



Combination of light quality and melatonin regulates the quality in mustard sprouts

Aolian Zhou^{a,1}, Jiayi Tang^{a,1}, Ying Li^{a,1}, Wenjuan Cheng^b, Xingwei Yao^b, Victor Hugo Escalona^c, Guiping Qian^d, Jie Ma^e, Xuena Yu^a, Huanxiu Li^a, Zhi Huang^a, Yi Tang^a, Fen Zhang^a, Zhifeng Chen^{f,**}, Bo Sun^{a,*}

^a College of Horticulture, Sichuan Agricultural University, Chengdu 611130, China

^b State Key Laboratory of Vegetable Biobreeding, Tianjin Academy of Agricultural Sciences, Tianjin 300192, China

^c Faculty of Agricultural Sciences, University of Chile, Santa Rosa 11315, Santiago 8820808, Metropolitan Region, Chile

^d Chengdu Jinniu District Center for Disease Control and Prevention, Chengdu 610037, China

^e Bijie Institute of Agricultural Science, Bijie 551700, China

^f College of Biology and Agriculture Technology, Zunyi Normal University, Zunyi 563000, China

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ABSTRACT

Mustard sprouts is a new form of vegetable product that is gaining attention due to its high content of health-promoting compounds such as glucosinolates. This study investigated the effects of different light qualities (white, red, and blue) alone and in combination with 100 $\mu\text{mol L}^{-1}$ melatonin on the growth and health-promoting substance content of mustard sprouts. The results showed that white light + melatonin treatment promoted the accumulation of glucosinolates in sprouts (compared with white light increased by 47.89%). The edible fresh weight of sprouts treated with red light + melatonin was the highest, followed by white light + melatonin treatment. In addition, the sprouts treated with blue light + melatonin contained more ascorbic acid, flavonoids, and total phenolics. Therefore, the combined treatment of light quality (especially white light) and melatonin can provide a new strategy to improve the quality of mustard sprouts.

1. Introduction

Mustard (*Brassica juncea*) is an annual herbaceous plant of the genus Brassica in the family Cruciferae that grows in the humid climatic conditions of southwestern China and is an economically important vegetable crop that can be eaten fresh or is commonly used as a processed variety of pickles (Sun et al., 2018). Notably, mustard is renowned for its abundance of soluble sugars, ascorbic acid, phenolic compounds, glucosinolates, and various other health-promoting compounds (Di et al., 2022). Glucosinolates and their degradation products serve as potent anticancer agents, impacting human health, plant defense against insects and diseases, and the flavor profile of cruciferous vegetables (Sun et al., 2021). While previous research predominantly focused on mature mustard plants, limited attention has been directed towards mustard sprouts. With increasing concerns regarding food nutrition and safety, fresh sprouts, characterized by their short production cycle and

nutritional richness, have gained popularity among consumers and the food industry, epitomizing contemporary green health vegetables. Mustard sprouts are widely embraced for their crisp texture, delightful taste, and high content of health-promoting phytochemicals such as glucosinolates. Remarkably, the glucosinolate content in mustard sprouts exceeds that of mature plants by over tenfold (Cano-Lamadri et al., 2023).

Light quality directly influences plant growth and chemical composition, thus serving as an external stimulus to enhance the yield and quality of vegetable foods (Cao, Wu, Shi, Li, & Zhang, 2023). For instance, red light has been shown to significantly enhance tomato seedling height, while blue light exerts a substantial inhibitory effect on tomato seedling growth (Wang et al., 2017). In cabbage seedlings, blue light has been found to elevate the ascorbic acid content (Kang et al., 2020). Furthermore, Chinese kale exhibits a substantial increase in total phenolics content under blue light, a trend also observed in pea

* Corresponding author at: College of Horticulture, Sichuan Agricultural University, 211 Huimin Road, Chengdu, Sichuan 611130, China.

** Corresponding author.

E-mail addresses: czf810@163.com (Z. Chen), bsun@sicau.edu.cn (B. Sun).

¹ These authors contributed equally.

seedlings, wherein blue light enhances both total phenolics content and antioxidant capacity (Liu et al., 2016; Qian et al., 2016). Regarding mustard sprouts specifically, it has been noted that the highest glucosinolate content occurs under white light, followed by red light and then blue light (Park, Park, Yeo, Kim, & Park, 2020). Additionally, blue light has been identified as a promoter of both the synthesis and accumulation of glucosinolates in broccoli sprouts (Xue et al., 2021). Moreover, it was found that red light irradiation was beneficial to the accumulation of glucosinolates before the harvest of Chinese kale sprouts (Qian et al., 2016). These findings underscore the intricate interplay between light quality and the composition of health-promoting compounds in various vegetable crops.

Melatonin is an adaptable, natural and strong antioxidant with a wide range of uses and has been associated with growth and development processes in plants (Arnao & Hernández-Ruiz, 2019). Simultaneously, it serves as a growth regulator capable of activating seed germination and seedling growth and contributes to the regulation of plant growth and development by enhancing the antioxidant defense system (Ali et al., 2023). Noteworthy is melatonin's efficacy in mitigating the degradation of chlorophyll and carotenoids (Wu et al., 2021), along with its role in secondary metabolism, inducing anthocyanin and flavonoid biosynthesis (Arnao & Hernández-Ruiz, 2019). Melatonin treatment increases anthocyanins content in cabbage and effectively enhances the total phenolics content and antioxidant activity (Zhang et al., 2016). Furthermore, melatonin's influence extends to triggering signaling cascade reactions and activating defense responses leading to the synthesis of glucosinolates. For example, broccoli treated with exogenous melatonin exhibited a higher total glucosinolates content, accompanied by the up-regulation of glucosinolate biosynthesis genes, including cytochrome P450 83A1 (CYP83A1), UDP-glycosyltransferase 74B1 (UGT74B1) (Xue et al., 2021). Additionally, it has been found that 100 $\mu\text{mol L}^{-1}$ melatonin treatment increased the content of total phenolics and total glucosinolates in mustard (Di et al., 2022). These findings underscore melatonin's multifaceted role in influencing diverse physiological processes and health-promoting compounds biosynthesis in vegetable crops.

Growing evidence suggests that the interaction of multiple factors can synergistically impact the growth of Brassica vegetables and the accumulation of secondary metabolites. Particularly noteworthy is the combined treatment of ultraviolet radiation and methyl jasmonate, which has been demonstrated to enhance the accumulation of glucosinolates and phenolics in broccoli sprouts (Moreira-Rodríguez, Nair, Benavides, Cisneros-Zevallos, & Jacobo-Velazquez, 2017). L-tryptophan and blue light both promoted the accumulation of carotenoids, and their combination further increased carotenoid content by 120% (Xiang, Qi, Hu, Wang, & Guo, 2023). Similarly, in the case of Chinese kale sprouts, a combination of glucose and gibberellic acid has been reported to increase glucosinolate content, leading to elevated levels of total phenolics and enhanced antioxidant activity (Miao et al., 2017). These findings collectively underscore the intricate interplay of factors influencing the biosynthesis of secondary metabolites in Brassica vegetables. However, there is a paucity of studies investigating the effects of light quality and melatonin on the growth and nutritional quality of cruciferous sprouts.

