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Mutual Effects of Zinc Concentration and Ratio of Red-Blue Light on Growth and Nutritional Quality of Flowering Chinese Cabbage Sprouts

Xiaopeng Li, Kaizhe Liu , Xinyang He, Yongkang Chen, Jiehui Tan, Haozhao Jiang , Rui He, Yamin Li and Houcheng Liu *

College of Horticulture, South China Agricultural University, Guangzhou 510642, China

* Correspondence: liuhch@scau.edu.cn; Tel.: +86-020-85-280

Abstract: The nutritional quality and biomass of various sprouts can be enhanced by Zn and red-blue light, especially the Brassica sprouts. However, the combined effects of this two on sprouts are rarely reported. In this study, different Zn concentrations (0, 1.74, 3.48, 10.43 and 17.39 mM) were combined with two ratios of red-blue light-emitting diodes (LEDs) (R: B = 1:2, 1R2B; R: B = 2:1, 2R1B, at 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, 14 h/10 h, light/dark) to investigate their mutual effects on the growth, mineral elements, and nutritional quality in flowering Chinese cabbage sprouts (FCCS). Fresh weight, dry weight, contents of organic Zn, soluble sugar, vitamin C, total flavonoids, total polyphenol, FRAP (ferric ion-reducing antioxidant power) and DPPH (radical inhibition percentage of 1,1-diphenyl-2-picrylhydrazyl) were significantly increased by Zn supplement (10.43 and 17.39 mM) and 2R1B, while hypocotyl length and moisture content were decreased remarkably by Zn supplement. Total glucosinolates contents in the sprouts increased dramatically under 2R1B compared with 1R2B, while photosynthetic pigments contents decreased. Heat map and principal component analysis showed that 2R1B + 17.39 mM Zn was the optimal treatment for the accumulation of biomass and health-promoting compound in FCCS, suggesting that a suitable combination of light quality and Zn supplement might be beneficial to zinc-biofortified FCCS production.



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Keywords: choy sum sprouts; zinc; light quality; biomass; glucosinolates

1. Introduction

Zinc is essential for maintaining the structure and form of protein molecules, and it is fundamental to their function as enzymes or structural proteins. Diverse physiological processes, including cell replication, are impacted by zinc deficiency [1]. It was estimated that 17.3% of the population was at risk of inadequate zinc intake worldwide, specifically the people who lived on grain [2,3]. Zinc is necessary for immune defense systems, growth, intestinal function and brain development [1]. Zinc supplementation appears to be a safe and low-cost way to support the optimal functions of the immune system, with the potential to reduce the risk and consequences of infection, including viral respiratory infections [4]. Zinc supplementation had positive results in acute respiratory infections [5]. Severe pre-existing zinc deficiency may predispose patients to a severe progression of COVID-19 [6]. Four COVID-19 outpatients at the age of 26–63 years were treated with 115–184 mg Zn/day for 10–14 days and all of these patients recovered [7]. In 2019, Acevedo-Murillo and colleagues [8] reported a quicker improvement in the clinical status of pneumonia pediatric patients receiving zinc supplementation. The combination of Zn^{2+} and zinc-ionophores such as pyrithione could increase the intracellular Zn^{2+} concentration and thus inhibit the replication of SARS-coronavirus (SARS-CoV) and equine arteritis virus in cell culture [9]. Nowadays, people may be willing to take over-the-counter supplements, including Zn-rich crops, to boost immunity due to the COVID-19 pandemic.

Zinc plays an irreplaceable role in regulating cell division and proliferation and promoting the growth and development of plants [10]. The biomass contents of Fe, vitamin C, and chlorophyll in two ecotypes (total of eight genotypes) of water spinach were improved by foliar application of Zn [11]. The key genes (*FaCHS*, *FaCHI* and *FaF3H*) in phenylpropanoid and flavonoid biosynthetic pathways in strawberries, which are responsible for the synthesis of ROS quenching compounds such as phenols and flavonoids, could be up-regulated by zinc supplement, and the contents of phenols, flavonoids and vitamin C in strawberry fruit increased by ZnSO₄ (50, 150 and 300 mg L⁻¹) in a drip irrigation system [12]. The content of Zn, bioaccessible Zn and Zn bioaccessibility in edible portions of soybean sprouts could be significantly enhanced by Zn (by 10 and 20 mg L⁻¹) [13]. The glucoraphanin content in broccoli sprouts was improved dramatically by 2 mmol L⁻¹ ZnSO₄ [14]. Thus, it is feasible to produce zinc-biofortified functional sprouts with Brassica vegetables.

Sprouts are the product obtained from seed germination and their development in water or another medium, harvested before the development of true leaves and which is intended to be eaten whole, including the seed [15]. Sprouts have been recognized as a functional food for the prevention of chronic diseases due to their rich phytochemicals, such as glucosinolates, vitamins and minerals [16–18]. The biomass and physiological quality of sprouts were affected by different ratios of red (R) and blue (B) light radiation too. Larger leaves and heavier fresh weight were found in red leaf lettuce when seedlings were grown under mixed R and B (R-B) than monochromatic R [19]. Higher B light (R20:B80) increased the contents of minerals and vitamins in Brassica microgreens compared with higher R light (R80:B20) [20]. Higher total desulfoglucosinolate content was observed in R compared with B (90 µmol m⁻² s⁻¹), whereas B was more effective in increasing phenolic compounds in the *Brassica juncea* sprouts [21]. The shoot lengths and fresh weights of R light-irradiated canola sprouts were higher than those exposed to W (white), B, and B + R (50 µmol m⁻² s⁻¹) [22], and the increase in accumulation of glucosinolates and phenolics was proved more effective under B light. The growth and phytochemicals of sprouts could also be prominently influenced by light intensity. In total, 50 µmol m⁻² s⁻¹ (PPFD, Photosynthetic Photon Flux Density) R: G (Green): B = 1:1:1 was found to be the optimal light intensity to enhance the growth of broccoli microgreens, while 70 µmol m⁻² s⁻¹ was more feasible to improve the phytochemicals [23].

Flowering Chinese cabbage (Choy sum) (*Brassica campestris* ssp. *chinesis* var. *utilis* Tsen et Lee.) is a leafy vegetable originally from southern China [24]. Positive effects of Zn supplement on the growth and nutrient quality of sprouts or microgreens have been verified [13,14,25], but long-term (more than 12 h) soaking [25] or spraying low concentration of foliar fertilizer Zn [13,14] is time-consuming and laborious. Meanwhile, R-B light has been used to improve the physiological qualities of sprouts [20]. However, few about the mutual effects of R-B light and Zn solution on the growth and physiology of sprouts have been reported. The contents of carotenoids, soluble protein, soluble sugar, vitamin C, total flavonoids, total polyphenol, total glucosinolates and organic Se in broccoli sprouts were dramatically improved through a combination of Se and light quality [26]. On this basis, in this study, seeds of flowering Chinese cabbage were soaked for a short time and then combined with R-B light to explore the effects of light and Zn on the growth, nutritional properties and Zn biofortification of the sprouts.

2. Materials and Methods

2.1. Plant Materials and Cultivation Conditions

The experiment was carried out in an artificial light plant factory at South China Agricultural University. Seeds of flowering Chinese cabbage were sterilized with 2% NaClO. Five copies of the seeds were soaked in the solution of ZnSO₄ (0, 1.74, 3.48, 10.43 and 17.39 mM) for 5 h. The seeds germinated and grown on filter paper (11 cm in diameter), which was on nonwoven fabric in plastic trays (32.5 × 24 × 4.5 cm) in growth chamber (25 ± 1 °C with 70 ± 10% relative humidity, 500 ± 100 µmol mol⁻¹ CO₂). After 7 days

growth in the dark, flowering Chinese cabbage sprouts (FCCS) were grown under two ratios of LEDs (Figure 1) (1R2B: R: B = 1:2, 2R1B: R: B = 2:1; R 660 ± 10 nm, B 440 ± 10 nm; $70 \pm 1 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD; light/dark = 14 h/10 h) for 5 days before harvest. The harvested sprouts were frozen, freeze-dried, milled and then stored at -80°C for further analysis. Three replicates were taken for analysis in each treatment, two filter papers for each replicate and 2.5 g (750 ± 10 seeds) on each filter paper.

