

Article

Growth and Quality of Purple Mustard Sprouts as Affected by Combined Light Quality and Sodium Selenite

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Highlights

What are the main findings?

- Red, white, and blue light differentially regulated growth, pigments accumulation, and antioxidant compounds and glucosinolates in mustard sprouts, respectively.
- Combined with sodium selenite, SeR showed the greatest gains in growth, SeW attained the highest total glucosinolates content, and SeB the highest antioxidant capacity.

What are the implications of the main findings?

- Targeted combinations of light quality and exogenous selenium can selectively enhance the yield or nutritional quality of mustard sprouts in controlled-environment production.
- Phenylpropanoid biosynthesis and linoleic acid metabolism are key pathways underlying the combined effects of light and selenium on health-promoting compounds.

Abstract

Mustard (*Brassica juncea*) sprouts are appreciated for their high levels of bioactive constituents, but how light quality and exogenous selenium interactively regulate their growth and nutritional properties is still poorly understood. In this study, we evaluated the individual and combined effects of red (R), white (W) and blue (B) light together with 100 $\mu\text{mol}\cdot\text{L}^{-1}$ selenium solution (SeR, SeW, SeB) on sprouts growth, health-promoting compounds, and metabolic profiles. Under single light quality treatments, red light favored growth, white light promoted photosynthetic pigment accumulation, and blue light most effectively enhanced antioxidant compounds and glucosinolates. Combined treatments further improved both yield and quality. SeR showed the greatest gains in growth indices; SeW achieved the highest total chlorophyll, total carotenoids, and total glucosinolates (the latter reaching 108.18 $\mu\text{mol g}^{-1}$ DW), and SeB attained the highest total selenium, soluble sugar, soluble protein, anthocyanins, ascorbic acid, flavonoids, total phenolics, and antioxidant capacity. Non-targeted metabolomics identified 1208 differential metabolites, with KEGG enrichment revealing that the phenylpropanoid biosynthesis pathway was significantly enriched across all selenium-treated groups. These findings demonstrate that targeted combinations of light quality and exogenous selenium can selectively enhance the growth or nutritional quality of mustard sprouts, offering practical guidance for their controlled-environment production.



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Keywords: antioxidants; glucosinolates; light quality; mustard sprouts; non-targeted metabolomics; sodium selenite

1. Introduction

Mustard (*Brassica juncea*) is a widely cultivated vegetable of the genus *Brassica* in the Brassicaceae family, possessing significant economic and nutritional value [1]. It is rich in health-promoting compounds such as glucosinolates, anthocyanins, ascorbic acid, flavonoids, and total phenolics, which may reduce the risk of cancer and obesity-related metabolic disorders [2]. Sprouts refer to edible young stems, tender shoots, or seedlings produced by the germination of seeds or other storage organs, with a growth cycle of approximately 4–10 days and harvested at the cotyledonary stage before true leaf emergence [3,4]. Compared to mature plants, mustard sprouts contain higher concentrations of nutrients per unit volume [5]. In recent years, as public concern for food safety and dietary health has grown, mustard sprouts have gained widespread attention due to their safe cultivation, lack of contamination, and rapid growth [4].

Light quality critically regulates plant growth, development, and the accumulation of secondary metabolites, especially glucosinolates and antioxidants. Recent studies have demonstrated that LED-based light management can effectively improve plant growth, physiological status, and quality-related traits under controlled conditions [6]. Different light qualities influence plant morphogenesis, photosynthesis, and secondary metabolism through photoreceptor-mediated signaling pathways [7]. Previous studies have shown that red light treatment significantly promotes plant height and biomass accumulation in cruciferous sprouts [8,9]. Blue light inhibits hypocotyl elongation in *Arabidopsis* through the cryptochrome (CRY) and phototropin (Phot) signaling pathways [10], while in *Brassica napus* it specifically activates the *HY5-MYB* transcriptional cascade to regulate the accumulation of antioxidants such as anthocyanins, ascorbic acid, and phenolics [11,12]. Furthermore, blue light regulates glucosinolates biosynthesis by affecting transcription factors such as *MYB28/34* [13,14]. Therefore, manipulating light quality is a promising approach to boost secondary metabolite accumulation in sprouts.

Selenium (Se) is an essential trace element for the human body, with various physiological functions including boosting immunity, preventing cardiovascular diseases, and acting as an antioxidant [15]. Approximately one billion people worldwide suffer from selenium deficiency, and consuming selenium-enriched foods is a safe and effective way to supplement selenium [16]. However, excessive Se intake may cause toxicity, so it is essential to monitor Se accumulation levels in fortified foods. Exogenous selenium can promote carbohydrate metabolism by upregulating the activity of sucrose synthase (SUS) and sucrose-phosphate synthase (SPS), thereby enhancing mungbean growth [17]. Additionally, exogenous selenium can enhance the antioxidant capacity of cruciferous vegetables and influence glucosinolates accumulation by regulating sulfur metabolism [18]. Studies have shown that selenocysteine methyltransferase (SMT) catalyzes the synthesis of Se-methylselenocysteine (SeMSC), which in turn promotes glucosinolates biosynthesis in broccoli [19].

Recent studies have shown that combining light quality with other factors can regulate vegetable growth and metabolism and enhance nutritional quality. For example, the combined treatments of light quality and melatonin promote the accumulation of phenolic compounds and glucosinolates in mustard sprouts [5], and the combination with calcium chloride also positively affects the quality of Chinese kale sprouts [20]. However, the

combined effects of different light qualities and exogenous selenium on sprouts growth and the accumulation of health-promoting compounds have not been systematically evaluated.

This study investigated the effects of different light qualities, both alone and in combination with selenium, on the growth of mustard sprouts and the accumulation of health-promoting compounds. By integrating non-targeted metabolomics analysis to elucidate the differential metabolic pathways among treatments, the study aims to provide a scientific basis for optimizing the light environment and improving the quality of mustard sprouts in production.

2. Materials and Methods

2.1. Plant Materials and Treatments

The test material was the purple mustard line 'ZJ-3'. Selected seeds were heat-treated with distilled water at 55 °C, then soaked in distilled water at 37 °C for 3 h before being sown in plastic seedling trays (33 cm × 26 cm × 4.5 cm) for hydroponic cultivation. The seedling trays were placed in adjustable-light incubators (Jiangnan Co., Ltd., Ningbo, China) and kept in the dark for 2 days. Subsequently, they were treated with red (660 nm), white (400–700 nm, 6500 K), or blue light (445 nm), respectively (Supplementary Figure S1), and were sprayed once daily at the end of the photoperiod with 8 mL of either 100 $\mu\text{mol}\cdot\text{L}^{-1}$ selenium solution (prepared from $\text{Na}_2\text{SeO}_3\cdot 5\text{H}_2\text{O}$, $\geq 98\%$ purity, Aladdin Biochemical Technology Co., Ltd., Shanghai, China, pH 8.5–8.8, no surfactant) or an equivalent amount of distilled water for 6 consecutive days, and the total Se input was 4.8 μmol per tray. Six treatment combinations were established: R (red light), SeR (red light + selenite), W (white light), SeW (white light + selenite), B (blue light), and SeB (blue light + selenite). Cultivation conditions: light intensity 80 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Supplementary Table S1), temperature 23 °C/18 °C, photoperiod 16 h light/8 h dark, relative humidity 80%, Daily light integral (DLI): 4.61 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, Density: 4 g of seeds per tray, Lamp-to-canopy distance: 37–38 cm. At day 9 after sowing, harvested seedlings were root-removed; the remaining tissues were frozen in liquid nitrogen and freeze-dried for physiological assays, while a subsample of fresh material was flash-frozen and kept at $-80\text{ }^{\circ}\text{C}$ for metabolomics.

