



Article

Effects of Different Combinations of Red and Blue Light on the Edible Organ Morphology and Quality of Buckwheat (*Fagopyrum esculentum* Moench) Microgreens

Jianlei Qiao, Zhongyang Li, Zheng Lv, Shuang Liu, Shanshan Chen * and Yucai Feng

College of Horticulture, Jilin Agricultural University, Changchun 130118, China; qiaojianlei@jlau.edu.cn (J.Q.); lizhongyang@mails.jlau.edu.cn (Z.L.); zhangyi@mails.jlau.edu.cn (Z.L.); lshuang@jlau.edu.cn (S.L.); fengyucai@jlau.edu.cn (Y.F.)

* Correspondence: chenshanshan@jlau.edu.cn

Abstract: Buckwheat microgreens are rich in nutrients and have a unique flavor that is favored by consumers. The light environment is closely related to the growth and development of the plant. In order to study the effects of treatments with different combinations of red and blue light on the edible organ morphology and nutritional quality of buckwheat microgreens, five experimental treatments were designed, with energy ratios of red light to blue light of 5:1 (R5B1), 3:1 (R3B1), 1:1 (R1B1), 1:3 (R1B3) and 1:5 (R1B5), respectively, and a white light treatment used as the control (CK). The results showed that different combination treatments of red and blue light had obvious effects on the growth of buckwheat microgreens. The hypocotyl length and main root length of buckwheat microgreens treated with a high proportion of red light (R5B1) were obviously higher than those of other treatment designs. However, contents of soluble protein, chlorophyll, rutin and total flavonoids in buckwheat microgreens showed an increasing trend with an increase in the proportion of blue light. Considering the fresh weight, dry weight and quality indexes of the edible organ, the combination of red light and blue light with a ratio of 1:1 was most suitable for buckwheat microgreen production. The results could provide a reference for the production of buckwheat microgreens.



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Keywords: red and blue light; buckwheat microgreens; edible organ; morphology; quality

1. Introduction

In recent years, there has been growing interest in vegetables that are abundant in bioactive compounds. Microgreens, known for their delicate, delicious, and nutritious qualities, are now being considered as a new “functional food” [1,2]. Buckwheat microgreens are a type of microgreen that is produced by the germination of buckwheat seeds, and are rich in nutrition and have a special flavor that is welcomed by many consumers of fresh vegetables in China. In addition, its stems and leaves are rich in vitamins, amino acids, rutin, minerals and other nutrients that the human body needs.

Light, as an important environmental factor for plant morphology and development, plays a crucial role in factory cultivation. The light environment is closely related to the growth and development of plants, and regulating the light environment well is a necessary condition for achieving high yield and high quality in agricultural factory production [3]. The emergence of Light Emitting Diode (LED) lights allows people to choose light of different wavelengths when regulating plant growth; moreover, the effects of light conditions on vegetables such as lettuce, peppers, tomatoes, and cucumbers have been extensively studied [4–6]. At present, artificial lighting in plant factories mostly adopts high-pressure sodium lamps, fluorescent lamps, etc.; however, LED as a lighting source has become a research hotspot at home and abroad. An LED is a solid-state semiconductor based on III–IV group compounds such as GaAs (gallium arsenide), GaP (gallium phosphide), and GaAsP (gallium arsenide phosphide), and its structure uses solid-state semiconductor

wafers as light-emitting substances; when a forward voltage is applied to both its ends, the carriers in the semiconductor wafers will be recombined to release excess energy, which triggers photon radiation, forms visible light, and directly converts electrical energy into light. In addition, LEDs have benefits such as small size, low energy consumption, extended lifespan, safety of use, energy saving, and environmental preservation in comparison to conventional lighting devices [7].

