Effects of LED light quality on the growth characteristics and nutritional quality of hydroponic arugula

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Abstract: The global planting area of arugula (*Eruca sativa* Mill.) is increasing because of the unique flavor of this species. Excessive application of chemical fertilizers and pesticides is common in open-field cultivation of arugula, which leads to the accumulation of pesticide residue and nitrates in the leaves. Currently, arugula is mostly consumed as raw produce, increasing the importance of pesticide and pest-derived food safety issues. To improve the food safety and yield of arugula, we evaluated the effects of light quality from different LED light sources on the growth characteristics and nutritional quality of arugula plants hydroponically grown in an artificially illuminated plant factory. The arugula plants were grown under artificial LED light sources with different ratios of red and blue LED chips (3:1, 5:1, 7:1, 9:1), a photoperiod of 12 h/d, and a light intensity of 200 μmol/(m^2·s). White light was used as a control. The height, stem thickness, and leaf width of the arugula plants were measured every 5 d to generate curves of growth characteristics at different growth periods and to analyze the dynamic light quality needs of arugula. After 30 d of growth, the arugula plants were harvested, and the patterns of the effects of light quality on the growth characteristics and nutritional quality were examined. The results showed that red light had a significantly positive effect on the height, stem thickness, biomass accumulation, chlorophyll content, and soluble protein content of arugula. The arugula plants were tallest under the light with 9:1 red and blue LED chips ratio, but the effect on the soluble protein content was not significantly different from that under the 7:1 ratio, and the stem thickness and biomass accumulation were lower under the 9:1 ratio. Under the 7:1 red and blue LED chips ratio, arugula presented the greatest values of stem thickness, dry weight, fresh weight ratio, chlorophyll content, and soluble protein content. In addition, blue light promoted the synthesis of vitamin C. The light quality involving a 3:1 red and blue LED chips ratio led to a stocky plant morphology, which improved the storage and transportation ability of arugula plants.
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1 Introduction

Arugula (*Eruca sativa* Mill.), an annual Brassicaceae herbaceous species, is distributed mainly in northeastern China and northern China[1]. The glucosinolate present in the leaves of arugula, which has a unique, pungent taste, is an important secondary metabolite common to Brassicaceae vegetables. Moreover, arugula has various medicinal functions, such as diuresis and anti-inflammatory functions[2], making it increasingly popular in cuisines. With strong drought resistance and tolerance to soil infertility, arugula has been widely planted in greenhouses in northern China[3].

The low temperatures during winter and spring and the low amount of sunshine hours in northeastern China result in low yields, high energy consumption, and poor energy-use efficacy with respect to arugula[4]. The application of pesticides in traditional open-field cultivation causes pesticide residue to remain on arugula plants, and excessive application of chemical fertilizers causes nitrates to accumulate in arugula leaves, the degradation products (nitrites and nitrosamines) of which can cause a host of food safety and health problems, such as methemoglobinemia and cancer[5]. Artificially illuminated plant factories, which provide closed crop growth environments making pesticide application unnecessary, are not limited by external factors such as weather, and hydroponic cultivation effectively replaces the need for chemical fertilizers and increases arugula yields while solving the food safety problems during the growth process, all of which enable year-round arugula production[6]. Traditional artificially illuminated plant factories mostly use incandescent lamps, high-pressure sodium lamps, metal halide lamps, and fluorescent lamps as light sources, which consume large amounts of energy, have a low photoelectric efficiency, and a short lifespan, so they have been gradually replaced by light-emitting diode (LED) light sources in recent years[7-9]. Compared with other light sources, LEDs are advantageous because of their small size, long life, and low heat generation[10]. Moreover, LED light source systems allow the control of light quality, making it possible to investigate the influence of monochromatic light or specific combinations of light sources on plant growth[11]. LED lights can therefore be used as important and effective test materials for the study of light quality in artificially illuminated plant factories.