2.2. Plant Height, Edible Fresh Weight, and Total Selenium

From each tray, 10 sprouts were randomly selected, and their heights were measured using a ruler. The mean height of the 10 sprouts was calculated as one replicate value ($n = 3$ trays per treatment). For fresh weight, the aboveground parts of an entire tray were weighed after root removal ($n = 3$ per treatment). Total selenium content was measured following the Chinese national standard GB 5009.93-2017 [21], with three biological replicates per treatment.

2.3. Soluble Sugars

One milliliter of sample extract, 0.5 mL of anthrone-ethyl acetate reagent, and 5 mL of concentrated sulfuric acid were mixed, boiled for 5 min, and finally the absorbance of the reaction mixture was measured at 630 nm [1].

2.4. Soluble Protein

Coomassie Brilliant Blue G-250 was added to 1 mL of the supernatant, and the absorbance was rapidly measured at 595 nm within 20 min after the reaction began [1].

2.5. Chlorophyll and Carotenoids

The sample powder was ground and extracted with acetone. Following extraction, the supernatant was filtered and analyzed using high-performance liquid chromatography

(HPLC). For HPLC analysis, 10 µL of sample was separated using a mobile phase of isopropanol and 80% acetonitrile-water at a flow rate of 0.5 mL/min [1].

2.6. Anthocyanins

An appropriate amount of sample was ground with 1% hydrochloric acid-methanol solution. The sample was diluted to 10 mL and left 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 [22].

2.7. Ascorbic Acid

The sample powder was first extracted with 1.0% oxalic acid solution, followed by centrifugation. Subsequently, each sample was filtered and analyzed by HPLC. The concentration of ascorbic acid was determined based on the absorbance value measured at 243 nm [1].

2.8. Flavonoids

Forty milligrams of sample powder was extracted with 50% ethanol and incubated at room temperature in the dark for 24 h. After centrifugation, 1.2 mL of the supernatant was mixed with 60 µL of 2% aluminum trichloride, 60 µL of 1 mol/L potassium acetate, and 1.68 mL of distilled water. After standing for 40 min, the absorbance was read at 415 nm [1].

2.9. Total Phenolics

Total phenolics were extracted using 50% ethanol as the solvent. The resulting supernatant was then mixed with Folin-Ciocalteu reagent. After a 3-min interval, saturated sodium carbonate solution was added, and the absorbance was recorded at 760 nm using a spectrophotometer [1].

2.10. Ferric Reducing Antioxidant Power (FRAP)

The extracted samples were mixed with the FRAP working solution and incubated at 37 °C. After incubation at 37 °C for 10 min, the absorbance was measured at 593 nm using a spectrophotometer, and the corresponding values were calculated [1].

2.11. Glucosinolates

One hundred milligrams of freeze-dried sample was heated in 5 mL of water for 10 min to form a liquid layer, which was then loaded onto a DEAE-Sephadex A-25 column. Glucosinolates were converted into their desulfated derivatives using aryl sulfatase. After this treatment, the desulfated glucosinolates were separated and quantified by HPLC [1].

2.12. Non-Targeted Metabolomics

Mustard sprout samples from each treatment group were ground in liquid nitrogen, and 100 mg was weighed, mixed with 1 mL of extraction solution (70% methanol containing 0.5 µg/mL 2-chlorophenylalanine as an internal standard), vortexed, sonicated for 30 min, allowed to stand at −20 °C for 30 min, and centrifuged at 4 °C and 12,000 rpm for 15 min. The supernatant was filtered through a 0.22 µm filter membrane before injection. Analysis was performed using an ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) system. The column was a Thermo Hypersil Gold C18 (2.1 mm × 100 mm, 1.9 µm) with a column temperature of 40 °C and a flow rate of 0.3 mL/min. The mobile phase consisted of solution A (5 mmol/L ammonium acetate in water) and solution B (acetonitrile), with gradient elution. Mass spectrometry was performed using an Orbitrap Exploris 120 mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) in both positive and negative ion modes, covering the *m/z* range

of 85–1250. Raw data were processed using MSDIAL (version 5.5, RIKEN Center for Sustainable Resource Science, Tokyo, Japan) software and compared against mzCloud, MassBank, METLIN, and an in-house database for metabolite identification. The total ion chromatograms of the QC samples showed good overlap, with a Pearson correlation coefficient > 0.99 , indicating stable and reliable data. After data normalization by Pareto scaling, orthogonal partial least squares discriminant analysis (OPLS-DA) combined with univariate analysis was used to screen for differentially expressed metabolites, with criteria of $VIP \geq 1.0$, $|\log_2 FC| \geq 1$, and $p < 0.05$. The differentially expressed metabolites were mapped to the KEGG database for pathway enrichment analysis, with a significance threshold of $p < 0.05$ based on Fisher's exact test [23].

2.13. Statistical Analysis

Statistical analysis was performed using SPSS Version 20 (SPSS Inc., Chicago, IL, USA). A two-way analysis of variance was conducted on the data, with light quality and selenium treatment as the two independent factors. Graphs were plotted 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. Results

3.1. Sprout Growth and Total Selenium

Compared with the W treatment, the R treatment stimulated mustard sprouts growth, whereas the B treatment suppressed it. Adding exogenous selenium to any light condition further enhanced growth. Among the three light-only treatments, the R treatment gave the greatest plant height and edible fresh weight, exceeding those of the W treatment by 15.54% and 24.10%, respectively. For the combined treatments, SeR, SeW, and SeB all significantly increased both parameters, with the SeR treatment showing the best performance, 7.33% greater plant height and 14.53% greater fresh weight than the R treatment alone (Figure 1A–C).

Under the different light quality treatments (without selenium), no significant differences in total selenium content were observed among the three light quality groups. The combined treatments significantly promoted total selenium accumulation; the total selenium content in the SeR, SeW, and SeB treatments was 12.22-fold, 9.15-fold, and 10.20-fold higher than that in the single light quality treatments, respectively, with the highest level observed in the SeB treatment ($18.57 \text{ mg kg}^{-1} \text{ DW}$) (Figure 1D).

3.2. Soluble Sugar and Soluble Protein

For soluble sugar, red light alone produced the greatest accumulation ($122.91 \text{ mg g}^{-1} \text{ DW}$), with the W treatment ranking second ($99.15 \text{ mg g}^{-1} \text{ DW}$). Adding selenite amplified this response across all backgrounds. Increases of 17.71%, 5.83%, and 102.20% were recorded in the SeR, SeW, and SeB groups relative to their respective light-only controls, with the SeB treatment reaching a peak of $177.45 \text{ mg g}^{-1} \text{ DW}$ (Figure 1E).