In this study, we investigated the effects of different light quality alone and in combination with melatonin treatments on the growth of mustard sprouts and health-promoting compounds, to provide a scientific basis for the light-environmental-chemistry conditioning techniques in the production of mustard sprouts and their quality improvement.

2. Material and methods

2.1. Plant material and treatments

The mustard cultivar 'Chuanbao 11' served as the material in this experiment, 9 g seeds were soaked in 40 mL distilled water or 100 μmol

L^{-1} melatonin for 24 h at room temperature, and then the seeds were evenly sown in a plastic seedling tray (33 cm \times 26 cm \times 4.5 cm) for hydroponics. Put it in adjustable light incubators (Jiangnan Co. Ltd., Ningbo, China) for 2 days of dark treatment, and then treat it with white light, red light and blue light respectively. The experiment includes six treatment methods: white light (W), blue light (B), red light (R), melatonin+white light (MW), melatonin+red light (MR) and melatonin+blue light (MB). The culture conditions included a light intensity of 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$, a temperature of 23 °C, a photoperiod of 16 h light and 8 h dark, humidity at and 70%. On the 9th day, the seedlings were collected and cut from the roots, and then frozen in liquid nitrogen. After freeze-drying, the samples were used for the determination of physiological indicators (Supplementary Fig. 1, Supplementary Table 1).

2.2. Growth parameters of sprouts

A total of 30 mustard sprouts were randomly selected for each treatment, with 10 plants per replicate. The plant height of sprouts was measured by ruler, and the fresh weight of single plant was measured by electronic balance. Fresh weight (fresh weight of the aboveground part after root removal) was repeated with the weight of a dish of mustard sprouts, and each treatment was repeated three times. Then the samples were placed in an oven at 105 °C for 30 min, and baked at 80 °C for 24 h to constant weight, and the dry weight of single plant was determined.

2.3. Soluble sugar content

Sample extract 1 mL, anthrone-ethyl acetate reagent 0.5 mL, concentrated sulfuric acid 5 mL, boiled for 5 min. Finally, the absorbance of the reaction mixture was measured at 630 nm (Sun et al., 2021).

2.4. Soluble protein content

Coomassie brilliant blue G-250 was added to 1 mL of the supernatant, and the absorbance was quickly measured at 595 nm within 20 min after the reaction began (Sun et al., 2018).

2.5. Chlorophyll and carotenoids content

The sample powder underwent grinding followed by extraction with acetone. After extraction, the supernatant was filtered and subjected to analysis using high-performance liquid chromatography (HPLC). For HPLC analysis, samples (10 μL) were separated using a mixture of isopropanol and 80% acetonitrile-water at a flow rate of 0.5 mL min^{-1} (Sun et al., 2021).

2.6. Ascorbic acid content

The sample powder was initially extracted with a solution of 1.0% oxalic acid, followed by centrifugation. Subsequently, each sample underwent filtration before being subjected to analysis using HPLC. The concentration of ascorbic acid was determined based on absorbance values measured at 243 nm (Sun et al., 2021).

2.7. Anthocyanins content

The appropriate amount of sample was weighed and grinded with 1% hydrochloric acid methanol. The sample was diluted to 10 mL and placed in the dark at 4 °C for 24 h. The absorbance of the supernatant was measured at 535 nm, and the anthocyanin content was calculated (Solfanelli, Poggi, Loreti, Alpi, & Perata, 2006).

2.8. Flavonoid content

Forty milligrams of sample powder was extracted in 50% ethanol and

incubated at room temperature for 24 h in the dark. A 1.2 mL aliquot of the supernatant was mixed with 60 μL of 2% aluminum trichloride, 60 μL of 1 mol L^{-1} potassium acetate, and 1.68 mL of distilled water after being centrifuged. The absorption was read at 415 nm after 40 min (Sun et al., 2021).

2.9. Total phenolics content

The extraction of total phenolics commenced with 50% ethanol, and the resulting supernatant was subsequently combined with Folin-Ciocalteu reagent. Following a 3-min interval, saturated sodium carbonate was introduced. The absorbance was then recorded at 760 nm using a spectrophotometer (Sun et al., 2021).

2.10. 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) assay

3 mL of ABTS^+ solution was combined with an aliquot of 300 μL from each extracted sample. After precisely 2 h, the absorbance was measured spectrophotometrically at 734 nm, followed by calculation of the value (Sun et al., 2021).

2.11. Ferric reducing antioxidant power (FRAP)

The extracted samples were mixed with the FRAP working solution and incubated at 37 $^{\circ}\text{C}$. After incubation for 10 min at 37 $^{\circ}\text{C}$, the absorbance was measured at 593 nm using a spectrophotometer, and the corresponding value was calculated (Sun et al., 2021).

