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Effects of red-blue light spectrum on growth, yield, and photosynthetic efficiency of lettuce in a uniformly illumination environment

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Abstract: This study comprehensively investigates the impact of varying red-to-blue light ratios on the growth of Spanish lettuce. The research considers various factors such as growth morphology, photosynthetic parameters, and chlorophyll fluorescence. Lettuce was cultivated in an environment with a photosynthetic photon flux density (PPFD) of 200 ± 20 μmol/m^2/s and a photoperiod of 16 h per day. The experiment incorporated eight distinct light treatment methodologies, with the red-to-blue light ratios ranging from 2:8 (R2B8) to 9:1 (R9B1). The data implies that during the initial 20 days of growth, groups exposed to a higher proportion of red light demonstrated superior growth. In particular, the R9B1 group exhibited the highest increase in plant height. The photosynthetic performance of leaves (net photosynthetic rate, stomatal conductance, and transpiration rate) showed a tendency to rise with a decreasing red-to-blue ratio within a particular range, peaking at R3B7. However, both the dry matter content and fresh weight were relatively lower under the R3B7 light quality ratio. The results indicate that cultivating lettuce under the R8B2 ratio led to optimal outcomes. This group significantly outperformed the other test groups in terms of weight and exhibited higher photosynthetic rates. Despite exhibiting lower stomatal conductance, this group reduced energy consumption and ultimately achieved the highest overall weight.

Keywords: leaf vegetables; hydroponic system; plant factories; uniformity of illumination

The integration of LED (light emitting diode) lighting and hydroponic systems has revolutionised modern agriculture. LEDs are beneficial due to their high energy efficiency, longevity, rapid response, affordability, and compactness (Nguyen et al. 2021). Additionally, LEDs are adept at producing monochromatic light, which is advantageous for plant growth, particularly in vertical farming. Light is a critical environmental factor for plant growth and development. Of various light-related factors, including light quality, intensity, and photoperiod, light quality exerts a more complex influence on plant growth. It significantly influences plant morphology, photosynthesis, and yield. Different light wavelengths affect diverse aspects of crop growth crop growth, such as seed germination, root and

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stem development, and the timing of flowering and fruiting (Johkan et al. 2012, Demotes-Mainard et al. 2016, Wei et al. 2023). Light modulates the activity of photosynthesis-related enzymes, thus affecting crop yield. Photosynthesis enables plants to transform light energy into vital energy for their survival and reproduction. However, plants do not utilise all available light efficiently in photosynthesis. Research has identified that pigment molecules are key players in photosynthesis, primarily absorbing light in the red and blue spectrums. Consequently, red and blue light are deemed crucial for plant growth. Red light, absorbed by plant photoreceptive pigments, affects various development aspects like stem elongation, chlorophyll content, root-to-shoot ratio, and photosynthesis (Li et al. 2017). Conversely, blue light can affect plant phototropism, stomatal opening, and leaf expansion (Hiyama et al. 2017, Kong and Zheng 2020, Wang et al. 2020). Green light penetrates leaves, enabling photosynthesis in lower leaves (Liu and Van Iersel 2021, Razzak et al. 2022). However, only green light is not conducive to plant growth. In Zhang et al.'s (2016) study on rice, it was found that adding 25% more green light to red and blue light is beneficial for increasing rice yields. The red-to-far-red light ratio (R/FR) also regulates plant growth (Lee et al. 2015, Zhang et al. 2020). Monochromatic light environments are not optimal for plants. Studies suggest that combining different light qualities is more beneficial for plant growth. Extensive research on the growth of plants, such as cucumber, tomato, pepper, and lettuce, under various red and blue light qualities, has revealed differing physiological responses and influences under different red-to-blue light ratios (Wollaeger and Runkle 2013, Hernández and Kubota 2014, 2016, Jeong et al. 2020).

With its high growth density, short cycle, and low energy requirements (Zhang et al. 2018), lettuce is a key vegetable crop in plant factories. Its high yield per land area, particularly in vertical farms, is significant because this vegetable is highly responsive to lighting conditions. Adjusting light quality notably influences lettuce's growth, yield, and quality. In Liu's research, varying red/blue (R/B) light ratios (4, 2, 1) were tested. Findings revealed a lower blue light proportion (R/B = 4) enhances lettuce yield, saccharide accumulation, and metabolic enzyme activity (Liu and Liu 2022). Similarly, a red and blue light blend promotes growth and photosynthesis (Amoozgar et al. 2017). Wang's study further indicated optimal growth conditions with a higher R/B ratio of 12, noting a peak in dry weight and leaf count without correlating it to the net photosynthetic rate (Wang et al. 2016). Research also demonstrates that increased blue light raises stomatal conductance in lettuce but hinders growth and lowers water use efficiency (Clavijo-Herrera et al. 2018). This phenomenon is attributed to increased stomatal number under heightened blue light exposure. Studies on red leaf lettuce show that augmenting blue light in red and blue light mixtures boosts growth (Samuolienė et al. 2020). Moreover, environments rich in red light enhance lettuce's fresh weight, chlorophyll, carotenoid content, and antioxidant capacity (Naznin et al. 2019). Under mixed lighting, red light dominance over 50% favours carbohydrate accumulation and nitrate breakdown, though excessive red light may distort leaf shape (Chen et al. 2021). Pennisi Giuseppina's study, exploring various R/B ratios (0.5, 1, 2, 3, 4), concludes that an R/B ratio of 3 optimises yield, chlorophyll and flavonoid content, and trace element absorption (Pennisi et al. 2019). Currently, research on supplemental lighting for plants primarily focuses on light quality, intensity, and photoperiod. However, when energy consumption is held constant, it is important to consider the parameter of light uniformity. High light uniformity promotes uniformity in plant maturation, whereas low light uniformity leads to inconsistent plant growth, which is detrimental to production. It is necessary to improve the uniformity of light to reduce the production cost of artificial plant factories. While improving the uniformity, it is also necessary to improve light energy utilisation to irradiate more light on the plants. Furthermore, in scientific research on plant growth, it is essential to ensure consistent overall plant development. This approach allows for a better reflection of the scientific validity of the experiment.

