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Effects of Supplemental Red and Far-Red Light at Different Growth Stages on the Growth and Nutritional Properties of Lettuce

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Abstract: The understanding of the supplementation scheme of red (R) and far-red (FR) light in the cultivation of leafy vegetables in plant factories with artificial lighting (PFALs) is still limited. This study investigated the effects of supplemental R and FR light at different plant growth stages on the morphology, growth characteristics, and nutritional properties of lettuce. Supplemented R + FR throughout the entire growth stages was beneficial for the growth of lettuce, for which the total fresh weight was increased by 53.76%, and it also enhanced the content of soluble sugars by 39.98% and vitamin E by 34.21%. The pre-supplementation of FR light followed by supplementation of R light at various growth stages not only increase the total fresh weight of lettuce by 26.10% but also ensured that most nutritional indices did not decrease, and it even increased the content of soluble sugars by 35.24% while decreasing the nitrate content by 31.52%. The scheme of pre-supplementation of R light followed by the supplementation of FR light promoted a more upright plant architecture in lettuce, which was advantageous for improving the cultivation density of lettuce in plant factories with artificial lighting, thereby enhancing the yield/m^2. Moreover, it could increase the vitamin E content of lettuce. The growth and nutritional properties of lettuce exhibit significant effects under different supplementation methods of R and FR light. In PFALs, the selection of different light supplement schemes also requires a careful balance between yield and quality. From an energy-saving perspective, the pre-supplementation of FR light followed by supplementation of R light at various plant growth stages is beneficial for lettuce production in PFALs.

Keywords: red light; far-red light; lettuce; nutrition; light supplement scheme

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1. Introduction

Plant factories with artificial lighting (PFALs) mainly focus on the production of leafy vegetables. Lettuce (*Lactuca sativa* L.) is the most widely consumed and cultivated leafy vegetable worldwide, which is rich in nutrients such as anthocyanins, ascorbic acid, dehydroascorbic acid, and vitamin E. The intake of these substances is beneficial for the formation of collagen in the human body, reduction in cholesterol levels, absorption of inorganic iron, prevention of neuronal and cardiovascular diseases, lowering the risk of diabetes, and exhibiting certain anti-cancer activities [1,2], making lettuce a key vegetable category in PFALs production. The remarkable benefits of PFALs are high resource use efficiency, high annual productivity per unit area, and production of high-quality plants without pesticides [3].

Light is the most important factor in vertical farming. With the widespread use of LED lighting, extensive research has been conducted on the fundamental effects of its primary spectral regions on plant growth, yield, and crop quality [4].

Red (R) (600–700 nm) light, as an artificial light source commonly used in PFALs, could increase the biomass, leaf area, leaf length, height and soluble sugars and reduce the nitrate content of leafy greens [5]. Far-red (FR) light (700–800 nm) could regulate plant morphology and photosynthetic capacity through the modulation of associated gene expression. These include adjusting leaf angle, promoting plant height, and increasing leaf area to optimize light absorption, ultimately leading to improved crop biomass [6,7]. As the photoreceptor of R and FR light, phytochromes (PHYs) are responsible for different plant light responses, such as germination, de-etiolation, shade avoidance, inhibition of stem and petiole elongation, leaf expansion and flattening, circadian rhythms, flowering, and branching [8].

Relevant studies have explored the effects of different ratios of R and FR light combined with composite light on plants in PFALs [9]. In recent studies, the effects of substituting blue (B) light with far-red (FR) and ultraviolet A (UVA) light at two growth stages were investigated in terms of their impact on the growth and quality of loose-leaf lettuce [10]. However, the understanding of the supplementation scheme of R and FR light at different growth stages of leafy vegetables in PFALs is still limited. This study investigated the effects of supplemental R and FR light at different growth stages on the morphology, growth characteristics, and nutritional properties of lettuce.

2. Materials and Methods

2.1. Plant Material, Growth Conditions, and Treatments

This study was conducted in an artificial light plant factory at the South China Agricultural University. The seeds of leaf lettuce (*Lactuca sativa* L. 'Green Butter') were sown in the sponge block, and the seedlings were cultivated with modified Hoagland nutrient solution under 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ white LEDs 10/14 h light/dark after germination. Within air temperature 24 ± 2 °C and 65–75% relative humidity, seedlings at the third-true leaf stage were transplanted to a hydroponic system 15 days after sowing.

Lettuce plants were cultivated in the hydroponic system (1/2 strength Hoagland nutrient solution, pH of 6.8 ± 0.2, electrical conductivity of 1.50 ± 0.05 $mS \cdot cm^{-1}$) at a density of 24 plants per plate (95 × 60 × 3 cm^3). Adjustable LED panels (Chenghui Equipment Co., Ltd., Guangzhou, China; 150 × 30 cm^2) containing white (peaking at 440 nm; W), red (660 ± 10 nm; R), and far-red (730 ± 10 nm; FR) LEDs were used as the light sources.