Soluble protein accumulation followed a different pattern under single-light conditions, peaking in the B treatment ($40.10 \text{ mg g}^{-1} \text{ DW}$) and declining slightly in the W treatment ($36.79 \text{ mg g}^{-1} \text{ DW}$). Selenium co-treatment elevated soluble protein across all treatments by 44.28%, 13.17%, and 10.99% in SeR, SeW, and SeB, respectively. The SeB treatment again recorded the highest value at $44.51 \text{ mg g}^{-1} \text{ DW}$ (Figure 1F).

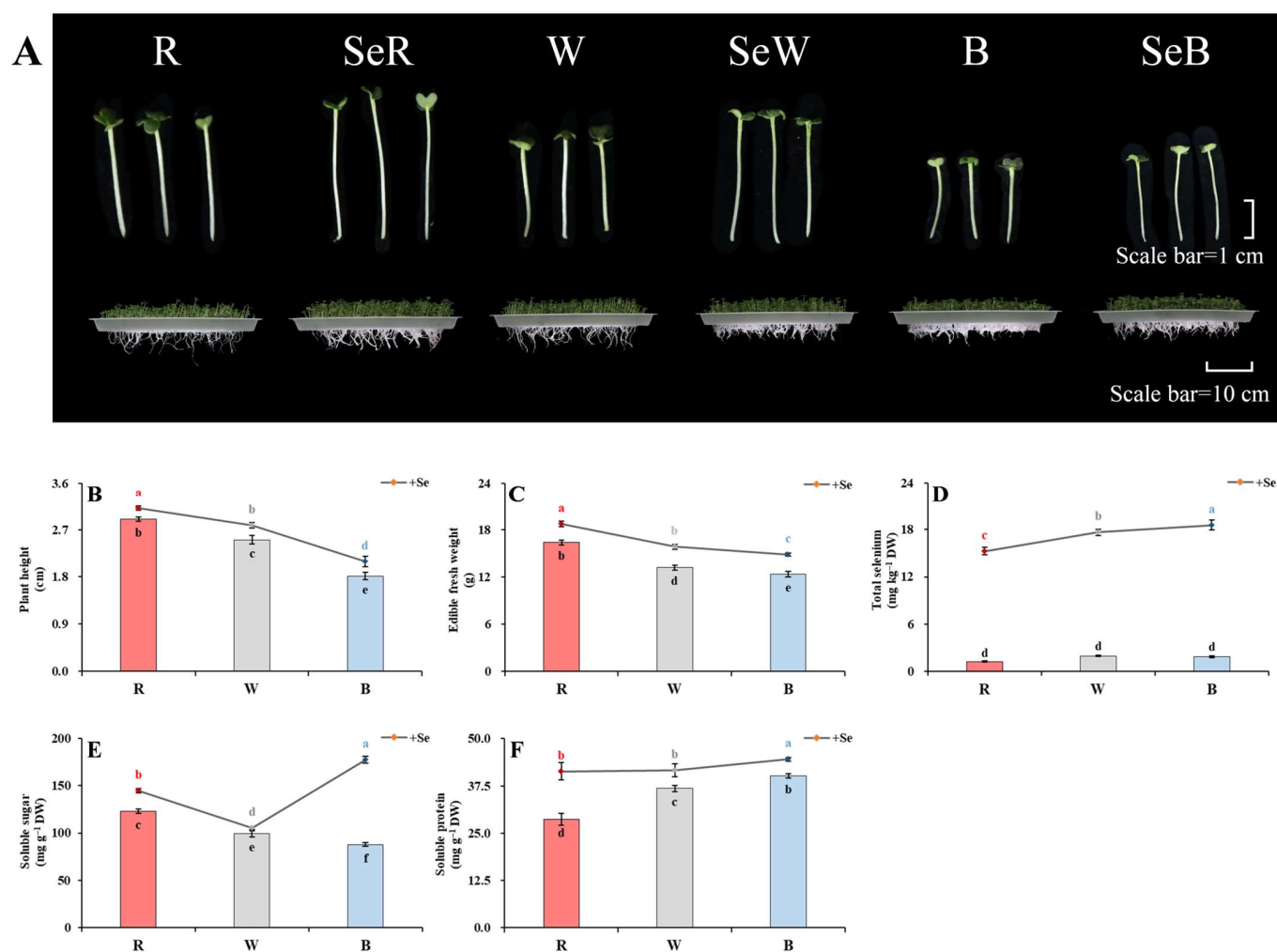


Figure 1. Growth morphology, corresponding physiological indicators and total selenium content of mustard sprouts among different treatments. (A) Growth morphology of mustard sprouts under red light (R), red light + 100 μ M sodium selenite solution (SeR), white light (W), white light + 100 μ M sodium selenite solution (SeW), blue light (B), and blue light + 100 μ M sodium selenite solution (SeB); (B) Plant height; (C) Edible fresh weight; (D) Total selenium; (E) Soluble sugar; (F) Soluble protein. Different letters above the bars denote significant differences among treatments (LSD test, $p < 0.05$).

3.3. Chlorophylls, Carotenoids, and Anthocyanins

Both chlorophyll a and b were detected in mustard sprouts (Figure 2A–C). The W treatment drove the greatest chlorophyll accumulation among single-light treatments, with total chlorophyll exceeding the R and B treatments by 15.34% and 12.24%, respectively. Selenium co-treatment further elevated all chlorophyll fractions. Relative to their single-light counterparts, chlorophyll a rose by 18.59%, 27.75%, and 24.79% in SeR, SeW, and SeB, while chlorophyll b increased by 20.41%, 29.65%, and 13.22%, and total chlorophyll by 19.39%, 28.56%, and 19.64%. The SeW treatment achieved the highest total chlorophyll content at 110.90 mg g⁻¹ DW.

Four carotenoids were quantified: lutein (most abundant), neoxanthin, β -carotene, and violaxanthin (least abundant) (Figure 2D–H). Under single-light conditions, lutein and β -carotene peaked in the W treatment; neoxanthin levels were comparable between the W and B treatments, both exceeding the R treatment; violaxanthin reached its maximum in the B treatment. Selenium co-treatment uniformly elevated lutein and β -carotene across all light backgrounds. Total carotenoid content was greatest in the W treatment, and selenium further increased it by 13.41%, 24.12%, and 6.54% in SeR, SeW, and SeB,

respectively. Additionally, the SeW treatment attained the highest total carotenoid content at $26.71 \text{ mg g}^{-1} \text{ DW}$.