The studies above identified optimal red-to-blue light ratios for lettuce growth in artificial lighting environments, predominantly underscoring that lettuce flourishes in red-light-rich settings. However, these experiments did not systematically vary the red-to-blue light ratios, precluding insights into how lettuce growth reacts under constant-gradient conditions of these ratios.

Additionally, the light environment's stability must consider the irradiation plane's uniformity. Numerous studies on supplemental lighting for plants frequently overlook the homogeneity of light distribution on the supplementary lighting plane. This negligence results in uneven growth patterns among plants within the same experimental group.

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In experiments involving supplemental lighting for plants, light intensity is typically quantified with numerical values. However, variations in light fixtures can result in uneven light distribution across different areas. Such non-uniformity potentially leads to inconsistent growth in lettuce within the same experimental group, thereby challenging the accurate representation of specific experimental conditions. The primary objective of this experiment is to examine the effects of various red-to-blue light ratios on the growth, photosynthesis, and yield of a Spanish lettuce cultivar under conditions of consistent high light uniformity in the supplemental lighting setting.

MATERIAL AND METHODS

Plant materials and growth conditions. The experiment was conducted at Shanghai Yingzhi Technology Co., Ltd. Spanish green lettuce (*Lettuce sativa* L. cv. Spanish Green) seeds in sponge cubes (25 mm × 25 mm × 25 mm) within a hydroponic system. Using two 4-tier plant cultivation racks (Figure 1), 42 lettuce plants were planted on each tier. Seed germination stage: the seeds are placed in a germination chamber for two days (no light, temperature 18 ± 2 °C, humidity: 70 ± 5%). Developmental stage: following which they were transferred to a supplementary light holder. This holder provided a photosynthetic photon flux density (PPFD) of 200 ± 20 μmol/m^2/s and a 16-h photoperiod for 38 days. Cultivate lettuce using the commercial vegetable formula nutrient solution developed by Otsuka in Japan, composed of the following components: $Ca(NO_3)_2 \cdot 4\ H_2O$: 76 g/L; KNO_3: 105 g/L; $MgSO_4 \cdot 7\ H_2O$: 36 g/L; $NH_4H_2PO_4$: 13 g/L; H_3BO_3: 2.8 g/L; $MnSO_4 \cdot 4\ H_2O$: 1.8 g/L; $ZnSO_4 \cdot 7\ H_2O$: 0.22 g/L; $CuSO_4 \cdot 5\ H_2O$: 0.05 g/L; $(NH_4)_2MoO_4$: 0.02 g/L; Fe-EDTA: 30 g/L. The electrical conductivity (EC) of the nutrient solution used for lettuce cultivation was maintained at 2.6 mS/cm, while the greenhouse temperature was controlled at 23 ± 2 °C. The CO_2 concentration in the laboratory was not controlled. Eight days post-transplantation to hydroponic tanks, four plants with uniform growth from each layer were selected for bi-daily height measurements.

Light treatments. A vertical four-layer light frame, constructed with steel shelves measuring [2.6 m (length) × 1.28 m (width) × 1.8 m (height)], was utilised in the experiment. Each layer was equipped with 12 evenly spaced LED tubes, each tube having a maximum power of 34 W. Each lamp tube can rotate axially. The LED tubes were fitted with lamp beads peaking in the red (660 nm) and blue bands (450 nm). Red and blue light emissions are uniformly regulated by a control box. When the photosynthetic photon flux density at the seedbed plane (30 cm below the lamp) reached 200 ± 20 μmol/m^2/s, the total output power was recorded at 225 W using a power meter. The power supply equipment for the plant supplemental lighting system used in this experiment is provided by Tianchang Fu'an Electronic Co., Ltd. (Tianchang, Chuzhou, China).

In this study, eight different ratios of red and blue light quality were exposed to varying ratios of red and blue LED light quality. These included: (1) R2B8 – with 20% red and 80% blue; (2) R3B7 – with 30% red and 70% blue; (3) R4B6 – with 40% red and 60% blue; (4) R5B5 – with 50% red and 50% blue; (5) R6B4 – with 60% red and 40% blue; (6) R7B3 – with 70% red and 30% blue; (7) R8B2 – with 80% red and

(A) (B)

Figure 1. (A) The actual picture of lamps and (B) the cultivation environment of lettuce 40 days after transplanting

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Table 1. The details of light treatments for the experiment

Experiment group	Performance period	PPFD (μmol/m²/s)	Red to blue light power ratio	
1	13:00–5:00 (16/8)	200	R2B8	20% red and 80% blue
2	13:00–5:00 (16/8)	200	R3B7	30% red and 70% blue
3	13:00–5:00 (16/8)	200	R4B6	40% red and 60% blue
4	13:00–5:00 (16/8)	200	R5B5	50% red and 50% blue
5	13:00–5:00 (16/8)	200	R6B4	60% red and 40% blue
6	13:00–5:00 (16/8)	200	R7B3	70% red and 30% blue
7	13:00–5:00 (16/8)	200	R8B2	80% red and 20% blue
8	13:00–5:00 (16/8)	200	R9B1	90% red and 10% blue

PPFD – photosynthetic photon flux density

20% blue; and (8) R9B1 – with 90% red and 10% blue. The detailed parameters of these lighting treatments are presented in Table 1.

To ensure uniform light intensity at a distance of 30 cm below the lamp, the experiment involved adjusting the spacing between lamps and rotating the angle of T8 tubes on both sides while measuring PPFD. Optimal light uniformity was obtained with a lamp spacing of 20 cm. Figure 2 illustrates the photosynthetic photon flux density distribution 30 cm beneath the lamp, with the lamp spacing maintained at 20 cm. Light distribution was conducted to verify the lamps' uniformity. The PPFD uniformity can be quantified using the following equation:

$$\text{PPFD uniformity} = \text{PPFD}_{min}/\text{PPFD}_{avg}$$

As lettuce is not cultivated around the seedling bed, the calculation is confined to the PPFD uniformity on the planting plane, which is found to be 84.11%. In this experiment, variations in light quality ratios are represented by the luminous power ratio of the luminaire. Specifically, the red-blue ratio refers to the output power ratio of the red and blue LED channels.