At present, the regulatory effect of combinations of red and blue lights on the morphology, development and nutritional quality of microgreens has received widespread attention internationally. Seo et al. [8] also found that different light condition treatments could affect the content of monomeric phenols in buckwheat sprouts and microgreens. Under red and blue light irradiation, the contents of rutin and chlorogenic acid in buckwheat microgreens were higher, while under blue light irradiation, the accumulation of anthocyanins in buckwheat microgreens was promoted. In addition, light condition stress has a certain impact on the expression of genes such as *FtDFR* and *FtANS*. Nam et al. [9] found that red light could promote the synthesis of flavonoids in the cotyledons of young buckwheat seedlings, while blue light could significantly inhibit the accumulation of C-glycosyl flavonoids in the plants. Tuan et al. [10] studied the regulation of white, blue and red light-emitting diodes on carotenoid content and synthesis-related enzymes in microgreens; the results showed that compared with blue light and red light, the amount of carotenoids in microgreens under white light treatment was 1282.63 µg/g DW, and the expression of synthesis-related enzyme genes was higher than that under blue and red light treatment. The study by Ban Tiantian et al. [11] found that under red light irradiation, the contents of vitamin C, protein, and anthocyanins in pea microgreens were the highest, while the content of amino acids increased by 312.55% compared with the control under blue light irradiation, and the quality and growth indices of microgreens improved by a ratio of R4B1 between red and blue light. Therefore, during the cultivation process, appropriate combinations of red and blue light should be selected to improve the growth, development, and nutritional quality of microgreens.

Although existing research indicates that technologies regulating combinations of red and blue light have good application prospects in agricultural production, research on buckwheat microgreens is not yet very complete. Therefore, this study used buckwheat microgreens as experimental materials, and set different light condition treatments to study the effects of different combinations of red and blue light condition treatments on the growth, nutritional quality, and metabolites of buckwheat microgreens. Through this research project, the impact of combinations of red and blue lights on the growth and nutritional quality of buckwheat microgreens is clarified, providing a reference for achieving the large-scale application of high-performance combinations of red and blue lights required for buckwheat microgreen production.

2. Materials and Methods

2.1. Experiment Materials

The experiment was conducted in Shengjian Ranch, Inner Mongolia, China in 2022. The tested buckwheat seeds were “Mongolian No.2” provided by Inner Mongolia Academy of Agricultural Sciences, with a thousand grain weight of 35.56 ± 0.05 g. Choose plump, mature, mechanically undamaged, and uniformly sized seeds for the experiment. The hypocotyl is the main edible part of buckwheat microgreens. The LED lights used in this study were produced by Shandong Guixiang Optoelectronics Co., LTD (Weifang, Shandong, China).

2.2. Seed Processing

Sterilization treatment: Soak the seeds in a 10% sodium hypochlorite solution for 0.5 h, and then rinse with clean water 5 times.

Soaking: at room temperature, water and seeds are mixed in a mass ratio of 4:1; the soaking time is 14 h.

Germination: Soaked seeds are placed in a seedling tray with a size of 32 cm × 25 cm × 5 cm, the bottom of the seedling tray is covered with moist gauze, and an amount of 20 g is

sowed in each seedling tray. Place the seedling tray in a dark environment for germination, with a temperature of 25 ± 1 °C, relative humidity of 85%, and a cultivation time of 24 h.

After the seeds sprout, transfer the seedling tray to the cultivation rack and perform different light treatments. The temperature of the cultivation room is 25 ± 1 °C and the relative humidity is 85%.

2.3. Light Condition Design

The experiment adopted a random block design, with the proportion of red and blue composite lights as variables, and selected red light at 660 nm wavelength and blue light at 450 nm wavelength as lighting sources. A total of 5 light condition treatments with different ratios of red and blue light were set up in the experiment, with white light as the control, and each treatment was repeated 3 times. The main parameters and proportion settings of the energy distribution in the combinations of red and blue light are shown in Table 1. Adjust the distance between the light source and the plant, use a plant light analyzer to adjust the ratio of red and blue light, and maintain the light intensity at $30 \mu\text{mol}/\text{m}^2 \cdot \text{s}^{-1}$ with a light period of 12 h/d. Spray distilled water evenly every 8 h. After germination, conduct light cultivation and harvest after 7 days of cultivation. The edible organ morphology development of buckwheat microgreens under these different light condition treatments is shown in Figure 1.