Light is the basic form of energy for plant growth and development. Plant photosynthesis utilizes the visible light spectrum (400-700 nm), and the absorbed light energy accounts for

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60-65% of the light energy causing physiological responses, with red-orange light (610-720 nm wavelengths) and blue-violet light (400-510 nm wavelengths) constituting the main peak-absorption areas[12]. LED light sources can emit monochromatic light needed for plant growth (e.g., blue light with a peak wavelength of 450 nm, red light with a peak wavelength of 660 nm). The combination of red light and blue LED light in certain ratios can result in the formation of an absorption peak spectrum that is essentially in line with the needs of plant photosynthesis and morphogenesis, with a light energy-use rate of 80%-90%, reflecting excellent energy savings and effectiveness[13].

In recent years, the effects of light quality, especially that involving the combination of red LED light and blue LED light, on plant growth, morphology, development, and photosynthesis have been extensively studied. The combination of red LED light and blue LED light has a positive effect on plant dry weight, fresh weight, soluble protein, and photosynthetic pigment accumulation, but in general, blue light reduces plant height, while red light promotes plant biomass accumulation[14]. Excessive amounts of red light have a negative impact on plant growth, and plant demand for red light and blue light is highly species specific. Wang et al. studied the effects of different ratios of red light to blue light (2:1, 4:1, 8:1, 12:1) on the morphological and photosynthesis characteristics of tomato seedlings and found that, as the red:blue ratio increased (until 8), the plant height, stem thickness, fresh weight, dry weight, seedling index, and G value (G=total plant dry weight/seedling age) of tomato seedlings increased, while the chlorophyll content decreased[15]. At different growth and development stages, the plant demands different combinations of blue light and red light changes. He et al. treated tomato seedlings at different growth periods with different ratios of red light to blue light (3:1, 5:1, 7:1) and white light and developed an LED light supplementation strategy for tomato seedlings: A light quality with a red:blue ratio of 7:1 should be adopted during the first two weeks after germination, which should be followed by irradiation with white LED light; light with a red:blue ratio of 3:1 was not suitable for the growth of tomato seedlings[16].

As a vegetable species that has recently increased in popularity, arugula has high edible and economic value because of its unique taste and flavor. Therefore, it has become valuable to explore the effects of environmental light on the growth characteristics and nutritional quality of arugula. In this study, we used artificial LED light sources with various red and blue LED chips ratios, i.e., 3:1 (R3B1), 5:1 (R5B1), 7:1 (R7B1), and 9:1 (R9B1), for growing arugula plants, with white LED light serving as the control (CK), to identify the optimal lighting conditions for arugula growth. The red and blue irradiance ratios of the above five light sources are 1.66 (R3B1), 2.54 (R5B1), 3.90 (R7B1), 5.32 (R9B1), and 0.84 (CK), respectively. The findings of this study can be used in designing intelligent light source systems in artificially illuminated plant factories and can provide a theoretical basis for establishing an optimal lighting strategy for arugula production.

2 Materials and methods

Arugula seeds were soaked in water for 5 h at 25°C, evenly spread on a layer of moistened, clean gauze on a seed pan, covered with another layer of moistened gauze, and then allowed to germinate in a 25°C thermostat incubator[17]. During the germination period, the seeds were rinsed twice daily with clean water and turned over after each rinsing. After forming uniform buds, the seeds were transferred to a seedling tray. Seedlings with two true leaves were then transferred to a hydroponic cultivation shelf system and subjected to different light treatments. The height, leaf width, and stem thickness of the plants were measured every 5 d, and the plants were harvested 30 d after transplanting, after which their growth characteristics and nutritional quality were determined.

The hydroponic cultivation shelf system used in this study had the following dimensions: 1200 mm (L)×500 mm (W)×2200 mm (H). The cultivation bed consisted of a cultivation table and a plate. The cultivation table had the following dimensions: 1000 mm (L)×300 mm (W)×70 mm (H). The plate had 20 planting holes, each of whose diameter was 20 mm (Figure 1). The cultivation bed was covered by an acrylonitrile-butadiene-styrene cover that was 4 mm thick. During the period of germination, the environment was set as follows: the temperature was 27°C±1°C in the photoperiod and 22°C±1°C in the dark period, the relative humidity was set as 75%±10%, and CO_2 was not controlled. Each light treatment included 20 plants, with three replicates.