Four light treatments were set as follows: entire growth stage under white LEDs (W), which was considered basal light; entire growth stage under basal light with supplemental R and FR light (A); the first 10 days of basal light with FR light and another 10 days with R light (FRR); and the first 10 days of basal light with R light and another 10 days with FR light (RFR). The radiation of the white LEDs was set as 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$; R and FR were set at the same radiation of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each light treatment set 3 repetitions; each plate (24 plants per plate) was defined as a repeat. All the light treatments were set as the same photoperiod, which was 10/14 h light/dark. The schematic diagram of the specific setup of the experiment and the ratios of R to FR and R to B can be seen in Figure 1 and Table 1.

2.2. Measurement of Plant Morphology and Growth Characteristics

Eight uniform plants were randomly selected from each light treatment to measure the indicators of the biometrics. The fresh weight of the shoots and roots was weighed separately by electronic balance. The leaf area, leaf length, and width (the sixth leaf of each plant) were measured by ImageJ 1.52 V (National Institutes of Health, Bethesda, MD, USA). To measure the dry weight, the samples were deactivated at 105 °C and weighed by electronic balance after tissue dehydration at 75 °C for 48 h. Before biochemical analysis, the shoot of lettuce was frozen in liquid N_2 and stored at −80 °C. Each biochemical index was performed with four analytical replicates.

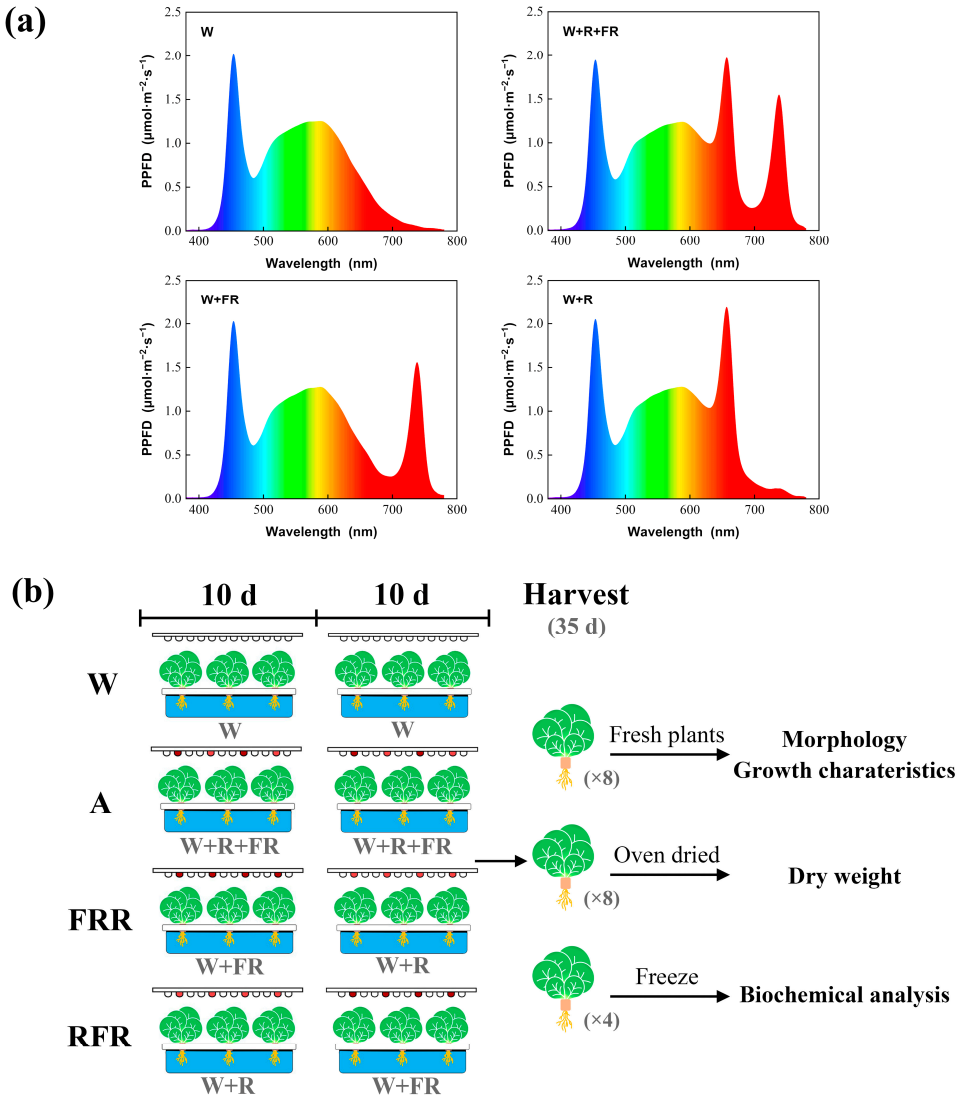

Figure 1. (**a**) Spectral composition of the light-emitting diode (LED) treatments measured at the top of the plant canopy and (**b**) the process of cultivation and harvesting.

Table 1. The ratios of R to FR and R to B in different spectral composition and light treatment.