Table 1. Main parameters and proportion settings of spectral energy distribution in the combinations of red and blue light.

Treatment	Light Condition	Light Spectral Energy Distribution	λ_p (nm)	Light Intensity ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)
W (CK)	Fluorescent light	White light	380~750 nm	30
R5B1	Red + Blue	Red:Blue = 5:1	Red light 660 nm Blue light 450 nm	30
R3B1	Red + Blue	Red:Blue = 3:1	Red light 660 nm Blue light 450 nm	30
R1B1	Red + Blue	Red:Blue = 1:1	Red light 660 nm Blue light 450 nm	30
R1B3	Red + Blue	Red:Blue = 1:3	Red light 660 nm Blue light 450 nm	30
R1B5	Red + Blue	Red:Blue = 1:5	Red light 660 nm Blue light 450 nm	30

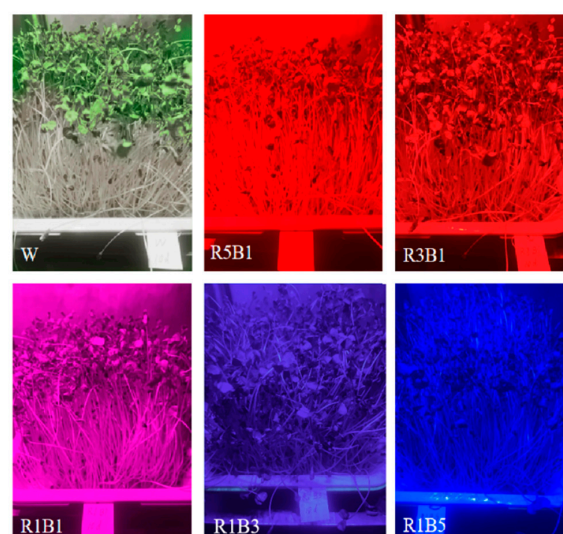


Figure 1. Morphological development of edible organs of buckwheat microgreens under different light conditions.

2.4. Measurement Indicators and Methods

2.4.1. Measurement of Growth Characteristics

After 7 days of cultivation under different lighting conditions, 30 plants were randomly selected from the different treatments. Measure the length of the hypocotyl and main root using a ruler, and the diameter of the hypocotyl using a vernier caliper. Measure the fresh weight of the entire plant and the fresh weight of edible organs using an electronic balance; after the measurement is completed, the plant and edible organs are packaged and marked and then sterilized in an oven at 105 °C for 15 min. Then, they are dried to a constant weight at 80 °C and weighed to obtain the dry weight of the entire plant and edible organs. Average values are used to represent test results.

$$\text{Edible rate(\%)} = \frac{\text{Fresh weight of edible organ}}{\text{Total fresh weight}} \times 100\%$$

2.4.2. Measurement of Quality Characteristics

The soluble protein content was determined using the Coomassie Brilliant Blue G-250 method [12]. The vitamin C content was measured using a spectrophotometer [13]. The chlorophyll content was determined via the ethanol acetone extraction method [14]. The content of rutin was determined by ethanol extraction spectrophotometry [15]. The total flavonoid content was determined by ethanol extraction spectrophotometry [16].

2.5. Data Processing and Analysis

The experimental data were processed and analyzed using Microsoft Office Excel 2016 and SPSS 19.0 statistical analysis software, and Tukey's post-hoc test was used to determine the significance of differences between each treatment ($p < 0.05$).

