

Article

Green Light Proportion Modulates Growth, Antioxidant Capacity, and Nutrient Utilization in Hydroponically Grown Lettuce Under Red–Blue Light

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Abstract

To investigate the effects of red–blue–green light ratios on the growth, photosynthesis, nutritional quality, and mineral nutrient accumulation of hydroponic lettuce, a controlled experiment was conducted using the deep flow technique (DFT) system. Four light treatments were applied under a constant photosynthetically active radiation of $350 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$: the red–blue light control (CK, R:B = 60:40) and 10% (T1), 20% (T2), and 40% (T3) green light substitution treatments. Growth, photosynthetic characteristics, quality and nutrient indexes were assessed. The results showed that 40% green light (T3) significantly enhanced biomass production, increasing the fresh and dry weights by 33.16% and 21.40%, respectively, compared with the CK (control). In addition, the soluble sugar and vitamin C contents increased by 61.79% and 13.43%, respectively, while the nitrate content decreased by 16.31%. T1 (10% green light) was the most effective at promoting antioxidant compound accumulation, resulting in 148.61% and 81.24% increases in the flavonoid and polyphenol contents. All green light treatments increased the net photosynthetic rate and transpiration rate while decreasing the intercellular CO_2 concentration and stomatal conductance. Green light supplements generally promoted phosphorus and magnesium uptake, and T3 remarkably enhanced the total accumulation of major mineral elements in shoots. In conclusion, 40% green light substitution optimizes both the biomass and nutritional quality of lettuce, whereas 10% substitution significantly promotes antioxidant accumulation. These findings provide valuable insights for optimizing light spectra in controlled environments for hydroponic lettuce production.

Keywords: lactuca; red–blue–green light; photosynthesis; nutrients; antioxidant



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1. Introduction

Light is a pivotal environmental factor regulating plant growth, development, and metabolic processes, serving as both an energy source for photosynthesis and a signal molecule modulating gene expression and physiological responses [1,2].

Lettuce (*Lactuca sativa* L.), a major leafy vegetable crop worldwide, is highly valued for its rapid growth, high yield, and rich nutritional composition, including vitamins, minerals, dietary fiber, and bioactive compounds beneficial to human health [3,4]. As a classic model crop for controlled-environment agriculture, it is widely cultivated in hydroponic systems and exhibits strong responsiveness to light quality manipulation owing to its short growth

cycle and leafy edible organ [4,5]. Increasing evidence indicates that the spectral composition of light exerts a greater influence on biomass accumulation and quality attributes than light intensity alone [5,6]. Both light intensity and spectral composition significantly modulate plant physiological and biochemical processes and ultimately shape plant growth and productivity. Red light (600–700 nm) is known to enhance photosynthetic electron transport and chlorophyll synthesis, while blue light (400–500 nm) regulates stomatal opening and the synthesis of secondary metabolites (e.g., vitamin C and polyphenols) [7–9]. Previous studies have confirmed that red light significantly mediates plant growth by regulating leaf expansion and leaf area development [10]. Accordingly, the combination of red and blue light (R:B) has been widely recognized as the basic light regime for hydroponic lettuce production [11–13]. However, many studies have focused on binary red–blue light combinations, neglecting the potential regulatory role of green light (500–600 nm) in lettuce growth and quality. It has been documented that combined red and blue light can improve photosynthetic acclimation in cucumber and lettuce leaves, promoting leaf expansion and chlorophyll accumulation and, hence, boosting plant growth and biomass accumulation [14].

Traditionally, green light was considered a “photographically inactive” spectral region due to its lower absorption by chlorophyll compared to red and blue light [15]. However, recent advances in plant photophysiology have revealed that green light possesses unique regulatory mechanisms [15–18]. Green light penetrates deeper into the plant canopy than red and blue wavelengths, promoting photosynthesis in lower leaf layers and alleviating canopy self-shading [15,16]. Moreover, green light signals are perceived through photoreceptor-mediated pathways involving cryptochromes and phytochromes, which influence stomatal behavior, intercellular CO₂ concentration, and ultimately photosynthetic carbon assimilation [17,18]. However, how green light interacts with red and blue light to regulate key physiological and metabolic processes, including antioxidant defense and nutrient acquisition, remains unclear in lettuce. Elucidating these mechanisms is essential for designing spectral strategies that enhance productivity and nutritional quality in controlled-environment hydroponic systems.

Green light exhibits species-specific regulatory roles in horticultural crops, offering critical insights for lettuce photophysiology research. In hydroponically grown cucumber (*Cucumis sativus*), supplementing 10–20% green light (500–600 nm) to red–blue backgrounds significantly elevates the fruit soluble sugar content and antioxidant capacity [19]. This is mechanistically attributed to the enhanced activity of sucrose synthase (SS) and phenylalanine ammonia-lyase (PAL), key enzymes governing sucrose metabolism and phenylpropanoid pathways. In spinach (*Spinacia oleracea*), green light supplementation upregulates nitrate reductase (NR) expression by 1.8-fold, thereby enhancing nitrate assimilation efficiency and reducing nitrate accumulation in edible leaves by 27% [20]. However, many studies predominantly focus on fruiting vegetables or broad-leaf greens, while research on green light ratio optimization in bolting-resistant loose-leaf lettuce remains scarce. “Yuefeng No. 1”, a representative cultivar with the Italian bolting-resistant trait, features a loose-leaf morphology and medium leaf size and is widely adopted in hydroponic vegetable production in South China. It may exhibit distinct photophysiological responses to green light compared with conventional lettuce varieties. Targeted investigations are thus imperative to decode its spectral sensing mechanisms and optimize light recipes for controlled environments.

The existing knowledge gaps highlight the need to further investigate the regulatory effects of red–blue–green (R:B:G) light spectra on hydroponically grown lettuce. Therefore, this study employed a deep flow technique (DFT) hydroponic system and evaluated four R:B:G light ratios, including a red–blue (R:B) binary spectrum as the control. The effects

of the different light treatments on the lettuce growth, photosynthetic performance, nutritional quality, antioxidant capacity, and mineral nutrient accumulation characteristics were systematically assessed. The objectives of this study were to: (i) elucidate the influence of the green light proportion on lettuce biomass accumulation and photosynthetic efficiency; (ii) identify the optimal green light ratio for enhancing nutritional quality and antioxidant capacity; and (iii) explore the mechanisms by which green light regulates nutrient uptake and utilization. The findings provide a theoretical basis and practical guidance for optimizing light environments and improving the efficiency of hydroponic lettuce production in controlled-environment agriculture.

2. Materials and Methods

2.1. Experimental Site and Materials

The experiment was conducted at the College of Horticulture, South China Agricultural University. Seeds of the loose-leaf lettuce (*Lactuca sativa* L.) cultivar “Yuefeng No. 1” were used as plant materials. This cultivar has Italian breeding traits of bolting resistance and is widely used for hydroponic production in South China. The seeds were bred, officially identified, and provided by the Vegetable Research Institute, Guangdong Academy of Agricultural Sciences.

The light environment was controlled by commercial LED dimming platforms (Huizhou Keda Technology Co., Ltd., Huizhou, China) equipped with 50 W LED panels. The red (peak wavelength: 658 ± 2.5 nm; FWHM: 10 nm), blue (peak wavelength: 458 ± 2.5 nm; FWHM: 10 nm), and green (peak wavelength: 523 ± 2.5 nm; FWHM: 10 nm) channels were independently adjustable, with a photosynthetic photon flux density (PPFD) range of $0\text{--}400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the canopy level. All channels were factory-calibrated with negligible spectral crosstalk between wavebands, ensuring high consistency between the actual spectral composition and the set R:B:G ratios. All panels with a 120° beam angle were placed in light-tight chambers to eliminate natural light interference and ensure uniform light distribution on the canopy. Before each experimental batch, the PPFD was verified at 9 evenly distributed points across the planting area using a calibrated quantum sensor, and the lamp height and output current were adjusted to maintain a canopy-level uniformity $> 90\%$ at the target total PPFD.

