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Effects of far-red light on growth, endogenous hormones, antioxidant capacity and quality of Lettuce

Lili Meng^{1,2,3}, Jiangfeng Song^{2,4*} , Degang Ni⁵ and Mohamad Anuar Kamaruddin³

Abstract

This study aimed to explore the effects of various intensities of far-red light on the growth performance, endogenous hormones, antioxidant indices, and overall quality of hydroponically cultivated lettuce. As the control treatment, a white LED emitting light at an intensity of 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ was utilized (referred to as CK with an R/FR ratio of 5.5), while two experimental treatments, FT1 (R/FR = 1.2) and FT2 (R/FR = 0.8), were established by adding different intensities of far-red light to the CK treatment. The results demonstrated that the application of far-red light, particularly in FT1, led to a significant increase in plant height, leaf area, and lettuce biomass, while simultaneously resulting in a notable reduction in leaf thickness. The content of indole-3-acetic acid (IAA) and abscisic acid (ABA) in response to far-red light treatments exhibited an initial increase followed by a subsequent decrease, with FT2 experiencing a significant decline. The gibberellin (GA_3) content in FT2 reached its peak on the 35th day, showing a substantial increase of 60.09% compared to CK. Far-red treatments were found to enhance peroxidase (POD) and catalase (CAT) activities, while significantly reducing superoxide dismutase (SOD) activity. In comparison to CK, FT1 exhibited a remarkable 134.33% increase in anthocyanin content. Both FT1 and FT2 significantly boosted vitamin C levels while reducing nitrite content. Additionally, the application of far-red light treatment significantly increased the alcohol and ester content in lettuce leaves. This study establishes a theoretical foundation for enhancing the quality and flavor of lettuce using different far-red light treatments.

Keywords Lettuce, Far-red light, Growth, Endogenous hormones, Antioxidant capacity, Quality

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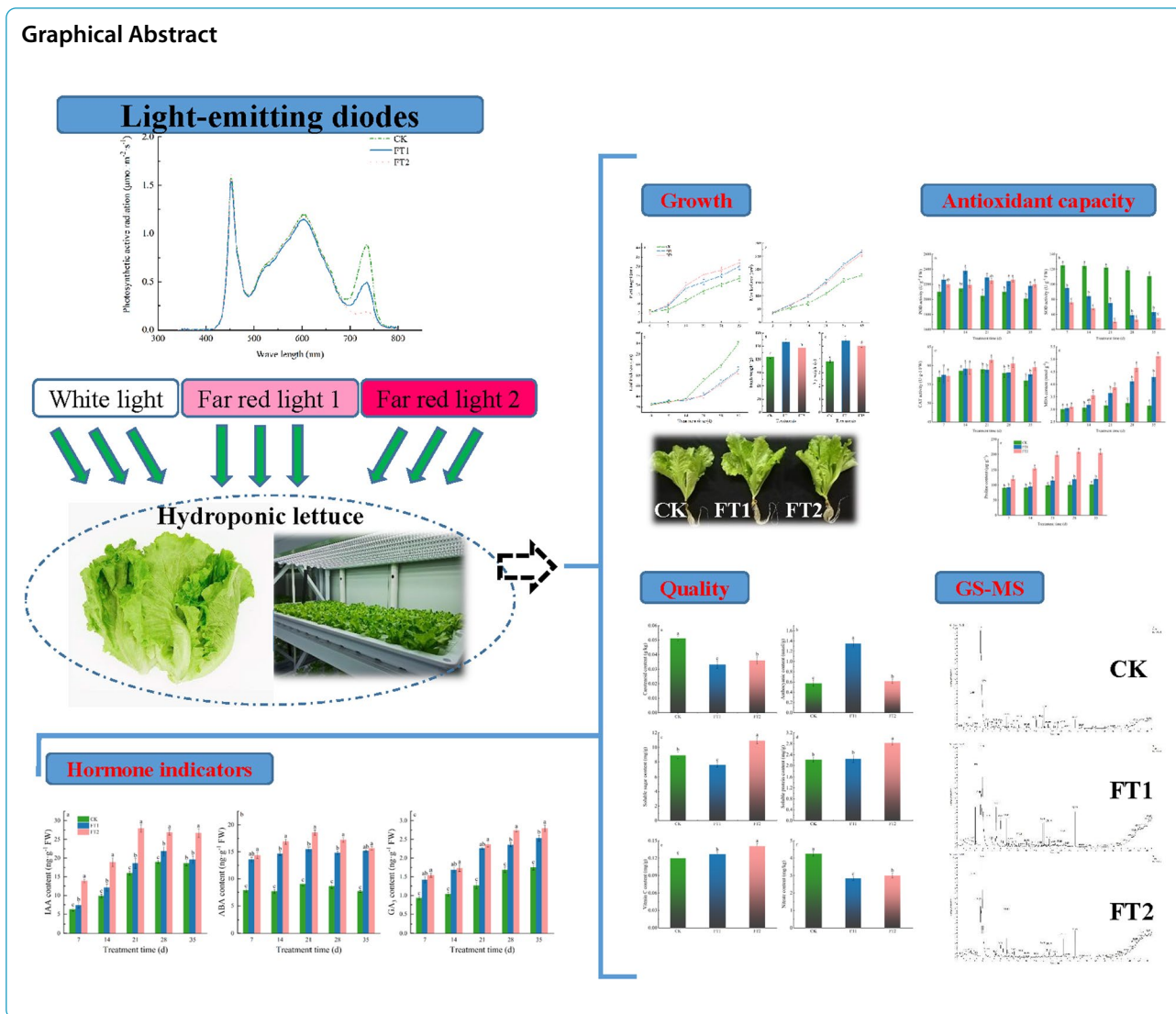
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Introduction

Light is an essential factor for plants among the various environmental factors, not only providing the energy needed for photosynthesis in which carbon dioxide and water were converted by light energy into organic matter and release oxygen (O_2) (Metallo et al. 2018; Wang et al. 2020), but also regulating the various processes of plant growth and development (Demotes-Mainard et al. 2016; Li & Kubota 2009). Light information was received and transmitted by the activity of photoreceptors (Burgie et al. 2014). Phytochrome A and phytochrome B are pigment proteins, which have a reversal effect on the absorption of red and far-red light and were also involved in photomorphogenesis and the regulation of plant development, regulating the process from seed germination to growth and fruiting by sensing the ratio of red to far-red light (R/FR) in the environment (Lund et al. 2007).