Spectral Composition and Light Treatment	R:FR			R:B		
	Spectral Composition	First 10 Days	Last 10 Days	Spectral Composition	First 10 Days	Last 10 Days
W	12.36	12.36	12.36	0.93	0.93	0.93
W + R	14.22	-	-	1.47	-	-
W + FR	1.44	-	-	0.93	-	-
W + R + FR	2.17	-	-	1.47	-	-
A	-	2.17	2.17	-	1.47	1.47
FRR	-	1.44	14.22	-	0.93	1.47
RFR	-	14.22	1.44	-	1.47	0.93

W, white; R, red; FR, far-red; B, blue; R:FR, the ratio of red to far-red; R:B, the ratio of red to blue; W + R, combination of white and red; W + FR, combination of white and far-red; W + R + FR, combination of white, red, and far-red; A, entire growth stage used basal light with supplemental red and far-red light; FRR, the first 10 days of white light with far-red light and another 10 days with red light; RFR, the first 10 days of white light with red light and another 10 days with far-red light.

2.3. Measurement of Pigment Content

Fresh lettuce samples (0.2 g) were kept in 8 mL of a 1:1 (v/v) acetone–alcohol solution for 24 h in the dark at 25 °C; after, the plant tissue was crushed. A UV spectrophotometer (Shimadzu UV-1780, Corporation, Kyoto, Japan) was used to determine the absorbance of the supernatant at 645 nm (OD645), 663 nm (OD663), and 440 nm (OD440). The chlorophyll and carotenoid concentrations were calculated as follows [11]:

$$\text{Chlorophyll a } (\text{mg}\cdot\text{g}^{-1}) = (12.7 \times \text{OD663} - 2.69 \times \text{OD645}) \times \text{V}\cdot\text{W}^{-1} \times 1000$$

$$\text{Chlorophyll b } (\text{mg}\cdot\text{g}^{-1}) = (22.9 \times \text{OD645} - 4.86 \times \text{OD663}) \times \text{V}\cdot\text{W}^{-1} \times 1000$$

$$\text{Total Chlorophyll } (\text{mg}\cdot\text{g}^{-1}) = (8.02 \times \text{OD663} + 20.20 \times \text{OD645}) \times \text{V}\cdot\text{W}^{-1} \times 1000$$

$$\text{Carotenoids } (\text{mg}\cdot\text{g}^{-1}) = (4.7 \times \text{OD440} - 0.27 \times \text{Total Chlorophyll}) \times \text{V}\cdot\text{W}^{-1} \times 1000$$

V is the volume of the extract and W is the weight of the sample.

2.4. Measurement of Soluble Sugar

The soluble sugar content was determined by anthrone–sulfuric acid colorimetry [12]. Fresh lettuce samples (0.5 g) were heated in a boiling water bath with 10 mL distilled water for 30 min; after, the plant tissue was crushed. The supernatant (0.1 mL) was mixed with 1.9 mL distilled water, 0.5 mL anthrone ethyl acetate, and 5 mL vitriol. After shaking to ensure proper mixing, soluble sugars were detected at 630 nm using a UV spectrophotometer.

2.5. Measurement of Soluble Protein

The soluble protein content of lettuce was determined according to the Coomassie brilliant blue G-250 dye method [13]. Approximately 0.5 g of fresh plant tissue was homogenized in 8 mL distilled water. The resulting homogenate was then centrifuged at 3000× g for 10 min at 4 °C. A total of 0.2 mL of the supernatant was mixed with 0.8 mL distilled water and 5 mL Coomassie brilliant blue G-250 (0.1 $\text{g}\cdot\text{L}^{-1}$) solution. After 5 min of incubation, the absorbance of the mixture was measured at 595 nm using a UV spectrophotometer.

2.6. Measurement of Nitrate Content

The nitrate content was determined using UV spectrophotometry [14]. About 1.0 g fresh lettuce tissue was homogenized in 10 mL distilled water and heated for 30 min in a water bath at a rolling boil. After filtering, the homogenate was transferred to a volumetric flask. Equal parts (0.4 mL each) of sample solution and 5% salicylic and sulfuric acids were added, and then 9.5 mL of the 8% NaOH was added. The amount of nitrate in this mixture was then determined using a UV spectrophotometer with a 410 nm wavelength.

2.7. Measurement of Vitamin C and Vitamin E

The vitamin C content was determined using molybdenum blue spectrophotometry [15]. Fresh lettuce samples weighing 0.5 g were finely ground into a pulp using 25 mL oxalic acid EDTA solution (w/v). The resulting mixture was then filtered, and 10 mL extract solution was combined with 1 mL phosphate–acetic acid, 2 mL 5% vitriol, and 4 mL ammonium molybdate. The determination of vitamin C content in this mixture was carried out using a UV spectrophotometer at a wavelength of 705 nm.