3. Result and Analysis

3.1. Effects of Different Combinations of Red and Blue Light on the Edible Organ Morphology Characteristics of Buckwheat Microgreens

The effects of different treatments of combinations of red and blue light on the hypocotyl length, hypocotyl diameter, and main root length of the buckwheat microgreens are shown in Figure 2. The longest hypocotyl length of the buckwheat microgreens was recorded under R5B1 treatment at 11.42 mm, significantly higher than that in control group W, followed by buckwheat microgreens under R3B1 treatment. From the graph, it can be seen that as the proportion of blue light in combinations of red and blue light gradually increases, the hypocotyl length shows a decreasing trend. Compared with the control group, the R1B5 treatment group reduced the hypocotyl length of the buckwheat microgreens by 30.1%, significantly inhibiting the increase in the hypocotyl length of the buckwheat microgreens, but the diameter of the hypocotyl reached its maximum value of 1.78 mm under R1B5 treatment. Compared with the control, except for the high-proportion red light treatments R5B1 and R3B1, other combinations of red and blue light treatments all increased the hypocotyl diameter of the buckwheat microgreens to varying degrees, showing an upward trend, and there was no significant difference between R5B1 and R3B1 compared to the control group, which indicates that adding a high proportion of blue light in combinations of red and blue light is beneficial for the lateral growth of the hypocotyl in buckwheat microgreens. Compared with the control group W, the main root length of the buckwheat microgreens under high-proportion red light R5B1 treatment reached its maximum value of 5.63 cm, significantly higher than other treatments; however as the proportion of blue light increased, the root length of the buckwheat microgreens was inhibited, and the main root length under R1B5 treatment was 2.86 cm, significantly lower than the 3.65 cm of the control group W, which indicates that a high proportion of red light in combinations of red and blue light can promote the growth of buckwheat microgreen roots, while increasing the proportion of blue light will inhibit root elongation.

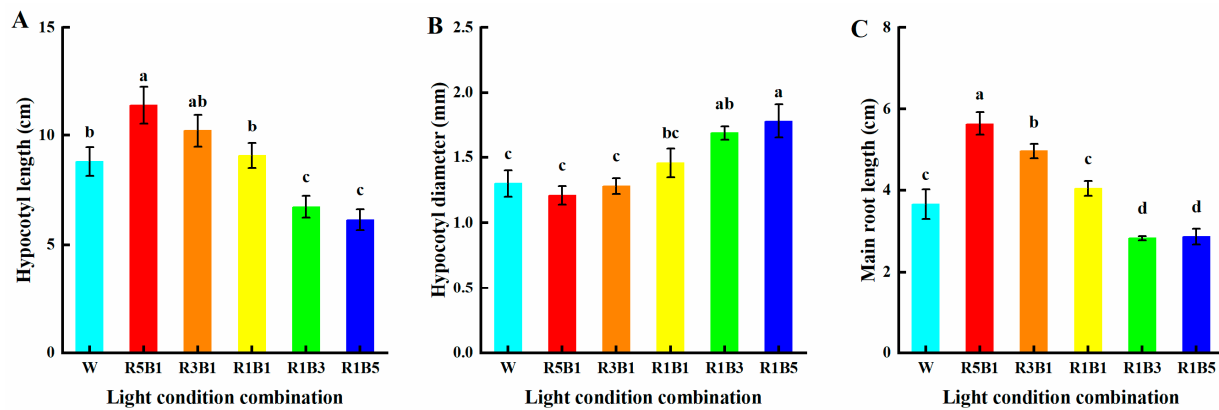


Figure 2. Effects of different combinations of red and blue light on hypocotyl length, hypocotyl diameter, and main root length of the buckwheat microgreens: (A) Hypocotyl length; (B) The diameter of the hypocotyl; (C) Main root length. Different letters represent significant differences between treatments for the same indicator when $p < 0.05$.

3.2. Effects of Different Combinations of Red and Blue Light on the Biomass of the Buckwheat Microgreens

The effects of different treatments of combinations of red and blue light on the biomass of the buckwheat microgreens is shown in Table 2. Among the six light condition treatments, the buckwheat microgreens treated with the highest proportion of red light, R5B1, showed the highest total fresh weight and fresh weight of edible organ, significantly higher than the control W. The total fresh weight and fresh weight of edible organ of R1B5, treated with the highest proportion of blue light, were the lowest. The total dry weight and dry weight of edible organ of the buckwheat microgreens treated with different combinations of red and blue light were significantly higher than in the control group W, and reached the maximum in R1B1 treatment.