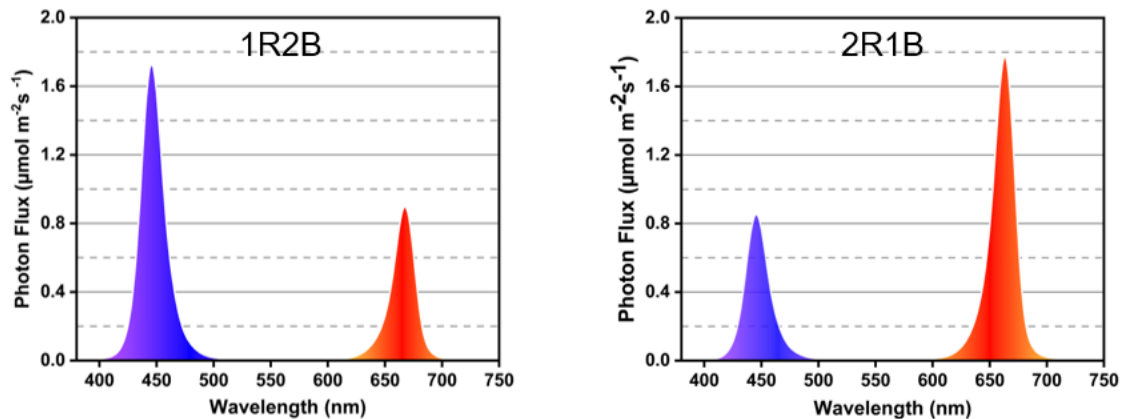


Figure 1. Photon flux density in the ten treatments. The spectral distribution was measured by a spectrometer (ALP-01, Asensetek, Taiwan).

2.2. Biometric Measurements

Fresh weight (FW), dry weight (DW) and sprouts' hypocotyl length (HL) were determined by an electronic analytical balance and a rule, respectively. Fifteen sprouts were randomly sampled from each treatment to obtain HL (1×15). Fresh sprouts were weighed to obtain the FW, then dried at 75°C for 72 h to obtain the DW and then to calculate the moisture content (MC). Ten sprouts were randomly sampled from each measurement, repeated eight times each treatment (10×8) to obtain FW and DW. Moisture content (%) = $(FW - DW)/FW \times 100\%$.

2.3. Pigment Content Determination

In total, 0.2 g of fresh sprout samples were dipped in 8 mL 95% ethanol (*v/v*) until turned white after 24 h in darkness. The absorbance of the extract solution was detected by the UV-spectrophotometer (UV-s) (Shimadzu UV-16A, Shimadzu, Corporation, Kyoto, Japan) at 649 nm (A649), 665 nm (A665) and 470 nm (A470). Concentrations of chlorophyll and carotenoid were calculated using the following formula [27]:

$$\text{Chlorophyll a (Chl a)} (\mu\text{g L}^{-1}) = 13.36 \times A_{665} - 5.19 \times A_{649}$$

$$\text{Chlorophyll b (Chl b)} (\mu\text{g L}^{-1}) = 27.43 \times A_{649} - 8.12 \times A_{665}$$

$$\text{Total Chlorophyll (Chl)} (\mu\text{g L}^{-1}) = 5.24 \times A_{665} + 22.24 \times A_{649}$$

$$\text{Carotenoids (Car)} (\mu\text{g L}^{-1}) = (1000 \times A_{470} - 2.13 \times \text{Chl a} - 97.64 \times \text{Chl b}) / 209$$

$$\text{Photosynthetic pigment (mg g}^{-1}\text{)} = \text{Photosynthetic pigment } (\mu\text{g L}^{-1}) \times 8 \text{ mL} \times 10^{-3} / 0.2 \text{ g}$$

All treatments were repeated three times.

2.4. Phytochemical Determination

Soluble sugar (SS) content was determined according to the anthrone-sulfuric acid colorimetry method [28]. Fresh sprout samples were extracted three times with 80% ethanol (8 mL for the first time and 2.5 mL for the other two times). The supernatants were added with activated carbon, then filtered after heating in a water bath at 80°C for 30 min. After that, the mixture was volume-controlled with 80% ethanol. In total, 0.2 mL supernatant, 0.8 mL ultrapure water (UPW) and 5 mL sulfuric acid anthrone reagent were blended, then bathed in boiling water for 10 min. At last, detected absorbance using the UV-s at 625 nm.

Total soluble protein (SP) content in sprouts was performed using the Coomassie brilliant blue G-250 dye method [29]. Samples were homogenized in 10 mL UPW. The

homogenate was centrifuged (3000 g). Then, 0.2 mL supernatant, 0.8 mL UPW and 5 mL Coomassie brilliant blue G–250 solution were combined. After 5 min, the SP content was determined by UV-s (595 nm).

Vitamin C (Vc) was performed by molybdenum blue spectrophotometry [30]. The samples were ground into pulp with oxalic acid EDTA solution (6.3 g L^{-1}). In total, 5 mL extracted solution was mixed with 0.5 mL metaphosphate-acetic acid, 1 mL vitriol (5%) and 2 mL ammonium molybdate solution (5%) (*w/v*). The absorbance of the mixed solution was read by UV-s (705 nm) 15 min later.

The samples were extracted with absolute ethanol, then centrifuged at 3000 g for 15 min. The supernatant was used for total phenolic compound (TP), total flavonoid (TF), eliminating rate of 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) and ferric ion-reducing antioxidant power (FRAP) determination.

Total phenolic compound (TP) content was detected with the method of Vernon [31] with slight modifications. In total, 1 mL extract was mixed with 0.5 mL foline-phenol (diluted twice), 1.5 mL 26.7% Na_2CO_3 (*w/v*) and 7 mL UPW for 2 h in darkness. Then determined by UV-s (760 nm).

Total flavonoid (TF) content was performed by the method of aluminum nitrate [32]. Sample solution was mixed with 5% NaNO_2 (*w/v*). After 5 min, 10% $\text{Al}(\text{NO}_3)_3$ (*w/v*) was added to the reaction solution, then added to 5% NaOH (*w/v*) 6 min later. After 10 min, the mixed solution was determined by UV-s (510 nm).

The measurement of the DPPH eliminating rate was according to the method by Musa et al. [33]. In total, $0.2 \mu\text{mol L}^{-1}$ DPPH solution and ethyl ethanol were added to extract solutions, then the absorbance A_i and A_j were detected (517 nm), respectively. The absorbance A_c was determined also at 517 nm by a mixture of ethyl ethanol and DPPH solution. ER of DPPH (Eliminating rate of DPPH free radical) was then calculated as follows: $\text{ER of DPPH (\%)} = [1 - (A_i - A_j) / A_c] \times 100\%$.

The FRAP determination was based on Benzie and Strain [34]. The supernatant was mixed with the FRAP reagent, and the mixture was heated in a water bath at 37°C for 10 min. The FRAP reagent was prepared by mixing 20 mM FeCl_3 solution, 10 mM TPTZ (2,4,6-tripyridyl-S-triazine in 40 mM HCl) and 300 mM acetate buffer (pH 3.6) in the proportion of 1:1:10 (*v: v: v*). The absorbance was then measured by the UV-s (593 nm).

Extraction and analysis of glucosinolates (GLs) were as follows description with minor modifications [35]. Freeze-dried samples were extracted with 4 mL methanol (70%) in a water bath at 75°C for 20 min, which was shaken every 10 min. Then barium acetate (0.4 mol L^{-1}) was added, and the mixture was centrifuged (4000 g) for 10 min, repeated twice more. All supernatants were then loaded onto a mini-column containing DEAE–Sephadex A–25 that had been conditioned with acetic acid and washed with imidazole formate. The column was washed with sodium acetate buffer after loading. The preparation was incubated overnight after the sulfatase solution was added. The sample solution was dissolved in UPW and filtered through a membrane filter ($0.22 \mu\text{m}$). HPLC analysis was performed with the liquid chromatograph (Waters e2695) and then controlled by software Waters Empower, $3.20 \mu\text{L}$ of sample solution were isolated on a reversed-phase C18 column. The mobile phase included acetonitrile (A) and distilled water (B), and the flow rate was set at 1.0 mL min^{-1} . Chromatograms were detected at 229 nm. The GLs were determined by their retention times and spectral data as compared by standards.

The contents of zinc (Zn), calcium (Ca), magnesium (Mg) and iron (Fe) in FCCS were determined by the atomic absorption spectrophotometry method [36].

All measurements were repeated three times.

2.5. Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) and interactive analysis by SPSS 26.0 (SPSS Inc., Chicago, IL, USA). Comparison of means was performed by Duncan's test ($p \leq 0.05$). Line charts and bar graphs were constructed with Origin 2018 software (Origin Lab, Northampton, MA, USA). The heat map was drawn by tBtools (<https://github.com/tBtools/tBtools>).

[//github.com/CJ-Chen/TBtools](https://github.com/CJ-Chen/TBtools), accessed on 20 August 2022). Multivariate principal component analysis (PCA) was carried out by XLSTAT 2019 software (Addinsoft, New York, NY, USA).