Far-red light was the regulator of plant morphology and photosynthetic capacity (Hitz et al. 2019; Tan et al. 2022). Some studies on the influence of far-red light on the morphological and photosynthetic parameters of plants have been reported. Increasing far-red light promotes the extension of plant cells, increasing leaf area and thus the efficiency of photosynthetic radiation use (Bae et al. 2017; Shibuya et al. 2016). Plants usually have elongated main stems and show increased apical dominance, while lateral branch growth is somewhat inhibited in a shaded environment with lower R/FR (Franklin 2008; Héraut-Bron et al. 2000). Far-red light in the plant canopy increases leaf length and decreases leaf width, with leaves narrowing compared to those in normal light (Zhang et al. 2019). The addition of far-red light to red and blue light significantly affects the growth and morphological structure of lettuce, increasing the dry and fresh shoot

weight, plant height, leaf area, and energy use efficiency of lettuce (Li et al. 2020). The addition of far-red light in red plus blue LEDs increased leaf fresh and dry weights of green leaf lettuce by 28% and 34%, respectively (Yan et al. 2020), plants grown under the sun-like LEDs exhibit larger leaf area, which may be due to some specific spectrum distributions (such as more far-red radiation), and consequently were favorable for light interception and therefore result in greater production (Zou et al. 2020).

Far-red light can also regulate hormone secretion in plants, such as indole-3-acetic acid (IAA), abscisic acid (ABA), and gibberellin (GA_3). It was reported that far-red light boosts IAA content and cell sorting hormone in tomatoes, promoting lateral branch activation and growth (Holalu et al. 2021; Islam et al. 2014). Increasing the proportion of far-red light during the day significantly increased the height of tomato seedlings and the hormone content of stems and leaves (Procko et al. 2016), but reduced chlorophyll content and photosynthetic rate (Procko et al. 2014). In angiosperms, low R/FR ratio light increased GA levels (Guo et al. 2022).

In addition, the change of R/FR in the environment can also affect the physiological metabolism and antioxidant properties of plants. It was found that the R/FR value was related to the pathway of inducing senescence and oxidative metabolism in wheat leaves (Humberto et al. 2006). The relationship between photosynthetic rate and R/FR value was found to be nonlinear (Leuchner et al. 2007). It was found that low R/FR could improve the salt tolerance of cucumber and alleviate the inhibition of salt stress on growth and photosynthesis, meanwhile, the addition of far-red light can also increase the content of osmoregulatory substances and antioxidant capacity in tomato leaves and roots (Wang et al. 2021).

Far-red light can also affect plant quality indicators. It was found that the R/FR ratio affects different steps of plant nutrition: absorption, assimilation under the organic form, and allocation to plant organs (Demotes-Mainard et al. 2016). The chlorophyll, soluble protein, soluble sugar, vitamin C, nitrate, flavonoid, polyphenol, anthocyanin, and other quality contents would be influenced by far-red light in the environment (He et al. 2021; Kim & Son 2022; Kim et al. 2020; Li et al. 2014). The soluble sugar content of the ripe tomato fruit was slightly decreased by longer exposure of the plants to far-red light (Zhang et al. 2019).

Above all, the investigations on the effects of adding different intensities of far-red light (with different R/FR values) on the growth performance, endogenous hormones, antioxidant indexes, and lettuce quality of lettuce were studied by using hydroponic lettuce as material, which would provide a theoretical basis for the efficient application of far-red light in plant factories.

Materials and methods

Experimental materials and the plant factory

The experimental site was located in the equipment institute of Jiangsu Academy of Agricultural Sciences ($118^{\circ}88'52''E$, $32^{\circ}04'12''N$) at the southern foot of Zijin Mountain in the eastern suburb of Nanjing, Jiangsu Province. The plant factory was converted from a 20-foot container (L×W×H: 5.9 m × 2.4 m × 2.4 m), with 5 cm polyurethane color steel plates laid inside, PVC flooring at the bottom and the installation of matching insulation doors to play the role of heat insulation.

Lettuce seeds were soaked and cleaned, sown in seedling sponge blocks, and placed in an artificial climate chamber with 12 h of light at 24 °C, 65% humidity, and 100 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ during the day and 12 h at 18 °C, 65% humidity at night. Lettuce plants with uniform growth were selected and randomly transplanted into the hydroponic tank of the plant factory at a cultivation density of 30 plants/ m^2 . The temperature in the container plant factory during day and night periods was set at 24 °C/18°C, humidity at 65%, and photoperiod at 12 h/d. The hydroponic nutrient solution was half Hoagland formula, including calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}$), potassium nitrate (KNO_3), ammonium nitrate (NH_4NO_3), potassium dihydrogen phosphate (KH_2PO_4), magnesium sulfate (MgSO_4), iron salt solution and trace element liquid, and its pH and electrical conductivity (EC) values were maintained at 6 ± 0.2 and 1.5 ± 0.1 mS/cm, respectively.

Experimental design

The experiment was carried out by deep liquid hydroponics, and the cultivation frame (L×W×H: 140 cm×60 cm×180 cm) was divided into 3 layers according to the height direction, and each layer was equipped with a different number of LED light sources, which were produced by Guangzhou Linong Lighting Technology Company. The test has a total of 3 light treatments.

The light intensity of the white LED was 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ with 2 peaks at 450 and 555 nm, using the white LED as a control (control treatment, CK, R/FR=5.5). The far-red treatment 1 (far-red treatment 1, FT1, R/FR=1.2) and far-red treatment 2 (far-red treatment 2, FT2, R/FR=0.8) were set up by adding different intensities of far-red light to CK. The spectra and light intensities of the three treatments were measured using a spectroradiometer (Model PS-100, Apogee, USA) (Fig. 1). Each treatment was planted with 15 lettuce plants and replicated three times.