The vitamin E content was determined using an enzyme-linked immunosorbent assay (ELISA) using the Plant Vitamin E ELISA Kit (Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) [16]. The vitamin E antigen was precoated on the microplate wells. The procedure involved adding the sample, standard solutions, and horse radish peroxidase (HRP)-labeled detection antibody in sequence, followed by incubation and thorough washing. Substrate was added for color development. Under the catalysis of peroxidase, it was converted into blue and then transformed into the final yellow color

under acidic conditions. The intensity of the color was positively correlated with the amount of vitamin E in the sample. The vitamin E content of this mixture was determined using an ELISA reader at 450 nm.

2.8. Measurement of Antioxidant Content and Antioxidant Activity

The polyphenol content was determined using the Folin–Ciocalteu assay [17]. A total of 0.5 g of freshly cut lettuce samples was extracted with 8 mL alcohol. The homogenate was centrifuged at 3000× *g* for 10 min at 4 °C after being left to stand for 30 min. In total, 1 mL supernatant was combined with 0.5 mL Folin phenol, 11.5 mL 26.7% sodium carbonate, and 7 mL distilled water. After two hours, the absorbance at 510 nm was measured with a UV spectrophotometer.

The total flavonoid content was determined using the aluminum nitrate method [18]. In a nutshell, 1 mL extract solution (extracted using the same procedure as outlined for polyphenols) was added to 11.5 mL 30% alcohol and 0.7 mL 5% $NaNO_2$. A total of 0.7 mL 10% $Al(NO_3)_3$ was added to the reaction solution after 5 min, and after another 6 min, 5 mL 5% NaOH was added. After 10 min, the absorbance at 760 nm was measured with a UV spectrophotometer.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging rate was determined following Tadolini et al.'s method [19]. A UV spectrophotometer was used to test the mixture's absorbance at 517 nm after 2.0 mL of the sample extract and 2.0 mL DPPH solution (0.0080 g DPPH in 100 mL alcohol) were mixed.

The ferric-reducing antioxidant power (FRAP) was determined following Benzie and Strain [20]. A solution containing 0.4 mL sample, obtained through the same extraction method as described for polyphenols, was combined with 3.6 mL of a solution consisting of 0.3 mol·L^{-1} acetate buffer, 10 mmol·L^{-1} 2,4,6-tripyridyl-S-triazine (TPTZ), and 20 mmol·L^{-1} $FeCl_3$ at a ratio of 10:1:1 ($v/v/v$). The resulting mixture was incubated at 37 °C for 10 min. Subsequently, the ferric-reducing antioxidant power (FRAP) of the mixture was measured at 593 nm using a spectrophotometer.

2.9. Statistical Analysis

Data were expressed as mean ± standard error (n = 4 replicates). Analyses of variance (ANOVA) followed by Duncan's multiple range test were conducted using IBM SPSS Statistics 25 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Plant Morphology, Growth Characteristics, and Pigment Content

Before the replacement of light treatment for FRR and RFR, lettuce had exhibited noticeable morphological differences under different light environments (Figure 2a). The treatment A and FRR, induced by far-red light, resulted in an overall larger plant size with more curled leaves, while the treatment W and RFR showed a flattened plant shape.

After 35 days of treatment, the morphology and growth characteristics of the lettuce were significantly influenced by the different light treatments (Figure 2b). The plant size of the lettuce in W was smaller than other treatments. The plant size of the lettuce in A was the largest, while the plant in FRR was flatter and the one in RFR was more upright.

Under different light treatments, there were significant differences in the biomass and growth characteristics of the lettuce (Figure 3a–d). Compared with W, the shoot dry weight, shoot fresh weight, leaf area, leaf length, and leaf width of the lettuce in A, FRR, and RFR significantly increased, and these factors of those who underwent the A treatment reached the maximum. Even though FRR and RFR were very different in morphology, they showed similar trends in growth characteristics.

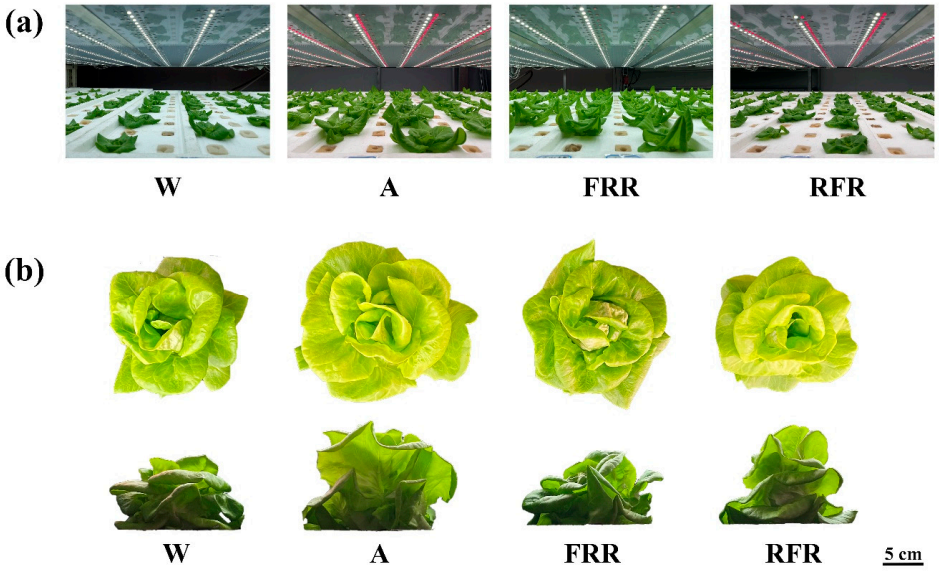

Figure 2. (**a**) The growth of lettuce at 10 days after transplant, before the replacement of light treatment for FRR and RFR, and (**b**) at 20 days after transplant by replacement of light treatment for FRR and RFR.