3. Results

3.1. Sprouts Growth

The growth of FCCS significantly affected by Zn concentration and R-B ratio (Table 1). The DW and FW of the sprouts under 2R1B were prominently higher than those under 1R2B, while with lower MC. The HL and MC of FCCS were significantly depressed by 10.43 mM and 17.39 mM Zn; nonetheless, the biomass was promoted remarkably. Compared with 0 mM Zn, HL and MC decreased by 20.5% and 1.3% under 1R2B 22.8% and 1.4% under 2R1B by supplement of 17.39 mM Zn, respectively. FW and DW in 1R2B + Zn 17.39 increased dramatically by 11.0% and 28.1% compared to 1R2B + Zn 0, respectively. The maximum increase of FW and DW was 17.3% (under 2R1B + Zn 10.43) and 30.7% (under 2R1B + Zn 17.39), which were 7.8% and 12.2% higher than 1R2B + Zn 17.39, respectively. There was significant interaction in the fresh weight of FCCS between light quality and Zn concentration.

Table 1. Growth of flowering Chinese cabbage sprouts (FCCS) under combination of Zn concentration and R-B ratio.

Treatment	R-B Ratio	Zn Concentration (mM)	Hypocotyl Length (cm)	Fresh Weight (mg/plant)	Dry Weight (mg/plant)	Moisture Content (%)
1R2B +Zn 0	1R2B	0	2.92 ± 0.41 a	29.50 ± 0.87 b	1.60 ± 0.05 d	94.81 ± 0.17 a
1R2B +Zn 1.74		1.74	2.78 ± 0.21 ab	29.38 ± 0.80 b	1.64 ± 0.05 cd	94.54 ± 0.12 ab
1R2B +Zn 3.48		3.48	2.67 ± 0.33 b	30.88 ± 0.44 ab	1.73 ± 0.04 c	94.38 ± 0.09 b
1R2B +Zn 10.43		10.43	2.44 ± 0.21 c	31.50 ± 0.50 a	1.87 ± 0.04 b	93.95 ± 0.15 c
1R2B +Zn 17.39		17.39	2.32 ± 0.27 c	32.75 ± 0.45 a	2.05 ± 0.04 a	93.58 ± 0.16 c
2R1B +Zn 0	2R1B	0	2.89 ± 0.08 a	30.10 ± 0.55 b	1.76 ± 0.05 b	94.14 ± 0.15 a
2R1B +Zn 1.74		1.74	2.86 ± 0.07 a	31.10 ± 0.85 b	1.82 ± 0.06 b	94.14 ± 0.10 a
2R1B +Zn 3.48		3.48	2.78 ± 0.08 a	31.90 ± 1.64 b	1.89 ± 0.05 b	94.09 ± 0.09 a
2R1B +Zn 10.43		10.43	2.47 ± 0.05 b	35.30 ± 5.84 a	2.28 ± 0.07 a	93.53 ± 0.14 b
2R1B +Zn 17.39		17.39	2.23 ± 0.05 c	32.40 ± 3.42 b	2.30 ± 0.07 a	92.86 ± 0.14 c
Significance						
L			NS	**	***	***
Zn			***	***	***	***
Zn*L			NS	*	NS	NS

All means in the table are expressed as mean ± standard error. Different letters in the same column indicate significant differences under Zn treatments by Duncan's multiple rang test ($p \leq 0.05$). NS, *, **, *** represent non-significant or significant at $p \leq 0.05$, 0.01 and 0.001 according to interactive analysis, respectively. R-B ratio means ratios of red and blue light. Zn = ZnSO₄·7H₂O solution, L = light quality.

3.2. The Mineral Element Content

Immersing seeds in Zn solution resulted in significant differences in mineral element content in FCCS (Table 2). With increasing Zn concentration, zinc content in the sprouts increased dramatically. The maximum zinc content was found in treatments of 17.39 mM Zn and was 1153.3% (under 1R2B) and 1142.9% (under 2R1B) higher than 1R2B + Zn 0 and 2R1B + Zn 0, respectively. Whereas with Zn concentration over 17.39 mM or 3.48 mM, a significant reduction in the contents of Ca, Mg and Fe emerged. The contents of Zn, Fe, Ca and Mg under 1R2B were higher than 2R1B (1.2%, 3.5%, 4.0% and 6.9% higher, respectively.). There was significant interaction in the Zn content of FCCS between light quality and Zn concentration.

Table 2. The content of mineral elements of flowering Chinese cabbage sprouts (FCCS) under combination of Zn concentration and R-B ratio.

Treatment	R-B Ratio	Zn Concentration (mM)	Zn (g kg ⁻¹ DW)	Ca (g kg ⁻¹ DW)	Mg (g kg ⁻¹ DW)	Fe (g kg ⁻¹ DW)
1R2B +Zn 0	1R2B	0	0.15 ± 0.00 e	11.90 ± 0.11 a	4.61 ± 0.05 a	0.14 ± 0.00 a
1R2B +Zn 1.74		1.74	0.33 ± 0.00 d	12.24 ± 0.27 a	4.73 ± 0.04 a	0.13 ± 0.00 ab
1R2B +Zn 3.48		3.48	0.58 ± 0.01 c	12.34 ± 0.07 a	4.37 ± 0.04 b	0.12 ± 0.00 b
1R2B +Zn 10.43		10.43	1.35 ± 0.04 b	12.11 ± 0.24 a	4.07 ± 0.04 c	0.11 ± 0.00 c
1R2B +Zn 17.39		17.39	1.88 ± 0.01 a	11.04 ± 0.20 b	3.88 ± 0.04 d	0.10 ± 0.00 c
2R1B +Zn 0	2R1B	0	0.14 ± 0.00 e	12.07 ± 0.54 a	4.24 ± 0.13 a	0.13 ± 0.00 a
2R1B +Zn 1.74		1.74	0.34 ± 0.01 d	11.88 ± 0.14 a	4.02 ± 0.26 ab	0.13 ± 0.00 ab
2R1B +Zn 3.48		3.48	0.58 ± 0.01 c	11.66 ± 0.13 a	3.83 ± 0.17 ab	0.12 ± 0.00 b
2R1B +Zn 10.43		10.43	1.43 ± 0.01 b	11.32 ± 0.13 ab	3.60 ± 0.18 b	0.11 ± 0.00 c
2R1B +Zn 17.39		17.39	1.74 ± 0.02 a	10.41 ± 0.37 b	3.63 ± 0.01 b	0.10 ± 0.00 d
Significance						
L			NS	**	***	*
Zn			***	***	***	***
Zn*L			**	NS	NS	NS

All means in the table are expressed as mean ± standard error. Different letters in the same column indicate significant differences under Zn treatments by Duncan's multiple rang test ($p \leq 0.05$). NS, *, **, *** represent non-significant or significant at $p \leq 0.05$, 0.01 and 0.001 according to interactive analysis, respectively. R-B ratio means ratios of red and blue light. Zn = ZnSO₄·7H₂O solution, L = light quality.

3.3. Pigment Content

Pigment content in FCCS was significantly affected by Zn concentration and R-B ratio (Figure 2 and Table 3). Compared with the treatment without Zn supplement, the contents of Chl a, Chl b, and Chl in the sprouts were distinctly promoted by 17.39 mM Zn under 1R2B (increased by 9.1%, 14.3% and 9.7%, respectively), while did not change significantly under 2R1B (Figure 2A,B,D). These pigment contents in sprouts under 2R1B obtained the largest reductions (by 16.7%, 16.1% and 15.6%, respectively) at 3.48 mM Zn supplement. Carotenoid (Car) content was detected to decrease in treatments of 1.74 mM Zn (under both R-B ratios) and 3.48 mM Zn (under 2R1B) (Figure 2C).

Table 3. The interaction effects on pigment content in flowering Chinese cabbage sprouts (FCCS).

Interaction	Chl a	Chl b	Car	Chl	Chl a/b	Chl/Car
L	***	***	***	***	NS	***
Zn	***	***	***	***	NS	*
L*Zn	**	NS	**	*	NS	***

NS, *, **, *** represent non-significant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively, according to interactive analysis. Zn = ZnSO₄·7H₂O solution, L = light quality.

Compared with 0 mM Zn, Chl a/b had no significant increase under 1R2B, however, Chl a/b did have a dramatic increase at 10.43 mM and 17.39 mM Zn (Figure 2E) under 2R1B. Under 1R2B, the minimum and maximum ratio of Chl/Car appeared in treatments of 3.48 mM and 17.39 mM Zn, while the maximum ratio of Chl/Car showed at 3.48 mM Zn under 2R1B (Figure 2F).

The contents of Chl a, Chl b and Car in the sprouts under 1R2B were dramatically higher than those under 2R1B (Figure 2A–C and Table 3). However, the maximum Chl a/b and Chl/Car were both registered under 2R1B (Figure 2E,F).