Plants were cultivated in a deep flow technique (DFT) hydroponic system, and a half-strength Hoagland nutrient solution was used as the culture medium. The nutrient solution was renewed every 7 days, and continuous aeration was maintained to ensure root oxygen supply.

2.2. Experimental Design

The red–blue–green light ratio was set as the sole experimental variable, and four treatments were designed, as follows: CK (R:B = 60:40); T1 (R:B:G = 54:36:10); T2 (R:B:G = 48:32:20); and T3 (R:B:G = 36:24:40). To maintain a stable R:B ratio across all treatments, green light replaced red and blue light proportionally based on the baseline R:B ratio of 60:40. Specifically, for every 10% increase in the green light fraction, red light decreased by 6% and blue light decreased by 4%. The total PPFD at the canopy level was uniformly controlled at $350 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for all treatments. The peak wavelength and FWHM of each LED channel were factory-calibrated with no significant spectral crosstalk, which further ensured the reliability of the single-variable design.

The experiment was conducted across three independent temporal batches, and light treatments were randomly re-assigned to the four controlled-environment chambers at the start of each batch to eliminate potential chamber effects. Each chamber hosted one light treatment per batch to prevent spectral cross-contamination. A completely randomized

design (CRD) was adopted at the batch level. Each chamber contained three vertically stacked hydroponic layers, and each layer held 24 plants arranged in 3 rows $\times$ 8 columns, resulting in a total of 72 plants per treatment per batch. Plant spacing was set at 15 cm to avoid mutual shading. A side-view schematic diagram of the experimental setup is provided in Figure 1, demonstrating the chamber configuration, stacked cultivation layers, and overall experimental workflow. Detailed spectral parameters of the four treatments are summarized in Table 1.

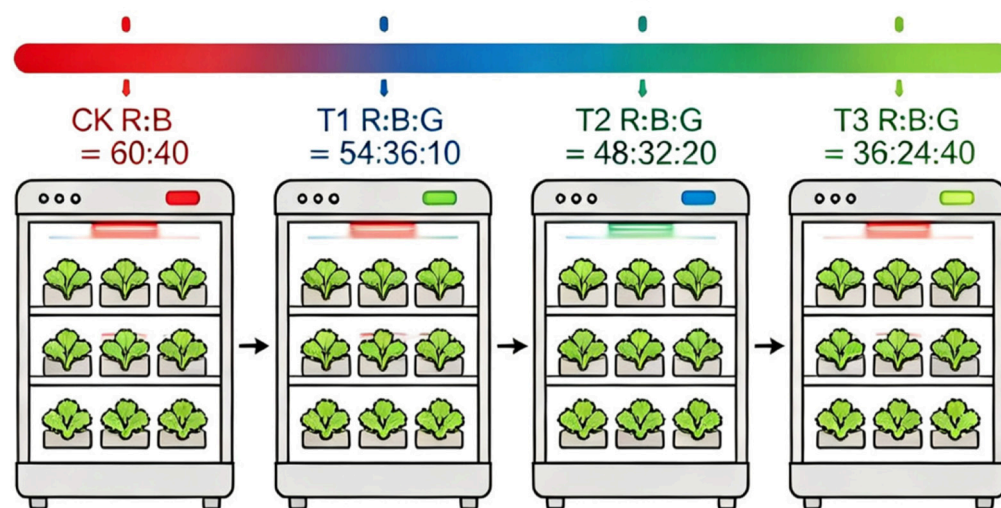


Figure 1. A schematic diagram of the experimental setup and sampling procedure. This side-view diagram shows the stacked hydroponic layers inside the controlled-environment chamber and the overall experimental workflow. Plants were cultivated hydroponically under controlled LED conditions. A completely randomized design was used across three independent batches, with one treatment per chamber per batch and 72 plants per treatment per batch (3 layers $\times$ 24 plants per layer, arranged in 3 rows $\times$ 8 columns). For each batch, 8 plants were randomly selected for growth measurements and 5 plants for physiological and biochemical assays, with the mean value per batch treated as one biological replicate ($n = 3$). The total PPFD was maintained at $350 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ across all treatments.

Table 1. The spectral parameters and experimental setup of the four light treatments. PPFD: photosynthetic photon flux density, measured at the canopy level.

Treatment	Red Light ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}/\%$)	Blue Light ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}/\%$)	Green Light ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}/\%$)	Total PPFD ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
CK	210/60	140/40	0/0	350
T1	189/54	126/36	35/10	350
T2	168/48	112/32	70/20	350
T3	126/36	84/24	140/40	350

Before sowing, seeds were germinated in darkness at $25\text{ }^{\circ}\text{C}$ for 48 h. A total of 384 uniformly germinated seeds were selected (96 seeds per treatment) to ensure enough seedlings of consistent growth for subsequent transplanting. The germinated seeds were sown into sterilized polyurethane sponge blocks ($2 \times 2 \times 2\text{ cm}$, 80% porosity) and placed in seedling trays with half-strength Hoagland nutrient solution. From day 0 to day 18 after sowing (seedling stage), all seedlings were cultivated in a unified growth chamber with a 12 h photoperiod, $25/20\text{ }^{\circ}\text{C}$ day/night temperature, 80% relative humidity, and a uniform PPFD of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by white LEDs.

On day 18 after sowing, healthy and pest-free seedlings at the three-leaf and one-heart stage with consistent growth were transplanted into the DFT hydroponic system. Plants

were harvested after 30 days of light treatment, at the marketable young leaf stage. In this study, functional leaves were uniformly defined as fully expanded, undamaged leaves with active photosynthetic capacity, which are the core organs for canopy carbon assimilation. At harvest, each plant had 16–20 functional leaves.

For growth indicators, 8 uniform plants with consistent growth were randomly selected from the 72 plants of each treatment per batch, and the mean value of the 8 plants was treated as one biological replicate value. For photosynthetic characteristic, photosynthetic pigment, nutritional quality, and antioxidant capacity assays, 5 uniform plants were randomly selected per treatment per batch, and the mean value of the 5 plants was treated as one biological replicate value. For mineral element determination, the 5 sampled plants per batch were pooled into one composite sample for analysis. Across three independent batches, the statistical sample size for all indicators was $n = 3$ (one value per batch).

The measured indicators included:

- (1) Growth indexes: number of functional leaves, maximum length and width of functional leaves, aboveground fresh and dry weights, and root fresh and dry weights;
- (2) Photosynthetic indexes: net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (Tr), and intercellular CO_2 concentration (C_i);
- (3) Quality indexes: soluble sugar content, soluble protein content, vitamin C (V_c) content, and nitrate content;
- (4) Photosynthetic pigment indexes: chlorophyll a, chlorophyll b, and carotenoid contents;
- (5) Antioxidant capacity indexes: total polyphenol content, total flavonoid content, DPPH free radical scavenging activity, and ferric ion-reducing antioxidant power (FRAP);
- (6) Mineral nutrient indexes: contents and total accumulations of total N, P, K, Ca, and Mg in shoots.

The experimental design and sampling procedure were as follows: Plants were cultivated hydroponically under controlled LED conditions. A completely randomized design was used, with three independent replicates per treatment and 24 plants per replicate plot. Five uniform plants were selected from each replicate for measurements. The total PPFD was maintained at $350 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ across all treatments.

2.3. Determination of Growth Indicators

Growth indicators were measured on the harvest day. Three independent experimental batches were included as biological replicates ($n = 3$), and 8 uniform plants were randomly selected from each treatment per batch for growth indicator determination, following the sampling criteria described in Section 2.2 [21].

In this study, functional leaves were uniformly defined as fully expanded, undamaged leaves with active photosynthetic capacity, which are the core organs contributing to plant canopy carbon assimilation. The growth indicators determined included the number of functional leaves, maximum length and width of functional leaves, aboveground fresh and dry weights, and root fresh and dry weights.