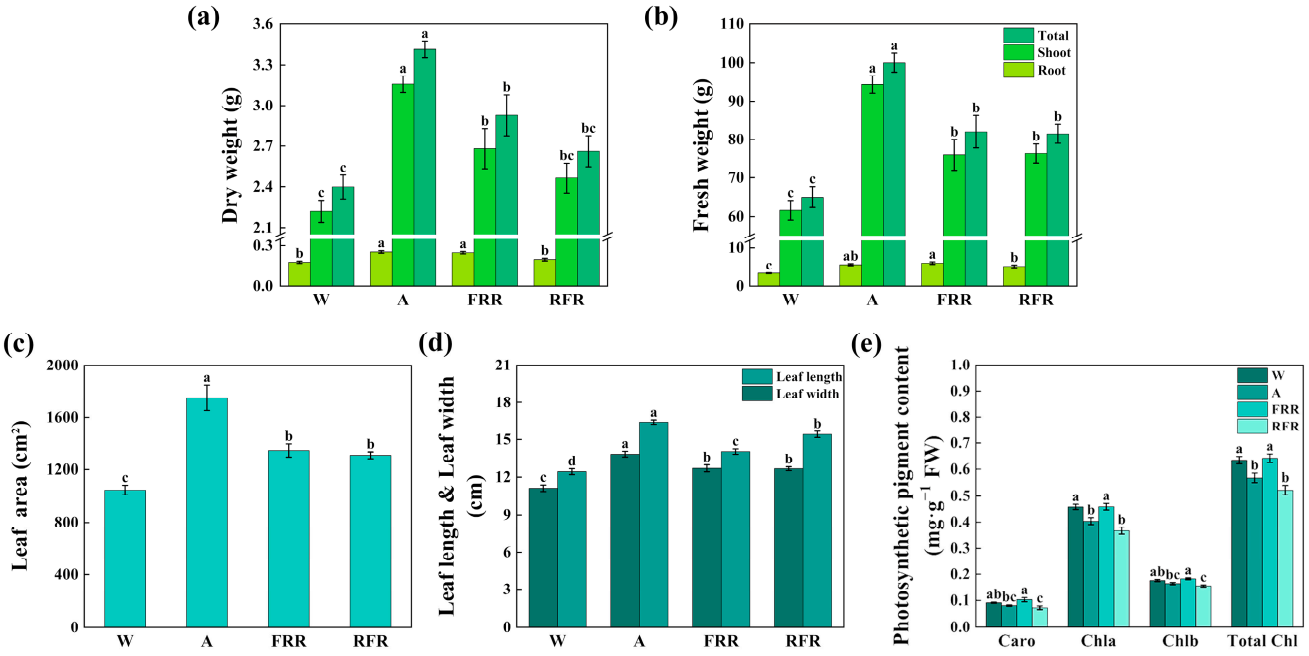

Figure 3. Effects of different light treatment on (**a**) total, shoot, and root dry weight, (**b**) total, shoot, and root fresh weight, (**c**) leaf area, (**d**) leaf length, leaf width, and (**e**) photosynthetic pigment content of lettuce. Different letters indicate significant differences ($p < 0.05$) (Duncan's multiple-range test).

Different light treatments impacted the photosynthetic pigment concentration, which included chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (total Chl), and carotenoid (Caro) (Figure 3e). Compared with W, the content of Chla and total Chl significantly decreased by 11.90% and 10.50% in treatment A, while there was not a statistically significant difference in the content of Chlb and Caro. Meanwhile, in comparison with W, there were significant reductions in the content of Caro, Chla, Chlb, and total Chl by 20.66%, 19.94%, 11.91%, and 17.71% treatment RFR.

3.2. Soluble Sugars, Soluble Protein, Nitrates, Vitamin C, and Vitamin E

The contents of soluble sugars (SSs), soluble protein (SP), nitrates, vitamin C (VC), and vitamin E (VE) in the lettuce were affected by different light treatments (Figure 4). Compared with W and RFR, there was a significant increase in the SSs content by 39.98% and 44.47% in treatment A and by 35.24% and 39.58% in FRR, respectively.