Meanwhile, the contents of Chl a, Chl, Car and Chl/Car were affected interactively by light quality and Zn concentration (Table 3).

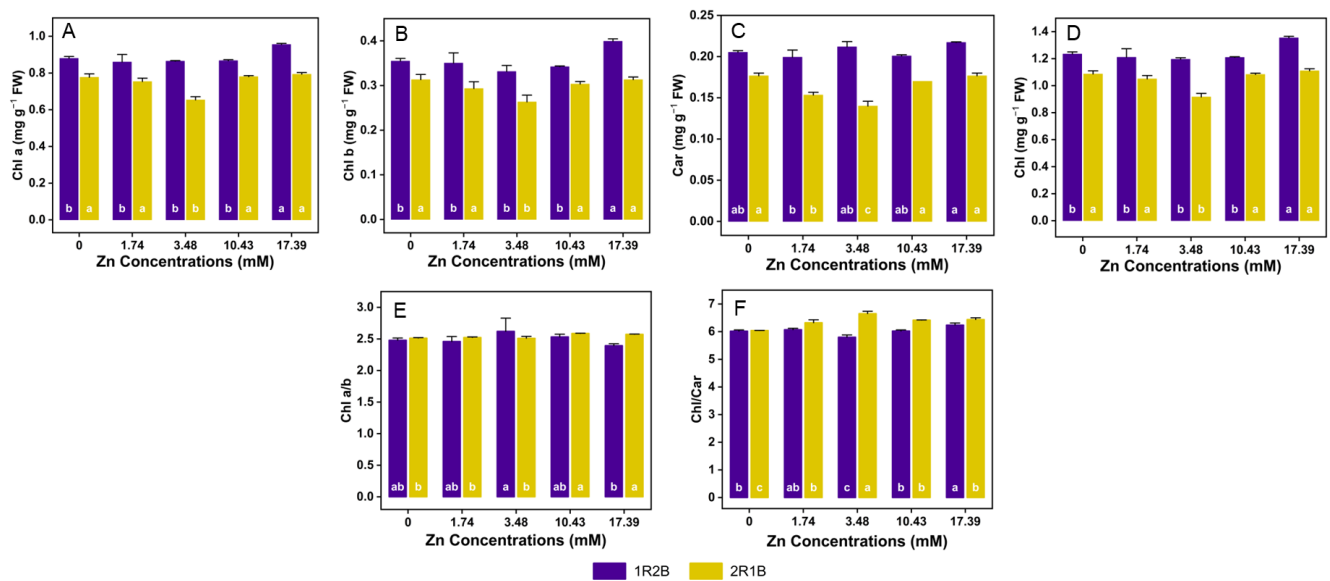


Figure 2. The contents of Chl a (A), Chl b (B), Car (C) and Chl (D), Chl a/b (E) and Chl/Car (F) in flowering Chinese cabbage (FCCS) sprouts grown under combination of Zn concentration and R-B ratio (ratios of red and blue light). The letters marked in all figures indicate the significance of the difference under Zn treatments ($p \leq 0.05$, Duncan's test). Chl a, Chl b, Chl, Car, Chl a/b and Chl/Car represent chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, ratio of chlorophyll a to chlorophyll b and total chlorophyll to carotenoids, respectively.

3.4. The Contents of Soluble Protein (SP), Soluble Sugar (SS) and Vitamin C (Vc)

Zn fertigation incurred distinct impacts on the contents of SP, SS and Vc in FCCS (Figure 3). At 3.48 mM Zn, the SP content in the sprouts reduced remarkably by 12.8% under 1R2B but increased significantly by 7.3% in 1.74 mM Zn under 2R1B (Figure 3A). With increasing Zn concentration, the SS content increased both under 1R2B and 2R1B (Figure 3B). The highest SS content under 1R2B and 2R1B were at 17.39 mM Zn, which increased by 1.42-fold and 1.31-fold compared with 0 mM Zn, respectively. The Vc content at 17.39 mM Zn was considerably higher than other Zn treatments under both 1R2B and 2R1B (up to 17.6% and 18.2% than 0 mM Zn, respectively.) (Figure 3C). The highest Vc content occurred at 1R2B + Zn 17.39.

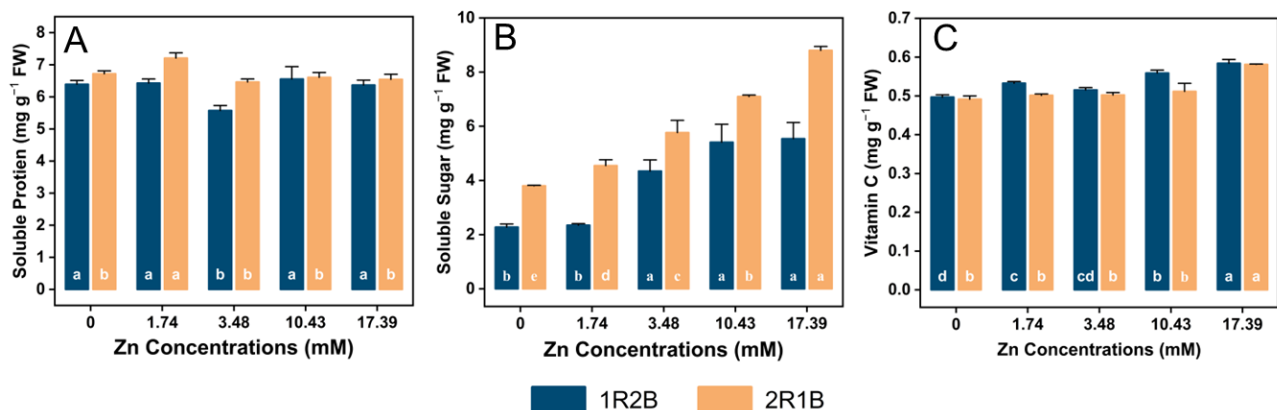


Figure 3. The contents of SP (soluble protein) (A), SS (soluble sugar) (B) and Vc (vitamin C) (C) of flowering Chinese cabbage sprouts (FCCS) grown combination of Zn concentration and R-B ratio (ratios of red and blue light). The letters marked in all figures indicate the significant difference under Zn treatments ($p \leq 0.05$, Duncan's test).

In FCCS under 2R1B, higher contents of SS and SP while a lower Vc content were detected (Figure 3A,B and Table 4). Light quality and Zn concentrations led to significant and independent differences in the contents of SS, SP and Vc (Table 4).

Table 4. The interaction effects of SP (soluble protein), SS (soluble sugar) and Vc (vitamin C) in flowering Chinese cabbage sprouts (FCCS).

Interaction	SP	SS	Vc
L	***	***	***
Zn	**	***	***
L*Zn	NS	NS	NS

NS, *, **, *** represent non-significant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively, according to interactive analysis. Zn = ZnSO₄·7H₂O solution, L = light quality.

3.5. Antioxidants Contents and Activities in Flowering Chinese Cabbage Sprouts (FCCS)

The radical inhibition percentage of DPPH, FRAP and contents TP and TF in FCCS were promoted by high concentrations of Zn (10.43 mM and 17.39 mM Zn). (Figure 4). DPPH was prominently increased by 12.9% at 10.43 mM Zn (under 1R2B) and increased by 9.1% at 17.39 mM Zn (under 2R1B) compared with 0 Zn, respectively (Figure 4A). FRAP was significantly improved by 39.6% (under 1R2B) and 67.1% (under 2R1B) at 17.39 mM Zn compared with 0 mM Zn, respectively (Figure 4B), which were the largest value under different light quality. The largest increases in TP appeared at 10.43 mM Zn under 1R2B and 17.39 mM Zn under 2R1B (increased by 25.8% and 37.1% compared with 0 mM Zn, respectively) (Figure 4C). The 17.39 mM Zn-treated sprouts had 25.3% and 106.8% higher TF contents than 0 Zn-treated under 1R2B and 2R1B, respectively (Figure 4D).