Light source heat dissipation design. Extended use of LED lamps can result in the accumulation of heat. To enhance heat dissipation, experiments were conducted by adding cross-flow fans to each layer of the lamp rack on the side.

Measurement of lettuce morphology. Starting from the 8th day after sowing, measurements of lettuce were initiated. Use a ruler to measure plants'

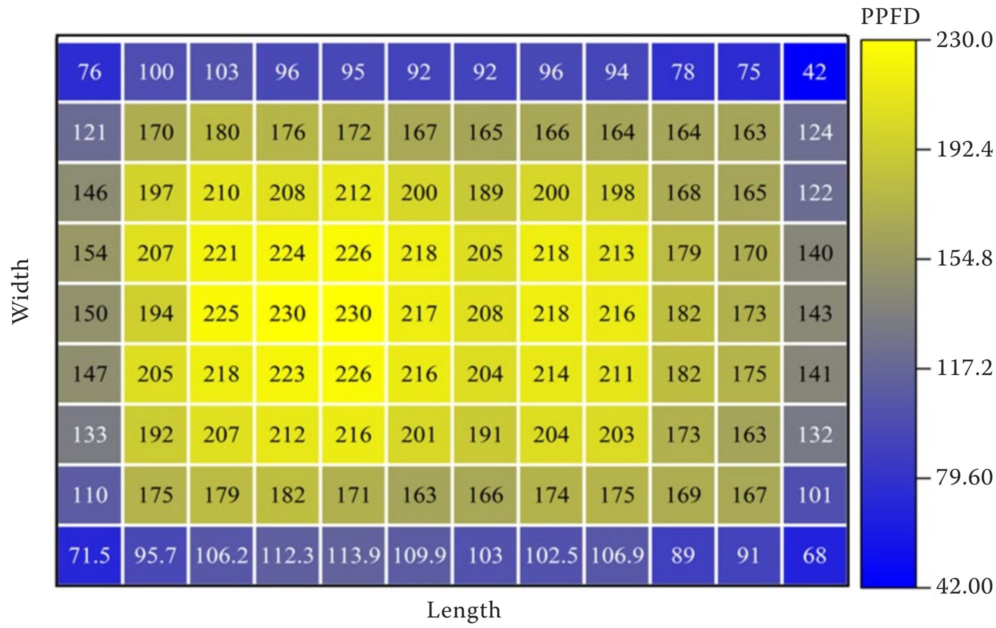

Figure 2. Light intensity distribution at 30 cm below the luminaire plane. PPFD – photosynthetic photon flux density

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height, length, and width, and select the outer layer of expanded lettuce plants with photosynthetic activity for measurement. Four samples from each experimental group underwent measurement. This data was recorded every two days until the 20th day. Subsequently, on the 20th day and after that, the leaf length and width of lettuce were measured every 5 days. On the 40th day, the plants' fresh weight (FW) was measured using an electronic balance. The plants were then placed in an oven at 105 °C for deactivation, followed by a two-day drying period at 80 °C to determine the dry weight (DW). The dry matter content (DMC) of the plants was calculated using the formula below:

$$DMC = \frac{DW}{FW} \times 100\%$$

Measurement of uniformity of lighting fixtures. The uniformity of lighting in the seedbed was assessed using a plant light analyser. PPFD value was measured at a distance of 30 cm below the lamp plane, aggregating data from 108 test points to calculate light uniformity.

Measurement of photosynthetic fluorescence in lettuce. The photosynthetic parameters of lettuce were gauged using a portable photosynthesis meter (LI-6400, Li-COR, Lincoln, USA). The largest fully expanded leaf was selected for measuring photosynthetic characteristics, including net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s) and intercellular CO_2 concentration (c_i). Each plant underwent measurement thrice. Chlorophyll fluorometer parameters in lettuce leaves were measured with a portable chlorophyll fluorometer (PAM-2500 WALZ, Nuremberg, Germany). After a 30-min dark adaptation, the lettuce plants were measured for photosynthetic parameters. The light intensity of the built-in light source of the photosynthesis meter was set to 200 μmol/m^2/s, and the value of the photosynthetic rate of the tested plants was recorded when it stabilised. Subsequent measurements included maximum quantum yield (F_v/F_m) and PSII maximum quantum efficiency (F_v'/F_m'). The ΦPSII parameter was calculated using the ($F_m' - F_s$) ratio to F_m' (Fu et al. 2012).

Statistical methods and tools. Each experimental group consisted of four plants, and all growth traits and intrinsic plant parameters were analysed using SPSS 22 (IBM, Inc., Chicago, USA). Significant differences among treatments were indicated by distinct letters at $P < 0.05$ by Duncan's multiple range test.

RESULTS AND DISCUSSION

Effect of R/B ratios on the morphology of lettuce. In examining the impact of light quality on Spanish lettuce, the study focused on the plant's entire growth cycle. Figure 3A illustrates the plant height growth data from days 8 to 18 of sowing. An increase in the proportion of red light significantly influences lettuce height. Notably, on day 16, the R9B1 group exhibited a 57.39% greater height than the R2B8 group. However, by day 18, the increase in lettuce height plateaued. This plateau can be attributed to the spreading of leaves, ensuring adequate light absorption as the plant grows. During days 20 to 40, a high proportion of red light (R9B1 and R7B3) positively impacted lettuce height. On day 40, plants under R9B1 and R7B3 treatments were 22.41–29.31% taller than those under the R4B6 treatment, whereas no significant height difference was observed in other groups (Figure 3B). The measurement of lettuce seedling height in our experiments revealed that a higher proportion of red light (R/B > 1) in the early stages of growth significantly benefits lettuce seedling development; the height of lettuce seedlings consistently increased with a higher red-to-blue ratio. Previous research has established that light quality during the seedling stage affects seedling growth and harvest weight (Johkan et al. 2010, Yan et al. 2019). Our findings corroborate this, indicating that lettuce is heavier when the proportion of red light exceeds blue light, peaking at a red-to-blue ratio of 8:2 (R8B2). For leaf length and width growth, the trends among different experimental groups showed minimal variance (Figure 3C, D), indicating that light quality did not significantly affect leaf morphology at a PPFD of μmol/m^2/s.