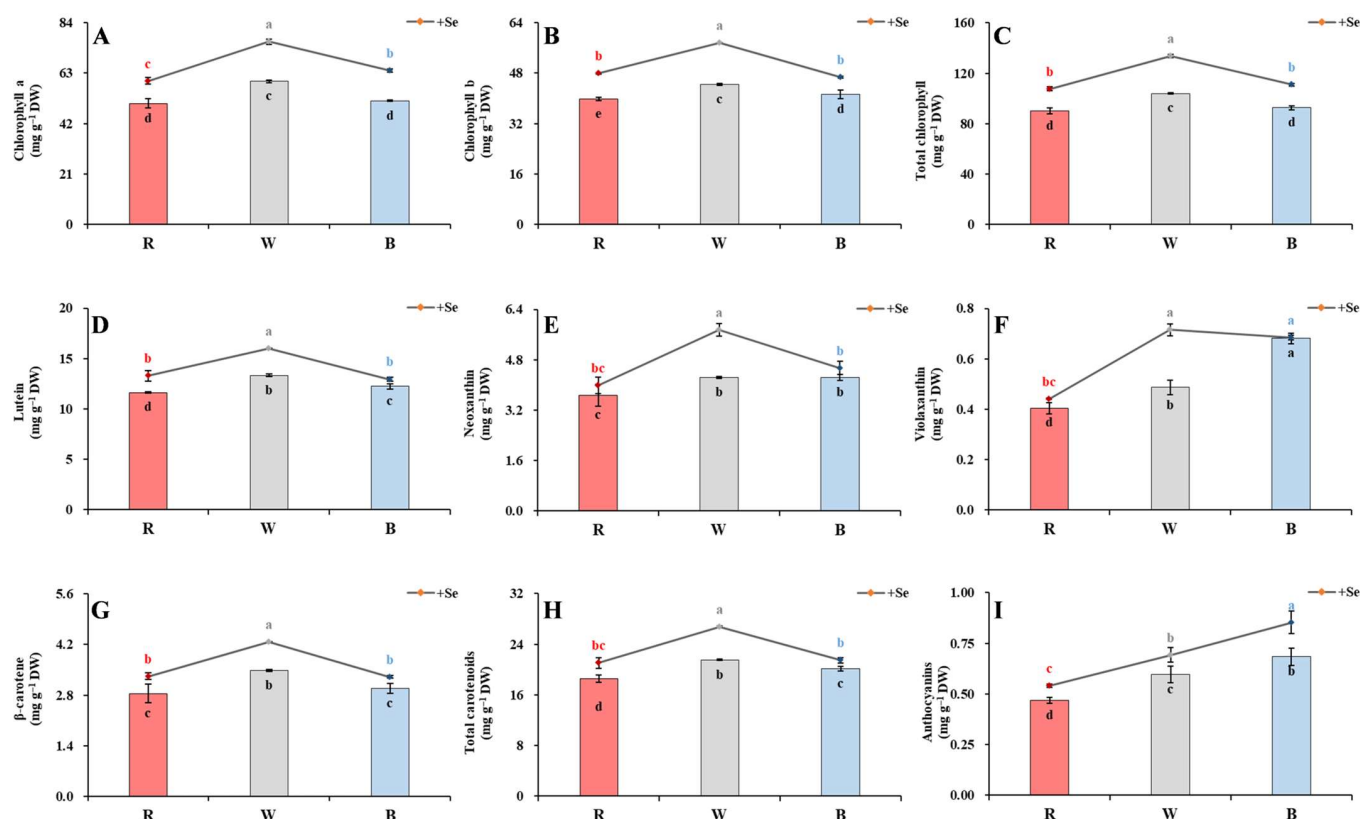


Figure 2. Chlorophylls, carotenoids and anthocyanins content in mustard sprouts among different treatments. (A) chlorophyll a; (B) chlorophyll b; (C) total chlorophyll; (D) lutein; (E) neoxanthin; (F) violaxanthin; (G) β -carotene; (H) total carotenoids; (I) anthocyanins. Different letters above the bars denote significant differences among treatments (LSD test, $p < 0.05$).

Regarding anthocyanins, the B treatment stimulated accumulation relative to the W treatment (+14.72%), whereas the R treatment had the opposite effect (−21.36%). All three selenium co-treatments promoted anthocyanin synthesis, with gains of 15.01%, 16.12%, and 24.72% over the R, W, and B controls, respectively; the SeB treatment reached the highest content at $0.85 \text{ mg g}^{-1} \text{ DW}$ (Figure 2I).

3.4. Antioxidant Content and Antioxidant Capacity

Among single-light treatments, the B treatment maximized ascorbic acid content ($4.50 \text{ mg g}^{-1} \text{ DW}$), while the W treatment yielded the lowest value ($3.87 \text{ mg g}^{-1} \text{ DW}$). Selenium co-treatment produced modest but consistent increases of 6.83%, 17.36%, and 5.80% in SeR, SeW, and SeB relative to their light-only controls. Additionally, the SeB treatment reached the highest ascorbic acid content at $4.76 \text{ mg g}^{-1} \text{ DW}$ (Figure 3A).

The B treatment similarly maximized flavonoids and total phenolics accumulation among single-light treatments (1.59 and $11.30 \text{ mg g}^{-1} \text{ DW}$, respectively). Selenium co-treatment further elevated both parameters across all treatments, with the SeB treatment recording the highest flavonoid and total phenolic contents—8.54% and 11.01% above the B-only control, respectively (Figure 3B,C). The antioxidant capacity of the SeB treatment, as measured by FRAP, also reached its peak at $182.11 \mu\text{mol g}^{-1} \text{ DW}$ (Figure 3D).

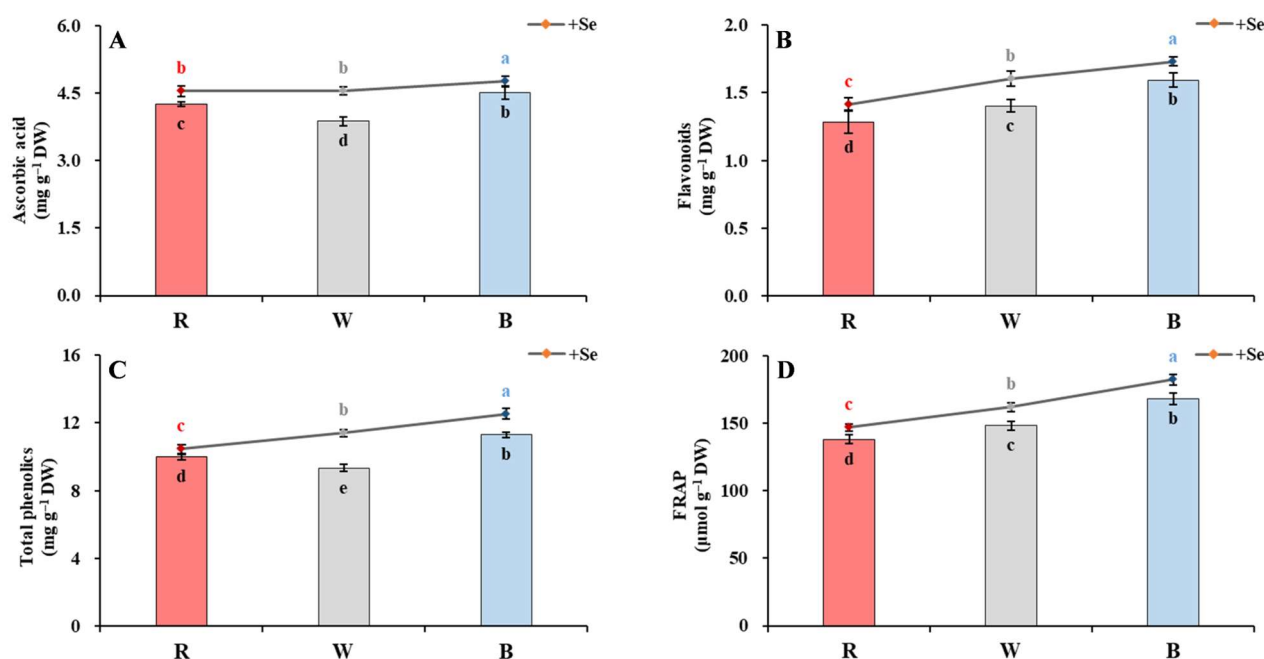


Figure 3. Antioxidant content and antioxidant capacity of mustard sprouts following different treatments. (A) ascorbic acid; (B) flavonoids; (C) total phenolics; (D) FRAP. Different letters above the bars denote significant differences among treatments (LSD test, $p < 0.05$).