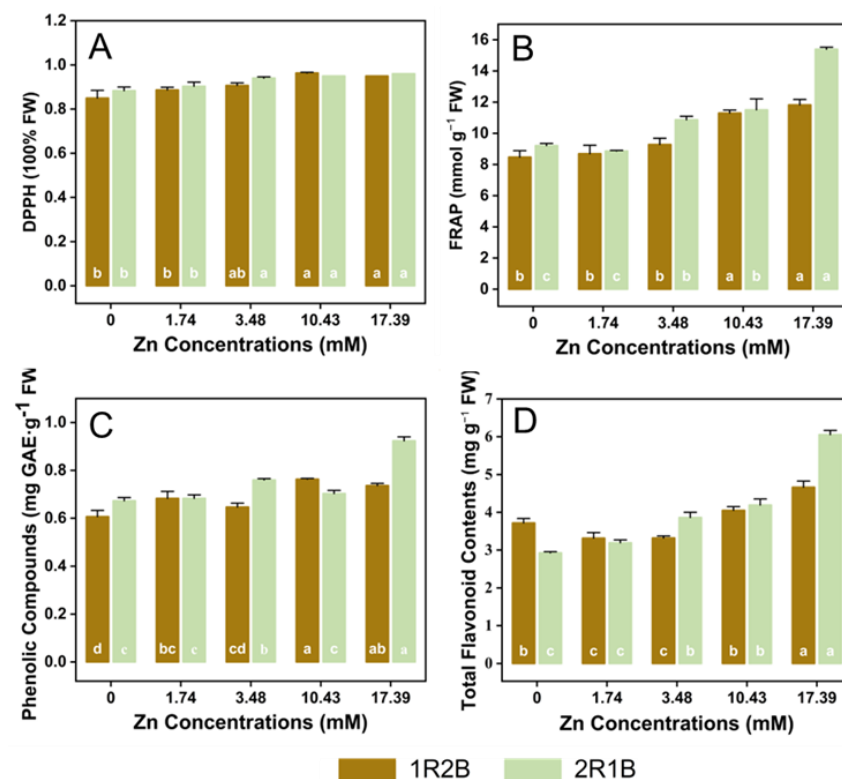


Figure 4. The DPPH (radical inhibition percentage of 1,1-diphenyl-2-picrylhydrazyl) (A), FRAP (ferric ion-reducing antioxidant power) (B) and contents of TP (total phenolic compounds) (C) and TF (total flavonoid) (D) in flowering Chinese cabbage sprouts (FCCS) grown under combination of Zn concentration and R-B ratio (ratios of red and blue light). The letters marked in all figures indicate the significant difference under Zn treatments ($p \leq 0.05$, Duncan's test).

There were no significant influences of light quality on DPPH, while significant impacts on FRAP, TP and TF. (Table 5). Meanwhile, higher FRAP and contents of TP and TF were exhibited under 2R1B than 1R2B. (Figure 4B–D)

Table 5. The interaction effects of DPPH (radical inhibition percentage of 1,1-diphenyl-2-picrylhydrazyl), FRAP (ferric ion-reducing antioxidant power), TP (total phenolic compounds) and TF (total flavonoid) in flowering Chinese cabbage sprouts (FCCS).

Interaction	DPPH	FRAP	TP	TF
L	NS	***	***	**
Zn	***	***	***	***
L*Zn	NS	***	***	***

NS, *, **, *** represent non-significant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively, according to interactive analysis. Zn = ZnSO₄·7H₂O solution, L = light quality.

In general, the antioxidant capacity of FCCS could be prominently improved by 10.43 mM and 17.39 mM Zn under the R-B ratio (Figure 4A–D). FRAP, contents of TP and TF in 2R1B +Zn 17.39 were 30.2%, 21.0% and 30.0% higher than the maximum value under 1R2B, respectively. Except for DPPH, light quality and Zn concentration had significant interaction effects on the contents of TP, TF and FRAP in the sprouts (Figure 4 and Table 5).

3.6. The Glucosinolates (GLs) Contents

There were nine GLs determined in FCCS, which included five aliphatic glucosinolates (AGLs) (PRO, GRA, SIN, GNA and GBN) and four indole glucosinolates (IGLs) (4-HGBS, GBS, 4-MGBS and NGBS) (Figure 5). GNA, GRA and GBN were the predominant GLs in FCCS, accounting for 52.1%, 19.4% and 13.8% of the total GLs content, respectively (Figure 6). The majority synthesis of GLs in FCCS was hampered after a Zn furnishing (Figure 6). However, 10.43 Mm Zn caused a dramatic increase in the GNA contents under two light qualities (by 41.0% under 1R2B and 56.2% under 2R1B, respectively) (Figure 6D).

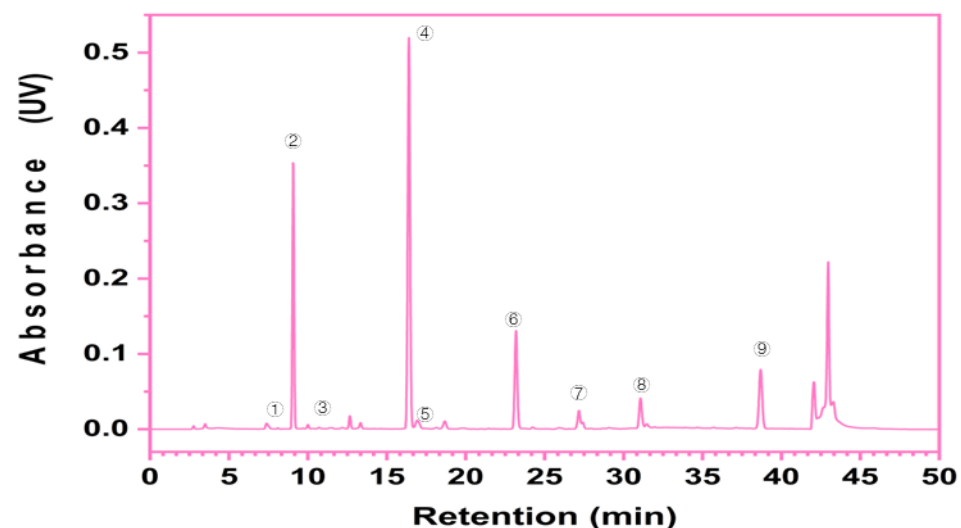


Figure 5. HPLC of identified glucosinolates from flowering Chinese cabbage sprouts (FCCS). ① PRO: Progoitrin; ② GRA: Glucoraphanin; ③ SIN: Sinigrin; ④ GNA: Gluconapin; ⑤ 4-HGBS: 4-Hydroxy-Glucobrassicin; ⑥ GBN: Glucobrassicinapin; ⑦ GBS: Glucobrassicin; ⑧ 4-MGBS: 4-Methoxy-Glucobrassicin; ⑨ NGBS: Neoglucobrassicin; 40–50 min is the time for an elution.