As depicted in Figure 4A, at the conclusion of the seedling stage (20 days), Spanish lettuce exhibited the most significant increase in leaf length under the R8B2 light condition, showing a 29.35% enhancement compared to the R3B7 and R4B6 conditions. Regarding leaf width, R7B3 emerged as the superior condition, presenting a 41.18% increase over R4B6. Figure 4B illustrates that, during the growth and maturity phase (40 days), the R3B7 and R6B4 experimental groups demonstrated the most pronounced leaf length improvement, with R3B7 outperforming R7B3 by 14.67%. Overall, this result reflects that the lettuce growth status of the R8B2 experimental group was most favourable before lettuce transplantation, but the difference in leaf morphology decreased after transplantation.

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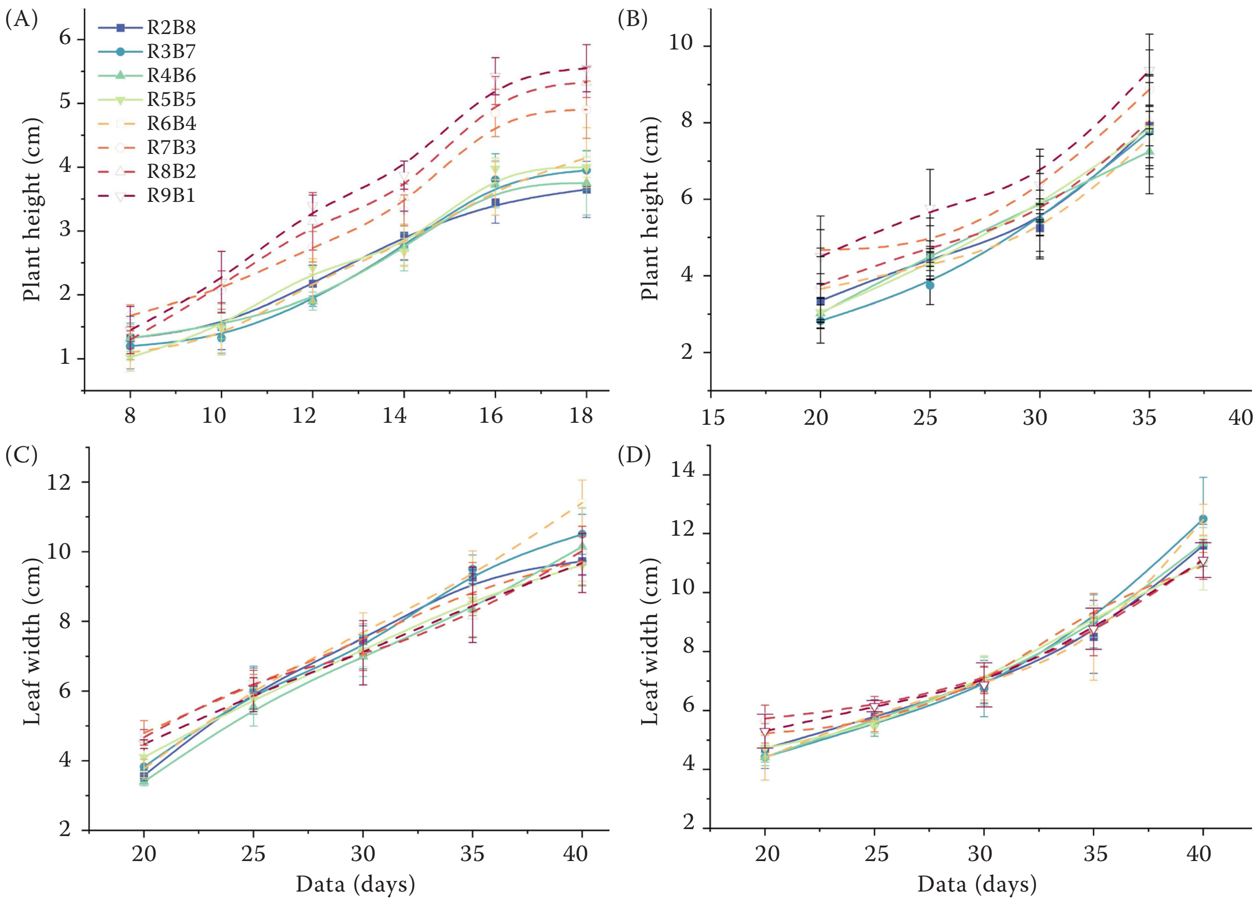

Figure 3. The plant height and growth of Spanish lettuce (A) before 18 days and (B) after planting under different light quality, and (C) the leaf width and (D) leaf length growth after planting. R2B8 – 20% red and 80% blue; R3B7 – 30% red and 70% blue; R4B6 – 40% red and 60% blue; R5B5 – 50% red and 50% blue; R6B4 – 60% red and 40% blue; R7B3 – 70% red and 30% blue; R8B2 – 80% red and 20% blue; R9B1 – 90% red and 10% blue