Table 2. Effects of different combinations of red and blue light on the biomass of the buckwheat microgreens.

Light Treatment	Total Fresh Weight/mg	Total Dry Weight/mg	Fresh Weight of Edible Organ/mg	Dry Weight of Edible Organ/mg	Edible Rate/%
W	261.50 ± 3.82 ^b	17.44 ± 0.79 ^c	212.67 ± 1.53 ^c	14.23 ± 0.57 ^d	81.33 ± 0.88 ^c
R5B1	273.61 ± 2.14 ^a	21.24 ± 0.57 ^b	243.56 ± 2.08 ^a	17.15 ± 0.18 ^b	89.05 ± 0.09 ^a
R3B1	268.28 ± 1.31 ^{ab}	21.23 ± 0.42 ^b	235.32 ± 4.51 ^a	16.02 ± 0.18 ^c	87.70 ± 1.18 ^a
R1B1	264.48 ± 3.07 ^{ab}	23.77 ± 0.89 ^a	223.04 ± 3.61 ^b	19.27 ± 0.29 ^a	84.31 ± 0.51 ^b
R1B3	251.53 ± 2.47 ^c	21.54 ± 0.44 ^b	205.11 ± 3.97 ^{cd}	17.66 ± 0.43 ^b	81.49 ± 0.61 ^c
R1B5	246.08 ± 3.52 ^c	20.95 ± 1.13 ^b	200.68 ± 2.52 ^d	17.06 ± 0.11 ^b	81.58 ± 0.45 ^c

Different letters represent significant differences between treatments for the same indicator when $p < 0.05$.

The highest edible rate of the buckwheat microgreens, under the R5B1 treatment, reached 89.05%, which was significantly higher than the control treatment. There was no significant difference between the edible rate of the two high-proportion blue treatment groups R1B3 and R1B5 and the control group. It can be seen that, compared with the control group, as the proportion of red light increases, the edible rate of buckwheat microgreens significantly increases.

3.3. Effects of Different Combinations of Red and Blue Light on the Nutritional Quality of Buckwheat Microgreens

The effects of different treatments of combinations of red and blue light on the contents of soluble protein, vitamin C, chlorophyll, and carotenoids in the buckwheat microgreens are shown in Figure 3. The soluble protein content under R1B1, R1B3, and R1B5 treatments was significantly higher than in the control W, with R1B5 treatment reaching a maximum

value of 4.98 mg/g FW. Different proportions of combinations of red and blue light all increased the content of soluble protein in buckwheat microgreens, with only R5B1 treatment showing no significant increase in soluble protein compared to the control. The results showed that the soluble protein content increased with increases in blue light ratio.

