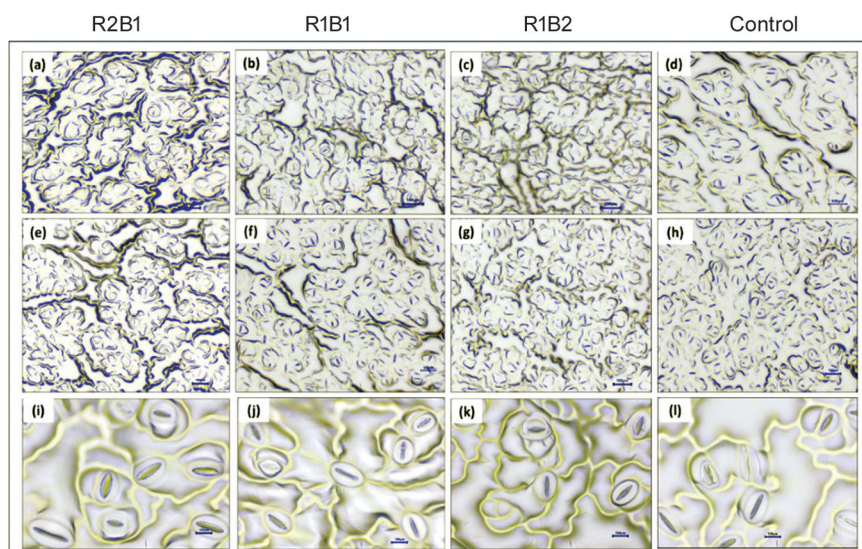


Fig. 2 Schematic representation of the stomatal structure on the adaxial and abaxial surfaces of pak choi cv. Choko leaves. (a), (b), (c) and (d) are images of the adaxial side of leaves; (e), (f), (g), (h) are images of the abaxial surface of leaves; (i), (j), (k), (l) are images taken at 400X magnification.

while red light influences photosynthesis-related stomatal behaviour, impacting stomatal density and size, especially on the abaxial surface (Zeiger *et al.* 1983, Matthews *et al.* 2020, Zhou *et al.* 2020). Stomatal responses to light are generally classified into two types, those that are quickly opened by blue light, independent of photosynthesis, and those that depend on photosynthesis under R2B1 and R1B2 treatments resulting in significantly higher stomatal densities on both surfaces, with R2B1 treatment showing the highest density. The abaxial surface had higher stomatal density than the adaxial surface. Overall higher red-light proportion leads to greater stomatal responses, compared to higher blue-light ratios (Lanoue *et al.* 2018, Lawson *et al.* 2018). Stomatal parameters and their intensity on the leaf surface had direct correlation with photosynthesis, gas exchange parameters and respiration, which directly influence the assimilation, net assimilation and quality of the produce

(Huang *et al.* 2021).

The stomatal density findings also align with previous observations of red light's role in enhancing photosynthetic activity. For example, stomatal density on the abaxial leaf surface was significantly higher in treatments with more red light (R2B1), which supports improved gas exchange and water vapour release. Increased stomatal density and pore size on the abaxial surface are advantageous, as they facilitate CO₂ uptake without excessive water loss. Physiological parameters and yield.

Different light spectral treatments impact different physiological parameters in plants, specifically net photosynthetic rate, stomatal conductance, transpiration rate, and water-use efficiency (Table 1). Among the treatments, R2B1 exhibited the highest values across all parameters, with a net photosynthetic rate of 22.26 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$, stomatal conductance of 0.58 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$, transpiration rate of 7.24 $\text{mol}/\text{m}^2/\text{s}$ and water use efficiency (WUE) of 72.67. Compared with the control, R2B1 showed approximately a 1.64-fold increase in photosynthetic rate, 1.87-fold increase in stomatal conductance, 1.28-fold higher transpiration rate, and a 1.44-fold improvement in WUE, demonstrating its superior photosynthetic efficiency and water-use performance under this spectral combination. This treatment outperformed others, indicating a more efficient photosynthetic and water-use response under this specific light spectrum. In comparison, R1B1 and R1B2 treatments showed moderate values. Treatment R1B1 has a photosynthetic rate of 19.29 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ and lower stomatal conductance and transpiration than R2B1, along with slightly reduced WUE. R1B2 treatment, with intermediate values for all

Table 1 Effect of light spectral treatments on physiological and yield parameters