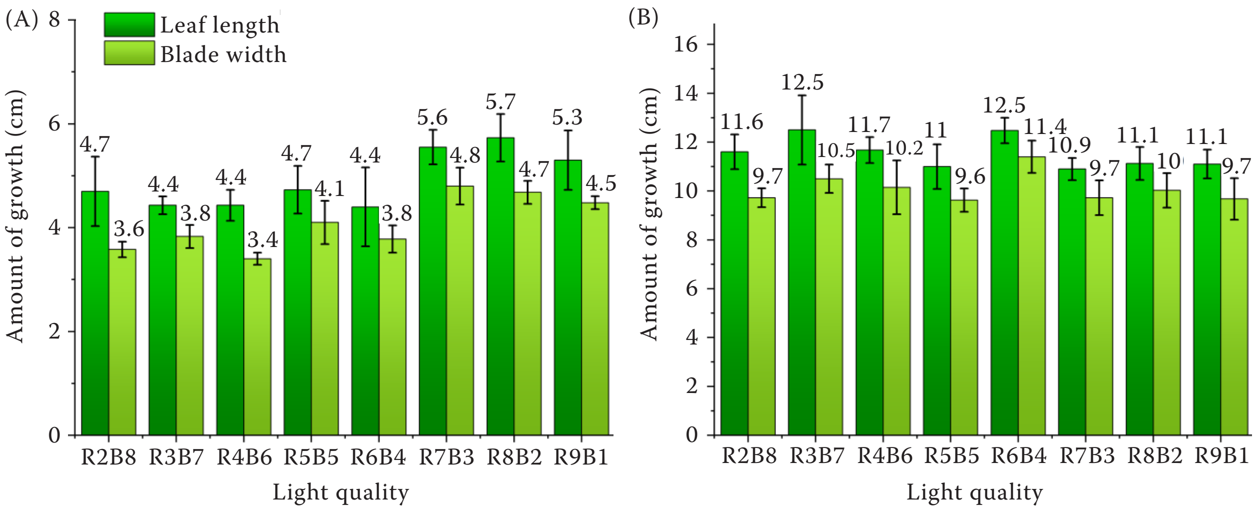

Figure 4. Effect of different ratios of red and blue light on leaf length and width of Spanish lettuce, (A) days 20 and (B) days 40. R2B8 – 20% red and 80% blue; R3B7 – 30% red and 70% blue; R4B6 – 40% red and 60% blue; R5B5 – 50% red and 50% blue; R6B4 – 60% red and 40% blue; R7B3 – 70% red and 30% blue; R8B2 – 80% red and 20% blue; R9B1 – 90% red and 10% blue

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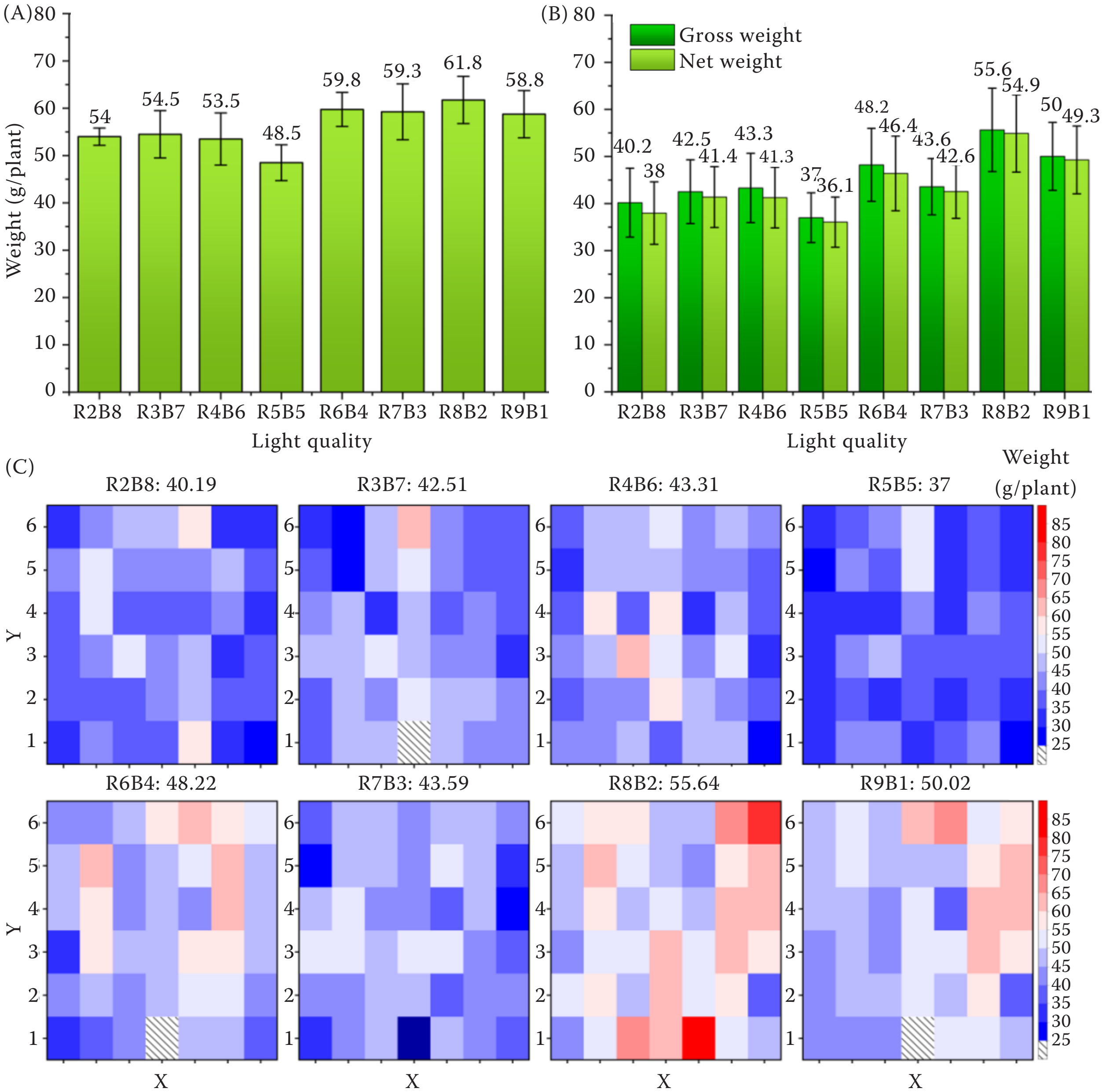

Figure 5. The effect of different LED light quality ratios on the weight of Spanish lettuce. (A) indicates the weight of the tested plants per layer; (B) reflects the average weight of the overall experimental group, and (C) is the weight of each lettuce in the spatial position of each experimental group. R2B8 – 20% red and 80% blue; R3B7 – 30% red and 70% blue; R4B6 – 40% red and 60% blue; R5B5 – 50% red and 50% blue; R6B4 – 60% red and 40% blue; R7B3 – 70% red and 30% blue; R8B2 – 80% red and 20% blue; R9B1 – 90% red and 10% blue

Plant weight and dry matter content. To ensure experimental accuracy, the fresh weight of all plants was measured. Figure 5A illustrates the weight of Spanish green lettuce in each group, with the R8B2 group showing 27.3% higher weight than the R5B5 group. No significant weight differences were observed among the R3B7, R6B4, R7B3 and R9B1 experimental groups. As depicted in Figure 5B, an increase in the proportion of red light in the red to blue correlated with a rising trend in lettuce weight. In the R8B2-treated plants, the average weight peaked at 55.64 g, marking a 53.08% increase compared to the R5B5 group. Figure 5C presents the weight distribution of each lettuce plant under varying light mass ratios, where each small square indicates the weight of the corresponding lettuce.