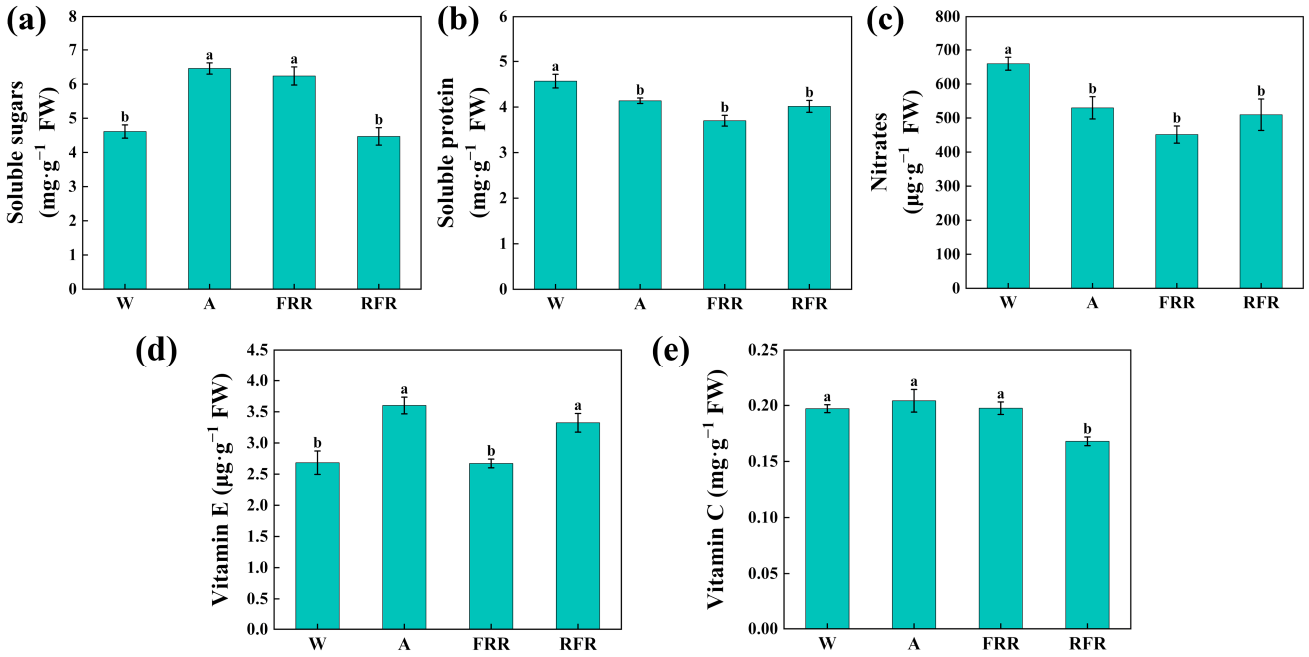

Figure 4. Effects of different light treatment on (**a**) soluble sugars (SSs), (**b**) soluble protein (SP), (**c**) nitrates, (**d**) vitamin C (VC), and (**e**) vitamin E (VE) of lettuce. Different letters indicate significant differences ($p < 0.05$) (Duncan's multiple-range test).

The content of SP significantly decreased in treatments A, FRR, and RFR compared to W by 13.83%, 22.95%, and 16.38%, respectively. A similar trend was seen in nitrate levels: the nitrate concentration in treatments A, FRR, and RFR significantly decreased by 20.09%, 31.52%, and 22.70%, respectively, in comparison to W.

There was no statistically significant difference in the contents of VC and VE between treatment W and FRR. The VE content significantly increased by 34.21% in treatment A compared to W. There was a 14.79% reduction in the VC content, while there was a 23.87% increase in the VE content in RFR compared to W.

3.3. Antioxidant Content and Antioxidant Activity

In treatment A, the contents of total phenolics (TPs) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) in the lettuce were not statistically different from those in W (Figure 5). However, there was a significant decrease in the total flavonoid (TF) content and ferric-reducing antioxidant power (FRAP) by 55.95% and 35.41%, respectively. The TF content and FRAP decreased by 48.55% and 12.53%, respectively, in FRR compared to W. Significant reductions in TPs, TF, DPPH, and FRAP were observed in treatment RFR compared to W, with values of 21.75%, 59.03%, 19.02%, and 22.86%, respectively.