2.4. Determination of Photosynthetic Characteristics

Photosynthetic characteristics were measured using a TARGAS-1 portable photosynthesis system (PP Systems, Amesbury, MA, USA) at 9:00–11:00 a.m. on the 27th day after transplanting. During the measurement, the leaf temperature was controlled at 25°C , the reference CO_2 concentration was set to $400 \mu\text{mol}\cdot\text{mol}^{-1}$, and the leaf-to-air water vapor pressure deficit was maintained at 1.2–1.5 kPa, which were consistent with the environmental conditions of the growth chambers. Consistent with the definition of functional leaves above, the 4th to 5th fully expanded functional leaves from the top of each plant were selected for measurement. The measured parameters included the net photosynthetic rate

(Pn), stomatal conductance (Gs), transpiration rate (Tr), and intercellular CO₂ concentration (Ci) [22].

2.5. Determination of Quality Indicators

The soluble sugar content was determined using the anthrone colorimetric method [23], the soluble protein content was determined using the Coomassie brilliant blue method [24], and the vitamin C (Vc) content was determined using the molybdenum blue colorimetric method [25]. The nitrate content was determined using the sulfanilic acid–salicylic acid colorimetric method [26].

2.6. Determination of Antioxidant Capacity Indicators

The polyphenol content was determined using the Folin–Ciocalteu method [27], and the flavonoid content was determined using the aluminum nitrate method [28]. The DPPH free radical scavenging activity and FRAP antioxidant capacity were determined according to previous methods [29,30].

2.7. Determination of Mineral Elements

Lettuce dry samples were ground into a fine powder for mineral element determination. The contents of major mineral elements were determined referring to the protocols described in “Soil and Agricultural Chemistry Analysis” [31]: total nitrogen was assayed using the H₂SO₄–H₂O₂ digestion–Kjeldahl method; total phosphorus was determined via the H₂SO₄–H₂O₂ digestion–molybdenum antimony anti-colorimetry method; total potassium was measured by the H₂SO₄–H₂O₂ digestion–flame photometry method; calcium and magnesium were analyzed using the ashing–atomic absorption spectrophotometry (AAS) method; and zinc and iron were determined by AAS.

Nutrient accumulation per plant was calculated using the following formula:

$$\text{Nutrient accumulation (mg} \cdot \text{plant}^{-1}) = \text{Nutrient content (mg} \cdot \text{g}^{-1}) \times \text{Shoot dry weight (g} \cdot \text{plant}^{-1})$$

2.8. Statistical Analysis

Statistical analyses employed one-way analysis of variance (ANOVA) with the light treatment as the fixed factor, and Duncan’s multiple range test in SPSS (v19.0, IBM Corporation, Armonk, NY, USA) at the $\alpha = 0.05$ significance level. Each treatment contained 3 biological replicates corresponding to three independent experimental batches, with each replicate value representing the mean of multiple plants sampled within one batch. The experimental unit for statistical analysis was the independent batch rather than individual plants. Data visualization was executed in GraphPad Prism 8 (v8.0.2) to generate publication-ready figures.

3. Results

3.1. Effects of Red–Blue–Green Light Ratios on Lettuce Growth

Lettuce growth characteristics (biomass accumulation, leaf morphology, and plant architecture) significantly differed among treatments with the spectra of red–blue–green light ratios, with T3 outperforming the CK (Figure 2). Among the treatments, T3 significantly improved the aboveground and root biomass compared with the CK, indicating a promoting effect on plant growth. Conversely, T1 suppressed biomass accumulation, and there was no significant difference between T2 and the CK (Figure 2A–D). Specifically, the shoot fresh (69.71 g·plant^{−1}) and dry (2.78 g·plant^{−1}) weights in T3 were increased by 33.16% and 21.40%, respectively, when compared to the CK. Likewise, the root fresh (6.96 g·plant^{−1}) and dry (0.52 g·plant^{−1}) weights were increased by 10.30% and 4.00% when compared with the CK. The leaf number of T3 was similar to that of the CK, while T1 and T2 had fewer

leaves (Figure 2E). In addition, the maximum functional leaf of T3 exhibited a greater length and width than that of the CK, while T1 showed reduced values and T2 showed slight increases (Figure 2D,E). Collectively, these results indicate that supplementing red–blue light with 40% of green light can exert a synergistic effect, promoting lettuce biomass accumulation and increasing the length and width of the maximum functional leaves.

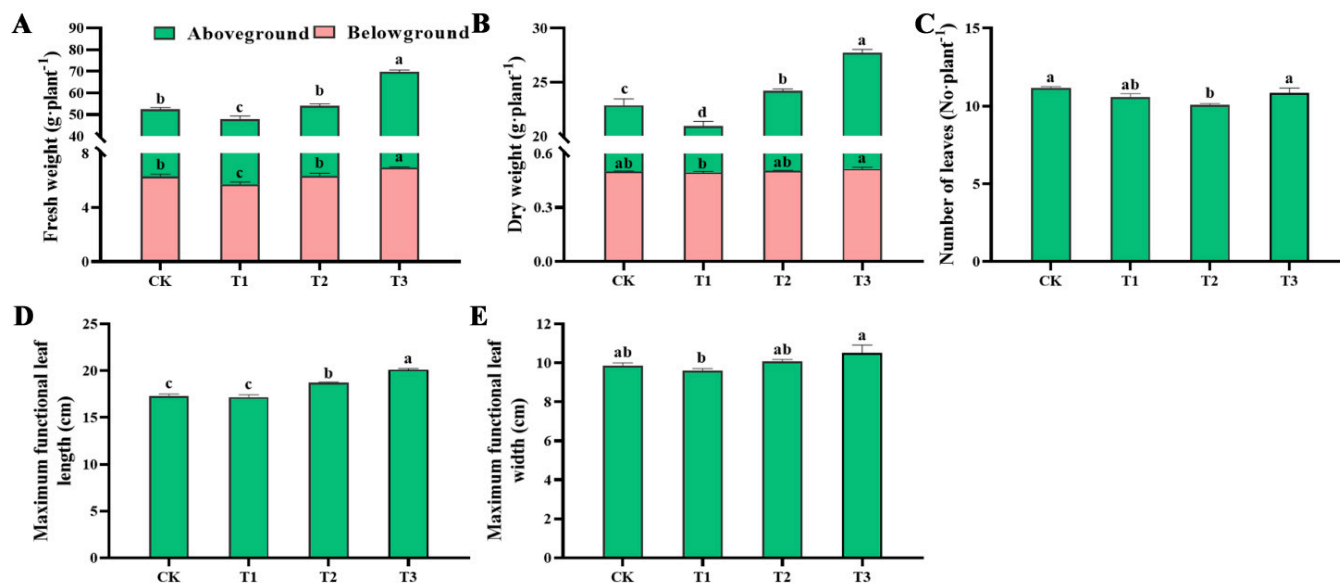


Figure 2. Effects of red–blue–green light quality ratios on lettuce growth. (A). Fresh weight. (B). Dry weight. (C). Number of leaves. (D). Maximum functional leaf length. (E). Maximum functional leaf width. Data indicated are means $\pm$ SD. Different letters above columns indicate significant differences between treatments ($p < 0.05$).

3.2. Effects of Red–Blue–Green Light Ratios on Chlorophyll

Low and moderate green light (T1, T2) significantly promoted photosynthetic pigment accumulation compared to the CK, while high green light (T3) showed no significant difference when compared to the CK (Figure 3). The chlorophyll a contents in T1 and T2 were $0.72 \text{ mg}\cdot\text{g}^{-1}$, representing an increase of 24.14% compared to the CK ($0.58 \pm 0.04 \text{ mg}\cdot\text{g}^{-1}$) (Figure 3A). Likewise, the chlorophyll b contents in both T1 and T2 were $\sim 0.21 \text{ mg}\cdot\text{g}^{-1}$, representing an increase of 16.67% compared to the CK (Figure 3B). The carotenoid content was increased by 25.00% and 18.75% in T1 and T2, respectively, compared with the CK, while T3 showed no significant difference from the CK (Figure 3D). These findings suggest that the addition of 10–20% green light to red–blue light promotes the accumulation of photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoids, in lettuce.