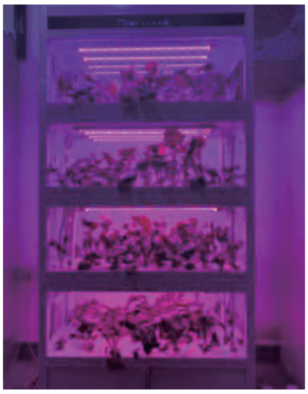


Figure 1 Hydroponic cultivation shelf system

The nutrient solution used was provided by Guangdong Aoma Agricultural Technology Co., Ltd., China, and its formula is listed in Table 1. The nutrient solution was stored in a tank at the bottom of the hydroponic shelf system. The circulation and aeration of the nutrient solution were achieved through a pump, which lifted the nutrient solution to the nutrient solution tray on the top layer of the hydroponic shelf system through a plastic tube. The trays on different shelves were connected through polyvinyl chloride (PVC) pipes, which allowed the nutrient solution to flow through to each shelf and then back to the nutrient solution tank at the bottom. The pH of the nutrient solution was maintained at 6.0-6.5. At the initial stage of seedling transplantation, the electrical conductivity (EC) value was maintained at 500-600 μS/cm and was increased to 1000-1200 μS/cm after 10 d.

Table 1 Nutrient solution formula

Component	Content/mg·L^{-1}
$NH_4H_2PO_4$	115.00
$MgSO_4·7H_2O$	493.00
$[-CH_2N(CH_2COONa)CH_2COO]_2Fe$	20.00-40.00
H_3BO_3	2.86
$MnSO_4·4H_2O$	2.13
$ZnSO_4·7H_2O$	0.22
$CuSO_4·5H_2O$	0.08
$(NH_4)_2MoO_4·4H_2O$	0.02
$Ca(NO_3)_2·4H_2O$	945.00
KNO_3	607.00

The arugula plants were grown under five LED lighting conditions: light produced by four different red:blue light chips ratios (R3B1, R5B1, R7B1, R9B1) and white light (CK). Their spectra are shown in Figure 2. The LED light sources were manufactured by Shanghai Chenhua Technology Co., Ltd., and had alternating-current power supplies. The photoperiod was set to 12 h/d, which was controlled by the automatic control system of the hydroponic shelf system. Illumination was provided from 9:00 to 21:00 daily, at an intensity of 200 μmol/(m^2·s).

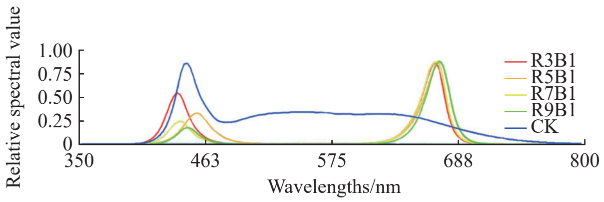


Figure 2 Spectra of light sources

2.1 Measurement

2.1.1 Growth characteristics

Arugula plants were harvested 30 d after transplanting. The plant height and stem thickness were measured with a Vernier caliper. The fresh weight of the edible portion was measured with a 0.001g-precision electronic balance (Kunshan Ante Measuring Equipment Co., Ltd., China), after which they were dried in an oven at 105°C for 3 h, followed by 80°C for 72 h until constant weight. The dry weight of the edible portion was subsequently weighed.

2.1.2 Nutritional quality

The chlorophyll content was determined with a chlorophyll measuring device (TYS-4N, Beijing Jinkelida Electronic Technology Co., Ltd., China), and the vitamin C concentration was measured through liquid chromatography (LC)[18]. Specifically, an appropriate amount of a thoroughly-mixed sample was added to a 50 mL volumetric flask, to which 40 mL of metaphosphoric acid solution (20 g/L) was then added. The resulting solution was sonicated for 30 min and reduced to a volume of 50 mL, from which the sample was first filtered through a 0.22 μm filter and then subjected to LC.

The soluble protein content was determined using the Coomassie Brilliant Blue G-250 method[19]. Specifically, approximately 0.5 g of sample tissue was placed into a centrifuge tube, to which 4 mL of 0.9% sodium chloride solution was then added. The protein was leached for 2 h at 37°C, after which the supernatant was collected through centrifugation (4500 r/min for 5 min). A total of 0.1 g of Coomassie Brilliant Blue G-250 was dissolved in 50 mL of 95% ethanol; the solution and 100 mL of 85% phosphoric acid solution were then mixed together, after which the solution was brought to a volume of 1000 mL with distilled water. The supernatant and distilled water were mixed together until the final volume reached 10 mL; this solution was subsequently used as a test sample. The mixture of 0.1 mL of the sample and 5 mL of Brilliant Blue G-250 solution was incubated at room temperature for 2 min, after which its absorbance at 595 nm was measured.