Parameter	R2B1	R1B1	R1B2	Control
Net photosynthetic rate ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)	22.26 \pm 0.82 ^a	19.29 \pm 0.33 ^c	20.14 \pm 0.29 ^b	13.55 \pm 0.11 ^d
Stomatal conductance ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)	0.58 \pm 0.02 ^a	0.34 \pm 0.03 ^c	0.39 \pm 0.01 ^b	0.31 \pm 0.01 ^c
Transpiration rate ($\text{mol}/\text{m}^2/\text{s}$)	7.24 \pm 0.03 ^a	4.11 \pm 0.18 ^c	5.97 \pm 0.11 ^b	5.67 \pm 0.02 ^b
Water use efficiency (WUE)	72.67 \pm 0.56 ^a	69.78 \pm 0.33 ^b	68.66 \pm 0.48 ^c	50.62 \pm 0.58 ^d
Plant fresh weight (g)	113.61 \pm 0.28 ^a	92.28 \pm 0.31 ^b	97.46 \pm 0.55 ^{ab}	85.11 \pm 0.48 ^c
Plant dry weight (g)	6.98 \pm 0.06 ^a	5.44 \pm 0.02 ^b	5.73 \pm 0.02 ^b	4.29 \pm 0.04 ^c
Total carotenoid content	0.36 \pm 0.001 ^a	0.35 \pm 0.002 ^{ab}	0.35 \pm 0.004 ^{ab}	0.29 \pm 0.001 ^c
Chlorophyll b	0.25 \pm 0.001 ^a	0.23 \pm 0.002 ^b	0.25 \pm 0.001 ^a	0.22 \pm 0.002 ^c
Chlorophyll a	1.54 \pm 0.01 ^a	1.36 \pm 0.03 ^b	1.54 \pm 0.02 ^a	1.27 \pm 0.01 ^c
Total chlorophyll content	1.79 \pm 0.02 ^a	1.59 \pm 0.01 ^b	1.79 \pm 0.01 ^a	1.49 \pm 0.02 ^c

Values expressed in mean \pm SE (n = 6) and the following different letters in columns are significantly different at the 0.05 level of significance by Tukey's multiple comparisons test letters. Treatment details are given under Materials and Methods.

parameters, suggests a less efficient response than R2B1 but a higher response than R1B1 and the control. The control treatment demonstrated the lowest photosynthetic rate (13.55 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), stomatal conductance (0.31 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), transpiration rate (5.67 $\text{mol}/\text{m}^2/\text{s}$) and WUE (50.62), underscoring the positive impact of spectral light treatments on physiological functions and efficiency in plants. The R2B1 treatment promoted a more effective photosynthetic response and water-use efficiency, as confirmed by earlier studies showing red light's impact on photosynthesis rates (Inada *et al.* 1976, Matthews *et al.* 2018). The R2B1 treatment achieves the highest fresh and dry weights (113.61 g and 6.98 g, respectively), indicating the most supportive conditions for plant growth, likely due to increased photosynthetic rate and stomatal conductance, which enhance nutrient absorption and gas exchange. R1B1 and R1B2 showed intermediate fresh (92.28 g and 97.46 g) and dry weights (5.44 g and 5.73 g), which suggests they support moderate growth. The Control, however, produces the lowest fresh (85.11 g) and dry weights (4.29 g), reflecting the most restricted growth conditions, potentially due to lower photosynthetic and water use efficiencies.

Marked differences were observed in pigment accumulation under different light spectra (Fig. 3). The R2B1 treatment recorded the highest values for all pigments, including chlorophyll a (1.54 mg/g FW), chlorophyll b (0.26 mg/g FW), total chlorophyll (1.79 mg/g FW), and total carotenoids (0.36 mg/g FW). The R1B2 treatment followed, with chlorophyll a (1.54 mg/g FW) and total chlorophyll (0.89 mg/g FW) also elevated compared to control, though slightly lower than R2B1 in carotenoid accumulation. Moderate pigment levels were

observed in R1B1, while the lowest values occurred in the control (chlorophyll a-1.27; chlorophyll b-0.22; total chlorophyll-1.49; carotenoids- 0.29 mg/g FW). The R2B1 treatment exhibited the highest photosynthetic pigment content and superior net photosynthetic and transpiration rate, reflecting enhanced photosynthetic efficiency. This indicates that the optimised red–blue spectral ratio in R2B1 effectively promotes pigment biosynthesis and improves carbon-assimilation and water-use dynamics in pak choi (Matthews *et al.* 2018).

Association between different parameters: The correlation matrix amongst various physiological and stomatal parameters, revealed significant relationships between them, i.e. photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), water-use efficiency, and guard cell dimensions (Table 2). The net photosynthetic rate has a strong, significant positive correlation with WUE ($r = 0.97$), suggesting that higher photosynthetic rate aligns with more efficient water use. Photosynthetic rate is also highly correlated with guard cell arc length ($r = 0.89$) and guard cell arc width ($r = 0.92$), implying a link between photosynthetic activity and stomatal structure.