3.3. Effects of Red–Blue–Green Light Ratios on Lettuce Photosynthetic Characteristics

Compared with the CK, T1–T3 significantly increased the net photosynthetic rate (Pn) and transpiration rate (Tr) while significantly decreasing the intercellular CO_2 concentration (Ci) and stomatal conductance (Gs). The maximum values of the Pn and Tr were observed in T1, representing increases of 12.3% and 15.7%, respectively, compared with the CK (Figure 4A,C). With the increase in the green light proportion, the Pn and Tr gradually declined; however, the Pn under T3 remained significantly higher than that of the CK by 7.8% (Figure 4B,D). In contrast, the Ci and Gs exhibited the opposite trend and reported minimums in T1 and maximums in T3, although all treatments remained significantly lower than the CK. Notably, there was no positive correlation between the single-leaf net photosynthetic rate and whole-plant biomass. T1 had the highest Pn, but its aboveground fresh weight and dry weight were 8.7% and 6.1% lower than those in the CK, respectively.

The single-leaf Pn in T3 was 4.1% lower than that in T1, but its aboveground fresh weight and dry weight were 33.16% and 21.40% higher than those in the CK, respectively, which were the highest among all treatments (Figure 4A). These results indicate that green light regulates lettuce biomass accumulation, through multiple physiological processes beyond single-leaf photosynthetic performance, including photosynthate partitioning and canopy-level light utilization.

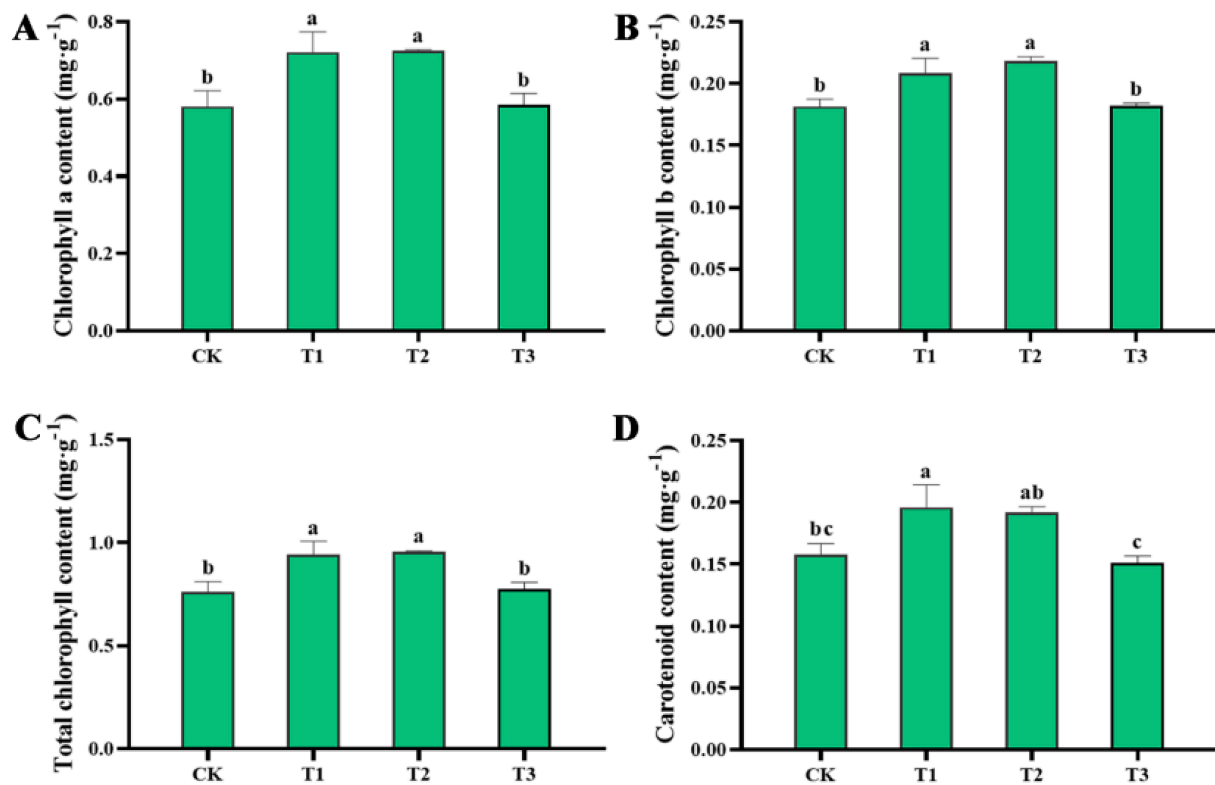


Figure 3. Effects of red–blue–green light quality ratios on chlorophyll contents of lettuce. (A). Chlorophyll a content. (B). Chlorophyll b content. (C). Total chlorophyll content. (D). Carotenoid content. Data indicated are means $\pm$ SD. Different letters above columns indicate significant differences between treatments ($p < 0.05$).

3.4. Effects of Red–Blue–Green Light Ratios on Lettuce Quality

Compared with the CK, green light treatments significantly regulated the nutritional quality of the lettuce, with clear dose-dependent responses in the soluble sugar, soluble protein, Vc, and nitrate contents (Figure 5). The soluble sugar content increased gradually with the increase in the green light proportion. T2 and T3 significantly increased the soluble sugar content by 33.22% and 61.79%, respectively, compared with the CK, while T1 showed no significant difference from the CK (Figure 5A). In contrast, the soluble protein content exhibited a decreasing trend under green light treatments, with the most pronounced reduction observed in T3 (Figure 5B). For the Vc content, T1 and T3 showed significant increases of 6.35% and 13.43% relative to the CK, respectively, whereas the lowest value was detected in T2 (Figure 5C). All green light treatments reduced the nitrate content compared with the CK, with T3 and T2 showing greater decreases of 16.31% and 15.36%, respectively, followed by T1 with a 5.49% reduction (Figure 5D).

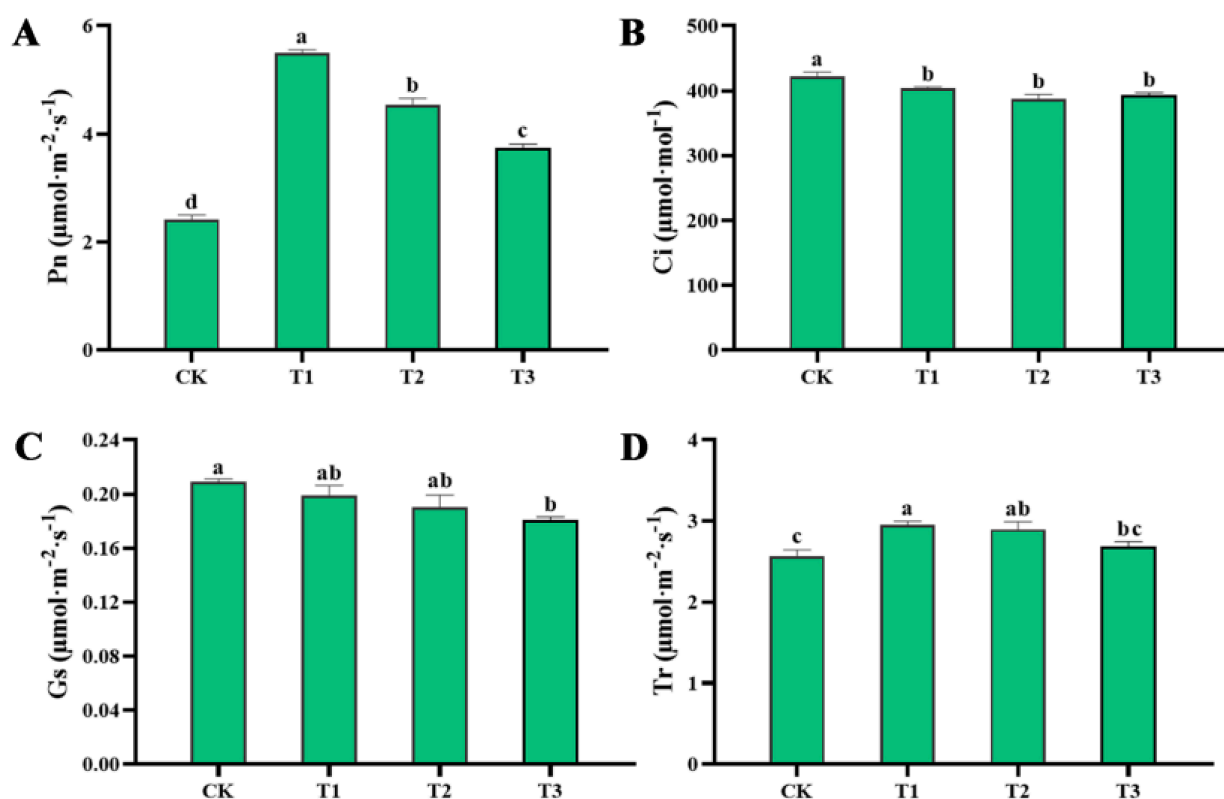


Figure 4. Effects of red-blue-green light quality ratios on photosynthetic pigment content of lettuce. (A). Pn value. (B). Ci value. (C). Gs value. (D). Tr value. Data indicated are means $\pm$ SD. Different letters above columns indicate significant differences between treatments ($p < 0.05$).