2.2 Statistical analysis

The data was processed using Excel, with results shown as mean±SD. Univariate analysis was performed via SPSS 22.0 (IBM, Inc., Chicago, IL, USA), and Duncan's method was used for multiple comparisons. The significance level was set to p<0.05.

3 Results and discussion

After the arugula seedlings were transplanted to the hydroponic shelf system, the plant height, stem thickness, and leaf width were measured every 5 d, and curves of the growth characteristics at different growth stages were plotted on the basis of these data (Figure 3).

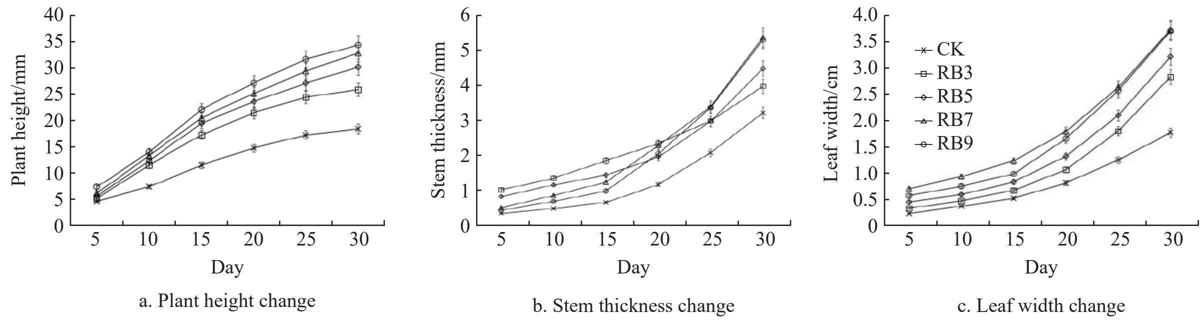


Figure 3 Changes in growth characteristics of arugula plants at different growth periods

The changes in the height of arugula showed that red light had a significantly positive effect on arugula growth at all growth stages. The height of arugula plants in the R9B1 treatment group was always greater than that of other groups, and that in the red and blue LED light treatment groups was significantly greater than that in the CK group. The positive effects of red light on the height of arugula plants gradually decreased with increasing plant growth, as indicated by the decreasing slope of the curve. At the middle and late growth stages, the effects of red light on arugula were reflected more in the increase in biomass accumulation, as revealed by the change in leaf width. Red light also had a significantly positive effect on leaf width, but the leaf width of the arugula plants in the R7B1 treatment group was greater than that in the R9B1 treatment group. This likely occurred because, at the initial growth stage, excessive red light (R9B1) caused plant spindling, which affected the biomass accumulation of arugula plants in that group, causing the leaf width of those plants to be narrower than that in the R7B1 treatment group but still wider than that in the R5B1, R3B1, and CK groups. In the case of stem thickness, the changes were rather complicated. The stem thickness in the red and blue LED light treatment groups was significantly greater than that in the CK group. During the growth period of 0-20 d, the plant stem thickness of the R3B1 treatment group was the greatest, while the plant height in this treatment group was lower than that in the R5B1, R7B1, and R9B1 treatment groups. After 20 d, the stem thickness of the plants in the R7B1 and R9B1 groups surpassed that in the R3B1 group at an accelerating rate (a steep slope of the curve), and the plant stem thickness of the plants in the R7B1 group was always greater than that in the R9B1 group. However, at harvest (30 d after transplanting), the plant stem thickness was similar between the R7B1 and R9B1 groups, as shown in Figure 4. Before day 20, the stem thickness of the plants in the R5B1 treatment group was always between those in the other groups; greater than those in the R7B1, R9B1, and CK treatment groups; but smaller than that in the R3B1 treatment group. After day 20, the pattern was the opposite.