Stomatal conductance has a robust correlation with GAL ($r = 0.97$, $p < 0.05$), indicating that guard cell length may influence gas exchange efficiency. Stomatal conductance also showed a strong association with transpiration rate ($r = 0.79$), suggesting that as stomatal conductance increases, so does transpiration, reflecting a close relationship between gas and water exchange parameters in plants. While transpiration rate correlates highly with left guard cell width ($r = 0.93$) and right guard cell width ($r = 0.82$), it has a weaker relationship with WUE ($r = 0.14$), suggesting that

Table 2 Correlation matrix among physiological and stomatal parameters

Parameter	Pn	gs	E	WUE	Pore W	Left GW	Right GW	Pore L	GAL	GAW	FW	DW
Pn	1.000											
gs	0.777	1.000										
E	0.353	0.794	1.000									
WUE	0.977*	0.643	0.144	1.000								
Pore W	0.355	0.672	0.341	0.307	1.000							
Left GW	0.098	0.535	0.934	-0.114	0.024	1.000						
Right GW	-0.231	0.386	0.825	-0.433	0.225	0.885	1.000					
Pore L	-0.36	0.281	0.489	-0.486	0.623	0.428	0.781	1.000				
GAL	0.895	0.972*	0.642	0.802	0.651	0.352	0.157	0.092	1.000			
GAW	0.929	0.944	0.669	0.829	0.475	0.424	0.145	-0.051	0.977*	1.000		
FW	0.874*	0.984*	0.717	0.762	0.601	0.452	0.241	0.111	0.993	0.985	1.000	
DW	0.952*	0.931	0.575	0.876*	0.549	0.295	0.043	-0.06	0.987	0.988	0.979	1.000

*for $p < 0.05$; Pn, Net photosynthetic rate; gs, Stomatal conductance; E, Transpiration rate; Pore W, Pore width; Left GW, Left guard cell width; Right GW, Right guard cell width; Pore L, Pore length; GAL, Guard cell arc length; GAW, Guard cell arc width; FW, Plant fresh weight; DW, Plant dry weight.

increased transpiration does not necessarily improve water-use efficiency. The guard cell dimensions, such as GAL and GAW, are notably interconnected, with a significant positive correlation ($r = 0.97$ for GAL and GAW, $p < 0.05$), highlighting the potential role of these stomatal structures in coordinating physiological responses.

The fresh weight (FW) and dry weight (DW) exhibit strong positive correlations with several parameters, indicating their close relationship with plant physiological and environmental factors. Fresh weight showed significant positive correlations with net photosynthetic rate ($r = 0.87$) and stomatal conductance ($r = 0.98$), suggesting that higher photosynthesis and stomatal activity contribute substantially to greater plant fresh mass. Dry weight similarly correlates strongly with ($r = 0.95$) and exhibits a marked relationship with water use efficiency ($r = 0.87$), highlighting that both photosynthetic activity and efficient water use are crucial for biomass accumulation in dry weight. The close association between GAL and GAW ($r = 0.97$, $p < 0.05$) emphasises their role in synchronizing physiological responses (Shimazaki *et al.* 2007, Cubo Ribas 2021). The results emphasise the impact of red and blue light ratios on stomatal morphology, physiology and photosynthetic efficiency, which are crucial for understanding plant growth and productivity. Previous studies have consistently shown that red light influences stomatal opening by modulating osmotic potential through ion (e.g. K^+) and higher sugar accumulation within guard cells (Shimazaki *et al.* 2007, Lawson 2009, Ballard *et al.* 2019). Red light activates proton pumps in the plasma membrane, which drives potassium influx and water movement, resulting in stomatal opening, as highlighted by Serrano *et al.* (1988) and Tominaga *et al.* (2001).

The significant correlations observed in Table 2 supports the influence of guard cell morphology on gas exchange. Guard cell arc length (GAL) and width (GAW) correlate positively with net photosynthesis and stomatal conductance, indicating that guard cell dimensions impact gas exchange efficiency and water use (Ando and Kinoshita 2018). These findings are consistent with research showing that guard cell size and structure play a role in optimising stomatal function, which directly affects photosynthesis and transpiration rates (Assmann and Shimazaki 1999, Lawson 2009). Moreover, the positive correlations between physiological and stomatal parameters suggested that certain structural adaptations in guard cells, such as increased arc length and pore size, align closely with enhanced photosynthetic capacity and gas exchange efficiency (Kaiser and Kappen 2000, Kaiser *et al.* 2018). This structural and functional integration appears especially prominent under red-dominant light conditions, supporting that targeted light quality can strategically improve stomatal efficiency and by extension, plant productivity.

The light spectral treatments significantly influence stomatal characteristics and physiological responses in plants. These observations suggested that light spectrum management could be leveraged to optimise stomatal function, targeting specific wavelengths for either short-

term stomatal response (with blue light) or more sustained photosynthetic performance and resource efficiency (with red light). By refining light conditions to include a strategic mix of red and blue light, we can potentially boost plant growth, enhance CO_2 assimilation, and increase WUE particularly valuable in controlled environments where maximising biomass and growth efficiency is essential. Notably, the R2B1 treatment resulted in the highest net photosynthetic rate, stomatal conductance, transpiration rate, and water-use efficiency, indicating optimal light conditions for plant growth. The findings also highlight the critical role of stomatal structure, with strong correlations between stomatal dimensions and physiological metrics, underscoring their importance in enhancing gas exchange and water regulation. Future studies could explore dynamic light modulation strategies or integration with AI-based control systems for real-time optimisation.

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