3.5. Glucosinolates

Seven glucosinolates were identified in mustard sprouts: three aliphatic (sinigrin, gluconapin, progoitrin) and four indolic (4-hydroxyglucobrassicin, glucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin) (Figure 4A–J). Aliphatic forms dominated total glucosinolate content across all treatments, and the trends in total aliphatic and total glucosinolates were tightly coupled throughout the experiment. For aliphatic glucosinolates, the W and B treatments consistently produced higher sinigrin, gluconapin, and progoitrin levels than the R treatment. The SeW treatment amplified this further, raising sinigrin, gluconapin, and progoitrin by 38.62%, 37.61%, and 18.86%, respectively, relative to the W-only control (Figure 4A–C).

Among the indolic forms, 4-hydroxyglucobrassicin was 2.51- and 2.65-fold higher in the W and B treatments than in the R treatment. Glucobrassicin showed an even more pronounced response, reaching 10.48- and 4.74-fold the R- and B-treatment values in the W treatment. 4-Methoxyglucobrassicin and neoglucobrassicin were most abundant in the B treatment, and their levels in the SeB treatment increased by a further 43.06% and 34.94% over the B-only control (Figure 4E–H).

At the total level, both aliphatic and total glucosinolates peaked in the B treatment, with the R treatment yielding the lowest values. Total indolic glucosinolates were highest in the W and B treatments, significantly exceeding the R treatment. The SeR and SeW treatments further elevated aliphatic and total glucosinolates, whereas the SeB treatment had no significant effect on total glucosinolate content, which is possibly because blue light had already driven glucosinolate synthesis close to saturation. Among all treatments, the SeW treatment recorded the highest total glucosinolate content at $108.18 \mu\text{mol g}^{-1} \text{DW}$ (Figure 4D,I,J).

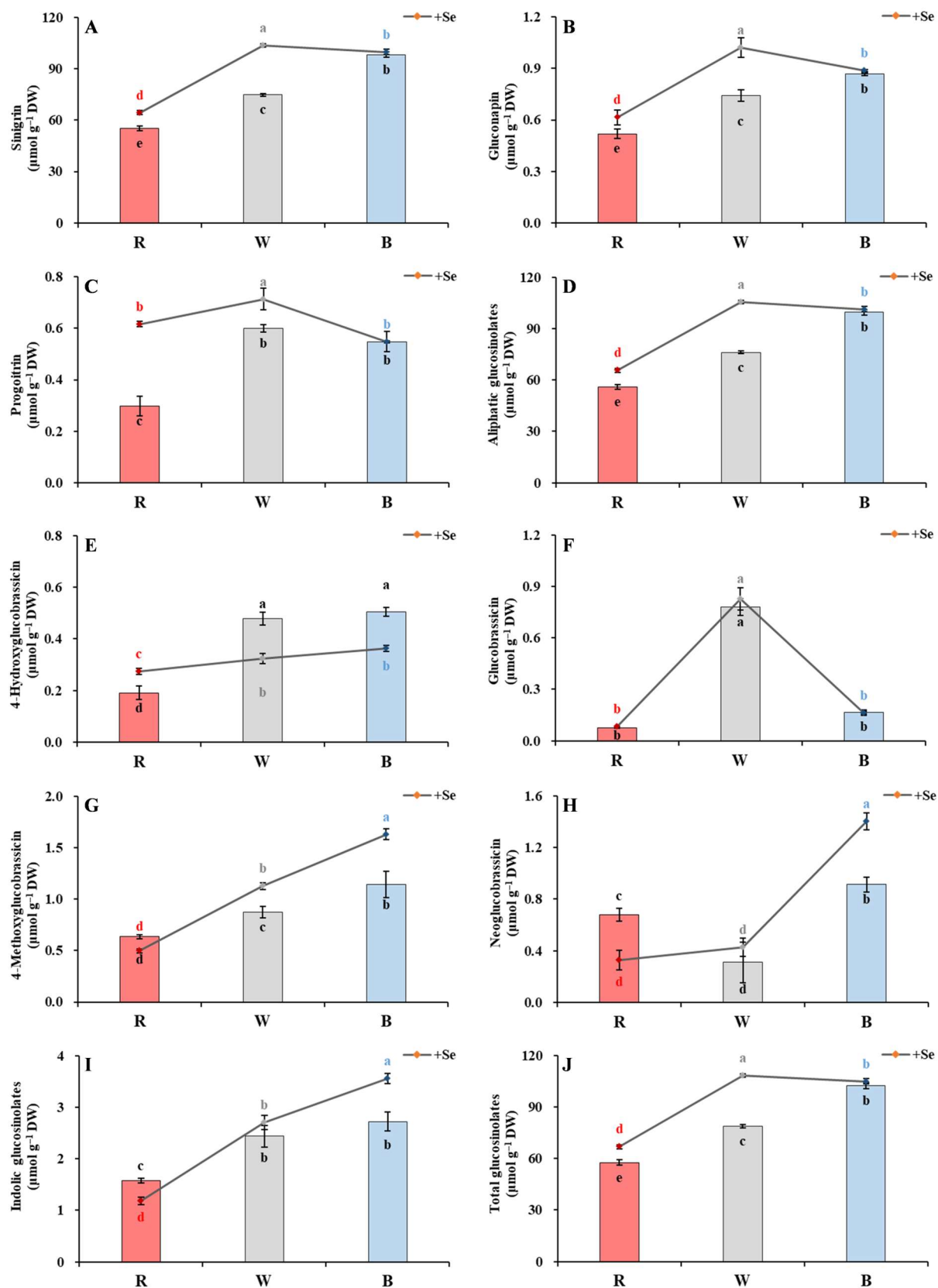


Figure 4. Glucosinolates content in mustard sprouts among different treatments. (A) sinigrin; (B) gluconapin; (C) progoitrin; (D) aliphatic glucosinolates; (E) 4-hydroxyglucobrassicin; (F) glucobrassicin; (G) 4-methoxyglucobrassicin; (H) neoglucobrassicin; (I) indolic glucosinolates; (J) total glucosinolates. Different letters above the bars denote significant differences among treatments (LSD test, $p < 0.05$).