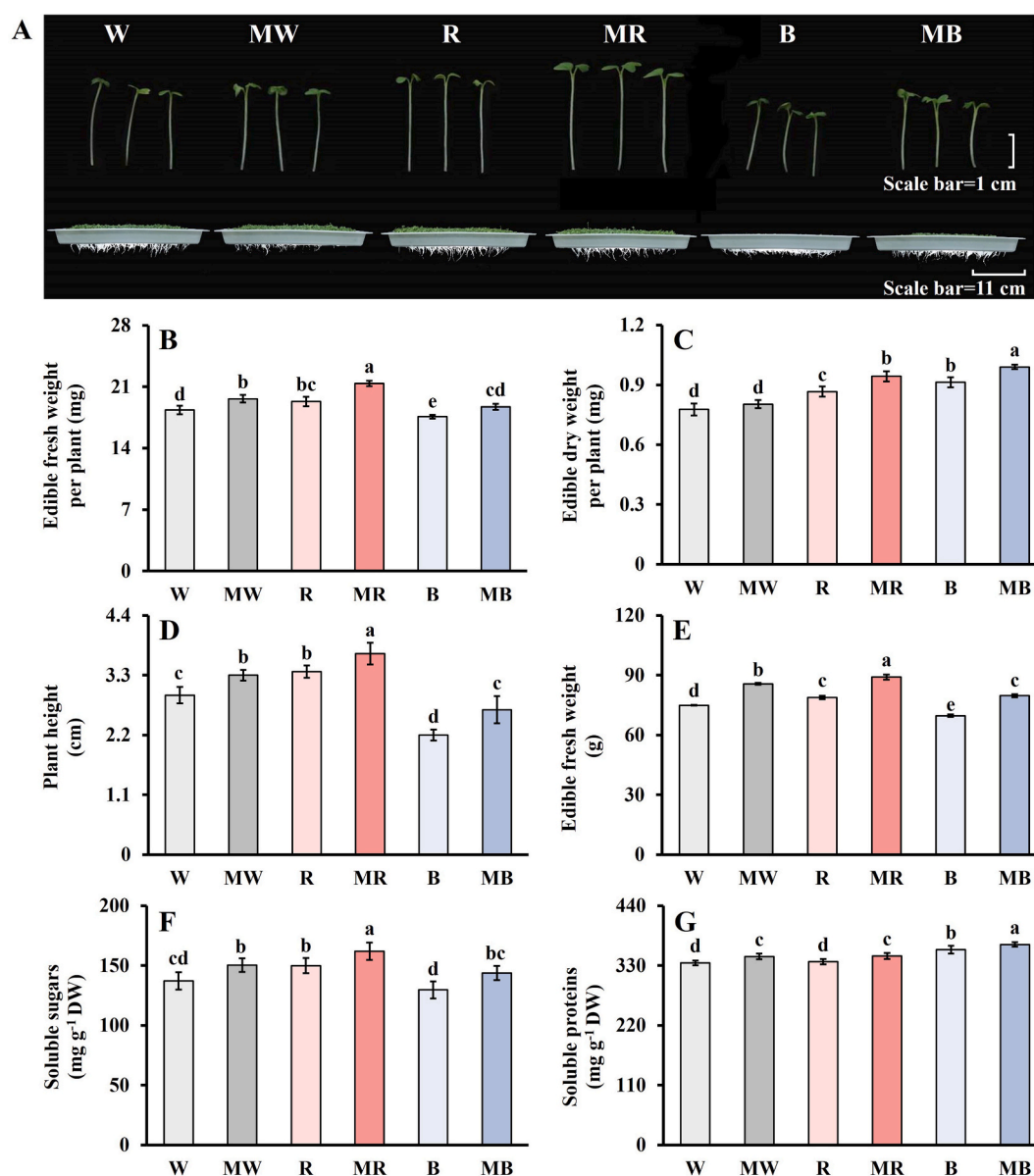


Fig. 1. Growth morphology and related physiological indexes under different treatments in mustard sprouts. A: The growth morphology in mustard sprouts under the treatment of white light (W), white light and melatonin (MW), red light (R), red light and melatonin (MR), blue light; B: fresh weight per plant in mustard sprouts; C: dry weight per plant in mustard sprouts; D: plant height in mustard sprouts; E: Fresh weight in mustard sprouts; F: soluble sugar content; G: soluble protein content. Different letters in the figure indicate statistically significant differences among treatments ($P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.12. Glucosinolates content

100 mg of the lyophilized sample was heated in 5 mL of water for 10 min to produce a liquid layer, which was then applied to a DEAE-Sephadex A-25 column. The glucosinolates were transformed into desulfated counterparts with aryl sulfatase. Following this process, the desulfated glucosinolates were separated and assessed through HPLC (Sun et al., 2021).

2.13. Statistical analysis

SPSS version 20 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Two-way analysis of variance was performed on the data. Create diagrams using Origin 8.5.0 (Origin Lab Corporation, Northampton, MA, USA). Principal component analysis (PCA) was performed using SIMCA 14.1 Demo software (Umetrics, Malmö, Sweden).

3. Result

3.1. Sprout growth

R treatment promoted the growth of mustard sprouts, while B treatment inhibited its growth. The combination of light quality and

melatonin further promoted the growth of mustard sprouts (Fig. 1A). Among different light quality treatments, the mustard sprouts treated with R had the highest edible fresh weight per plant, plant height and edible fresh weight, which were 9.85%, 53.03% and 13.14% higher than those of B treatment, respectively (Fig. 1B, D, E). In the combined treatment of light quality and melatonin, MW, MR and MB treatments significantly increased the edible fresh weight, plant height and edible fresh weight of mustard sprouts. The edible fresh weight, dry weight, plant height and edible fresh weight of MR treatment were the highest, which were 1.11, 1.10 and 1.13-fold of R treatment, respectively. In addition, compared with W and R treatments, the edible dry weight per plant increased significantly under B treatment, which increased by 17.60% and 5.39%, respectively, compared with W and R treatments (Fig. 1C). MB treatment further increased the edible dry weight per plant of mustard sprouts, which was 1.08-fold that of B treatment.

3.2. Soluble sugar and soluble protein

Under different light qualities, R (149.94 mg g⁻¹ DW) treatment had the highest soluble sugar content, followed by W (137.20 mg g⁻¹ DW) treatment, and B (129.65 mg g⁻¹ DW) treatment was the lowest. Under the combined treatment of light quality and melatonin, compared with W, R and B treatments, MW, MR and MB treatments further promoted