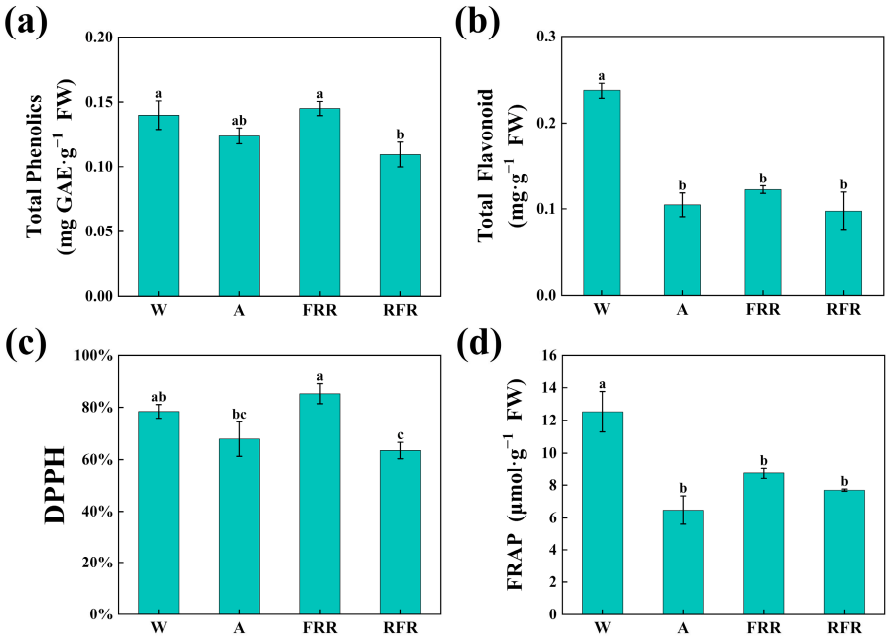

Figure 5. Effects of different light treatment on (**a**) total phenolics (TPs), (**b**) total flavonoid (TF), (**c**) 2,2-diphenyl-1-picrylhydrazyl (DPPH), and (**d**) ferric-reducing antioxidant power (FRAP) of lettuce. FW indicates fresh weight. Different letters indicate significant differences ($p < 0.05$) (Duncan's multiple-range test).

4. Discussion

Plant biomass was influenced by not only light quantity, such as intensity, but also light quality in terms of wavelength composition that affects plant growth and morphology. The FR light provided by LEDs affected the morphology of lettuce leaf and canopy [6] and increased the leaf length, leaf width, and projected canopy size. These increases in leaf dimensions and canopy size resulted in the enhanced interception of incident light, ultimately leading to an increase in the biomass of lettuce [21]. Supplementing different intensities of FR light on white light could increase the plant height and shoot dry weight of lettuce [22]. When the plants detected a decrease in the ratio of R to FR in their environments (low R:FR), it triggered shade avoidance responses, developing shade avoidance morphology [23]. Under different ratios of R and FR light (0.7, 1.2, 4.1, and 8.6), lettuce cell division was promoted, and increments in biomass, leaf length, and leaf area were observed compared with the treatment under fluorescent lamps [24]. Supplemental treatments combining R and FR light have promoted the biomass of broccoli, cabbage microgreens [25], and tomato [26]. In the present study, treatments A, FRR, and RFR, which involved the supplementation of FR light, demonstrated similar results in terms of increases in the fresh weight, dry weight, leaf length, leaf width, and leaf area of lettuce (Figure 3). This study involved four different ratios of R:FR of 12.36 (W), 2.17 (W + R + FR), 1.44 (W + FR), and 14.22 (W + R), respectively (Table. 1). At the beginning of the light treatment, as the R:FR ratio decreased, the lettuce in treatment A and FRR exhibited a more upright canopy (Figure 2a). In the late stage with light replacement, the R:FR ratio of treatment FRR increased from 1.44 to 14.22 and the initially more erect plant shape gradually became flattened, whereas the R:FR ratio changed from 14.22 to 1.44 in treatment RFR, and the plant shape showed the opposite trend (Figure 2b). These findings suggest that the formation of shade avoidance morphology in lettuce plant might be induced by a lower R:FR ratio.

Light, as the energy source for plant photosynthesis, plays a crucial role in regulating chlorophyll synthesis. Adding FR to different blue + red light radiation (R:B ratios of 5:1 and 1:1), of which the ratio of R to FR reduced from 235.2 to 5.0 and 140.0 to 3.0, reduced the specific Chl content in 'Rex' and 'Cherokee' lettuce by 10–20% [27]. Similarly, supplementing with 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ FR light resulted in a significant decrease in the

content of lettuce Chl a + b [28]. With the addition of FR light in white light, the R:FR ratio reduced from 51.70 to 12.76, and the 'Red Butter' lettuce also underwent significant reductions in its levels of Chla, Chlb, and Caro [29]. A similar phenomenon was observed in 'Green Oak Leaf' lettuce, where the R:FR ratio decreased from 57.72 to 0.73 [30]. The continuous supplementation of FR light for 10 days before harvest reduced the content of Chl and Caro in lettuce, as observed in both treatment A and RFR (Figure 3e), which had a low R:FR ratio. Despite the decrease in photosynthetic pigments, the biomass of the lettuce in treatment A and RFR significantly increased compared to W (Figure 3a,b), which might be attributed to the Emerson effect [31]. R light also played an important role in plant growth. The combination of R and B light at a certain intensity (R:B ratios of 3:1, 1:1, and 1:3) could enhance the Chl content in lettuce, rocket [32], and rapeseed [33]. Although FRR underwent 10 days of FR light exposed after transplantation, the photosynthetic pigment content still remained at the same level as treatment W, with the R:B ratio increasing from 0.93 to 1.47 (Figure 3e).