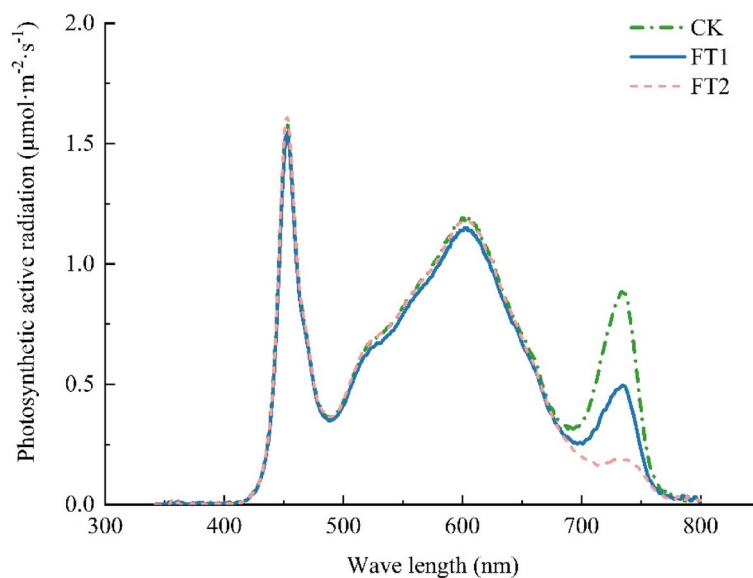


Fig. 1 Light intensity and spectral distribution of three processed led treatments

Measurement indicators and methods

Determination of growth indicators of lettuce

The relative index measurement was started when the lettuce plants were planted for 7 days, and the measurement was carried out every 7 days. The maximum leaf area of lettuce was measured with a portable leaf area measuring instrument (YMJ-A, Beijing Yaxinli Instrument, China); three measuring points were selected on the leaves at the same location (avoiding the leaf veins) and a spiral micrometer (standard single-use type Ltd.) to measure leaf thickness and take the mean value; 35 d after transplanting, lettuce was randomly sampled from three plants per treatment. The plants were cleaned, and dried with absorbent paper and the above-ground parts were taken to determine the fresh mass with an accuracy of 0.01 g. The samples with the determined fresh mass were put into an oven, killed at 105 °C for 15 min and dried at 60 °C for 48 h and the dry mass was determined with an accuracy of 0.001 g.

Determination of hormone and antioxidant indicators of lettuce

Randomly selected leaf samples from the same section of lettuce for each treatment were mixed and then rapidly frozen in liquid nitrogen, followed by storage in a freezer at -80 °C. The extraction, purification, and measurement of IAA, ABA, and GA₃ hormones followed standard procedures using enzyme-linked immunosorbent assay (ELISA).

Randomly selected leaf samples from each treatment of lettuce were used for enzyme activity measurements,

with three mixed samples taken for each treatment. Proline content was determined using the ninhydrin colorimetric method, superoxide dismutase (SOD) was measured using the nitro blue tetrazolium method, malondialdehyde (MDA) was quantified using the thio-barbituric acid method, peroxidase (POD) activity was assessed using the guaiacol method, and catalase (CAT) levels were determined using the ultraviolet absorption method.

Determination of quality index and volatile components of lettuce

The carotenoid content was determined by colorimetric method, the anthocyanin content was determined by ultraviolet spectrophotometry, the soluble protein content was determined by coomassie brilliant blue colorimetric method, the soluble total sugar content was determined by anthrone sulfate method, the vitamin C content was determined by molybdenum blue colorimetric method and the nitrite content was determined by ultraviolet spectrophotometry.

The content of volatile substances in lettuce leaves was determined by gas chromatography-tandem mass spectrometry (TSQ 8000 EVO, Thermo Fisher, USA) (Gao et al. 2018).

Data analysis

The obtained results were analyzed statistically with the Co-Stat software version 6.400 (Copyright © 1998–2008 Co-Hort Software). The statistical treatments include an analysis of the variances (ANOVA) followed by a

comparison of the means with the Least Significant Difference (LSD) test ($P \leq 0.05$). The principal component analyses (PCA) using XLStat software were performed by analyzing the correlation between applied treatments and the measured parameters.

Results and analysis

Effects of different ratios of red and far-red light on the growth of lettuce leaves

Plant height and leaf area of seedlings were two important morphological indicators. As shown in Fig. 2, with the increase of far-red light treatment intensity, the overall plant height, maximum leaf area, fresh weight, and dry weight of lettuce plants all increased compared with the control. After the 7th day, the plant heights of FT1 and FT2 treatments were significantly increased compared with CK treatments, and FT1 and FT2 had the largest increases on the 14th day, which were 57.25% and 70.81%, respectively.

The leaf is the main organ for photosynthesis in higher plants, mainly by regulating the transmission of light in the mesophyll tissue for photosynthesis. From the 14th day, the leaf area of FT1 and FT2 in the far-red treatment showed a significant increase trend compared with the CK treatment, and the increase in FT1 and FT2 was the largest on the 35th day, which was 51.01% and 45.44%, respectively, and the difference between the FT1 and FT2 treatments was not significant. Before the 14th day, there was no significant difference between the leaf thicknesses of each treatment. After the 14th day, the leaf thickness of FT1 and FT2 treatments showed a significant downward trend compared with the CK treatment. On the 35th day, the leaf thicknesses of FT1 and FT2 treatments were 90 μm and 88 μm , respectively, which were decreased significantly by 35.71% and 37.14% compared with that of CK.

Plant fresh and dry biomass weight is the accumulation of assimilated photosynthesis products, which is an important index to measure plant growth. On the 35th