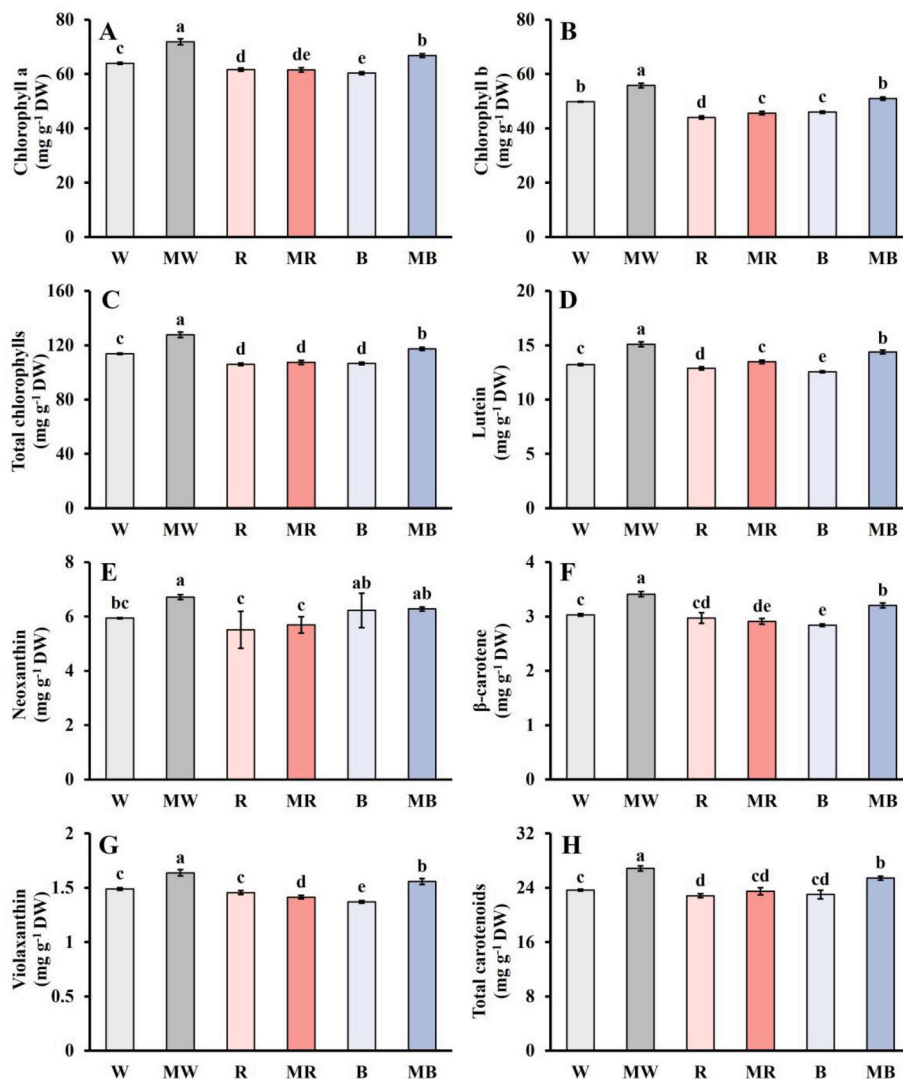


Fig. 2. Chlorophylls, carotenoids content under different treatments in mustard Sprouts. A: chlorophyll a; B: chlorophyll b; C: total chlorophylls; D: lutein; E: neoxanthin; F: β-carotene; G: violaxanthin; H: total carotenoids.

the increase of soluble sugars content, which increased by 1.10-fold, 1.08-fold, and 1.11-fold, respectively (Fig. 1F).

Under different light qualities, B (359.18 mg g⁻¹ DW) treatment had the highest soluble protein content, followed by R (336.89 mg g⁻¹ DW) treatment. Under the combined treatment of light quality and melatonin, compared with W, R and B treatments, MW, MR and MB treatments further promoted the increase of soluble proteins content, and content all increased by 1.03-fold (Fig. 1G).

3.3. Chlorophyll and carotenoids

Two chlorophylls and four carotenoids were detected in mustard sprouts (Fig. 2A-H). Among the different light quality treatments, the highest total chlorophylls content was found in the W treatment, which increased by 7.41% and 6.89% over the R and B treatments, respectively. Under the combined treatment of light quality and melatonin, the total chlorophyll content in MW and MB treatments was 1.12 and 1.10-fold higher than that in W and B treatments, respectively (Fig. 2C). Among them, the chlorophyll *a* content and the total chlorophyll content changed in the same trend, and its content was higher than that of R and B treatments under W treatment, and under the combined treatment of light quality and melatonin, the chlorophyll *a* content in MW and MB treatments increased further, and its content was 1.12 and 1.11-fold of that of W and B treatments, respectively (Fig. 2A). As for the changes in chlorophyll *b* content, the chlorophyll *b* content was significantly higher under both W treatment than R and B treatments. Under the combined treatment of light quality and melatonin, chlorophyll *b* content further increased in MW, MR, and MB treatments, which were 1.12, 1.04, and 1.11-fold higher than W, R, and B treatments, respectively (Fig. 2B).

There were four carotenoids detected in mustard sprouts. Lutein was the most abundant carotenoid in mustard sprouts, followed by neoxanthin and β -carotene, while violaxanthin had the lowest content among the four carotenoids (Fig. 2D-G). Under different light qualities,

both lutein and violaxanthin contents were highest in the W treatment (13.21 mg g⁻¹ DW, 1.49 mg g⁻¹ DW), their contents were further increased in the MW treatment under the combined treatment of light quality and melatonin, and compared with the W treatment, the contents of lutein and violaxanthin in the MW treatment were increased by 1.14 and 1.10-fold, respectively (Fig. 2D, G). Melatonin also promoted neoxanthin and β -carotene accumulation under white light, the content of which were 1.13-fold higher than that without melatonin treatment, respectively (Fig. 2E, F). Among the different light quality treatments, the total carotenoids content of mustard sprouts under W treatment was the highest, reaching 23.67 mg g⁻¹ DW, but there was no significant difference compared to B treatment (Fig. 2H). Under the combined treatment of light quality and melatonin, the total carotenoids content under MW and MB treatments further increased 1.13 and 1.11-fold more than W and B treatments, respectively (Fig. 2H).

3.4. Ascorbic acid

Under different light qualities, B (7.25 mg g⁻¹ DW) treatment had the highest ascorbic acid content. Under the combined treatment of light quality and melatonin, compared with W, R and B treatments, MW, MR and MB treatments further promoted the increase of ascorbic acid content, and their content increased 1.14, 1.07, and 1.08-fold, respectively. The highest content of ascorbic acid was 7.80 mg g⁻¹ DW under MB treatment (Fig. 3A).

3.5. Antioxidant content and antioxidant capacity

Under different light qualities, the contents of anthocyanins, flavonoids and total phenolics in B treatment were the highest (0.27, 12.20, 8.28 mg g⁻¹ DW) (Fig. 3B-D). In the combined treatment of light quality and melatonin, MW, MR and MB treatments significantly increased the contents of anthocyanins, flavonoids and total phenolics. Among them,

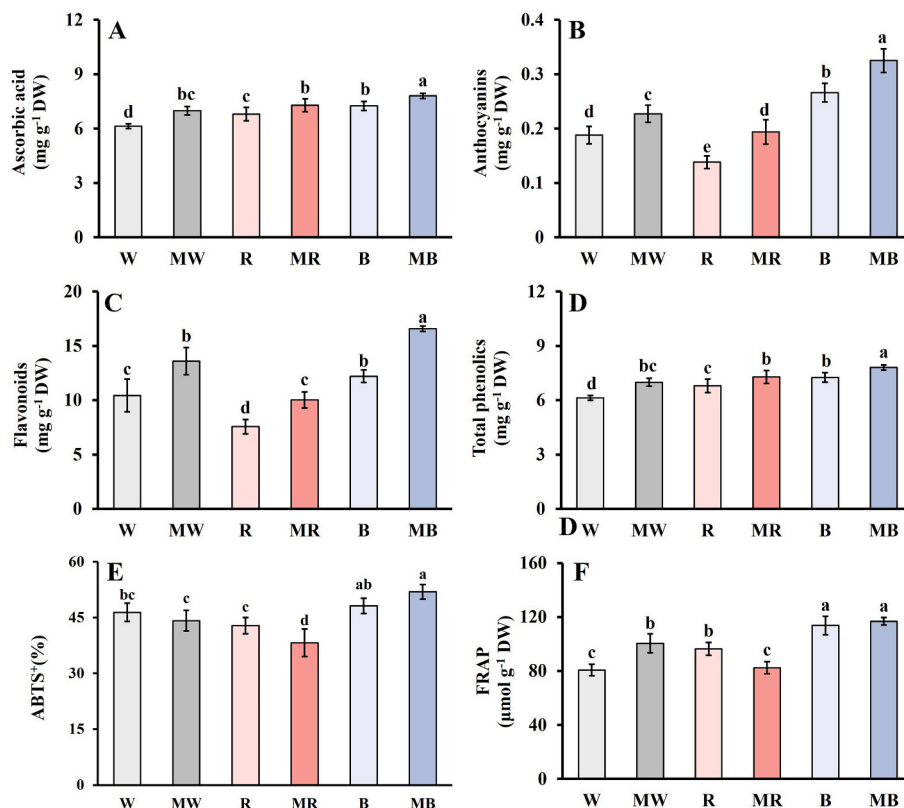


Fig. 3. Main antioxidant content and antioxidant capacity level under different conditions in mustard sprouts. A: ascorbic acid; B: anthocyanins; C: flavonoids; D: total phenolics; E: ABTS⁺; F: FRAP.

the mustard sprouts treated with MB had the highest contents of anthocyanins, flavonoids and total phenolics, which were 1.22, 1.36 and 1.08-fold higher than those of B treatment, respectively. Both ABTS⁺ and FRAP levels reached the maximum in MB treatment (Fig. 3E, F).