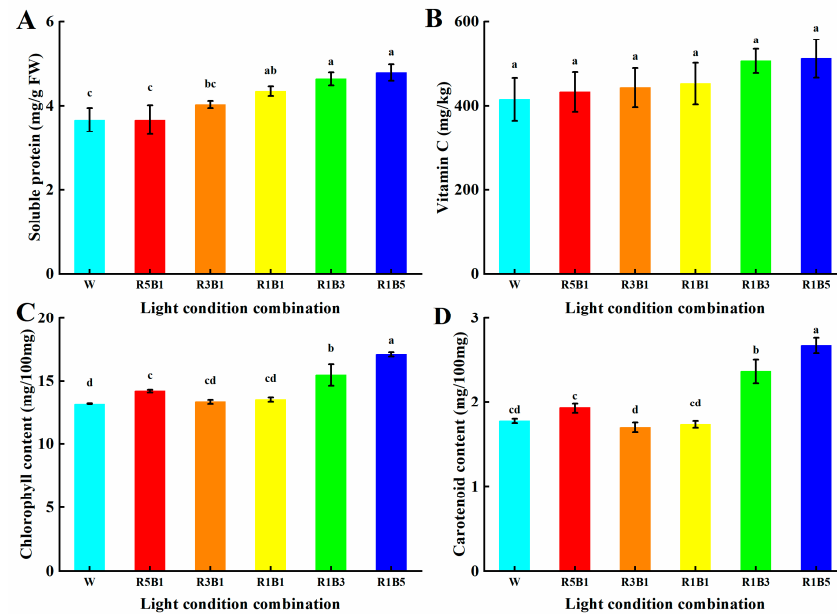


Figure 3. Effects of different combinations of red and blue light on the contents of soluble protein, vitamin C, chlorophyll, and carotenoids in the buckwheat microgreens: (A) Soluble protein; (B) Vitamin C; (C) Chlorophyll; (D) Carotenoids. Different letters represent significant differences between treatments for the same indicator when $p < 0.05$.

Various proportioned combinations of red and blue light treatments all increased the content of vitamin C in the buckwheat microgreens. The highest content of vitamin C in the buckwheat microgreens, under R1B5 treatment, reached 511.2 mg/kg, and the content of vitamin C showed an upward trend with increases in blue light ratio; the vitamin C content in the buckwheat microgreens under R5B1 treatment was 432.5 mg/kg, which was still higher than the control group. There was no significant difference between each treatment and the control.

Different treatments of combinations of red and blue light had different effects on the chlorophyll content and carotenoid content of the buckwheat microgreens. Compared with the control W, each combination of red and blue light increased the chlorophyll content of the buckwheat microgreens. Among all treatments, the chlorophyll and carotenoid contents under R1B5 treatment were the highest of any group, reaching 17.09 mg/100 mg and 2.67 mg/100 mg, respectively; the chlorophyll content increased by 29.8% compared to the control. The contents of chlorophyll and carotenoids under different light condition treatments showed a similar trend of change, all increasing with increases in blue light ratio.

3.4. Effects of Different Combinations of Red and Blue Light on the Content of Secondary Metabolites in Buckwheat Microgreens

The effects of different treatments of combinations of red and blue light on the content of rutin and total flavonoids in the buckwheat microgreens are shown in Figure 4. When the buckwheat microgreens were harvested after seven days of light cultivation, different combinations of red and blue light had different effects on the rutin content in the buckwheat microgreens. The rutin content in the high-proportion red light treatments, R5B1 and R3B1, was significantly lower than in the control, and the rutin content in the R5B1 treatment was the lowest at 45.96 mg/g, which decreased by 21.7% compared to the control; there was no significant difference between the R1B1 treatment and the control

group. As the proportion of blue light in combinations of red and blue light increased, the rutin content in the buckwheat microgreens also increased significantly; the rutin content in the R1B3 and R1B5 treatments was significantly higher than that in other treatment groups, with the highest rutin content, in the R1B5 treatment, reaching 64.45 mg/g, which was 15.6% higher than the control group.

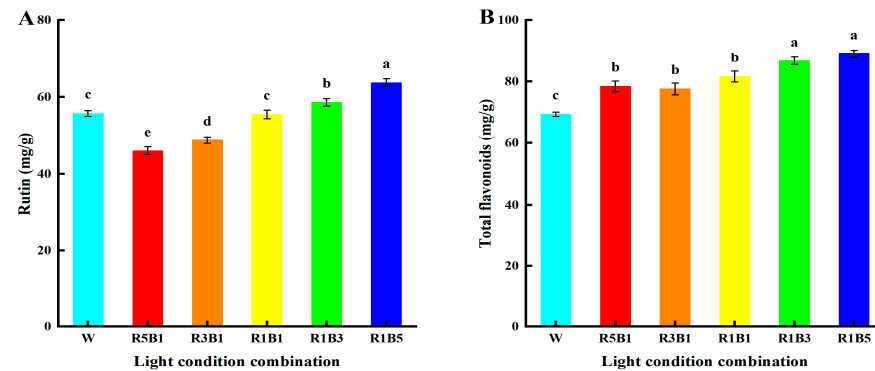


Figure 4. Effects of different combinations of red and blue light on the contents of rutin and total flavonoids in the buckwheat microgreens: (A) Rutin; (B) Total flavonoids. Different letters represent significant differences between treatments for the same indicator when $p < 0.05$.