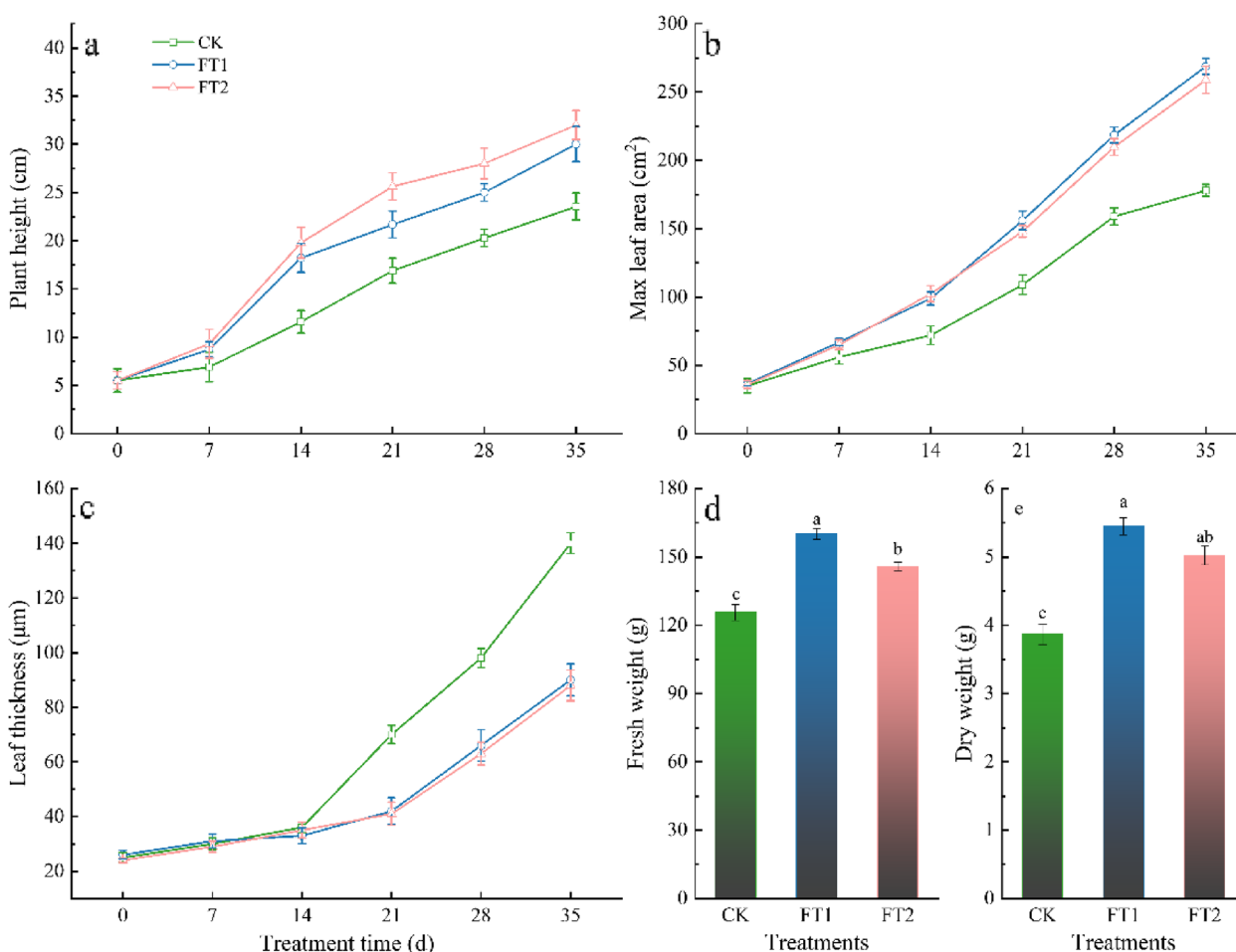


Fig. 2 Effects of different ratios of red light and far-red light on the growth index of lettuce

day, the fresh weight of lettuce treated with FT1 and FT2 was significantly increased by 27.39% and 16.09% compared with the CK treatment; the dry weight of lettuce treated with FT1 and FT2 was significantly increased by 40.83% and 29.72% compared with the CK treatment. It could be seen that the growth and yield of lettuce could be promoted by adjusting the proportion of red light and far-red light in the artificial light source.

Effects of different ratios of red and far-red light on the hormone content of lettuce leaves

The hormone content levels in plants are closely related to the growth and development of plants. As shown in Fig. 3, the far-red treatment had a certain effect on the IAA content in lettuce leaves. It could be seen from Fig. 3a that the IAA content of the far-red treatment was higher than that of the control treatment at the same treatment time, and the increase ranged from 46.15 to 120.72%. Among them, the IAA content of FT2 was the highest on the 21st day, reaching 27.89 ng·g⁻¹ FW. On the 28th day, the IAA content of FT2 was second. With the prolongation of treatment time, the content of IAA showed a trend of first increase and then a slight decrease. On the 35th day, the IAA content of FT2 was significantly higher than that of the control group by 43.61%, and the IAA content of FT1 was not significantly different from that of CK treatment.

As shown in Fig. 3b, the ABA content of CK treatment increased slowly and wave-like with the prolongation of treatment time, and the difference was not significant. The ABA content of far-red treatment showed a trend of rapid increase in the early stage and slowdown in the later stage. Under the same treatment time, the ABA content of lettuce treated with far-red was higher than that of the control treatment, and the increased range was from 5.23 to 122.61%. Among them, the ABA content of FT2 and FT1 was the highest on the 21st day, reaching 18.55 ng·g⁻¹ FW and 15.46 ng·g⁻¹ FW, which were significantly

increased by 106.38% and 72.01% compared with the control, respectively, meanwhile, on the 14th day, the ABA content of CK was the lowest, which was 7.65 ng·g⁻¹ FW.

As shown in Fig. 3c, with the prolongation of treatment time, the GA₃ content in lettuce leaves treated with FT2 and FT1 was significantly higher than that in the control treatment, with an increased range of 39.67-86.39%. Before the 21st day, the difference between FT2 and FT1 treatment was not significant. The difference between FT2 and FT1 treatment increased and reached a significant level ($P \leq 0.05$) on the 28th day. The GA₃ content of FT2 and FT1 was the highest on the 35th day, reaching 2.79 ng·g⁻¹ FW and 2.53 ng·g⁻¹ FW, which were significantly increased by 60.09% and 44.95% compared with CK treatment, and the difference between groups was significant.

Effects of different ratios of red and far-red light on the antioxidant system of lettuce leaves

When a plant is in adversity, a series of special organic compounds will be produced by the antioxidant defense system in the body, such as peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and osmotic regulation substances including proline. They have catalytic activity and are highly selective, which can be used to remove reactive oxygen species in plants to protect plant cells and reflect the stress resistance including salt, cold, and other factors of plants (Ahres et al. 2021; Wang et al. 2021).