3.6. Glucosinolates

Three aliphatic glucosinolates and four indolic glucosinolates were detected in mustard sprouts (Fig. 4A-J). Among them, aliphatic glucosinolates account for most of the total glucosinolates content in mustard sprouts. There was no significant change in the content of sinigrin, gluconapin and progoitrin under different treatments, but under the combined treatment of light quality and melatonin, MW treatment

further promoted the increase of its content. The content of sinigrin, gluconapin and progoitrin in MW treatment was 1.49-fold, 1.48 and 1.31-fold that of W treatment, respectively (Fig. 4A-C).

4-methoxyglucobrassicin is a relatively abundant indolic glucosinolates in mustard sprouts, and its content did not change significantly under different light quality treatments. However, under the combined treatment of light quality and melatonin, MW promoted the increase of its content, which was 1.18-fold that of W treatment (Fig. 4D). Under different light qualities, the content of 4-hydroxyglucobrassicin was the highest in B treatment, which was 28.57% higher than that in W treatment. Under the combined treatment of light quality and melatonin, the content of 4-hydroxyglucobrassicin under MR treatment increased significantly, which was 1.41-fold that of R treatment (Fig. 4E). There

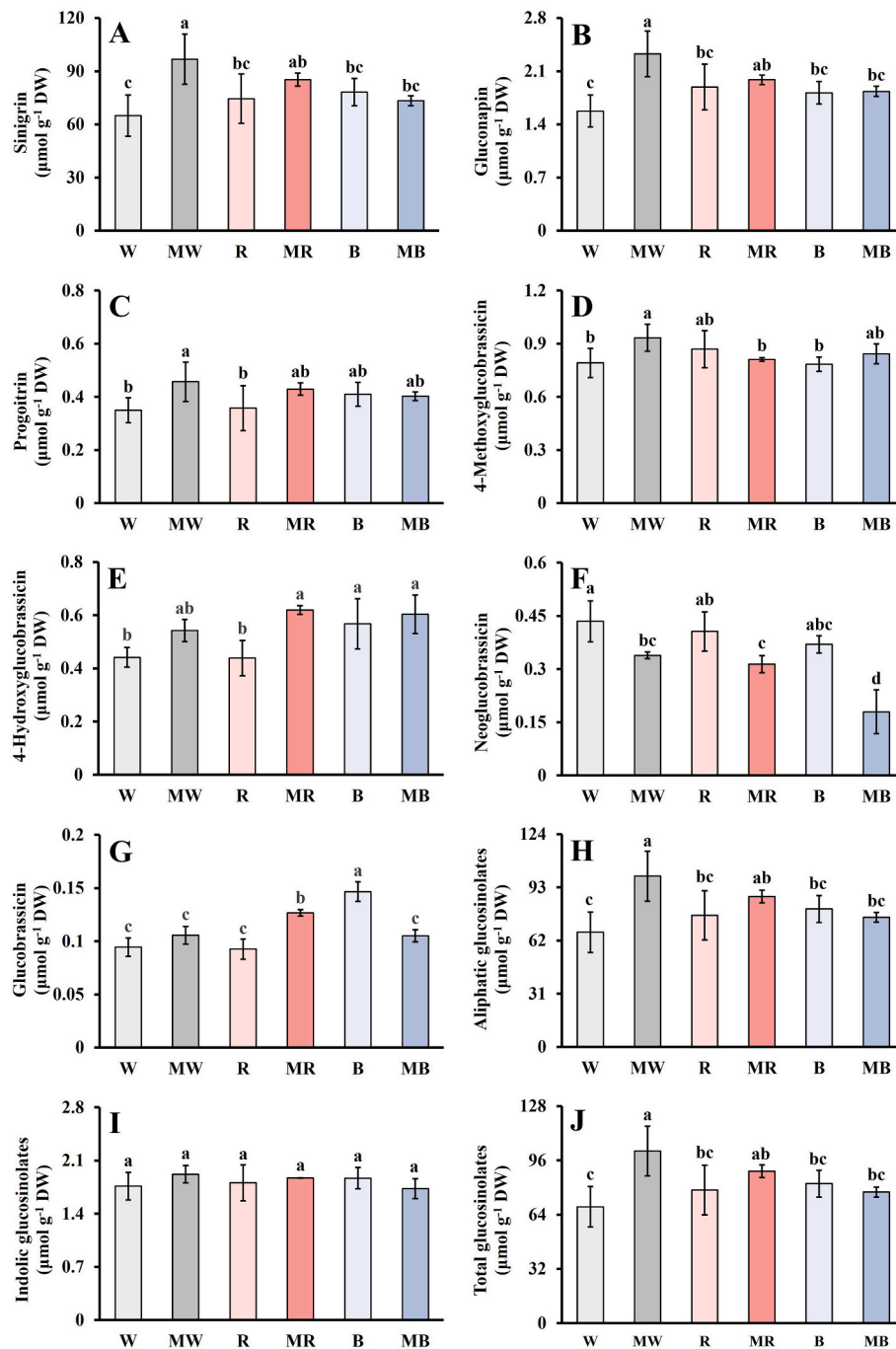


Fig. 4. Glucosinolates content under different treatments in mustard sprouts A: sinigrin; B: gluconapin; C: progoitrin; D: 4-methoxyglucobrassicin; E: 4-hydroxyglucobrassicin; F: neoglucobrassicin; G: glucobrassicin; H: aliphatic glucosinolates; I: indolic glucosinolates; J: total glucosinolates.

was no significant change in neoglucobrassicin content under different light quality treatments (Fig. 4F). The content of glucobrassicin under B treatment increased by 55.32% compared with W treatment. Under the combined treatment of light quality and melatonin, the content of glucobrassicin under MR treatment was 1.37-fold that of R treatment (Fig. 4G).

The change of aliphatic glucosinolates content was consistent with the change trend of total glucosinolates content (Fig. 4H, J). Under different light quality treatments, the contents of total aliphatic glucosinolates and total glucosinolates did not change significantly. Under the combined treatment, the aliphatic glucosinolate content and total glucosinolate content of MW treatment were 1.49 and 1.48-fold higher than those of W treatment, respectively. There was no significant difference in the content of total indolic glucosinolates under different treatments (Fig. 4I).