On the seventh day of light cultivation of the buckwheat microgreens, compared with the control group W, different combinations of red and blue light significantly increased the total flavonoid content in the buckwheat microgreens. Among them, R1B5 treatment showed the greatest improvement, which was 15.9% higher than the control group. The comparison of total flavonoid content among the other treatments is R1B3 > R1B1 > R3B1 > R5B1. The total flavonoid content of R5B1 treatment was 77.66 mg/g, which was also significantly higher than in the control W. As the proportion of blue light in combinations of red and blue light increased, the content of total flavonoids in the buckwheat microgreens showed a gradually increasing trend.

4. Discussion

Different combinations of red and blue light have a positive effect on the growth of buckwheat microgreens, leading to elongation of the hypocotyl and significant increases in main root length, edible fresh weight, and edible rate. This experiment found that during the growth of buckwheat microgreens, compared with the control group under white light (W), treatment groups R5B1 and R3B1, with high proportions of red light, significantly increased the hypocotyl length of the buckwheat microgreens. It is speculated that red light can enhance the activity of plant photosensitizers, thereby promoting cell division in the stem and causing an increase in hypocotyl length, which is due to the conversion of two different structures in the photosensitizers, Pfr and Pr, in the plant after red light irradiation, causing changes in endogenous hormones and promoting the growth of its hypocotyl [17]. The study found that blue light inhibited the elongation of hypocotyls in seedlings, while red light had the opposite effect [18], which is consistent with the results of this experiment. In the growth and development of buckwheat microgreens, adding a high proportion of blue light, such as in treatments R1B5 and R1B3, to red and blue light combinations will significantly inhibit the elongation of the hypocotyl of the buckwheat microgreens, but significantly increase the diameter of the hypocotyl. Previous studies have shown that light can inhibit the growth of sprouts, causing sprouts to transition towards a shorter and thicker type, and the inhibitory effect of blue light is most significant [9]; the results of this experiment are almost consistent with the above, and the mechanism of this change may be due to the change in endogenous hormone content caused by blue light. However, studies have also shown that blue light can promote the elongation and development of hypocotyls in microgreen vegetables, which may be related to differences

in light quality responses among different plant varieties [19]. The fresh weight and dry weight of microgreen vegetables can indicate the level of yield and efficiency, and fresh weight is a major indicator reflecting the economic benefits of microgreen vegetables, as well as an important indicator of plant health and material metabolism. Previous studies have shown that combinations of blue and red light reduce the fresh and dry weight of cucumber seedlings as the blue light ratio decreases [20]. This experimental study found that using combinations of red and blue light with a high proportion of red light to treat buckwheat microgreens resulted in a significant increase in fresh weight compared to the control and other treatments, reaching its maximum value, which may be due to the fact that red light can improve photosynthetic rate and increase carbohydrate accumulation [21]. However, under the treatments with a high proportion of blue light among combinations of red and blue light, the buckwheat microgreens showed the opposite effect, significantly inhibiting the accumulation of carbohydrates, and previous reports have shown that blue light can regulate some key enzymes in the tricarboxylic acid cycle, especially pyruvate kinase, which has a regulatory effect on yeast metabolism. It can significantly increase the dark respiration rate of mitochondria, leading to a decrease in biomass.