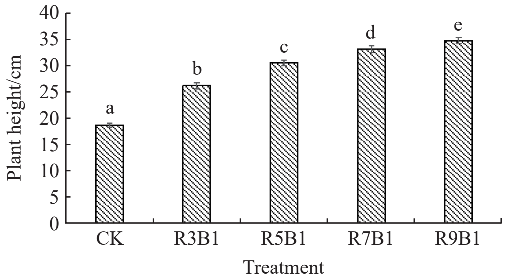
In this study, red light had a positive effect on the height, stem thickness, and leaf width of arugula plants at different growth periods, which is consistent with the conclusion of Wang and Zhang about the effects of red and blue LED light on the growth of brassica chinensis, leaf lettuce, and spinach[20,21]. It was also found that, at the initial growth stage, a greater proportion of red light led to arugula spindling, which was not conducive to subsequent biomass accumulation. However, the indices under this lighting condition were still significantly greater than those of plants grown under white LED light, as has also been reported for lettuce and cabbage seedlings[22,23]. The R7B1 lighting combination had significantly positive effects on the various growth characteristics of arugula plants at different growth periods, with less spindling than that induced by R9B1. The R3B1 lighting combination resulted in dwarf-type arugula plants within 20 d after transplanting, which would be beneficial for subsequent transplanting and transportation of arugula plants.

a. CK b. R3B1 c. R5B1 d. R7B1 e. R9B1

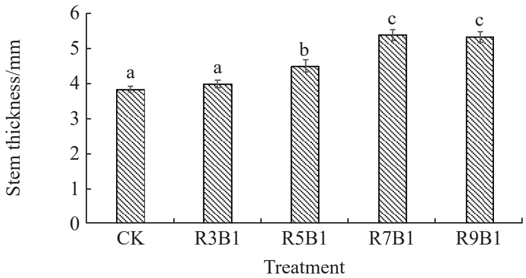
Figure 4 Growth of plants in each treatment group at 30 d after transplanting

3.1 Effects of light quality on plant height

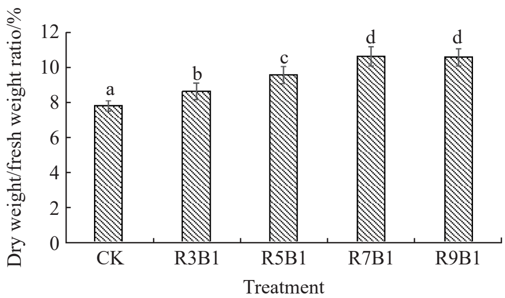
As shown in Figure 5a, light quality had a significant effect on the height of arugula. As the proportion of red light increased, the plant height increased. The heights of the plants in the treatment groups were significantly greater than the height of plants in the CK group, by 40%, 63%, 77%, and 86%, and were significantly different from each other, indicating that red light has a positive effect on the height of arugula plants. As the proportion of red light



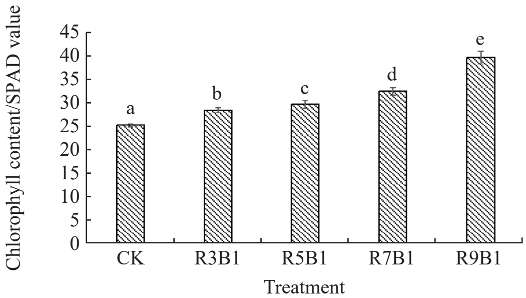
a. Effect of light quality on plant height



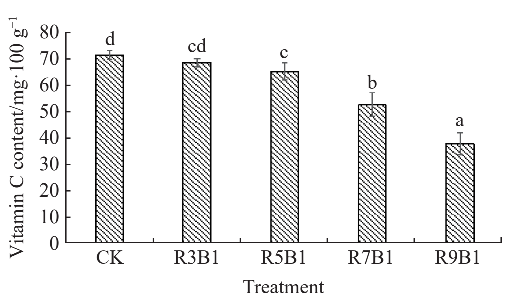
b. Effect of light quality on stem thickness