It could be seen from Fig. 4a that with the prolongation of treatment time, the POD activity of CK treatment showed a slight fluctuation trend, and the overall change was not large; the POD activity of FT2 and FT1 treatment first increased slightly and then decreased slowly, but was always higher than that of CK treatment; on the 14th day, the POD activity of FT1 reached the highest value of 2380 U·g⁻¹ FW, which was significantly higher

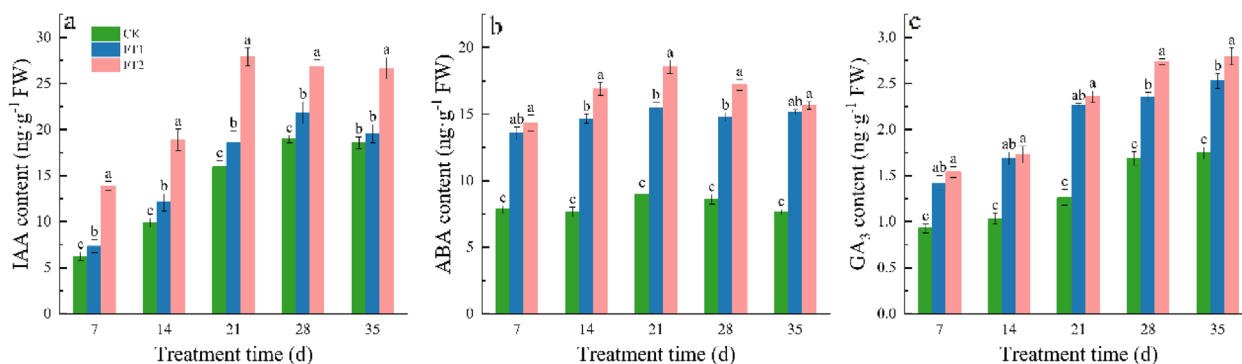


Fig. 3 Effects of different ratios of red light and far-red light on the hormone content of lettuce

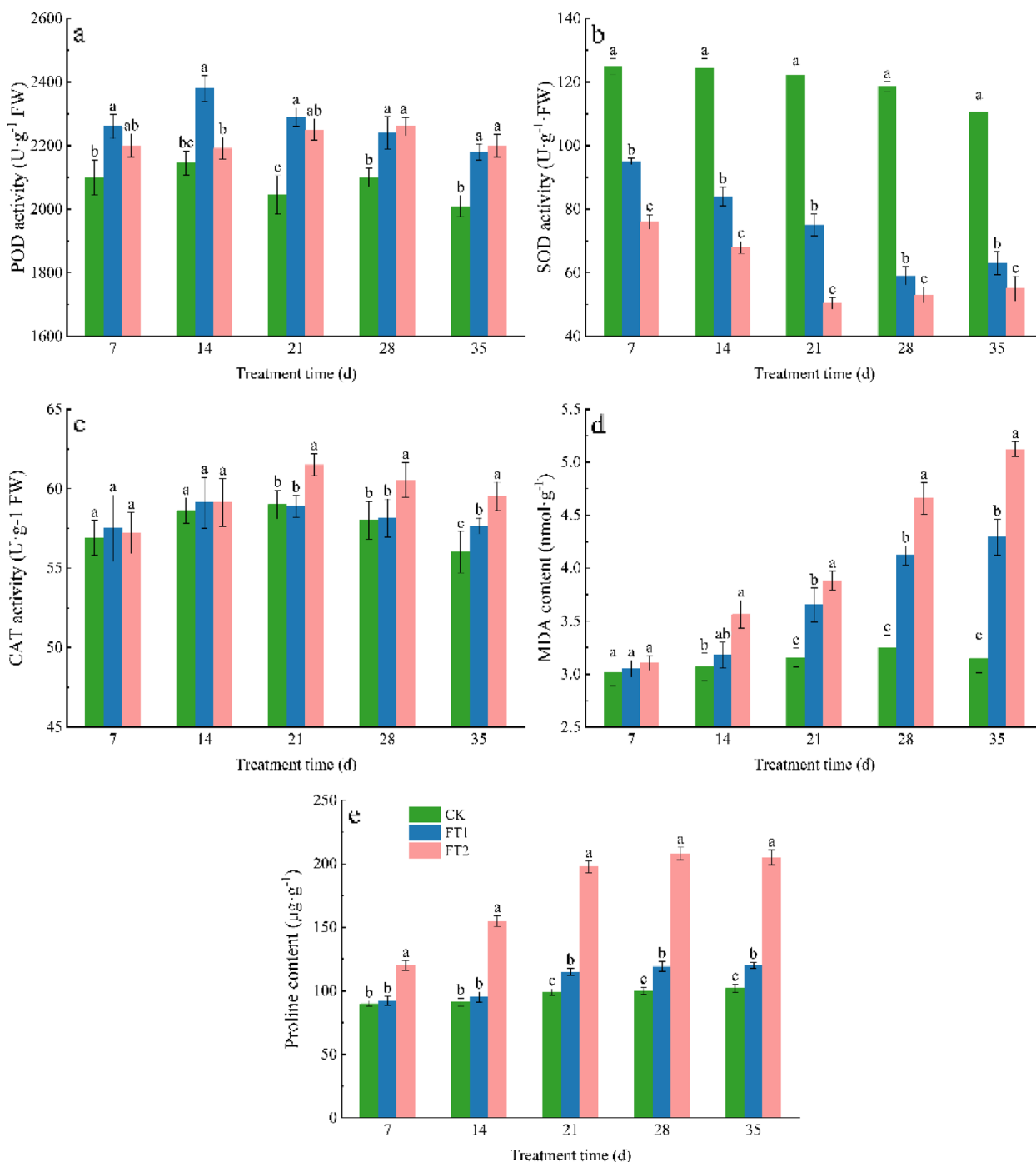


Fig. 4 Effects of different ratios of red light and far-red light on the leaf antioxidant system of lettuce

than that of CK treatment by 10.95%; on the 28th day, the POD activity of FT2 reached the highest value of 2260 U·g⁻¹ FW, which was significantly higher than that of CK treatment by 7.62%.

It could be seen from Fig. 4b that with the prolongation of treatment time, the SOD activity of CK treatment

showed a slow decline trend, the highest was 125.64 U g⁻¹ FW, the lowest was 110.64 U g⁻¹ FW, and was always significantly higher than that of FT1 and FT2 treatment. On the 21st day, the SOD activity of FT2 was the lowest at 50.41 U·g⁻¹ FW, which was significantly lower than that of CK treatment by 58.76%; the SOD activity of FT1

treatment on the 28th day was the lowest at $59.24 \text{ U}\cdot\text{g}^{-1} \text{ FW}$, which was significantly lower than that of CK by 50.27%.