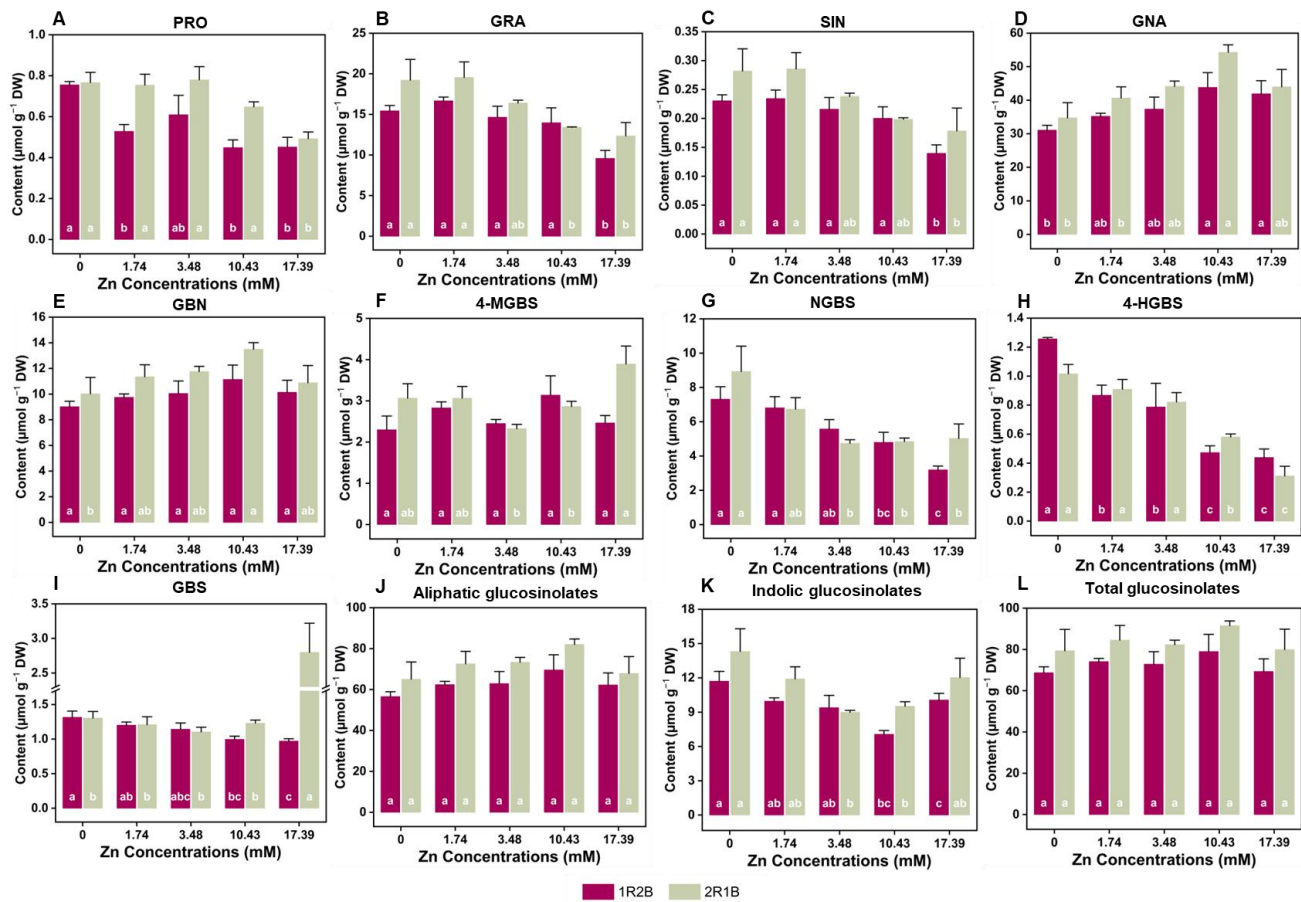


Figure 6. The contents of individual glucosinolate ((A) PRO, (B) GRA, (C) SIN, (D) GNA, (E) GBN, (F) 4-MGBS, (G) NGBS, (H) 4-HGBS, (I) GBS), aliphatic glucosinolates (AGLs) (J), indolic glucosinolates (IGLs) (K) and total glucosinolates content (TGLs) (L) in flowering Chinese cabbage sprouts (FCCS) grown under combination of Zn concentration and R-B ratio (ratios of red and blue light). The letters marked in all figures indicate the significant difference under Zn treatments ($p \leq 0.05$, Duncan's test).

The GBN contents were also improved by 10.43 mM Zn (increased by 23.6% and 34.7% under 1R2B and 2R1B, respectively) but had a dramatic difference only under 2R1B (Figure 6E). There was no significant difference in the contents of PRO, GRA and SIN under both 1R2B and 2R1B (Figure 6A–C). Contents of NGBS and 4-HGBS in the sprout were remarkably reduced by Zn solutions under two light qualities (Figure 6G,H). Distinct rising content of GBS (increased by 114.7%) emerged at 2R1B + Zn 17.39, as an obvious reduction in other concentrations of Zn was observed under both 1R2B and 2R1B (Figure 6I). The 4-MGBS contents had no prominent difference after Zn soaking (Figure 6F). The IGLs contents decreased by Zn treatments, and with the minimum value at 1R2B + Zn 10.43 (reduced by 39.6% compared with 1R2B + Zn 0) (Figure 6K). There was no remarkable influence on the contents of AGLs and TGLs (total glucosinolates) in the sprouts with Zn treatments under the same light quality (Figure 6J,L).

Most kinds of GLs (PRO, GRA, SIN, GNA, GBN, GBS and 4-MGBS) contents were enhanced outstandingly by 2R1B compared with 1R2B, with the exception of 4-HGBS content, which was inhibited by 2R1B (Figure 6 and Table 6). The contents of AGLs and GLs were also increased dramatically by 2R1B compared with 1R2B (Figure 6 and Table 6).

Table 6. The interaction effects of individual glucosinolate contents and total glucosinolate content in flowering Chinese cabbage sprouts (FCCS).

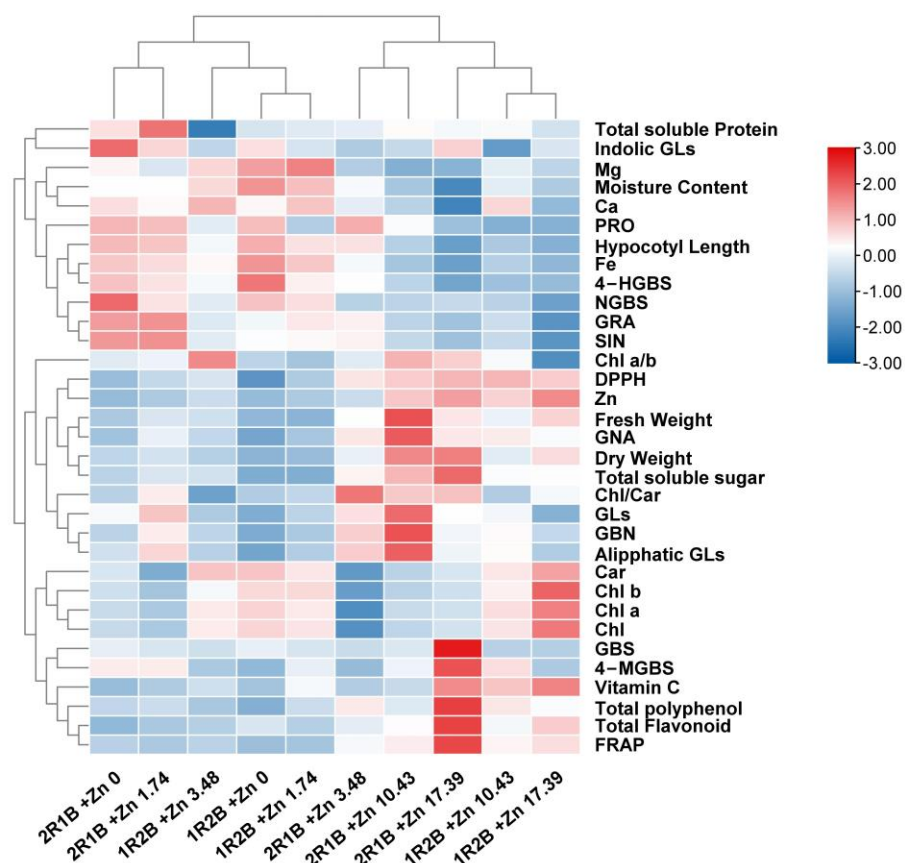
Interaction	PRO	GRA	SIN	GNA	4-HGBS	GBN	GBS	4-MGBS	NGBS	AGLs	IGLs	GLs
L	***	*	*	*	NS	*	***	*	NS	*	NS	*
Zn	***	***	***	**	***	NS	***	NS	***	NS	**	NS
L*Zn	NS	NS	NS	NS	NS	NS	***	*	NS	NS	NS	NS

NS, *, **, *** represent non-significant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively, according to interactive analysis. Zn = $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ solution, L = light quality.

The contents of seven kinds of GLs (PRO, GRA, SIN, GNA, GBN, GBS and 4-MGBS) were affected by light quality significantly, while the contents of seven kinds of GLs (PRO, GRA, SIN, GNA, 4-HGBS and GBS) were affected by Zn supplement. Light quality and Zn had an interactive effect on the contents of GBS and 4-MGBS (Table 6).

3.7. Heatmap Analysis

The heatmap was made to obtain an integrated view of agronomic traits on the nutritional and functional profile of choy sum sprouts in response to light quality and Zn supplementary (Figure 7).

**Figure 7.** Cluster heat map analysis of flowering Chinese cabbage sprouts (FCCS) grown under combination of Zn concentration and R-B ratio (ratios of red and blue light). Results are visualized using a false color scale, with blue and red indicating a decrease and an increase in the response parameters, respectively.

The heatmap displayed that 10.43 mM and 17.39 mM Zn under 1R2B and 2R1B had similar impacts on choy sum sprouts, and they were also classified into the same cluster as 2R1B + Zn 3.48, indicating the similarity of their effects. The organic Zn content, antioxidant characters (TP, TF, FRAP and DPPH), and biomass (fresh and dry weight) were improved, while the contents of Ca, Fe, Mg and five GLs (PRO, 4-HGBS, NGBS, GRA and SIN), as

well as moisture content and hypocotyl length, were inhibited by high Zn concentrations. Thus, higher Zn concentrations (10.43 mM and 17.39 mM Zn) were benefited for improving the antioxidants and biomass of the sprout but had negative influences on hypocotyl elongation and contents of some mineral elements and GLs in choy sum sprout. Pigment contents were influenced by light quality. The 1R2B led to higher contents of Car, Chl a, Chl b, Chl than 2R1B. The other characters of choy sum sprouts did not classify into one final cluster within the same light quality, which might be due to the mutual effects of light quality and Zn concentrations on the growth, mineral elements and nutritional quality. Additionally, the map showed that most phytochemicals under 2R1B were higher than those of 1R2B (Figure 7).