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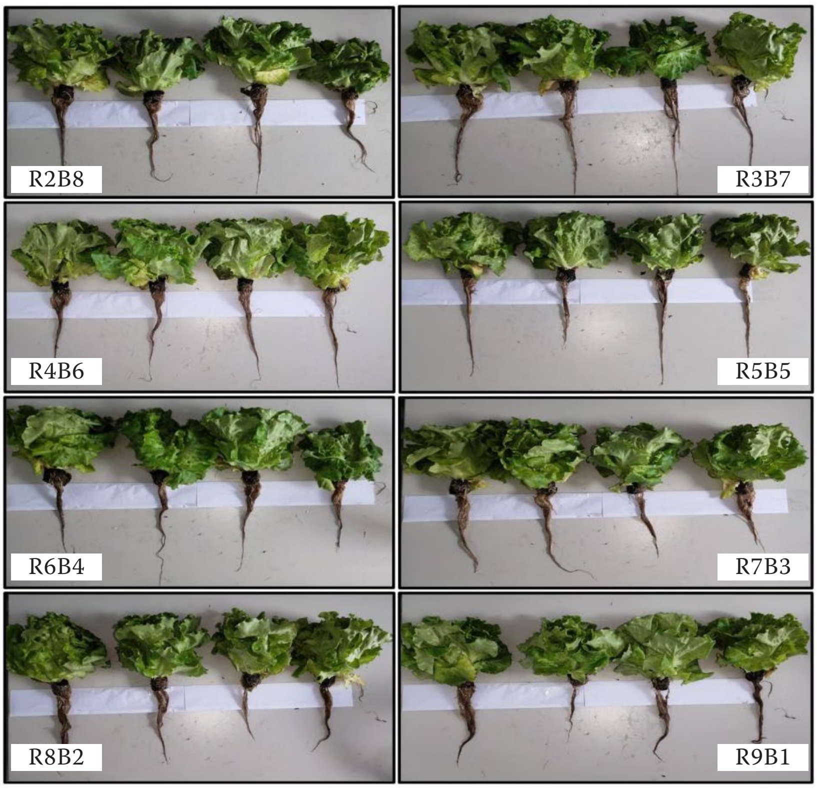

Figure 6. Growth of Spanish lettuce under different light quality ratios (40 days). R2B8 – 20% red and 80% blue; R3B7 – 30% red and 70% blue; R4B6 – 40% red and 60% blue; R5B5 – 50% red and 50% blue; R6B4 – 60% red and 40% blue; R7B3 – 70% red and 30% blue; R8B2 – 80% red and 20% blue; R9B1 – 90% red and 10% blue

A darker red colour signifies a heavier weight, while a bluer colour indicates a lighter weight, highlighting that R8B2-treated plants are generally heavier than those in the other groups. The plant growth status in each experimental group on day 40 is showcased in Figure 6.

The test plants were dehydrated, and their dry matter content was calculated by dividing their dry weight by fresh weight for each experimental group. Figure 7 reveals that the R7B3 group had the highest dry matter content at 4.58%. The R8B2 group's dry matter content stood at 4.27%, which is not significantly different from the R7B3 group, which had the highest value. The lowest dry matter content was observed in the R3B7 experimental group at 3%. This indicates that a light source rich in red light is beneficial for accumulating dry matter in lettuce.

Effect of R/B ratios on photosynthesis in lettuce. Figure 8 demonstrates the influence of various red-to-blue light mass ratios on Spanish green lettuce's photosynthetic parameters (P_n, T_r, c_i, g_s). Across the eight experimental groups, P_n peaked in the R3B7 group, showing no significant difference from the R2B8, R4B6, R5B5 and R8B2 groups. The remaining three groups differed significantly from these five. Snowden et al. (2016) observed that variations in red-to-blue light ratios did not significantly affect the net photosynthetic rate of lettuce under 200 μmol/m^2/s, aligning with our findings. Regarding transpiration rate, the R3B7 group achieved the highest rate, differing significantly only from the

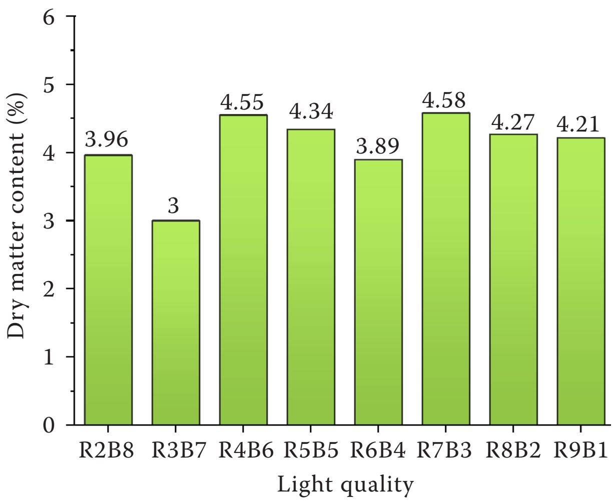

Figure 7. Dry matter content of lettuce under different red-to-blue ratios. R2B8 – 20% red and 80% blue; R3B7 – 30% red and 70% blue; R4B6 – 40% red and 60% blue; R5B5 – 50% red and 50% blue; R6B4 – 60% red and 40% blue; R7B3 – 70% red and 30% blue; R8B2 – 80% red and 20% blue; R9B1 – 90% red and 10% blue