3.7. PCA

The first component (PC1) and the second component (PC2) explained 40.9% and 25.7% of the data changes, respectively (Fig. 5A). PC1 can distinguish between melatonin treatments and not, while PC2 can distinguish between different light quality treatments. According to the results of the loading plot, the indicators related to the growth and quality of mustard sprouts were mainly distributed in the first and fourth quadrants, indicating that the growth and quality of mustard sprouts were closely related to the combined treatment of light quality and melatonin. Among them, the MW treatment group had a greater impact on total chlorophylls, total carotenoids, indolic glucosinolates, aliphatic glucosinolates, and total glucosinolates, while plant height and edible

fresh weight per plant were affected by the MR treatment group. In addition, the MB treatment group and soluble protein, anthocyanin, flavonoid, ascorbic acid and total phenolics were distributed in the first quadrant, indicating that these five indicators were affected by the combined treatment of blue light and melatonin (Fig. 5B).

3.8. Analysis of correlation

Soluble sugar content was positively correlated with edible fresh weight per plant (0.98), plant height (0.93) and edible fresh weight (0.95). Soluble protein content was positively correlated with anthocyanins (0.94), ascorbic acid (0.90), flavonoids (0.82) content and FRAP (0.82), but negatively correlated with neoglucobrassicin content (-0.84). The total chlorophylls content was positively correlated with the total carotenoids content (0.97). In addition, the content of aliphatic glucosinolates was positively correlated with the content of sinigrin, and the content of total glucosinolates was positively correlated with the content of sinigrin (1.00), gluconapin (0.97) and progoitrin (0.91) (Fig. 6).

3.9. Analysis of variance

The results of variance analysis showed that the effect of melatonin on soluble protein, chlorophyll *a*, violaxanthin, ascorbic acid, 4-hydroxyglucobrassicin and glucobrassicin of mustard sprouts was greater than that of light quality. The interaction between light quality and melatonin had greater effects on plant height, edible fresh weight, soluble sugar, chlorophyll *b*, total chlorophylls, lutein, β -carotene, violaxanthin, total carotenoids, anthocyanins, ABTS⁺, sinigrin, gluconapin, progoitrin, 4-

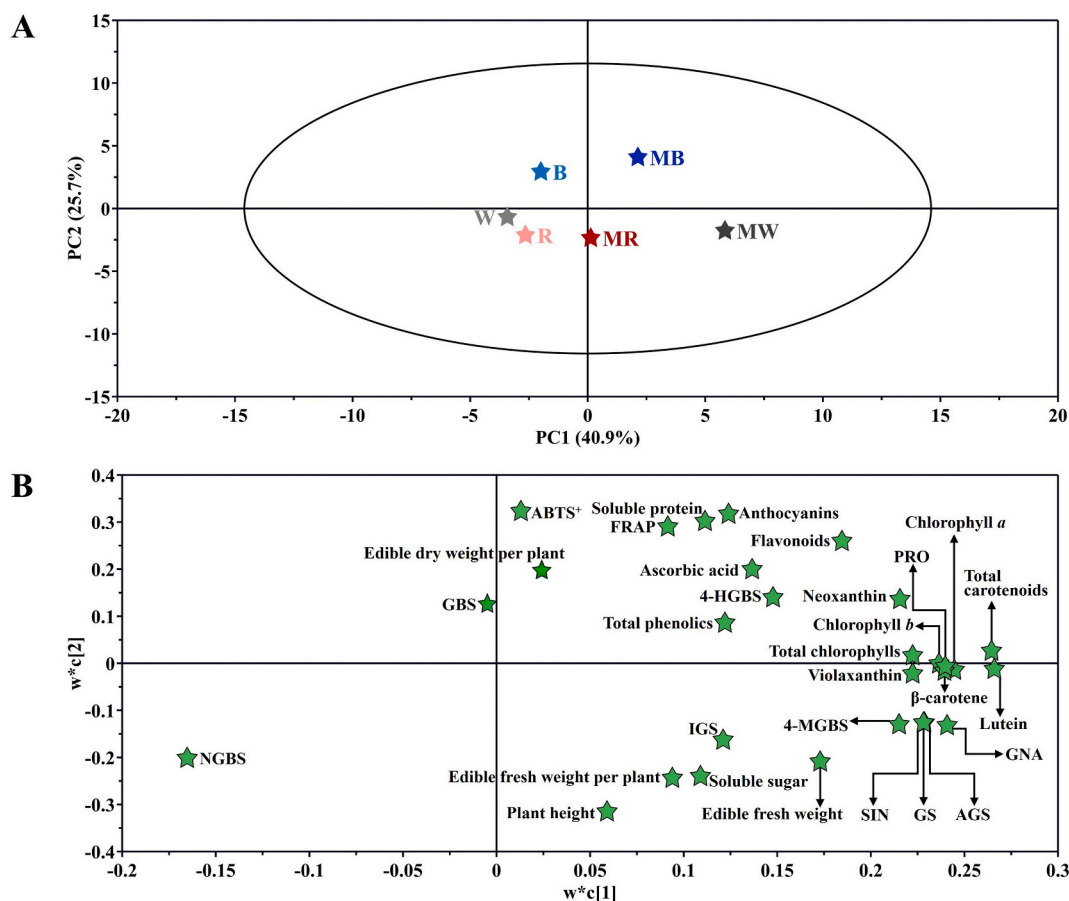


Fig. 5. Principal component analysis under different treatments in mustard sprouts. A: PCA score plot; B: loading plot. SIN: sinigrin; GNA: gluconapin; PRO: progoitrin; NGBS: neoglucobrassicin; GBS: glucobrassicin; 4-MGBS: 4-methoxyglucobrassicin; 4-HGBS: 4-hydroxyglucobrassicin; AGS: total aliphatic glucosinolates; IGS: total indolic glucosinolates; GS: total glucosinolates.