In plants, most enzymes belong to the soluble proteins, which have relatively large molecular weights and complex structures that can produce various reactions, and can also regulate plant growth and development, resistance to diseases and pests, maturity, and aging, etc.; therefore, they play a unique role in biomass, and their content is a key indicator of plant metabolism, whereas soluble protein is easily absorbed by the body, which is an important indicator of good nutritional quality [22]. It is known that blue light can promote mitochondrial dark respiration and provide a carbon source for organic components, promoting protein synthesis; this phenomenon is closely related to the stress resistance response of plants [4]. This experiment showed that the soluble protein content of the buckwheat microgreens is highest under higher proportions of blue light treatment. Li et al. found that the soluble protein content was highest in the leaves of seedlings exposed to blue light, which is consistent with the conclusion of this experiment [23]. Vitamin C is a very important vitamin that is essential for the human body. Since the human organism cannot synthesize vitamin C, vitamin C is considered an essential dietary micronutrient and needs to be ingested through diet [24]. Su et al. found a positive correlation between blue light irradiation and vitamin C content in pea seedlings, which was higher than other light quality irradiation treatments [25]. Ioannidi et al. [26] conducted experimental studies on tomatoes and found that increases in vitamin C content in tomato fruits may be due to external stress causing the expression of key enzymes in the vitamin C synthesis pathway in the fruit. In plants, galactolactone dehydrogenase (GLDH) can directly catalyze the conversion of galactose esters to vitamin C [27], and there is evidence to suggest that blue light can enhance the activity of GLDH, thereby promoting the accumulation of vitamin C [28]. Chlorophyll is the material basis of photosynthesis and has an important impact on the photosynthetic efficiency of plants. Carotenoids have a certain protective effect on photooxidation and can improve resistance to light inhibition. Zhang et al. [29] found that under blue light treatment, the chlorophyll content of *Toona sinensis* seedlings was significantly higher than that of the control and other treatments. Studies have shown that under blue light conditions, the chlorophyll content in pea leaves significantly increases [30]. Lobiuc et al.'s study [31] showed that at the molecular level, blue light upregulates the gene expression of *MgCH*, *GluTR*, and *FeCH*, regulating the synthesis of enzymes involved in chlorophyll biosynthesis and thus promoting chlorophyll accumulation.

Rutin and total flavonoids are important secondary metabolites in buckwheat microgreens. Rutin is a representative flavonoid compound [32], a plant-synthesized phenolic substance; its effects include promoting blood circulation and removing blood stasis, lowering blood lipids, lowering blood sugar, diuresis, anti-cancer activity, enhancing hypoxia resistance, and improving immune function [33]. The results of this experiment indicate that the contents of rutin and total flavonoids in the buckwheat microgreens treated with the highest proportion of blue light (R1B5) are significantly higher than in the control group

(W) and other treatments with different combinations of red and blue light. After four days of exposure to blue light, the maximum rutin content was observed in the sprouts [34], consistent with the results of this experiment. Previous research has shown that compared to red light or fluorescent lamps, blue light can be used to increase flavonoid levels in common buckwheat malt [35]. Liu et al. [36] found that under blue light conditions, the flavonoid content in the leaves of honeysuckle seedlings is the highest. Nam et al. [9] found that blue light promoted the total flavonoid content in buckwheat malt, while red light showed the opposite effect, which could be due to the synthesis of flavonoids being sensitive to changes in light quality. Furthermore, blue light effectively enhances flavonoid accumulation by upregulating the expression of pathway genes [37], thus leading to the promotion of synthesis and accumulation of total flavonoid in buckwheat microgreens.

5. Conclusions

The growth condition under red–blue light combinations was better than that under white light in buckwheat microgreens production. A high proportion of red light treatment is conducive to increasing the weight of the edible organ of buckwheat microgreens, while increasing the proportion of blue light can improve the nutritional quality of buckwheat microgreens to a certain extent. Considering the fresh weight, dry weight and quality indexes of the edible organ, the combination of red light and blue light with a ratio of 1:1 was most suitable for buckwheat microgreen production. The results can provide a reference for the production of buckwheat microgreens.

Author Contributions: J.Q. conceived and designed the experiments. Z.L. (Zheng Lv) and Y.F. performed the experiments. S.L. analyzed the data. Z.L. (Zhongyang Li) wrote the manuscript. J.Q., Z.L. (Zhongyang Li) and S.C. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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