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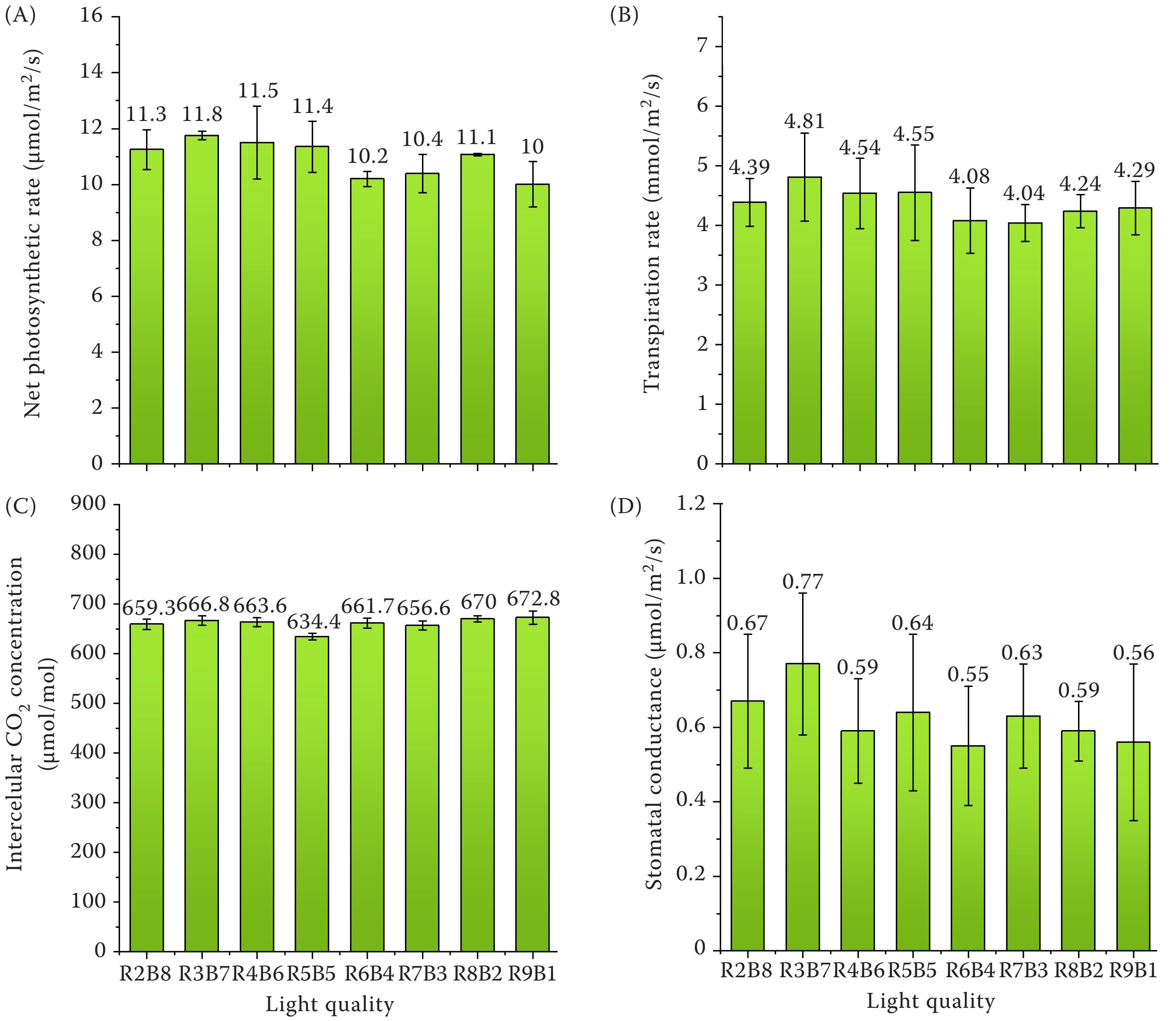

Figure 8. These four figures represent the variations in photosynthetic parameters: (A) net photosynthetic rate; (B) transpiration rate; (C) intercelular CO_2 concentration, and (D) stomatal conductance of Spanish green lettuce at different R/B (red-to-blue) ratios. The vertical line under the letter indicates the error. R2B8 – 20% red and 80% blue; R3B7 – 30% red and 70% blue; R4B6 – 40% red and 60% blue; R5B5 – 50% red and 50% blue; R6B4 – 60% red and 40% blue; R7B3 – 70% red and 30% blue; R8B2 – 80% red and 20% blue; R9B1 – 90% red and 10% blue

R6B4 and R7B3 groups. Following the R3B7 group, there was a slight decrease in stomatal conductance with increasing red light, but the reduction was not significant; this indicates that under the conditions of this experiment, stomatal conductance was not found to be influenced by photosynthesis. Regarding intercellular CO_2 levels, the R5B5 group recorded the lowest value, with no significant difference observed between the R3B7, R4B6, R8B2, and R9B1 groups. Previous studies, such as those by Snowden et al. (2016), indicate that blue light can enhance stomatal conductivity (Zheng and Van Labeke 2017).