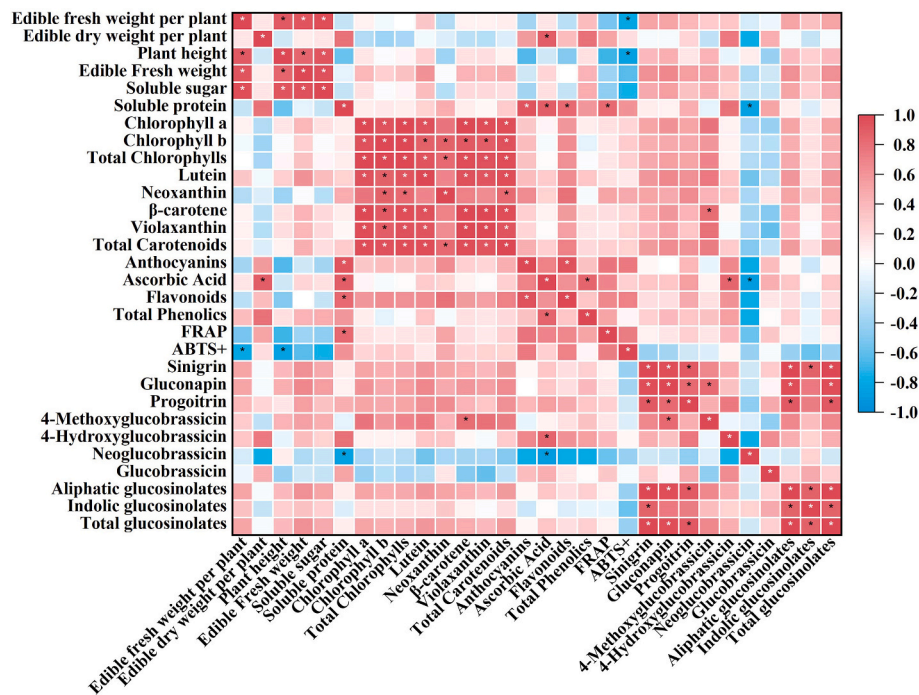


Fig. 6. Correlation plot of the correlations between health-promoting compounds and antioxidant capacity in mustard sprouts. All correlations in the figure reflect the absolute values of Pearson correlation coefficient above the threshold ($P > 0.05$).

methoxyglucobrassicin, neoglucobrassicin, total aliphatic glucosinolates, total glucosinolates than single factor (Supplementary Table 2).

4. Discussion

Mustard sprouts boast high nutritional value and are well-suited for mass production (Cano-Lamadri et al., 2023). Recently, combined treatment involving various factors has emerged as an effective method for enhancing the growth of cruciferous plants (Moreira-Rodríguez et al., 2017; Wang et al., 2017). In this study, we investigated the effects of different light quality alone and in combination with melatonin treatments on the growth of mustard sprouts and health-promoting compounds.

The yield and benefit can be seen from the plant height, fresh weight and dry weight of sprouts. Fresh weight is a main index to reflect the economic benefit of sprouts, and it is also an important index for the robustness of sprouts and the metabolism of substances in plants (Pierik & Ballaré, 2021). Our study revealed that red light positively influenced sprout growth, resulting in increased plant height and fresh weight, whereas blue light had the opposite effect. Specifically, tomato seedlings exhibited the greatest height under red light (Izzo, Mele, Vitale, Vitale, & Arena, 2020), whereas the growth and height of tomato seedlings were hindered when exposed to blue light. Phytohormones are closely related to plant height and elongation, and red light promotes the activity of photosensitive pigments as well as increases gibberellin content, thus favoring cell elongation growth and stem elongation, while blue light may inhibit stem elongation by decreasing growth hormone content and altering cell wall properties (Wang et al., 2023; Xu et al., 2018). Additionally, our investigation revealed that the application of melatonin enhanced the plant height and fresh weight of mustard sprouts under different light qualities. This aligns with the observed promotion of lentil and brassica sprout growth with melatonin treatment (Yolanda et al., 2015). Melatonin is an important biological molecule that plays a vital role in plant growth and development. It promotes the growth of roots and hypocotyls and increases seedling biomass (Ali et al., 2023). Notably, melatonin's role in promoting cucumber seed germination was attributed to its antioxidant effect, coupled with its hydrophilic and

lipophilic properties, facilitating easy penetration into seeds and enhancing their antioxidant capacity, thereby bolstering seed vigor and growth (Posmyk, Balabusta, Wiczorek, Sliwinska, & Janas, 2009). The promotion of mustard sprout growth by melatonin may be linked to its stimulation of metabolic activities before germination, thus facilitating sprout growth.

Soluble sugars and soluble proteins, byproducts of plant photosynthesis, serve as vital energy storage compounds and key indicators of vegetable nutritional quality, reflecting the overall carbon and nitrogen metabolism level in plants (Jakobek, 2015). Light quality directly affects the accumulation of carbohydrates in plants by regulating photosynthetic metabolism. Our study showed that red light was beneficial to the accumulation of soluble sugar in mustard sprouts, while blue light was beneficial to the accumulation of soluble protein. Red light, being the primary wavelength absorbed in photosynthesis, enhances the photosynthetic rate by inducing photosensitizing pigments, thus regulating sucrose metabolizing enzymes and subsequently increasing the products of photosynthesis favoring the accumulation of soluble sugars (Xu, Cheng, Zhu, Zhang, & Wang, 2011). Compared with other photosynthetic products, protein as a macromolecule requires more energy than other photosynthetic products, and the blue light region has higher photon energy (Moe, Morgan, & Grindal, 2002). Therefore, it is speculated that the synthesis of blue light catalytic protein is likely to depend on light quality energy. Blue light, another essential spectral region for plant photosynthesis, amplifies plant respiration by accelerating metabolism, leading to the synthesis of organic acids and nitrogen-containing compounds, thereby promoting soluble protein synthesis (Moe et al., 2002). Furthermore, the combination of different light qualities and melatonin increased the contents of soluble sugar and soluble protein in mustard sprouts. These findings align with previous research indicating that melatonin supplementation enhances soluble sugars, soluble proteins, and ultimately improves cabbage biomass and growth (Hasnain, Zafar, Usman, Zhang, & Elansary, 2023). Moreover, melatonin treatment of cotton boll leaves has been reported to enhance the production of soluble sugars, contributing to increased resistance to drought stress (Khattak et al., 2022). These results underscore the multifaceted influence of both light quality and melatonin on the metabolic pathways

leading to the accumulation of essential compounds in mustard sprouts.

Chlorophylls and carotenoids, essential pigments for photosynthesis, exhibit potent antioxidant properties and are crucial for maintaining human health (Sun et al., 2023; Xie et al., 2023). In addition, carotenoids have functions in the protection and enhancement of chlorophylls in de-etiolated plants (Meier, Tzfadia, Vallabhaneni, Gehring, & Wurtzel, 2011). During the differentiation of etioplasts into chloroplasts, the coordinated and co-localized synthesis of carotenoids and chlorophylls is required for normal photomorphogenic development (Meier, Tzfadia, Vallabhaneni, Gehring, & Wurtzel, 2011). This study found that most of the total carotenoid content was positively correlated with the total chlorophylls content. At the same time, our study found that chlorophylls and carotenoids were highest in white light under different light qualities. These findings align with previous research indicating that white light significantly boosts the biosynthesis of chlorophylls and carotenoids in comparison to blue and red light (Cheng et al., 2023). Rape grown under white light also demonstrated higher carotenoid content (Frede, Schreiner, & Baldermann, 2019). At the same time, melatonin further promoted the accumulation of chlorophyll and carotenoids in mustard sprouts under white and blue light. In kiwifruit seedlings, melatonin increased chlorophyll content and was associated with enhanced chlorophyll synthesis and decelerated decomposition (Liang et al., 2019). Similarly, melatonin treatment elevated the total carotenoid content in pepper seedlings and modulated the composition of key carotenoids in the carotenoid metabolic pathway (Li et al., 2022). Given their susceptibility to environmental factors, photosynthetic pigments can benefit from melatonin's antioxidant properties, effectively mitigating the degradation of chlorophylls and carotenoids and improving overall photosynthetic efficiency (Wu et al., 2021). Interestingly, melatonin did not increase the content of chlorophyll and carotenoids under red light. It may be due to the long time of red light will seriously damage the photosynthesis of mustard sprouts, thus affecting the synthesis and accumulation of photosynthetic pigments.