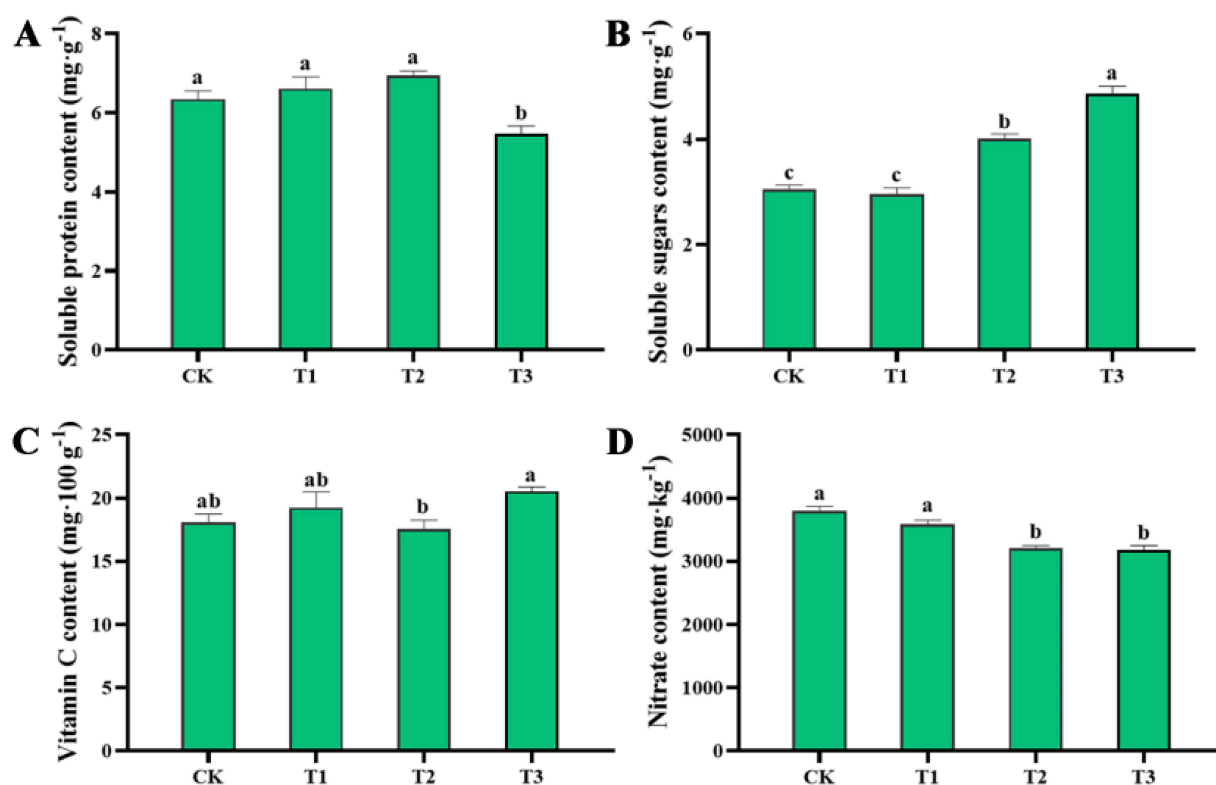


Figure 5. Effects of red-blue-green light quality ratios on lettuce quality. (A). Soluble protein content. (B). Soluble sugar content. (C). Vitamin C content. (D). Nitrate content. Data indicated are means $\pm$ SD. Different letters above columns indicate significant differences between treatments ($p < 0.05$).

These results indicate that 40% green light substitution (T3) improves overall nutritional quality by increasing soluble sugar and Vc contents and reducing nitrate accumulation, while 10% substitution (T1) slightly enhances Vc contents and reduces nitrate with no significant effect on soluble sugar and protein.

3.5. Effects of Red–Blue–Green Spectra on Antioxidant Capacity of Lettuce

Antioxidant systems play a critical role in plant adaptation to environmental stimuli [24]. In this study, the flavonoid content in T1 increased significantly by 148.61% ($2498.29 \text{ mg}\cdot\text{kg}^{-1}$) compared with that in the CK. In contrast, the flavonoid contents in T2 and T3 were reduced by 11.59% and 25.57%, respectively, compared to those in the CK (Figure 6A). All green light treatments altered the polyphenol accumulation. The results demonstrated that the polyphenol contents increased by 81.24%, 64.29% and 35.71% in T1, T2 and T3, respectively, compared to the CK, as presented in Figure 6B. Likewise, a significant increase was noted in the FRAP antioxidant capacity, with increases of 26.67% and 6.67% in T1 and T2, respectively, compared to the CK, while a decrease of 6.67% was observed in T3 (Figure 6C). In contrast, the DPPH scavenging rate showed no difference between the CK (19.11) and T3 (19.27) but was significantly reduced in T1 and T2 by 26.32% and 31.58%, respectively, when compared to the CK (Figure 6D). These results indicate that 10% green light (T1) synergistically promotes lettuce flavonoid and polyphenol synthesis, enhancing the FRAP antioxidant capacity. With an increasing green light proportion, the promotion of polyphenols weakens, while the inhibition of flavonoids strengthens.