3.8. Principal Component Analysis (PCA)

The prominent overall influences of the combination of Zn and light quality on growth, phytochemical composition and mineral element of choy sum sprouts were evaluated by PCA (Figure 8). In total, 75.95% of the total observed variance with the first two principal components (PCs) was captured by the computed model. PC2 and PC1 explained 49.62% and 26.33%, respectively (Figure 8). Choy sum sprout in the left quadrants (10.43~17.39 mM Zn treatments) and down quadrants (the 2R1B treatment) were characterized by higher biomass and nutritional composition. Zn was prominently correlated with DPPH, FRAP, TP and TF in terms of antioxidant quality, DW and FW, indicating a significant effect of Zn on the antioxidant quality and biomass (DW and FW) of choy sum sprouts. The results also showed a better effect of 2R1B on the morphology and physiology of the sprouts than those of 1R2B, which was verified by the heat map analysis that the contents of most phytochemicals under 1R2B were lower than those under 2R1B.

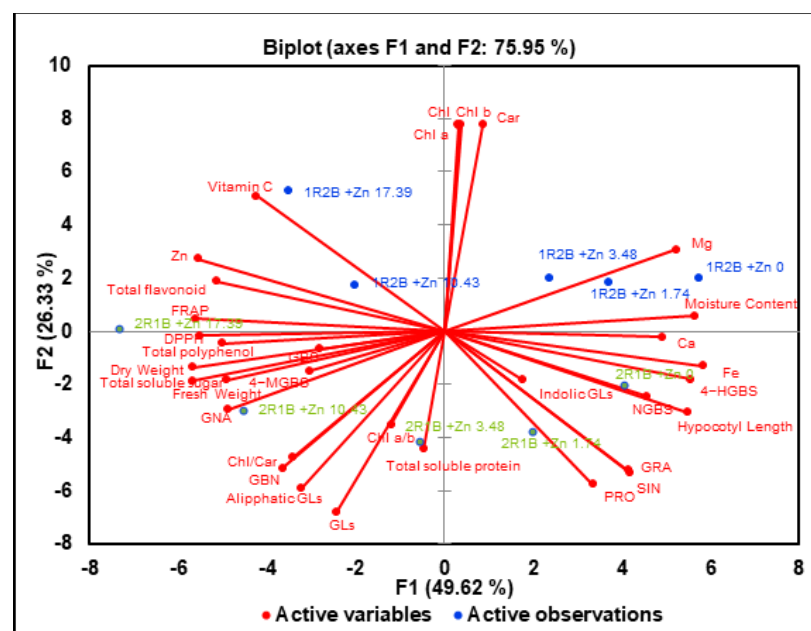


Figure 8. Principal component analysis of flowering Chinese cabbage sprouts (FCCS) grown at combination of Zn concentration and R-B ratio (ratios of red and blue light).

4. Discussion

4.1. Effects of Zn Concentrations on the Growth of Flowering Chinese Cabbage Sprouts (FCCS) under Two Ratios of R-B

Light is one of the most important environmental factors for plants. Light provides photons that can activate discrete developmental pathways to affect plant growth (e.g., leaf area, thickness, stem length) through photoreceptors, including phytochrome and cryptochrome [16]. The lower-yielding capacity of FCCS under 1R2B is presently demonstrated

in comparison to 2R1B. Park et al. [22] proved that R light was more conducive to the growth of sprouting canola, and the hypocotyls length and shoot dry weight of mustard microgreens were significantly higher under R75%: B25% than R25%: B75% ($220 \mu\text{mol m}^{-2} \text{s}^{-1}$, TPDF). In this study, with Zn supplement, compared with 1R2B, DW and FW of the sprouts cultivated under 2R1B significantly increased, and HL also increased at three Zn concentrations (1.74, 3.48 and 10.43 mM) (Table 1). Interestingly, B light significantly stimulated the water content of broccoli sprouts compared with R light [37], which was similar to the higher moisture content in choy sum sprouts under 1R2B (Table 1).

Zinc plays a key role in the structural and catalytic functions of numerous enzymes [38], and it is considered an important micronutrient for plant growth [38,39]. The positive effect of Zn soaking seeds on the growth of sprouts or microgreens (e.g., pea, soybean, arugula) had verified by Yuan et al. [25], Zou et al. [13] and Gioia et al. [40]. In this study, Zn treatments increased dramatically the biomass (FW and DW) of choy sum sprouts but shortened significantly the HL (Table 1). Edible portion increase in HL and FW when grown under $10 \mu\text{g mL}^{-1}$ Zn solution, compared to complete deprivation of Zn supplementation (i.e., irrigated only with distilled water), was demonstrated with soybean sprouts, increased by 16% (HL) and 19% (FW), respectively [13]. However, with increases of Zn concentration in culture solutions from 30 to $100 \mu\text{g mL}^{-1}$, the sprouts showed significant reductions in HL of 17–42% [13]. It suggested the inhibitory effect of concentrated Zn solution on sprouts. Nevertheless, the pea sprouts' fresh weight and plant height were promoted significantly by $10\text{--}60 \text{ mg L}^{-1}$ seeds soaking-Zn solution [25]. Thus, different responses of plant species of sprouts to zinc supplements are different. Zn treatments result in decreasing moisture content in FCCS (Table 1). Similarly, Se ($100 \mu\text{mol L}^{-1} \text{Na}_2\text{SeO}_3$) significantly reduced the moisture content of broccoli sprouts [26], underscoring the negative effect of microelement supplements on the moisture content of sprouts.

4.2. Effects of Zn Concentrations on the Mineral Element Content in Flowering Chinese Cabbage Sprouts (FCCS) under Two Ratios of R-B

Light quality has crucial effects on element contents in plants, especially the monochrome B/R light and their combinations [41–44]. Short-term monochromatic B light or its percentage in different light spectrum compositions have positive effects on the mineral element contents in plants [43,45–47], whereas R light or its higher percentage in R-B lighting increased certain mineral nutrients in marigold [48], lettuce [49] and basil [50]. In the current work, higher contents of Zn, Fe, Mg and Ca are under 1R2B compared with 2R1B (Table 2). These four elements attained comparable content changes found in mustard 'Red Lace' microgreens [51] and einkorn (*Triticum monococcum* L.) seedlings [52] under a higher proportion of B light- combining R-B light, similarly the content of Zn Ca and Mg in broccoli sprouts [26]. Thus, higher proportions of B/R light were more favorable to the accumulation of mineral elements in sprouts.

Seed priming with different minerals is an agronomic treatment within the biofortification method to improve the bio-available mineral concentration in edible plants [3]. Socking seeds with Zn solution has been proven an efficient way for the accumulation of edible Zn in sprouts [3,13,25,40]. Zn content in FCCS increased remarkably by Zn treatment and did not have a peak (Table 2). Sprouting pea-, broccoli- and sunflower-soaked ZnSO_4 solution also continuously accumulated Zn without peak [3,25]. In the presence of Zn (20 mg L^{-1}), contents of Ca and Mg in arugula, red cabbage and red mustard microgreens distinctly increased, while Fe decreased [40]. The uptake of Ca, Mg and Fe in lettuce increased in response to 15 g m^{-2} Zn in field soil [53]. Compared to conventionally cultivated crops, nutrient imbalances and deficiencies were not allowed to be expressed within the short time between sowing and harvest of microgreens because of their short growth cycle, while the absorbed Zn is transported from roots to shoots at these early growth stages [54]. Besides, in the presence of concentrated Zn solution, plants are prone to up-regulation of the expression of Zn transporters and a complex network of Zn detoxification mechanisms to overcome this stress [55]. These led to a sustained surge of Zn contents in FCCS after

Zn treatment. However, 10.43 and 17.39 mM Zn resulted in a dramatic reduction in the contents of Ca, Mg and Fe in the sprouts (Table 2). These negative effects were due to the antagonism effects between Zn and Ca, Mg and Fe [56] (Table 2).

4.3. Effects of Zn Concentrations on the Photosynthetic Pigment Contents in Flowering Chinese Cabbage Sprouts (FCCS) under Two Ratios of R-B

B (425–490 nm) and R light (610–700 nm) were widely considered the most efficient light spectra for plant photosynthesis [57]. Different ratios of B-R light are crucial constituents to the current mode of combining light to explore their influence on photosynthetic pigments' content [58–60]. Wavelengths of R light perfectly fit the absorption peak of chlorophylls and phytochromes, which makes it more effective in improving photosynthesis compared to B light [61]. Nonetheless, B light, which was strongly absorbed by carotenoid pigments (lutein and β -carotene), was reported to increase the chlorophyll content of plant [62]. Non-heading Chinese cabbage seedlings attained higher contents of Chl a, Chl b, Chl and Car under B light than R light ($80 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD for 60 days) [63], and accumulation of Chl was reported in *Brassica juncea* sprouts in response to B light [21], and in this study, the photosynthetic pigment contents in choy sum sprouts under 1R2B were higher than those under 2R1B (Figure 2A–D). The chlorophyll contents of mustard microgreens and chlorophyll and carotenoid contents of kale microgreens did not significantly change, while carotenoid contents of mustard microgreens declined prominently under 75% B: 25% R ($220 \mu\text{mol m}^{-2} \text{s}^{-1}$, TPFD.) compared with 25% B: 75% R [51]. Therefore, pigments' content response to R and B light might be highly species-dependent.