It could be seen from Fig. 4c that the changes in CAT activity in each treatment were less affected by different treatments, and with the prolongation of treatment time, the CAT activity showed a stable wave-like trend. Before the 14th day, there was no significant difference in CAT among the treatments; on the 21st day and 28th day, the CAT content of FT2 treatment was significantly increased by 4.24% and 4.38% compared with CK, and the CAT content of FT1 treatment was not significantly different from CK treatment; CAT content was increased significantly by 2.95% and 6.27% compared with that of CK.

It could be seen from Fig. 4d that there was not a big change in the MDA content of CK and the MDA content of lettuce of far-red light treatments shows a general upward trend. On the 21st day, the MDA content of FT2 treatment was significantly increased by 16.17% compared with CK. On the 35th day, the MDA content of FT2 and FT1 treatments reached the maximum value of $4.29 \text{ nmol}\cdot\text{g}^{-1}$ and $5.12 \text{ nmol}\cdot\text{g}^{-1}$, which were significantly increased by 36.19% and 62.67% compared with that of CK.

It could be seen from Fig. 4e that there was no big change in the proline content of CK and slightly raised in the later period. The proline content of FT1 treatment had risen slightly in the later stage and the proline content was significantly increased by 16.16%, 19.02%, and 17.65% compared with CK on the 21st day, 28th day, and 35th day. With the processing time prolonged, the proline content of FT2 treatment increased greatly and it reached the highest value of $208.84 \mu\text{g}\cdot\text{g}^{-1}$, which was increased by 108.84% than that of CK on the 28th day.

Effects of different ratios of red and far-red light on the quality index of lettuce leaves

It could be seen from Fig. 5 that the trends of quality indicators of lettuce leaves varied among different far-red light treatments. Compared with CK, the carotenoid content of FT1 and FT2 treatments decreased significantly by 35.36% and 29.75% and differed significantly between groups; the anthocyanin content of FT1 treatment increased significantly by 134.33%; the soluble sugar content in FT1 treatment decreased significantly by 14.50%; the soluble sugar content in FT2 treatment increased significantly by 22.73%; the soluble protein content in FT2 treatment increased significantly by 27.59% and there was no significant difference between the protein content of FT1 and CK treatments. The vitamin C content of FT1 and FT2 was significantly increased by 5.80% and 17.35% than that of CK and the difference between groups was

significantly different. The nitrate content of FT1 and FT2 was significantly decreased by 33.43% and 29.55% than that of CK and the difference between groups was significantly different.

Effects of different ratios of red and far-red light on the volatile flavor compounds of lettuce leaves

As could be seen from Table 1, a total of 32 volatile flavor substances, including 11 aldehydes, 5 alkanes, 4 alcohols, 3 olefins, 3 esters, 3 ketones, 1 ether, 1 carboxylic acid and 1 aromatic hydrocarbon, were detected in the lettuce leaves of each treatment using GC-MS, with the largest content of aldehydes and the largest variety of components, followed by alcohols, with the two categories together accounting for more than 70% of the total.

There were 74.17% aldehydes, 7.07% alkanes, 11.09% alcohols, 1.14% olefins, 2.69% esters, 2.76% ketones, 0.26% ethers, 0.58% carboxylic acids and 0.25% aromatic hydrocarbons in lettuce leaves of CK treatment. The substance with the highest content was 2-Hexenal (60.42%) and the substance with the lowest content was Cedrene (0.24%).

There were 44.05% aldehydes, 5.20% alkanes, 31.05% alcohols, 1.35% olefins, 13.64% esters, 3.29% ketones, 0.26% ethers, 0.51% carboxylic acids and 0.65% aromatic hydrocarbons in lettuce leaves of FT1 treatment. The substance with the highest content was 2-Hexen-1-ol, (E)- (26.91%) and the substance with the lowest content was Cedrene (0.04%).

There were 49.09% aldehydes, 5.67% alkanes, 36.91% alcohols, 0.98% olefins, 4.19% esters, 2.05% ketones, 0.19% ethers, 0.42% carboxylic acids and 0.50% aromatic hydrocarbons in lettuce leaves of FT2 treatment. The substance with the highest content was 2-Hexen-1-ol, (E)- (32.83%) and the substance with the lowest content was Cedrene (0.03%).

Compared with CK, the 2-Hexenal content of lettuce leaves in FT2 and FT1 treatment was decreased by 50.96% and 62.63%, the 2-Hexen-1-ol, (E)- content of lettuce leaves in FT2 and FT1 treatment was increased by 284% and 215%, the 3-Hexen-1-ol, acetate, (E)- content of lettuce leaves in FT1 treatment was increased by 22.23 times.

Discussion

Different far-red light intensities were proven to have distinct effects on both plant morphology and biomass. In this study, three treatments of different LEDs used showed distinct light spectra and they are different in many aspects. This experiment compared the growth of lettuce plants under three treatments of LEDs that had different R/FR ratios. It is conspicuous the lettuce plants grown under low R/FR showed greater plant size,