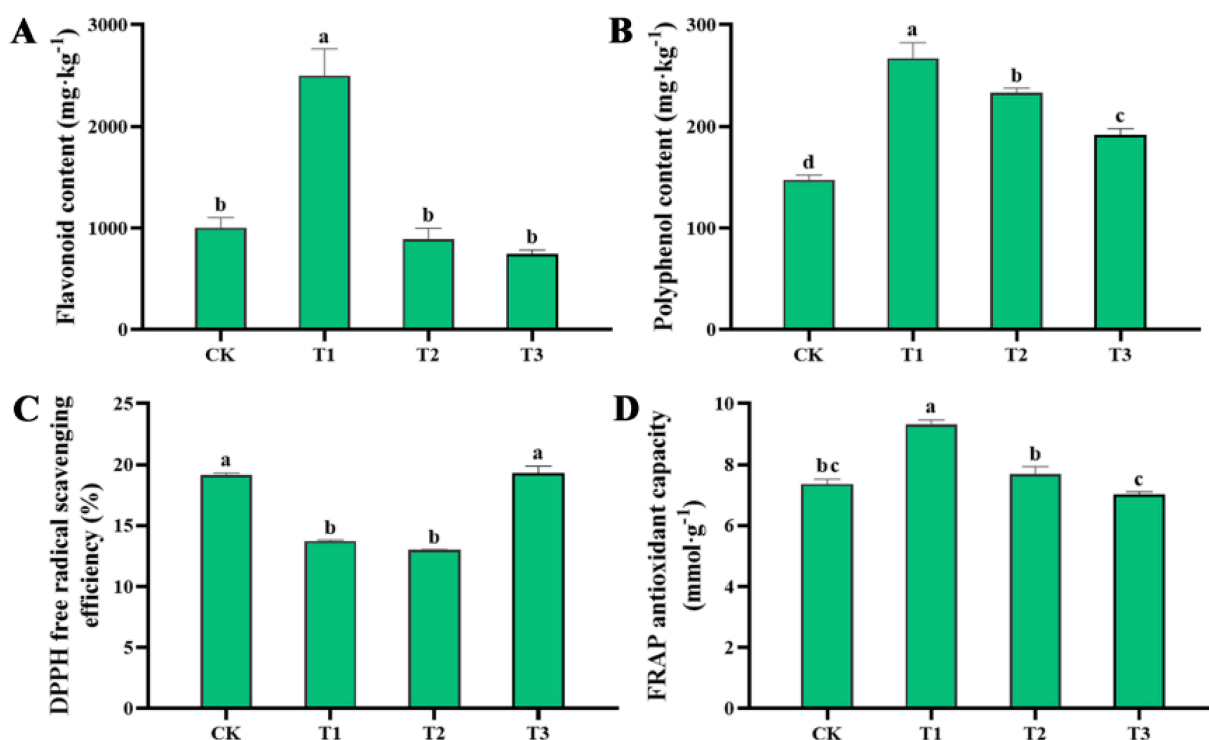


Figure 6. Effects of red–blue–green light quality ratios on antioxidant capacity of lettuce. (A). Flavonoid content. (B). Polyphenol content. (C). DPPH free radical scavenging rate. (D). FRAP antioxidant capacity. Data indicated are means $\pm$ SD. Different letters above columns indicate significant differences between treatments ($p < 0.05$).

3.6. Effects of Red–Blue–Green Light Ratios on Nutrient Absorption Characteristics of Lettuce

The mineral element contents in the lettuce were significantly regulated by the red–blue–green light ratios, as presented in Figure 7.

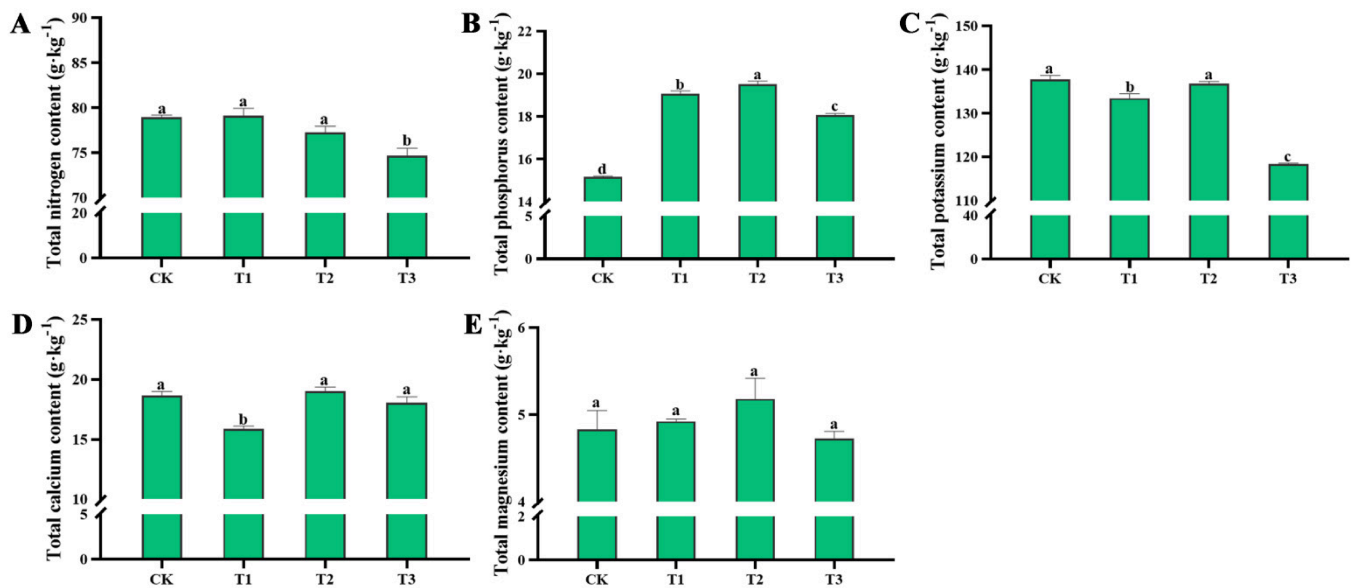


Figure 7. Effects of red–blue–green light quality ratios on nutrient absorption characteristics (mineral element contents) of lettuce. (A). Total nitrogen content. (B). Total phosphorus content. (C). Total potassium content. (D). Total calcium content. (E). Total magnesium content. Data indicated are means $\pm$ SD. Values are means $\pm$ SE ($n = 5$). Different letters above columns indicate significant differences between treatments ($p < 0.05$). Absolute difference of this indicator is small, and statistical significance is attributed to low coefficient of variation and high experimental precision.

The phosphorus (P) content was significantly higher in all green light treatments compared with that in the CK and showed a trend of first increasing and then decreasing with the increase in the green light proportion. Specifically, T1, T2 and T3 significantly increased the P content by 28.0%, 32.7% and 18.7%, respectively, with the maximum value observed in T2 (Figure 7B). For the total N and K, T2 showed no significant difference from the CK, while T3 exhibited significantly reduced values by 5.7% and 14.3%, respectively (Figure 7A,C). The calcium (Ca) content was significantly decreased by 14.9% in T1 but significantly increased by 3.7% in T3 relative to the CK, with no significant difference observed between T2 and the CK (Figure 7D). No significant difference in the magnesium (Mg) contents was detected among the treatments (Figure 7E).

These results indicate that green light supplementation consistently promotes P absorption (reflected by the tissue P concentration), while the responses of the N, K and Ca contents to light spectra vary with the green light proportion, and the Mg content is not affected by light spectral changes.

3.7. Effects of Red–Blue–Green Light Ratios on Nutrient Accumulation Dynamics of Lettuce

The total accumulation of essential mineral elements is not only a comprehensive result of plant growth and nutrient absorption but also an important indicator reflecting plant nutrient status. Our results showed that the different light spectra significantly affected the mineral element accumulation in lettuce, as presented in Figure 8.

The P accumulation increased gradually with the increase in the green light proportion, and all green light treatments showed significantly higher values than the CK. Specifically, T1, T2 and T3 significantly increased the P accumulation by 9.1%, 25.0% and 50.0%, respectively, with the maximum value detected in T3 (Figure 8B). For the total N, K and Mg, T1 showed significantly lower or comparable values to those in the CK, while T2 and T3 exhibited significantly elevated accumulations, with the highest values observed in T3. Specifically, T3 increased the N, K and Mg accumulation by 23.5%, 18.5% and 38.7%, respectively, compared with the CK (Figure 8A,C,E). Similarly, the Ca accumulation was

significantly reduced by 22.1% in T1 and significantly increased by 17.6% in T3, with no significant difference between T2 and the CK (Figure 8D).