Excessive Zn could inhibit chlorophyll synthesis in tobacco leaves [10]. Zinc treatment (400 mg L^{-1}) declined carotenoid contents in wheat sprouts [38]. However, with a low dose of Zn, the pigments' contents increased. The chlorophyll content of pea sprouts with Zn soaking ($10\text{--}60 \text{ mg L}^{-1}$ Zn) on seed remarkably increased compared to Zn deprivation (0 mg L^{-1}) [25], and chlorophyll content of water spinach could be improved by foliar application of Zn (0.05% (*w/v*) ZnSO_4) [11]. In this study, the contents of Chl a, Chl b, Chl and Car reduced in the 3.48 mM Zn sprouts while increased in the 17.39 mM Zn sprouts (Figure 4A–D and Table 3). Since most kinds of the Brassicaceae are Zn hyperaccumulation plants, these were probably related to heavy zinc tolerance gene (HMA) response to an extremely high concentration of Zn solution [64].

There were different interaction effects of light quality and Zn on the photosynthetic pigment in the sprouts; a higher proportion of B light combined with a higher Zn concentration synergistically promoted the synthesis of photosynthetic pigment in FCCS, while higher proportion of R light combined with lower Zn concentration impacted antagonistically on the photosynthetic pigment in the sprouts (Figure 2A–D and Table 3).

4.4. Effects of Zn Concentrations on the Phytochemical Content in Flowering Chinese Cabbage Sprouts (FCCS) under Two Ratios of R-B

Combining R and B light could improve nutritional metabolites in sprouts (e.g., Vc, TE, TP) [16,65,66]. In FCCS, except for that Vc contents reduced under 2R1B compared with 1R2B, the contents of SP, SS, TP and TE, FRAP improved dramatically under 2R1B (Figures 3 and 4). The contents of SS and SP in non-heading Chinese cabbage under B light were higher than those under R light ($80 \mu\text{mol m}^{-2} \text{s}^{-1}$) [63], but 'Green Oak Leaf' lettuce under R-B had higher SS content compared with monochromatic B or R light [PPF (Photosynthetic Photon Flux): $133 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$] [67]. Synthesis and accumulation of hexose and D-glucose, which were Vc precursors [68], could be enhanced by B light; thus, there was higher Vc content under 1R2B (Figure 3C).

Compared with 25%B: 75%R, flavonols content in 'Red Lace' mustard microgreens did not differ, while promoted dramatically in 'Red Russian' kale microgreens under 75%B: 25%R ($220 \mu\text{mol m}^{-2} \text{s}^{-1}$, TPFD.) [51]. The higher TP and TE accumulation and free radical scavenging capacity were recorded in 2R: 1B ($120 \mu\text{mol m}^{-2} \text{s}^{-1}$) treatment in the green basil microgreens while in 1R: 2B treatment in the red basil microgreens at 17 days after sowing [69]. In this study, higher FRAP and contents of TP and TE were exhibited in choy

sum sprouts under 2R1B than 1R2B (Figure 4). These might be attributed to the positive correlation between antioxidant activity and TF content and their close dependence on light conditions [16] (Figure 8).

Supplemental Zn could not only improve bio-available Zn contents [13,40,70] but also increase phytochemical content in plants [71,72]. The production of SS, SP and Vc, DPPH, TP and total antioxidants in peanut sprouts were promoted by ZnCl_2 during germinating [72]. In the sprouting pea, soaking and spraying Zn ($20\text{--}50\text{ mg L}^{-1}$) significantly increased the contents of SS, SP, Vc and TP, total antioxidative capacity [25]. In this study, a comparable enhancement of SS, SP and Vc contents and antioxidant capacity was found in choy sum sprouts under the Zn supplement (Figures 3 and 4).

Even though the contents of proteins, Vc, TP in durum wheat ('Karim') sprouts increased after Zn soaking, the reducing sugar contents and DPPH were reduced [37]. Meanwhile, the free-radical scavenging activity (FRSA) in broccoli and pea sprouts decreased by Zn ($10\text{--}30\text{ mg L}^{-1}$) [3]. The effects of Zn on the quality of Brassicaceae sprouts might be due to treatment methods or plant species.

Although accumulation of SS, SP and Vc in choy sum sprouts were distinctly affected by Zn and light quality, there was no interaction effect between Zn and light quality (Table 4). There were noticeable synergistic effects between light and Zn on FRAP and the contents of TP and TF (Table 5), the antioxidant in sprouts was improved by the Zn supplement and 2R1B synergistically (Table 5).

4.5. Effects of Zn Concentrations on the Glucosinolates (GLs) Content in Flowering Chinese Cabbage Sprouts (FCCS) under Two Ratios of R-B

GLs are secondary metabolites derived from amino acids, invariably present in Brassicaceae, which have huge health and agricultural benefits [73]. B light was beneficial for GLs accumulation more than R light. The higher concentrations of total aliphatic GLs (AGLs), indolic GLs (IGLs) and total GLs (TGLs) in sprouting broccoli microgreen were found under the 20% B: 80% R light ($250\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$) treatment, more than under 5% B: 85% R [74]. The highest and lowest component of TGLs in broccoli sprouts appeared at 5R5B and 5R0B, which experienced radiation of different light quality (5R0B, 5R1B, 5R3B, 5R5B) ($30\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$) [75]. In research of combined R-B light at different ratios (10:0, 8:2, 5:5, 2:8 and 0:10) ($150\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$), the AGLs content of Chinese kale sprout increased by 100% B light compared with R light [76]. However, higher TGLs contents in seven rapeseed cultivars sprouts were found under high B light (HB: 31.7% B: 66.3% R), but two were found under low B light (LB: 14.8% B: 81.3% R) [77]. Additionally, higher AGLs contents in sprouting broccoli microgreens were under ratios of 8:2 (R-B) than those under 2:8 [76]; in this study, higher GLs contents were found under 2R1B than 1R2B (Figure 6). Lower TGLs contents in canola sprouts were found under B/R ($50\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$) light than monochrome B and R [22]. Thus, monochromatic B light might be more conducive to the accumulation of GLs than monochromatic R light, and an appropriate proportion of B at combining R-B light was more beneficial to the accumulation of GLs.

Application of Zn during plant growth has proved positive in affecting GLs in plants [14,78,79]. Increased GBS and NGBS contents in rape seedlings under ZnCl_2 solution ($1 \times 10^{-4}\text{ M} - 1 \times 10^{-5}\text{ M}$) after cultivating for 2, 4 and 8 days [78]. Sprayed 2 mmol L^{-1} ZnSO_4 solution on broccoli sprouts significantly increased the TGLs contents by 58.2% [14]. In in vitro liquid medium culture 1 mg L^{-1} ZnSO_4 caused an accumulation of TGLs in microshoots of *Nasturtium officinale* (watercress) [79]. In this study, the contents of most GLs in choy sum sprouts decreased in 10.43 and 17.39 mM Zn treatments; there were negative effects of high Zn concentration on GLs in choy sum sprouts (Figure 6). Intriguingly, Zn treatments hampered the accumulation of most kinds of GLs (Figure 6) but not the contents of AGLs and TGLs. These were because that GNA, the highest content of glucosinolate among all kinds of GLs, significantly increased (Figure 6D). This was similar to the contribution of GNA to rapeseed after a ZnCl_2 supplement [80].

5. Conclusions

In this study, the positive effects of Zn supplement and 2R1B on the growth and nutrient quality of flowering Chinese cabbage sprouts were verified. In flowering Chinese cabbage sprouts, biomass (dry weight and fresh weight) and the contents of organic Zn, soluble sugar, vitamin C, total flavonoid, total phenolic compound, FRAP and DPPH were dramatically increased by concentrated Zn supplement and 2R1B, indicated that soaking seeds with high concentration Zn in the short term and higher R light could improve biomass and nutrient quality of sprouts. Glucosinolates in the sprouts were dramatically higher under 2R1B than under 1R2B. The significant interaction effects of light quality and Zn on FRAP, total phenolic compound, total flavonoid, carotenoids, chlorophyll a, organic Zn and fresh weight were found on the sprouts. There was higher biomass and quality of sprouts in 2R1B + Zn 17.39 mM treatment, which might render a suitable combination of light quality and Zn supplements a significant tool for improving the growth and nutrient quality of flowering Chinese cabbage sprouts.

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