Ascorbic acid, a vital component of the plant's antioxidant defense system, plays a critical role in regulating cell division and expansion (Foyer & Noctor, 2011). In addition, ascorbic acid is also one of the essential nutrients for the human body, and most of the ascorbic acid in people's daily life comes from vegetables, so ascorbic acid is a key indicator for evaluating the quality of vegetables (Foyer & Noctor, 2011). Our study revealed that mustard sprouts exhibited the highest ascorbic acid content under blue light compared to white and red light conditions. These findings are consistent with a prior investigation which reported the highest ascorbic acid content in broccoli sprouts under blue light treatment relative to white and red light (Zhuang, Huang, Li, Xiao, & Guo, 2022). Blue light has been shown to enhance plant photosynthetic capacity, promote the synthesis and accumulation of hexose and D-glucose-precursors of ascorbic acid and stimulate ascorbic acid synthesis. Specifically, blue light induces the expression of genes involved in ascorbic acid biosynthesis in cabbage seedlings, leading to an increase in ascorbic acid content (Kang et al., 2020). Melatonin treatment can increase the content of ascorbic acid, and enhance the antioxidant capacity (Zheng, Liu, Liu, Liu, & Zheng, 2019). This study also found that melatonin increased the ascorbic acid content of mustard sprouts under different light qualities. It shows that there may be a complex interaction between melatonin and light quality, thus promoting the synthesis and accumulation of ascorbic acid.

Phenolic compounds are abundant phytochemicals in plants, including anthocyanins, flavonoids and total Phenolics (Park et al., 2023). Phenolic compounds are involved in plant defense and contribute to the formation of plant color (Liu et al., 2016). At the same time, due to their antioxidant properties and potential health improvements, they are also an integral part of the human diet (Liu et al., 2016). The content of phenolic compounds is an important index to measure the quality of sprouts, and the content of phenolic compounds in sprouts is different due to different light conditions. This study found that blue light promoted the increase of anthocyanin, flavonoid and total phenolics

content in mustard sprouts, and its effect was better than that of white light and red light, indicating that blue light is beneficial to the synthesis of phenolic compounds in mustard sprouts. Previous studies have also shown that the accumulation of phenolic compounds is closely related to blue light. Blue light documented to elevate anthocyanin content in broccoli sprouts and tomato seedlings (Hernández, Eguchi, Deveci, & Kubota, 2016; Zhuang et al., 2022). At the same time, blue light showed a promoting effect on the total flavonoid content of buckwheat sprouts, while red light showed the opposite effect (Nam, Kim, & Eom, 2018). Blue light is more conducive to the improvement of phenolic compounds content and antioxidant capacity in Chinese kale sprouts and pea sprouts (Liu et al., 2016; Qian et al., 2016). The antioxidant capacity of plants is closely related to the content of antioxidant substances and their synergistic effect. The results of this study showed that blue light treatment not only promoted the accumulation of phenolic substances in mustard sprouts, but also enhanced the antioxidant capacity of mustard sprouts. Melatonin also modulates key enzymes of phenolic metabolism, such as inducing phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) expression, enhancing PAL and CHS activity, inhibiting polyphenol oxidase activity, and regulating the expression levels of related genes. In addition, melatonin as a strong antioxidant can effectively improve the phenolic compound content and antioxidant activity of broccoli sprouts (Miao, Zeng, Zhao, Wang, & Wang, 2020). This study found that the combined treatment of blue light and melatonin further increased the content of phenolic compounds and antioxidant capacity in mustard sprouts, indicating that there was a complex interaction between blue light and melatonin, which was conducive to the improvement of phenolic compounds content and antioxidant capacity in mustard sprouts.

Glucosinolate, a secondary metabolite primarily found in cruciferous plants, contributes to the diverse aromas and flavors of cruciferous vegetables (Sun et al., 2021). In addition, glucosinolates, as the main secondary metabolites and important functional components in mustard sprouts, also play an important role in phytochemical defense and anticancer activity (Sun et al., 2021). The content of glucosinolates is affected by many factors. This study found that there was no significant difference in the content of total glucosinolates in mustard sprouts under single light quality, but the combined treatment of white light and melatonin increased the content of total glucosinolates in mustard sprouts. The accumulation of glucosinolates is different due to factors such as light quality and plant species. For instance, blue light promoted the accumulation of glucosinolates in Chinese cabbage by inducing the expression of key genes in glucosinolate biosynthesis and regulating the activity of enzymes related to glucosinolate biosynthesis (Mao et al., 2022). Continuous white light irradiation is more conducive to the synthesis and accumulation of glucosinolates in broccoli (Demir, Sarikamis, & Seyrek, 2023). Notably, the combined treatment of white light and melatonin in our study further promoted the accumulation of glucosinolates. The possible reason is that the effect of white light as a compound light combined with melatonin on mustard sprouts is more complex, resulting in delayed contact between glucosinolates and myrosinase, promoting the preservation of cell integrity, thereby increasing the content of glucosinolates.

5. Conclusions

This study shows that different light qualities alone and in combination with melatonin have different effects on the morphogenesis of mustard sprouts and the accumulation of health-promoting compounds. White light + melatonin had the highest total chlorophyll, total carotenoid and total glucosinolates content, red light + melatonin had the highest plant height, fresh weight and soluble sugar content, blue light + melatonin had the highest soluble protein, antioxidant level, ascorbic acid and total phenolics content. In the future, we will further study the molecular mechanism of how light quality and melatonin regulate the growth of mustard sprouts and promote the content of healthy

compounds.

CRedit authorship contribution statement

Aolian Zhou: Writing – original draft, Investigation. **Jiayi Tang:** Writing – original draft, Investigation. **Ying Li:** Writing – original draft, Data curation. **Wenjuan Cheng:** Investigation. **Xingwei Yao:** Data curation. **Victor Hugo Escalona:** Data curation, Conceptualization. **Guiping Qian:** Data curation. **Jie Ma:** Funding acquisition, Data curation. **Xuena Yu:** Investigation. **Huanxiu Li:** Funding acquisition, Data curation. **Zhi Huang:** Investigation, Funding acquisition. **Yi Tang:** Data curation. **Fen Zhang:** Funding acquisition, Data curation. **Zhifeng Chen:** Writing – review & editing. **Bo Sun:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2024.101560>.

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