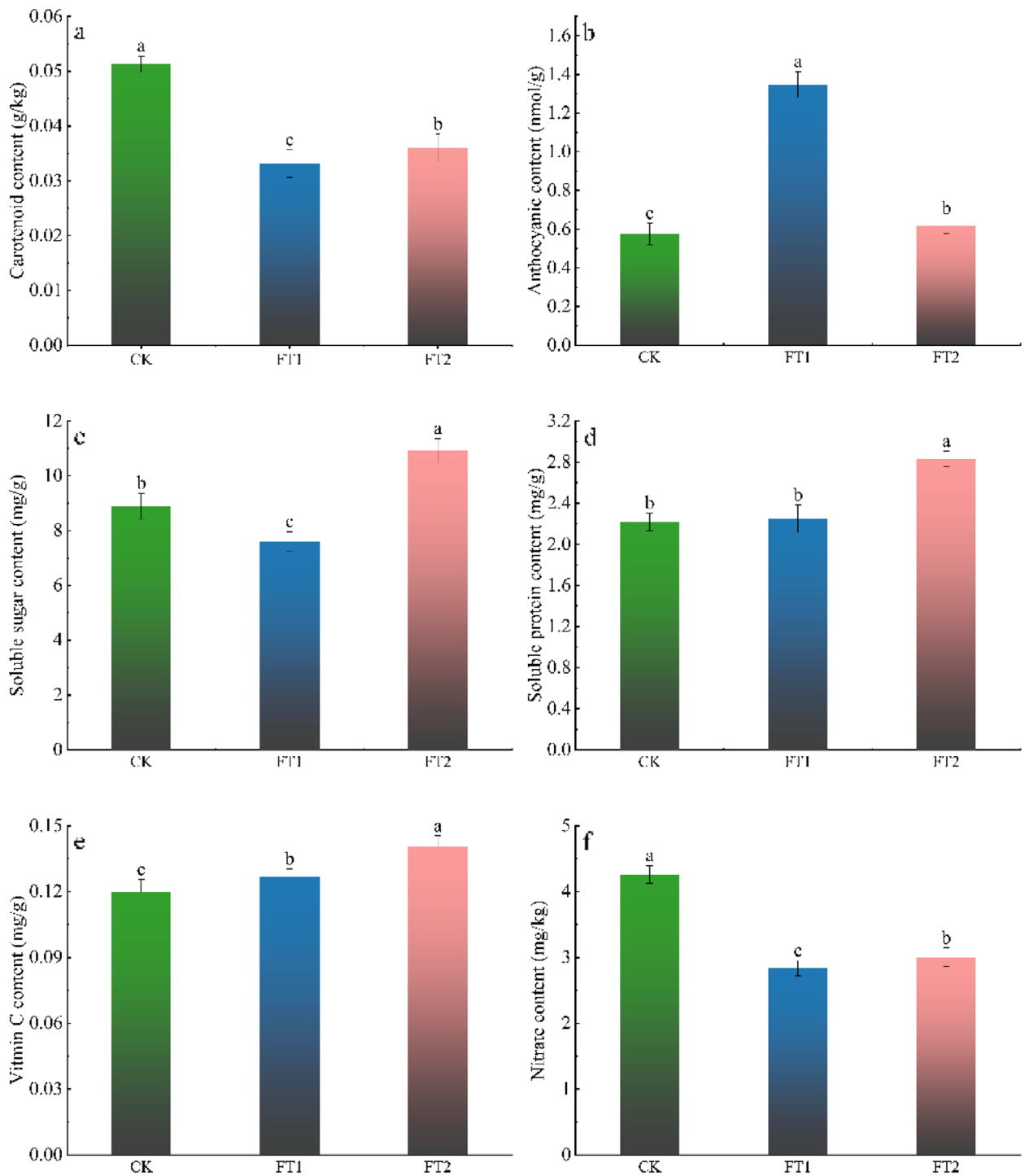


Fig. 5 Effects of different ratios of red light and far-red light on leaf quality of lettuce

leaf area and biomass accumulation compared with the lettuce under CK LEDs. FT2 and FT1 treatments improved lettuce biomass weight, mainly because of the enhancements in leaf area, and height, which were

useful to capture more light energy for photosynthetic capacity, and consequently resulted in more biomass accumulation.

Table 1 Effects of different light treatments on main volatile components and contents of lettuce

Categories	Volatile compounds	Retention time	Formula	relative content %		
				CK	FT1	FT2
Aldehydes	2-Hexenal	5.38	C ₆ H ₁₀ O	60.42	22.58	29.63
	Benzaldehyde	7.89	C ₇ H ₆ O	1.74	2.79	0.86
	2,4-Heptadienal, (E,E)-	8.76	C ₇ H ₁₀ O	1.78	1.84	1.45
	Benzeneacetaldehyde	9.96	C ₈ H ₈ O	2.50	7.01	3.75
	Nonanal	11.41	C ₉ H ₁₈ O	2.98	5.11	9.49
	Decanal	13.88	C ₁₀ H ₂₀ O	0.97	0.74	0.70
	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	14.31	C ₁₀ H ₁₆ O	2.22	1.66	1.00
	cis,cis-7,10,-Hexadecadienal	21.21	C ₁₆ H ₂₈ O	0.43	0.88	0.77
	cis,cis,cis-7,10,13-Hexadecatrienal	23.64	C ₁₆ H ₂₆ O	0.25	0.23	0.13
	Dodecanal	20.59	C ₁₂ H ₂₄ O	0.46	0.74	0.75
	Tetradecanal	22.60	C ₁₄ H ₂₈ O	0.42	0.47	0.57
	Alkanes	Dodecane	13.70	C ₁₂ H ₂₆	1.35	0.36
Tetradecane		18.24	C ₁₄ H ₃₀	1.41	0.34	0.37
1,3-di-n-Propyladamantane		20.19	C ₁₆ H ₂₈	2.84	2.37	1.48
Oxirane, tetradecyl-		24.49	C ₁₆ H ₃₂ O	0.92	1.62	3.15
Decane, 2-methyl-		22.30	C ₁₁ H ₂₄	0.56	0.50	0.43
Olefins	Bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene	16.05	C ₁₁ H ₁₀	0.62	0.85	0.64
	Benzocycloheptatriene	16.45	C ₁₁ H ₁₀	0.28	0.46	0.31
	Cedrene	18.69	C ₁₅ H ₂₄	0.24	0.04	0.03
Alcohols	2-Hexen-1-ol, (E)-	5.75	C ₆ H ₁₂ O	8.54	26.91	32.82
	1-Octanol	10.66	C ₈ H ₁₈ O	1.00	1.50	1.97
	Phenylethyl Alcohol	11.80	C ₈ H ₁₀ O	0.99	2.20	0.90
	1-Nonanol	13.12	C ₉ H ₂₀ O	0.55	0.44	1.21
Esters	3-Hexen-1-ol, acetate, (E)-	9	C ₈ H ₁₄ O ₂	0.39	9.07	0.34
	2-Heptenoic acid, ethyl ester, (E)-	23.19	C ₉ H ₁₆ O ₂	0.88	0.63	0.53
	Phthalic acid, hex-3-yl isobutyl ester	27.26	C ₁₈ H ₂₆ O ₄	1.42	3.95	3.31
Ketones	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	19.43	C ₁₃ H ₂₂ O	0.25	0.24	0.15
	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethyl ethyl)-	19.78	C ₁₄ H ₂₀ O ₂	1.57	2.56	1.64
	1,8(2H,5H)-Naphthalenedione, hexahydro-8a-methyl-, cis-	20.99	C ₁₁ H ₁₆ O ₂	0.93	0.50	0.27
Ethers	Thiophene, 2,5-bis(1,1-dimethyl ethyl)-	16.54	C ₁₂ H ₂₀ S	0.26	0.26	0.19
Carboxylic acid	Oxiranoctanoic acid, 3-octyl-, cis-	18.48	C ₁₈ H ₃₄ O ₃	0.58	0.51	0.42
Aromatic hydrocarbons	Naphthalene, 1,7-dimethyl-	18.81	C ₁₂ H ₁₂	0.25	0.65	0.50