Light can affect the synthesis of the primary and secondary metabolites in plants. The supplementation of R light promoted the accumulation of hexose, sucrose, and VC in lettuce, though it reduced the nitrate content [34]. Supplementing FR light on R and B light (R:B ratios of 9:1) significantly enhanced the SSs content in lettuce [35], though it decreased the SP content [36]. The supplementation of R light on W light (increasing the R:FR ratio from 57.72 to 133.72) significantly increased the SSs content, though it decreased the nitrates content. Similarly, the supplementation of FR light on W light (decreasing the R:FR ratio from 57.72 to 0.73) significantly increased the SSs content [30]. The supplementation of R and FR light at different growth stages significantly decreased the contents of SP and nitrate in lettuce (Figure 4b–c). The SSs content in lettuce under treatment A and FRR was negatively correlated with the nitrate contents (Figure 4a,c); this might be due to the accumulation and degradation mechanisms of nitrates [37].

Light, as a signal, has an impact on the synthesis of vitamin E. As a lipophilic antioxidant compound belonging to the vitamin E group, tocopherol is exclusively synthesized by plants [38]. In high light stress for 24–60 h, there was a slight increase in the α-tocopherol, γ-tocopherol, and total tocopherols of Arabidopsis [39]. R light has increased the α-tocopherol content in basil [40]. The supplementation of UV-A has also increased the α-tocopherol content of mustard microgreens [41]. When combining R with B, FR and UV also enhanced the α-tocopherol content of sprouted seeds [42]. In our recent study, we observed that supplementing with FR led to decrease in the content of α-tocopherol in kale, while there was a significant increase in the content of γ-tocopherol (unpublished results). Treatment A and RFR significantly increased the VE contents in lettuce, which involved the supplementation of FR light 10 days before harvest (Figure 4e). These might be attributed to the addition of FR light or the low R:FR ratio that caused the enhancements of γ-tocopherol in lettuce, which is the predominant tocopherol in VE of green leaf lettuce [43]. Thus, the biosynthesis of VE was not only dependent on changes in light quality and intensity but also on the crop type and its growth stage.

Light, as one of the fundamental environmental factors, could influence the antioxidant content and activity of plants. The preharvest supplementation of R light significantly enhanced the phenolic compounds and free radical scavenging activity in lettuce [44]. With the increase in the red light intensity, the antioxidant content and activity of pak choi enhanced [45]. The supplementation of R light for 10 days before harvest (A and FRR) increased the TPs content and DPPH in lettuce (Figure 5). However, with the addition of FR light, the antioxidant content and activity in lettuce significantly declined, showing a decreasing trend with the reducing R:FR ratio [46]. Similar trends were observed in the supplemental lighting experiments at different growth stages, where the treatment with additional FR light during the last 10 days before harvesting consistently resulted in reduced levels of antioxidant content and activity [10]. With the addition of FR light in white light, the 'Red Butter' lettuce also underwent reductions in its levels of TPs and TF [29]. TPs showed a decreasing trend with increasing far-red fractions in butterhead

lettuce [35]. These might be attributed to the supplementation of FR light that caused the plant to devote more energy to growing rather than producing secondary metabolites like polyphenols and flavonoids. Thus, treatments A and RFR, which have low R:FR ratios of 2.17 and 1.44 after light replacement, exhibited decreased levels of TPs, TF, DPPH, and FRAP compared to treatment W (Figure 5).

5. Conclusions

The application of different supplementation schemes of R and FR light at different growth stages significantly influenced the morphology and chemical constituents of lettuce. The supplementation scheme of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ R and FR light throughout the entire growth stage (treatment A) was beneficial for the improvement in the growth of lettuce, which also enhanced the content of soluble sugars and vitamin E. The pre-supplementation of FR light followed by the supplementation of R light before harvest (treatment FRR) could not only increase lettuce biomass but also make sure that there were no decreases in most nutritional indices, as well as enhancing the soluble sugars content while minimizing the nitrate content. The scheme of the pre-supplementation of R light followed by the supplementation of FR light (treatment RFR) promotes a more upright plant architecture in lettuce, which is advantageous for improving the cultivation density of lettuce in plant factories with artificial lighting, thereby enhancing the unit area yield. Moreover, it could increase the vitamin E content of lettuce. The utilization of different supplementation schemes of R and FR light at different growth stages provides a fresh perspective for the high-quality and efficient production of vegetables in plant factories with artificial lighting. The selection of different light supplement schemes also requires a careful balance between yield and quality. From an energy-saving perspective, we recommend the supplementation scheme of pre-supplementation with FR light followed by supplementation with R light at various growth stages, which is beneficial for lettuce production in PFALs. Further exploration is still needed to determine the optimal supplementation scheme in plant factories with artificial lighting.

Author Contributions: Conceptualization, methodology, validation, formal analysis, software, data curation, and writing—original draft, J.J.; methodology and validation, S.Z.; methodology and supervision, Y.H., R.H. and Y.L.; methodology and software, M.Z.; writing—review and editing, X.L.; conceptualization, methodology, resources, supervision, project administration, and funding acquisition, H.L. All authors have read and agreed to the published version of the manuscript.

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