3.6. Overview of Non-Targeted Metabolomics Analysis

To further elucidate the metabolic basis by which light quality and selenium regulate the quality of mustard sprouts, this study conducted non-targeted metabolomics analysis on six treatment groups, identifying a total of 1208 differential metabolites. Venn diagrams (Figure 5A–C) show that in the light quality main effect comparison groups (R vs. W, B vs. W, R vs. B), there were 37 shared differential metabolites across the three groups, with 30, 22, and 67 group-specific metabolites, respectively. In the selenium effect comparison groups (SeR vs. R, SeW vs. W, SeB vs. B), the three groups shared 50 differential metabolites; in the comparisons between selenium treatment groups (SeR vs. SeW, SeB vs. SeW, SeR vs. SeB), the three groups shared 30 differential metabolites. The differential metabolites encompassed various chemical classes, including fatty acids, flavonoids, phenolic acids, carbohydrates, and amino acids (Figure 5G).

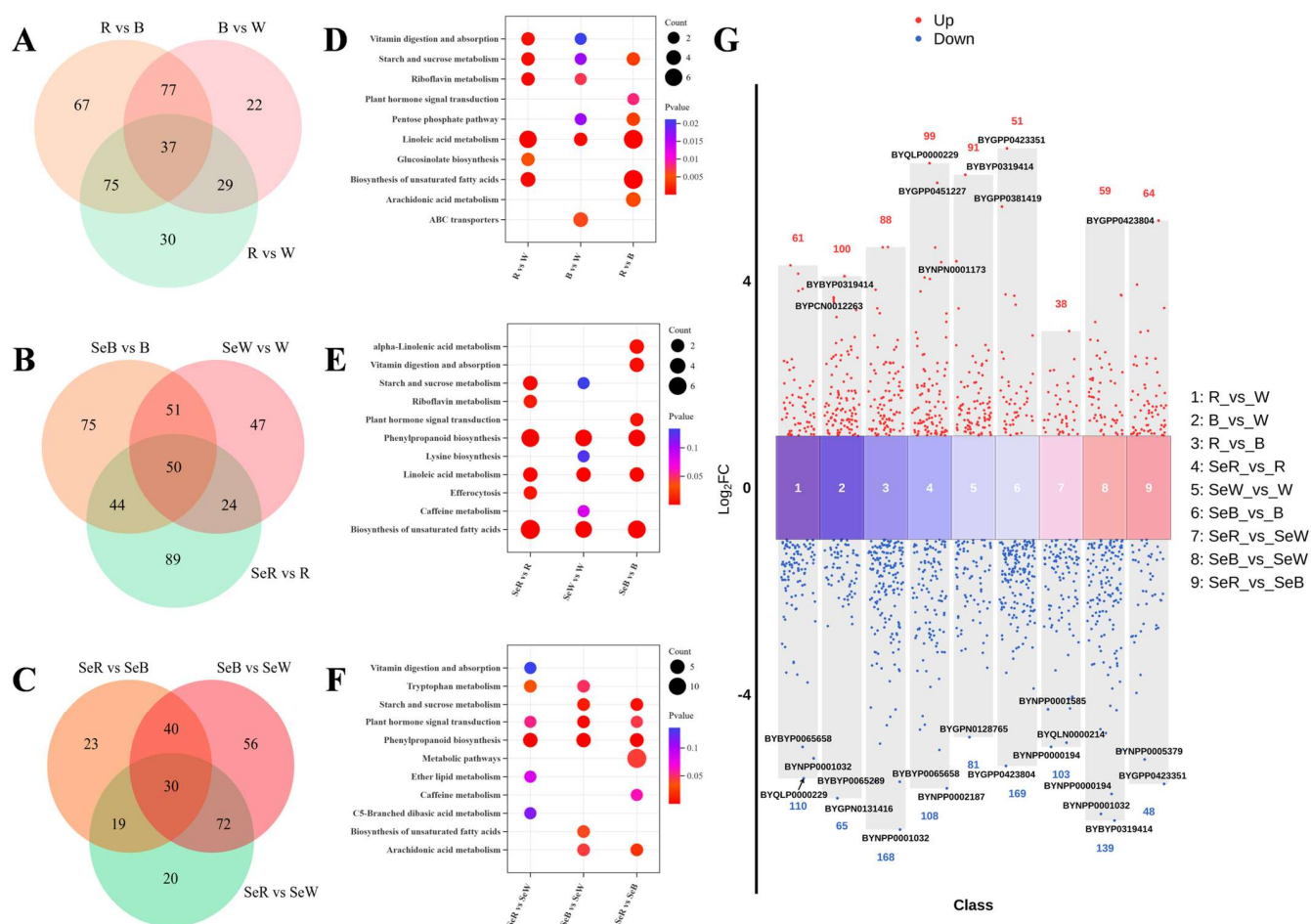


Figure 5. Comprehensive analysis of differential metabolites and KEGG pathway enrichment across treatment groups. (A) Venn diagram of differential metabolites among R vs. W, B vs. W, and R vs. B groups; (B) Venn diagram of differential metabolites among SeR vs. R, SeW vs. W, and SeB vs. B groups; (C) Venn diagram of differential metabolites among SeR vs. SeW, SeB vs. SeW, and SeR vs. SeB groups; (D–F) KEGG enrichment bubble plots for the light-only comparisons (D), selenium-effect comparisons (E), and selenium-treatment comparisons (F); (G) Multi-group Log₂FC distribution of differential metabolites across all nine comparison pairs.

KEGG pathway enrichment analysis indicated (Figure 5D–F) that, in the light quality main effect comparison groups, the glucosinolate biosynthesis pathway was strongly enriched in the R vs. W group (with glucobrassicin and sinigrin as the main differential metabolites), suggesting significant differences in glucosinolate metabolism between red

and white light treatments. In the selenium effect comparison groups (SeR vs. R, SeW vs. W, SeB vs. B), the phenylpropanoid biosynthesis pathway was highly enriched in all three groups (with cinnamaldehyde, coniferyl aldehyde, coniferin, and methyl cinnamate as the shared differential metabolites across groups), suggesting that the regulatory effect of selenium on phenylpropanoid metabolism may be universal across different light quality backgrounds. Notably, the unsaturated fatty acid synthesis and linoleic acid metabolism pathways were significantly enriched in multiple comparison groups.

3.7. Principal Component and Correlation Analysis

Principal component analysis (PCA) and correlation analyses were performed using treatment means ($n = 6$), and thus the results are interpreted as exploratory. PC1 and PC2 accounted for 56.4% and 23.5% of the total variance, respectively. Most indicators related to sprout quality and development fell within the second and third quadrants. B and SeB treatments strongly influenced soluble sugar, antioxidants, and glucosinolates, whereas SeW primarily affected photosynthetic pigments and total selenium. Plant height and edible fresh weight were mainly located in the fourth quadrant together with W, R, and SeR treatments, indicating their major impact on growth traits (Figure 6A,B).

The correlation heatmap (Figure 6C) shows that plant height and edible fresh weight are positively correlated (0.84), but both are negatively correlated with anthocyanins (-0.67 , -0.39), flavonoids (-0.61 , -0.33), and total glucosinolates (-0.67 , -0.50), suggesting that treatment groups with higher growth rates had relatively lower levels of functional components. Anthocyanins were positively correlated with flavonoids (0.89), total phenolics (0.81), and FRAP (0.96), and ascorbic acid was positively correlated with total phenolics (0.85). Additionally, carotenoids showed a strong positive correlation with total chlorophyll (0.96) (Supplementary Table S2).