Far-red light can also regulate leaf angle and increase plant height and leaf area, via expression of associated genes, to capture more light energy. far-red light regulates plant morphology and photosynthetic capacity. Many studies have shown that a lower R/FR is attribute to a higher height, larger leaf area, and more biomass (Franklin 2008; He et al. 2021; Lee et al. 2019; Li & Kubota 2009; Li et al. 2020). Leaf expansion was stimulated by far-red light, especially in younger plants and this contributed to capturing radiation for photosynthesis.

Far-red light treatment also had some effects on hormone signals in plants (Ialam et al. 2014). In this study, IAA showed an upward trend and slightly decreased at the end of growth, which was similar with previous

studies (Casal 2013). A low R/FR ratio can make most plants in different tissues and organs and at different developmental stages, auxin IAA content was increased, hormone transported from leaves to stems, lengthened internode distance, increased plant height in the shade-avoiding response of plants, auxin promotes the elongation of hypocotyl, petiole, and stem (Iglesias et al. 2018).

Phytochrome not only participates in the activity of plant physiological metabolism but also affects the activity of the antioxidant system. ABA, a plant stress hormone, induces a series of reactions to adverse conditions, which can make plants adapt to the changing environment (Kim et al. 2022). Previous studies have found that the addition of moderate far-red light can increase

ABA levels during cold acclimation of barley, which indicates that plants have a stronger stress response and adaptability, the expression profiles of key hormone metabolism-related genes were also consistent with the characteristics of hormone changes (Ahres et al. 2021; Islam et al. 2014). In this study, the ABA of FT1-treated plants always showed an increasing trend, but the ABA of FT2-treated plants decreased slowly at the later stage, the results showed that the stress resistance of FT2-treated plants gradually decreased and more far-red light could bring inhibition to some extent.

In this study, far-red treatment significantly increased GA₃ levels in lettuce, which was consistent with lower R/FR levels can promote GA levels in tomato seedlings, and promote stem elongation conclusion (Zhang et al. 2019). However, some researchers have found that far-red treatment can reduce the concentration of bioactive cytokinin and GA in the shoot tips of Japanese pears, and promote the growth of flower buds ahead of time (Ito et al. 2014). Increasing the far-red ratio at the end of the day can enhance the shoot elongation of Poinsettia and decrease the GA content in the body through the effect of photosensitive pigment on hormone metabolism (Islam et al. 2014). Therefore, different R/FR ratios have different regulatory effects on GA₃ levels in crops.

Plants in long-term shading environments tend to outgrow and have low resistance, but under adverse conditions, plants can resist external stress through the antioxidant enzyme system. The lower R/FR in this experiment led to a decrease in SOD activity and an increase in membrane lipid peroxidation, which was consistent with previous studies in cucumber (Shibuya et al. 2016; Zou et al. 2020). This phenomenon indicates that the anti-aging ability was weakened and the growth trend of the plant was accelerated under a long time for far-red light environment.

The MDA content of the three treatments increased steadily during the whole treatment period. the MDA content of FT2 treatment was the highest, which indicated that the free radical content was higher in the plant with the damage of low R/FR, the membrane of lettuce was damaged to a great extent, which was consistent with previous studies on Chrysanthemum (Yang et al. 2012).

Leaf quality properties highly correlate with the spectrum of growth radiation. In this experiment, the carotenoid content of FT2 treatment was significantly higher than that of FT1 treatment, indicating that additional far-red lighting has a trade-off relationship between fruit yield and carotenoid content (Kim & Son 2022); the synthesis of anthocyanins under FT2 treatment was inhibited, what was due to the changes in the level of light-sensitive pigmentation in R/FR, and then the expression of the key genes in the synthesis

of anthocyanin affects anthocyanin accumulation (Li et al. 2014), as previous study reported that anthocyanin accumulation in *Stellaria longipes* with R/FR value of 0.7 was significantly lower than that with R/FR value of 1.2 (Alokam et al. 2002).

Leaf protein and sugar under far-red light treatments were significantly higher than CK treatments, and this explain how the addition of far-red light affected the synthesis and absorption of carbohydrates and various amino acids with different R/FR values (He et al. 2021). When R/FR was 0.8, the vitamin C content of leaves was increased and the nitrate content of leaves was significantly decreased, which is consistent with the research conclusions of a previous study (He et al. 2021; Li & Kubota 2009). Far-red light treatment increased the contents of alcohol and ester volatile substances in lettuce leaves, which was similar to the results of previous studies on tomatoes (Kim et al. 2020; Wei et al. 2020). It can be seen that far-red light treatment can increase the yield and does not reduce the flavor quality of lettuce.

Conclusion

Far-red light treatments FT1 and FT2 significantly increased the plant height, leaf area, and dry and fresh weight of lettuce, and FT1 treatment was better, but far-red light also significantly decreased the leaf thickness of lettuce.

FT1 and FT2 increased the content of endogenous hormones in lettuce leaves, but the IAA and ABA content decreased with the treatment time, the content of GA₃ in FT1 and FT2 treatment showed an increasing trend. The POD activity of FT1 and FT2 increased first and then decreased, and the content of SOD decreased.

Far-red treatment decreased the content of carotenoid and nitrite in leaves and increased the content of vitamin C and volatile compounds of alcohols and esters in leaves. The content of anthocyanin in FT1 treatment was significantly increased.

In the future, studies can be carried out on the regulation of plant quality indexes by different far-red light intensities, especially functional components enrichment of plants including anthocyanin and antioxidants.

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Authors' contributions

LLM performed the experiments, data analysis, and write-up of the manuscript. JFS provided review and final edit of the manuscript. MAK provided review and polishes of paper. DGN carried out investigation and data analysis. All authors read and approved the final manuscript.

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Availability of data and materials

All necessary data is included in this paper.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing financial interest.

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