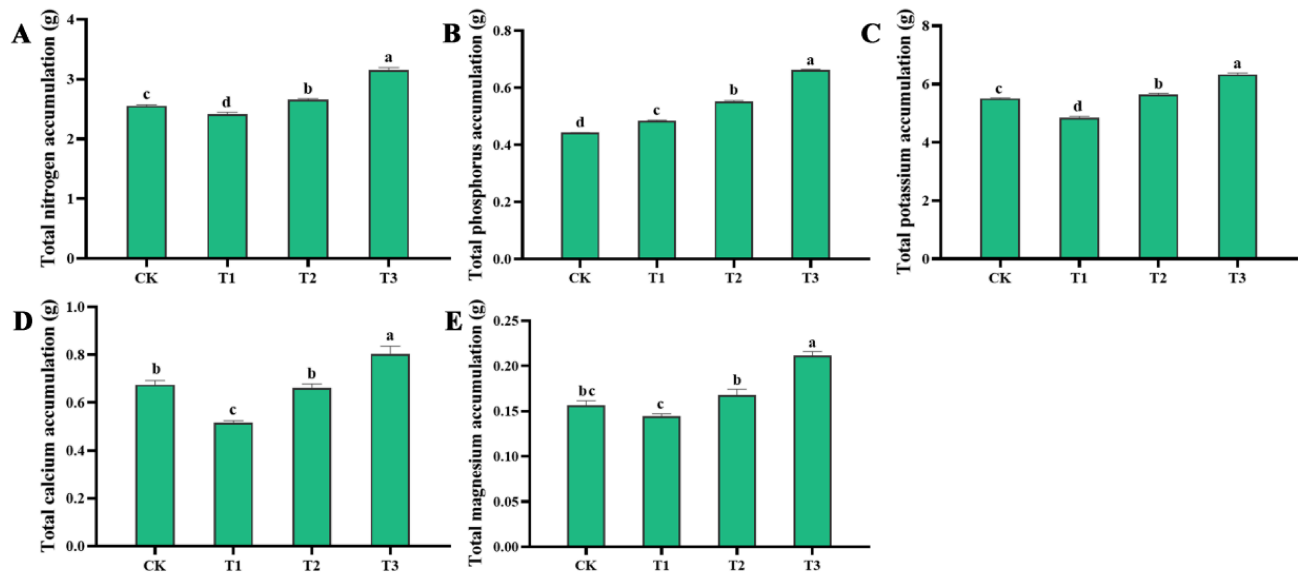


Figure 8. Effects of red–blue–green light ratios on mineral element accumulation in lettuce. (A). Total nitrogen content. (B). Total phosphorus content. (C). Total potassium content. (D). Total calcium content. (E). Total magnesium content. Unit of mineral accumulation is $\text{mg} \cdot \text{plant}^{-1}$, representing total mineral accumulation per plant. Different letters above columns indicate significant differences between treatments ($p < 0.05$).

These results indicate that a high proportion of green light (T3, 40%) most strongly enhances the aboveground nutrient accumulation of lettuce. This effect is attributed to the simultaneous promotion of lettuce biomass growth and nutrient absorption by high green light, which jointly increases the total nutrient accumulation per plant.

4. Discussion

This study aimed to explore the effects of different red–blue–green light spectra on the growth, quality, and mineral nutrient accumulation characteristics of hydroponic lettuce, addressing the current limited understanding of green light responses. Green light has traditionally been considered less photosynthetically efficient than red and blue light due to its lower absorbance by chlorophyll [15]. The results of this study demonstrated that a 40% proportion of green light combined with red–blue light significantly enhanced biomass accumulation, improved key quality attributes, and promoted mineral nutrient uptake and accumulation. These results highlight the synergistic role of green light with red and blue wavelengths and underscore its potential value in improving lettuce production in hydroponic culture.

Moreover, our results showed that 40% green light (T3) significantly promoted aboveground and root biomass accumulation, with aboveground fresh and dry weights (33.16% and 21.40%) higher than those of the CK (Figure 1). This growth promotion may be explained by two well-documented potential mechanisms: the unique deep-penetration ability of green light within the plant canopy [13], and its possible promoting effect on photosynthetic carbon assimilation efficiency [18]. In sweet pepper, green light mitigates the “shade effect” of upper leaves by promoting photosynthesis in lower leaf layers, thereby effectively increasing the total photosynthetic area of plants [16]. As reported in previous lettuce studies, green light may upregulate the activity of key photosynthetic enzymes (e.g., Rubisco) [15]. This finding is consistent with our observation that 40% green light (T3)

increased the Pn and Tr while decreasing the Ci, suggesting more efficient CO₂ utilization for photosynthesis (Figure 3).

Further analysis of the photosynthetic parameters based on the Farquhar model revealed that the increase in the Pn under green light treatments was primarily driven by non-stomatal factors [32]. Compared with the CK, all green light treatments (T1, T2 and T3) showed increased Pn values alongside reductions in the Ci and Gs, suggesting that the improvement in the photosynthetic efficiency is unlikely to stem from greater stomatal conductance or increased CO₂ availability but rather from enhanced biochemical performance, such as elevated Rubisco activity. This inference is consistent with a previous study reporting that green light induces the expression of Rubisco large-subunit genes in lettuce [18]. We note that these mechanistic explanations are reasonable inferences based on the published literature, as the present study did not directly measure the activity of photosynthetic enzymes or the expression of related genes. Interestingly, we observed an uncoupling between the leaf-level photosynthetic rate and whole-plant biomass: the 10% green light treatment (T1) exhibited the highest Pn but the minimum biomass, whereas 40% green light (T3) with a moderately lower Pn achieved the greatest biomass. Comparable results have been reported in relevant studies on light quality manipulation [33], which likely reflects the differential partitioning of photosynthates between primary and secondary metabolic pathways.

Notably, this growth-promoting effect on biomass exhibited a clear dose-dependent pattern. Treatment with 10% green light (T1) suppressed plant growth, whereas 40% green light (T3) yielded the most pronounced promotive effect (Figure 1). This finding is consistent with a previous study reporting that lettuce seedlings need a critical proportion of green light (30–40%) to sustain optimal growth based on a mixture design approach, and that any deviation from this range results in poorer growth performance [13]. Such distinct responses of lettuce, when compared with previously investigated conventional cultivars, further emphasize the necessity of developing light regimes tailored to specific cultivars [6,8].

Green light proportions markedly regulate the quality and antioxidant capacity of lettuce. The 40% green light treatment (T3) significantly improved the nutritional quality by increasing the soluble sugar (61.79%) and Vc (13.43%) contents while reducing the nitrate content by 16.31% (Figure 4). The increase in soluble sugar may be associated with enhanced sucrose metabolism; consistent with previous reports, green light may enhance sucrose synthase activity and thereby promote carbohydrate accumulation [16]. Likewise, the increase in the Vc content aligns with previous findings, which suggest that green light may upregulate genes (GME, GaLGDH) involved in ascorbic acid biosynthesis in lettuce [8]. Meanwhile, the reduction in the nitrate content is consistent with prior evidence indicating that green light could elevate nitrate reductase (NR) activity in spinach, which may potentially accelerate nitrate assimilation and reduce nitrate accumulation in edible tissues [34]. These findings suggest that appropriate proportions of green light improve the nutritional quality of lettuce by promoting carbohydrate and antioxidant compound synthesis.

The observed metabolic profiles offer a plausible explanation for the uncoupling between the single-leaf photosynthesis and biomass observed in 10% green light (T1). We speculate that the treatment with 10% green light (T1) may have acted synergistically with blue light to strongly stimulate the phenylpropanoid metabolic pathway, resulting in 148.61% and 81.24% increases in the flavonoid and polyphenol contents, respectively, compared with the control (Figure 5). The biosynthesis of these secondary metabolites requires substantial amounts of carbon skeletons, ATP and NADPH [35], which may greatly reduce the proportion of photosynthates available for vegetative growth. In addition, the

higher transpiration rate under T1 likely increases water loss and respiratory carbon consumption, further reducing the net accumulation of assimilates.

In contrast, 10% green light (T1) was the most effective at promoting antioxidant compounds, increasing the flavonoid and polyphenol contents by 148.61% and 81.24%, respectively (Figure 5). These responses are closely associated with the phenylpropanoid pathway. This finding is consistent with a previous study reporting that low-proportion green light may act synergistically with blue light to activate key enzymes, particularly phenylalanine ammonia-lyase (PAL), thereby potentially regulating the synthesis of phenolic compounds [8]. Since enzyme activity was not directly assayed in this study, this interpretation remains to be verified by targeted biochemical experiments. Moreover, the higher green light proportions (T2 and T3) reduced the relative proportion of blue light, which may have weakened this stimulatory effect and consequently suppressed flavonoid synthesis. Interestingly, while the FRAP antioxidant capacity was the highest in 10% green light (T1), no significant difference in DPPH radical-scavenging activity was observed between 40% green light (T3) and the CK, suggesting that antioxidant capacity is a complex trait shaped by the combined contributions of multiple antioxidant compounds, including phenolics, vitamin C, and carotenoids. The patterns of nutrient absorption and accumulation further support the observed differences in growth and quality among treatments (Figures 7 and 8).