3.8. Analysis of Variance

The results of the analysis of variance (ANOVA) indicate that light quality is the primary factor influencing the growth and quality of mustard sprouts, exerting a highly significant effect ($p < 0.01$) on all 28 parameters. Furthermore, its impact on plant height, edible fresh weight, total carotenoids, anthocyanins, total phenolics, flavonoids, and total glucosinolates was greater than that of selenium treatment. Selenium treatment had a significant effect on 26 parameters ($p < 0.05$), excluding glucobrassicin and neoglucobrassicin, and its effects on soluble sugars, soluble proteins, total selenium, total chlorophyll, and ascorbic acid were greater than those of light quality. The combined effect of light quality and selenium treatment reached a significant level for 23 indicators ($p < 0.05$), and its effects on soluble sugars, 4-hydroxyglucobrassicin, progoitrin, and neoglucobrassicin were greater than those of light quality alone, indicating that the combined treatments has a strong regulatory effect on these indicators (Supplementary Table S3).

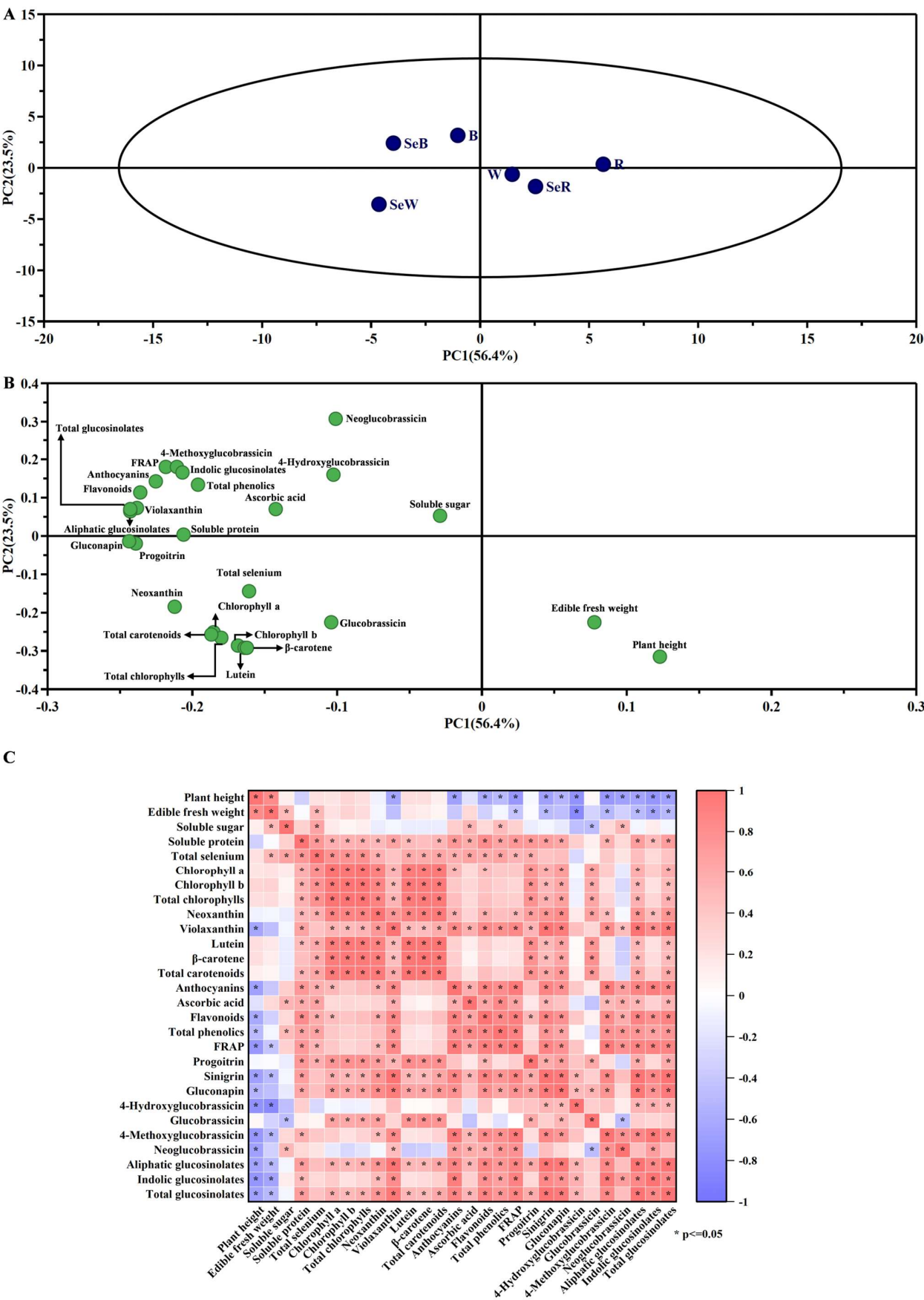


Figure 6. PCA and correlation analysis of antioxidant capacity and nutritional components in mustard sprouts across treatments. **(A)** Score plot; **(B)** loading plot; **(C)** correlation plot. Absolute Pearson coefficients with (* $p \leq 0.05$) are shown.

4. Discussion

Consumers strongly prefer mustard sprouts because of their rich nutritional profile and short cultivation period [24]. Modulating multiple environmental factors together has emerged as an effective strategy to enhance the growth, development, and quality of cruciferous vegetables [25]. Here, we systematically investigated the effects of different light qualities, both as standalone treatments and in combination with sodium selenite, on the growth and nutritional quality of mustard sprouts.

4.1. Role of Light Quality and Selenium in Regulating Mustard Sprout Growth

Plant height and edible fresh weight are important indicators for assessing the economic value and growth status of sprouts [5]. Growth differences in plants under different light quality environments result from their adaptation to the light environment [7]. Red light can promote the growth of hot pepper (*Capsicum frutescens*) seedlings by upregulating the expression of *Rubisco*-related genes and NADPH oxidase activity [26]. This study indicates that red light treatment promoted plant height and edible fresh weight in mustard sprouts, whereas blue light treatment exhibited an inhibitory effect, consistent with previous reports on Chinese kale sprouts [9]. Blue light primarily inhibits hypocotyl elongation through signaling pathways mediated by Phot1 and Cry1 [10]; this inhibitory effect may be associated with reduced IAA levels and altered expression of cell wall remodeling genes according to previous studies [27,28]. Furthermore, combined treatments of different light qualities with selenium further increased the plant height and edible fresh weight of mustard sprouts. The growth promoting effect of low concentration exogenous selenium has been confirmed in various crops [15]; the mechanism may involve selenium upregulating sucrose synthase (SUS) and sucrose phosphate synthase (SPS) activity at appropriate levels, thereby promoting carbohydrate synthesis and transport and accelerating vegetative growth [17]. In the combined treatments, the increase in soluble sugar content further indicates that exogenous selenium can enhance primary carbon metabolism to promote biomass accumulation. However, these concentration-based values should be interpreted alongside biomass per tray, as higher content does not necessarily mean higher total yield per tray.