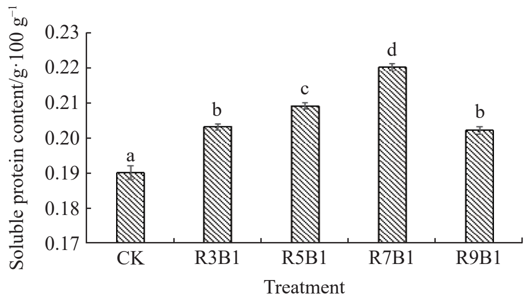
c. Effect of light quality on dry weight/fresh weight ratio



d. Effect of light quality on chlorophyll content



e. Effect of light quality on vitamin C content



f. Effect of light quality on soluble protein content

Note: A different letter in the same column indicates a significant difference by Duncan's multiple range test (p<0.05)

Figure 5 Effect of light quality on the growth characteristics and nutritional quality of arugula plants increased, the positive effects of red light on plant height gradually decreased.

3.2 Effects of light quality on stem thickness

Light quality also had a significant effect on stem thickness (Figure 5b). As the proportion of red light increased, the stem thickness increased first but then decreased, in the order of R7B1>R9B1>R5B1>R3B1>CK. Compared with those in the CK group, the plants in all the treatment groups had significantly thicker stems (by 4%, 17%, 40%, and 38%), and the thickness values were significantly different from each other.

3.3 Effects of light quality on the dry weight:fresh weight ratio of the edible portion

Light quality had a significant effect on the dry weight:fresh weight ratio of the edible portion, with a pattern similar to that seen for stem thickness. As the proportion of red light increased, the dry weight:fresh weight ratio increased but then decreased, in the order of R7B1>R9B1>R5B1>R3B1>CK. Compared with those in the CK treatment group, the plants in all the treatment groups had a significantly greater dry weight:fresh weight ratio (by 11%, 28%, 37%, and 36%), and the values were significantly different from each other.

3.4 Effects of light quality on chlorophyll content

As shown in Figure 5d, light quality had a significant effect on the chlorophyll content. As the proportion of red light increased, the chlorophyll content of arugula increased. The plants in the treatment groups contained significantly more chlorophyll than those in the CK treatment group (by 13%, 18%, 29%, and 58%), and the contents were significantly different from each other. The greater the proportion of red light was, the more profound the increase in chlorophyll content, indicating that red light has a significantly positive effect on the production of chlorophyll.

3.5 Effects of light quality on the vitamin C content

Light quality had a significant effect on vitamin C content. As the proportion of red light increased, the vitamin C content in the arugula plants decreased. The vitamin C contents in the plants in the R7B1 and R9B1 treatment groups were significantly lower than those in the CK treatment group, by 30% and 40%, respectively. The plants in the R3B1 treatment group had similar vitamin C content as those in the CK treatment group. Compared with that in the R5B1 treatment group, the vitamin C content in the plants in the R7B1 treatment group decreased, which reflected the most significant difference in vitamin C content in plants between the red and blue light treatment groups.

3.6 Effects of light quality on the soluble protein content

As shown in Figure 5f, light quality had a significant effect on the soluble protein content in the arugula plants, with a pattern similar to that for the stem thickness and dry weight:fresh weight ratio of the edible portion of the plants. When the red:blue ratio was below 7, red light had a positive effect on the soluble protein content; when the ratio was above 7, as the proportion of red light increased, the soluble protein content in the arugula plants decreased significantly, such that the soluble protein content in the plants in the R9B1 treatment group was not significantly different from that in the R3B1 treatment group. Moreover, the plants in the R5B1 and R7B1 groups had significantly more soluble protein than those in the CK group, by 10% and 16%, respectively.

In this study, we found that red light promoted the plant height of arugula, with the plants in the R9B1 treatment group growing the tallest, while blue light resulted in the opposite pattern. These findings are consistent with the results of Azad and Yang, who reported that red light increases the height of leaf lettuce and eggplant, while blue light inhibits the elongation of their stems[24,25]. The mechanism of action may involve blue light increasing the activity of indoleacetic acid (IAA) oxidase, which reduces the concentration of IAA and thus inhibits stem elongation. However, Tang et al. reached the opposite conclusion, finding that blue light promotes the elongation of tobacco seedling stems[26]. Blue light leads to stocky plants, and this morphology has a positive effect on yield, storage and transportation ability, and economic value[27-29]. The difference in the effects of red light and blue light on different crop species reflects the differences in the responses to light quality between different vegetable species[30].