Stomatal conductance is influenced by several factors, including the number, size, and pore dimensions of the stomatal (Franks and Beerling 2009). Both red and blue light trigger stomatal opening in plant leaves, with blue light being more quantum-efficient in stimulating stomatal aperture than red light. In this study, groups with a higher blue light proportion showed increased stomatal conductance and a corresponding rise in transpiration rate, leading to more water loss. This is consistent with findings by Clavijo-Herrera et al. (2018). The stomatal conductance reached a maximum value at R3B7. Regarding

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the photosynthetic rate, the highest g_s was recorded in the R3B7 group, which also exhibited a 17.4% higher photosynthetic rate than the R9B1 group. This indicates that the high blue light content in this experiment may promote the photosynthetic process and moderately promote stomatal opening. However, at the 40-day harvest point, R3B7 was not the most effective. These parameters alone do not predict the final biomass of the lettuce. The research conducted by Izzo et al. (2021) suggests that blue light influences the leaf thickness and chlorophyll content of lettuce, which is consistent with the trends presented in the experiment. Consequently, excessive blue light may lead to smaller, thicker leaves with a higher photosynthesis rate per unit area but reduces the total light energy absorbed by the plant (Dougher and Bugbee 2004, Kang et al. 2016). Wang et al. (2016) noted a similar trend in lettuce, with the photosynthetic rate decreasing as the red-to-blue ratio increased. This aligns with our findings that the photosynthetic rate in lettuce improved with a higher blue-light proportion, outperforming groups with a higher red-light proportion. However, there is no direct correlation between the photosynthetic rate of a single leaf and the weight of aboveground parts. Although the R8B2 illumination is not optimal for g_s and T_r, the increase in red light and an appropriate amount of blue light enables rapid growth of lettuce seedlings, leading to more significant growth potential in later stages. Further research is needed to elucidate the specific mechanisms behind these observations.

Effect of R/B ratios on chlorophyll fluorescence of lettuce. Chlorophyll molecules capture light energy and utilise it in three primary ways: conducting photochemical reactions, converting it to thermal energy, and emitting chlorophyll fluorescence. The assessment of chlorophyll fluorescence can gauge the efficiency of photochemical reactions and their associated heat dissipation (Tsai et al. 2019). Photochemical quenching parameters (qP and qL) indicate the proportion of open PSII reaction centres and the reduced state of the primary electron acceptor Q_A, calculated using different models (Kramer et al. 2004).

Table 2 shows that the photochemical quenching (qP and qL) peaked at R9B1 (0.860), followed by R8B2, and was the lowest in the R5B5 group. Among all groups, the R8B2 treatment displayed the highest F_v/F_m value (0.806), succeeded by the R3B7, R4B6, and R6B4 treatments in descending order. Additionally, the R8B2 treatment exhibited the highest F_v'/F_m' and ΦPSII values.

Chlorophyll molecules absorb light energy and revert to the ground state *via* three mechanisms: driving photosynthesis, dissipating as heat, or manifesting as chlorophyll fluorescence. Improvement in any one of those processes inversely affects the other two. The qP and qL values represent the proportion of PSII reaction centres in an open state and serve as indicators of the QA quinone's oxidation-reduction state (Hu et al. 2022). In this study, the R9B1 experimental group exhibited the highest qP and qL values, markedly surpassing those of other groups. Overall, qP and qL show a trend of initially decreasing and then

Table 2. Chlorophyll fluorescence parameters at different red-to-blue light mass ratios

	qP	qL	F_v/F_m	F_v'/F_m'	ΦPSII
R2B8	0.906 ± 0.001[c]	0.771 ± 0.003[c]	0.781 ± 0.007[cd]	0.75 ± 0.003[d]	0.589 ± 0.003[d]
R3B7	0.893 ± 0.001[d]	0.732 ± 0.002[d]	0.793 ± 0.005[b]	0.773 ± 0.002[c]	0.601 ± 0.002[d]
R4B6	0.906 ± 0.002[c]	0.758 ± 0.004[d]	0.787 ± 0.009[bc]	0.776 ± 0.001[b]	0.612 ± 0.002[c]
R5B5	0.874 ± 0.001[d]	0.697 ± 0.002[d]	0.759 ± 0.004[d]	0.749 ± 0.002[d]	0.584 ± 0.002[d]
R6B4	0.877 ± 0.002[d]	0.708 ± 0.002[d]	0.792 ± 0.009[b]	0.760 ± 0.003[d]	0.578 ± 0.004[d]
R7B3	0.900 ± 0.002[d]	0.725 ± 0.004[d]	0.758 ± 0.01[d]	0.756 ± 0.002[d]	0.600 ± 0.001[d]
R8B2	0.915 ± 0.001[b]	0.787 ± 0.003[b]	0.806 ± 0.008[a]	0.798 ± 0.001[a]	0.637 ± 0.001[a]
R9B1	0.948 ± 0.001[a]	0.860 ± 0.002[a]	0.775 ± 0.005[d]	0.761 ± 0.001[d]	0.627 ± 0.002[b]

Different letters indicate statistically significant differences at $P \leq 0.05$. qP – photochemical quenching based on the puddle model; qL – photochemical quenching based on the lake model; F_v/F_m – maximum quantum yield; F_v'/F_m' – PSII maximum quantum efficiency; ΦPSII – quantum yield of photosystem II; R2B8 – 20% red and 80% blue; R3B7 – 30% red and 70% blue; R4B6 – 40% red and 60% blue; R5B5 – 50% red and 50% blue; R6B4 – 60% red and 40% blue; R7B3 – 70% red and 30% blue; R8B2 – 80% red and 20% blue; R9B1 – 90% red and 10% blue

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increasing with the red/blue ratio increase. However, the overall average weight of the R9B1 group is still lower than that of the R8B2. ΦPSII, denoting the photochemical efficiency of PSII, helps estimate the electron transport rate through PSII. F_v'/F_m' and ΦPSII also reflect photochemical efficiency, and in this study, R8B2 outperformed other groups in these parameters. This aligns with R8B2's superior biomass at harvest, offering a potential explanation. F_v/F_m, F_v'/F_m', and ΦPSII parameters more reliably represent photosynthetic activity than qL. These parameters are measured after saturation light exposure in the measuring equipment; the measured results more accurately reflect the condition of the lettuce. Miao et al. (2016) observed that red light reduces PSII activity and electron transfer to PSI, but blue light can mitigate this effect. This phenomenon may explain the higher net photosynthetic rate in the R8B2 group compared to others. In conclusion, our findings recommend using red-light-rich lamps for supplementary lighting in the early stages of lettuce seedling growth. Additionally, creating an optimal environment (200 ± 20 μmol/m^2/s, 16/8 h, R8B2) proves beneficial for maximising the yield of Spanish green lettuce. This experiment offers valuable insights into the cultivation strategies of other lettuce cultivars.

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