Chlorophylls and carotenoids are essential pigments for plant photosynthesis, growth, and development, and are also important dietary antioxidants [20,29]. This study indicates that among the different light quality treatments, white light treatment resulted in the highest chlorophyll and carotenoid contents in mustard sprouts, and the levels of most carotenoids were significantly positively correlated with chlorophyll content, consistent with previous studies [5]. The combined treatments of light quality and selenium further promoted the accumulation of chlorophyll and total carotenoids. The previous study indicated that exogenous selenium may protect pigments from degradation by alleviating light-induced oxidative damage and maintaining the structural integrity of chloroplast membranes [30]. Simultaneously, the increase in total carotenoid synthesis is probably related to selenium-mediated upregulation of the carotenoid isomerase gene (*CRTISO*) expression in leaves [31].

4.2. Role of Light Quality and Selenium in Enhancing Antioxidant Compounds and Capacity

Plants naturally contain abundant antioxidants, including anthocyanins, ascorbic acid, flavonoids, and total phenolics, which together determine their antioxidant capacity [32]. In our study, blue-light treatment resulted in the highest levels of ascorbic acid, flavonoids, and total phenolics among all light-only conditions, indicating a strong overall antioxidant potential. Similar observations have been reported for Chinese kale sprouts and pea seedlings [33,34]. Blue light can upregulate the expression of transcription factors *HY5* and

MYB via the cryptochrome (CRY) signaling pathway, thereby specifically activating the gene transcription of key enzymes such as *PAL*, *CHS*, and *4CL* in the phenylpropanoid metabolic pathway, promoting the massive accumulation of flavonoids and phenolic acids [11,12,35]. Moreover, blue light stimulates ascorbic acid biosynthesis by inactivating the PAS/LOV photoreceptor that normally suppresses GDP-L-galactose phosphorylase [36].

Combined treatment with light and selenium further increased the levels of anthocyanins, ascorbic acid, flavonoids, total phenolics, and FRAP. Selenite metabolism can induce moderate oxidative stress [37], which was confirmed to trigger the defensive accumulation of secondary metabolites and upregulate the synthesis of phenolic and flavonoid compounds [38]. Concurrently, as an active site component of glutathione peroxidase (GPx), selenium helps maintain cellular redox homeostasis, providing a favorable biochemical environment for the cyclic regeneration of ascorbic acid [39]. Correlation analysis revealed positive correlations between anthocyanins and flavonoids (0.89), total phenolics (0.81), and FRAP (0.96), as well as between ascorbic acid and total phenolics (0.85). This indicates a high degree of coordination in the overall response of the antioxidant system, suggesting that antioxidant compounds may share a common metabolic basis under the combined regulation of light quality and selenium.

4.3. Role of Light Quality and Selenium in Modulating Glucosinolates Content

Glucosinolates are an important class of secondary metabolites in cruciferous vegetables, directly influencing the aroma and flavor of the edible parts [1], and playing a key role in plant biodefense and anticancer activity [40]. Their types and content are comprehensively regulated by various factors, including light quality and mineral elements [41]. In this study, four indolic and three aliphatic glucosinolates were detected in mustard sprouts, with aliphatic glucosinolates accounting for the vast majority of the total. Furthermore, the trends in total aliphatic glucosinolates and total glucosinolates content were highly consistent across all treatments. Among the different light qualities, blue light significantly elevated both indolic glucosinolates (e.g., 4-methoxyglucobrassicin and neoglucobrassicin) and aliphatic ones (e.g., sinigrin and gluconapin). Consequently, total glucosinolate content was highest under blue light and lowest under red light, which aligns with earlier reports that blue light promotes glucosinolate accumulation in broccoli sprouts and pakchoi [13,14]. Blue light differentially regulates the accumulation of various types of glucosinolates, which may be related to the selective regulation of downstream metabolic pathways by light signals [8,42,43]. Although the accumulation patterns of individual glucosinolate classes (e.g., aliphatic vs. indolic) vary with light quality, and specific combinations can be selected according to the target compound.

The effect of selenium on glucosinolate synthesis is closely related to its regulation of sulfur metabolism; by promoting sulfur absorption and transport, it provides a supply of sulfur precursors for glucosinolate synthesis [18]. Selenocysteine methyltransferase (BoSMT) significantly increases the content of glucosinolates in broccoli by catalyzing the synthesis of Se-methylselenocysteine (SeMSC) [19]. Furthermore, appropriate selenium treatment effectively increases the content of aliphatic and total glucosinolates in Chinese cabbage, while significantly reducing the content of indolic glucosinolates [44]. This study indicates that the promoting effect of selenium on glucosinolates depends on specific light quality. SeR and SeW treatments significantly promoted the accumulation of aliphatic and total glucosinolates, while SeB treatment had no significant effect on total glucosinolates content. This may be because blue light treatment had already induced the glucosinolate synthesis flux to a high level, and sulfur allocation in this pathway tended toward saturation. Therefore, there was no significant increase in total content after selenium treatment.

Notably, the content of indolic glucosinolates (especially 4-methoxyglucobrassicin and neoglucobrassicin) in the SeB treatment increased further compared to the B treatment.

Linoleic acid is a direct precursor of α -linolenic acid, which is converted into jasmonic acid (JA) via the LOX-AOS-AOC enzymatic cascade [45–47], and JA signaling has been shown to induce the accumulation of indolic glucosinolates in Brassicaceae plants [48], suggesting that the activation of lipid signaling pathways may be one of the potential upstream mechanisms by which light quality and selenium jointly regulate the accumulation of indolic glucosinolates. However, this hypothesis is derived from metabolomic data and literature, and awaits direct experimental validation.

The increased selenium accumulation in Se-treated sprouts calls for a balanced consideration of nutritional benefit and dietary safety. Although the observed Se levels (approximately 17–18 $\mu\text{g/g}$ DW) are far below the acceptable daily intake limits for adults (255 $\mu\text{g/day}$) when consumed in moderate portions, the safety margin may vary depending on Se speciation and individual consumption patterns [49]. We therefore suggest that future biofortification efforts should not only focus on enhancing Se content but also include routine safety assessments, such as speciation analysis and risk–benefit evaluation, to guide safe consumption recommendations.

5. Conclusions

Our findings reveal that light qualities, both individually and in combination with selenium treatment, have distinct effects on the morphology biomass and the accumulation of health-promoting compounds in mustard sprouts. The red light + selenium treatment may be suitable when biomass is the primary target, the white light + selenium treatment when pigment and total glucosinolate accumulation are prioritized, and the blue light + selenium treatment when selenium enrichment and antioxidant-related compounds are the main objective. The combined use of different light qualities and exogenous selenium can specifically enhance yield or quality, offering flexible strategies for mustard sprout production under the tested conditions. Further evaluation of selenium safety, sensory quality, and treatment costs would be needed before commercial application.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12070866/s1>, Figure S1: Spectrogram of white light, red light and blue light; Table S1: Spectral composition of white light, red light, and blue light; Table S2: Correlations among nutritional components and antioxidant capacity in mustard sprouts; Table S3: Estimated variance component ratios for growth indices, health-promoting compounds, and antioxidant capacity.

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