All green light treatments promoted the uptake and accumulation of P and Mg in lettuce. In terms of the nutrient contents, 40% green light (T3) significantly increased the P and Mg contents by 54.55% and 38.46%, respectively, compared with the CK. As essential components of ATP, nucleic acids and chlorophyll, P and Mg play critical roles in photosynthetic biochemistry [33–35]. Mg^{2+} not only stabilizes the chlorophyll structure but is also known to activate key enzymes involved in carbon fixation and energy metabolism, including Rubisco and enolase [36], while P is indispensable for ATP and NADPH production during the Calvin cycle [37]. It is reasonable to infer that the elevated tissue concentrations of these elements contribute to the biochemical efficiency of photosynthesis, which may partly explain the high photosynthetic performance observed in 40% green light (T3).

In terms of nutrient accumulation, the differences in the total mineral uptake further shaped the growth gap among treatments. Although T1 displayed a higher chlorophyll content and single-leaf photosynthetic rate (P_n), its relatively lower accumulation of major nutrients, particularly N, P and K, may have constrained the whole-plant photosynthetic capacity and sustained growth. In contrast, 40% green light (T3) achieved the highest accumulation of all major mineral elements (Figure 7), providing a stronger nutritional foundation for the synthesis of photosynthetic enzymes and long-term photosynthetic activity. From a biological perspective, the efficient acquisition of Mg and P in 40% green light (T3) further strengthened the overall photosynthetic advantage. The enhancement of both the nutrient concentration and total accumulation likely contributed to the 33.16% increase in biomass in 40% green light (T3), despite its lower chlorophyll content relative to T1 and T2 (Figure 2). Meanwhile, the high penetrability of 40% green light may facilitate light capture by the middle and lower canopy leaves, which potentially enhances total canopy photosynthesis and compensates for the slightly lower single-leaf P_n compared with 10% green light (T1).

The species specificity of green light's nutrient regulation is evident: while cucumber requires only 10–20% green light for optimal P and Mg uptake [34], 40% green light (T3) maximized mineral absorption in lettuce, which may reflect cultivar-dependent differences in photoreceptor-mediated responses. This aligns with a previous study, which demonstrated in other species that green light (523 nm) upregulates genes encoding PHT1 (phosphate transporters) and MRS2 (Mg^{2+} channels) in roots via cryptochrome 1-mediated

signaling [38]. Notably, the higher transpiration rate in 40% green light (T3) may have accelerated the mass flow of ions (e.g., Mg^{2+} , H_2PO_4^-) to shoots, thereby promoting nutrient accumulation (Figure 3). Unexpectedly, 40% green light (T3) also boosted Zn accumulation by 53.65% (Figure 9), potentially because Mg^{2+} facilitates Zn^{2+} uptake through interactive effects on ion channels, further supporting photosynthetic resilience [39]. However, this shift in nutrient status may have redirected metabolic flux: the soluble sugar content increased by 61.79% (Figure 4) at the expense of a 25.57% reduction in the flavonoid content (Figure 5), which aligns with findings in spinach showing that >30% green light suppresses PAL activity [20].

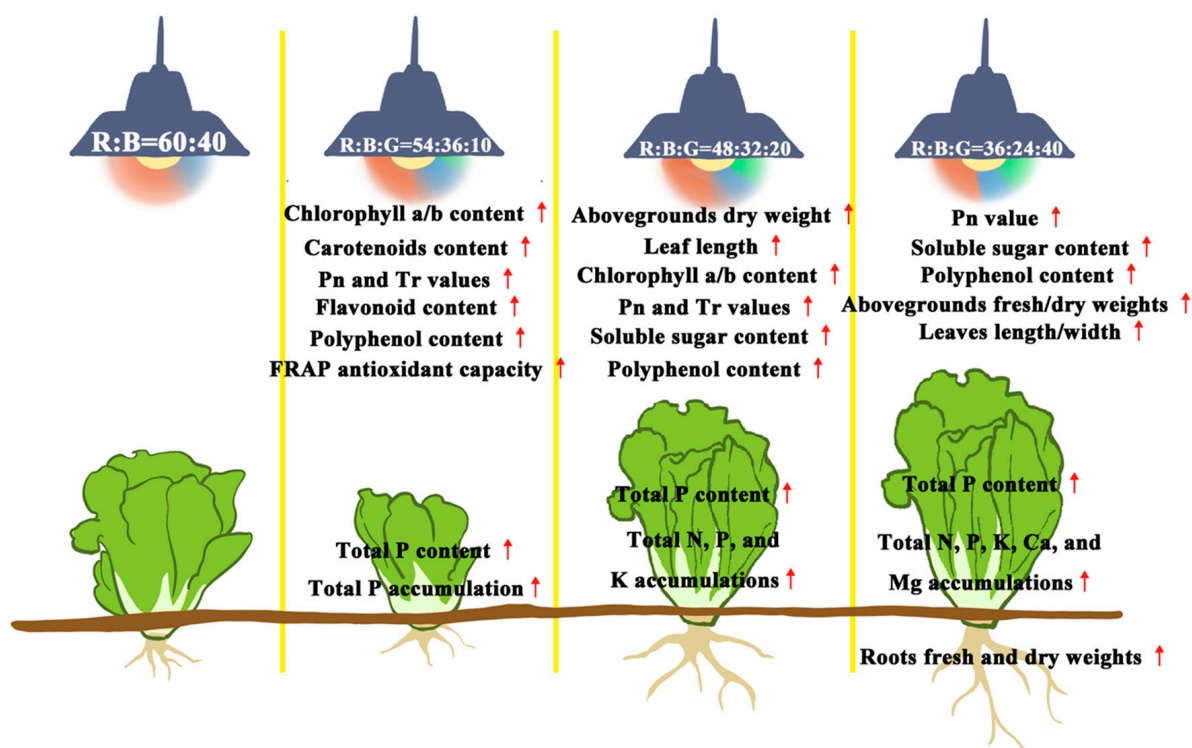


Figure 9. Schematic diagram of effects of different red–blue light ratios on growth, physiology, and mineral nutrition of hydroponic lettuce. Note: ↑ indicates increase.

To visually summarize the comprehensive regulatory effects of the red–blue–green light ratios on lettuce, a schematic diagram (Figure 9) was constructed, integrating key phenotypic, physiological, nutritional, and antioxidant-related responses across the four treatments. As shown, the red–blue binary control exhibited a moderate performance across all indices, with no distinct advantages. The 10% green light treatment (T1) achieved the highest flavonoid and polyphenol contents and an enhanced FRAP antioxidant capacity but inhibited biomass accumulation. The 20% green light treatment (T2) balanced soluble sugar elevation and nitrate reduction, with moderate nutrient absorption and accumulation. The 40% green light treatment (T3) achieved the highest biomass among all tested treatments, significantly increased the soluble sugar and Vc contents, reduced the nitrate content, and enhanced the accumulation of multiple aboveground nutrients (N, P, K, Ca, and Mg). This schematic diagram clearly illustrates the dose-dependent regulatory role of green light, providing a visual reference for precise light environment management in hydroponic lettuce and supporting targeted cultivation optimization.

Notably, this study did not directly determine the activities of key metabolic enzymes (e.g., Rubisco, NR, PAL) or photoreceptor-mediated signaling pathways. All mechanistic explanations above are reasonable inferences based on the published literature, and tar-

geted biochemical and molecular experiments are needed to verify the specific regulatory pathways in future research.

5. Conclusions

This study challenges the traditional view of green light as a less effective photosynthetic wavelength and demonstrates its crucial role in regulating growth and metabolic processes in hydroponic lettuce, specifically for commercial production. A green light proportion of 40% significantly enhanced lettuce plant growth, productivity, nutritional quality, and mineral accumulation, while 10% green light favored antioxidant accumulation through the stimulation of secondary metabolism. Collectively, these findings advance our understanding of green light value and function and provide a theoretical and practical framework for optimizing spectral management in controlled-environment lettuce production.

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