The effects of light quality on the stem thickness and dry weight:fresh weight ratio of the edible portion of arugula plants exhibited similar patterns. When the red:blue ratio was below 7, red light had a positive effect on the stem thickness, and the dry weight:fresh weight ratio and stem thickness of the plants in the R7B1 treatment group were significantly greater than those in the other treatment groups, the results of which are consistent with those of Liu et al.[31]. When the red:blue ratio was above 7, the stem thickness and dry weight:fresh weight ratio no longer significantly increased; however, they were still greater than those of plants in the R3B1, R5B1, and CK groups. When examining the effects of red light and blue light on the photosynthesis of lettuce, Wang et al.[32] drew a similar conclusion: When the red:blue ratio was below 8, red light significantly promoted the photosynthesis characteristics of lettuce, but when the ratio was above 8, the above effect became statistically insignificant. In the present study, we found that, at the early stage of arugula growth, excessive red light (R9B1) led to arugula spindling, causing the stem thickness and dry weight:fresh weight ratio of the edible portion of the plants in the R9B1 group to be lower than those in the R7B1 group at harvest, indicating that, at different growth periods, arugula has different light requirements. Thus, it is necessary to investigate the dynamic light requirements of arugula plants throughout the entire plant growth process.

We found that red light had a positive effect on the chlorophyll content in arugula, while blue light inhibited chlorophyll synthesis. These findings are consistent with the results of most previous studies. When investigating the effects of light quality on the growth of tomato seedlings, Pu et al. found that the total chlorophyll content of seedlings grown under blue light was significantly lower than that under red light or white light, and showed a higher value of chlorophyll a/chlorophyll b, indicating that plants grown under blue light often have characteristics similar to those of heliophytes, while the characteristics of plants growing under red light are similar to those of shade plants[33].

Vitamin C is an important index of vegetable nutritional quality. In this study, we found that the effects of light quality on the vitamin C content in arugula plants exhibited a pattern in which red light significantly inhibited the synthesis of vitamin C in arugula; however, the vitamin C content did not significantly differ between the R3B1 and R5B1 groups. When the red:blue ratio was greater than 5, the magnitude of the decrease in vitamin C content increased significantly. The difference in vitamin C content between the R5B1 and R7B1 groups was the most significant, which has implications for future studies on the effects of light quality on arugula vitamin C content. Most of the previous relevant studies showed that red light reduces plant vitamin C content, while blue light promotes vitamin C synthesis[34-37], likely because blue light can increase the activity of galactonolactone dehydrogenase, a key enzyme involved in the biosynthesis of vitamin C, thus increasing the plant vitamin C content[38].

The effects of light quality on arugula soluble protein content exhibited a pattern similar to that for stem thickness or the dry weight:fresh weight ratio of the edible portion of the plants. As the proportion of red light increased, the arugula soluble protein content increased; this was similar to the case of the vitamin C content but differed in that the turning point occurred under a red:blue ratio of 7:1 (R7B1). When the red:blue ratio was above 7, the soluble protein content decreased significantly, resulting in the difference between the R9B1 and R3B1 groups being statistically insignificant, while the edible portion dry weight:fresh weight ratio of the plants in the R9B1 group was still significantly greater than that in the R3B1 and R5B1 treatment groups. Blue light is generally considered to reduce plant soluble protein contents, the phenomenon of which is likely associated with the concentrations and activities of relevant enzymes[39,40], which would be consistent with the results of this study.

4 Conclusions

Red light has a significantly positive effect on the height, stem thickness, biomass accumulation, chlorophyll content, and soluble protein content of arugula plants, while blue light promotes the synthesis of vitamin C in these plants. Combinations of red light and blue light can significantly improve the growth characteristics and nutritional quality of arugula. Arugula presented the greatest stem thickness, dry weight:fresh weight ratio, chlorophyll content, and soluble protein content under the R7B1 light treatment. The R7B1 light treatment had significantly positive effects on various growth characteristics of arugula at different growth periods, while the R3B1 light treatment induced a stocky plant morphology during the first 20 d of growth, which is conducive to its transplanting and transportation. The above findings provide important light quality information and can serve as a theoretical basis for the study of light quality in artificially illuminated plant factories.

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