

Article

Differential Effects of Low Light Intensity on Broccoli Microgreens Growth and Phytochemicals

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Abstract: To produce high-quality broccoli microgreens, suitable light intensity for growth and phytochemical contents of broccoli microgreens in an artificial light plant factory were studied. Broccoli microgreens were irradiated under different photosynthetic photon flux density (PPFD): 30, 50, 70 and 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with red: green: blue = 1:1:1 light-emitting diodes (LEDs). The broccoli microgreens grown under 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had the highest fresh weight, dry weight, and moisture content, while the phytochemical contents were the lowest. With increasing light intensity, the chlorophyll content increased, whereas the carotenoid content decreased. The contents of soluble protein, soluble sugar, free amino acid, flavonoid, vitamin C, and glucosinolates except for progoitrin in broccoli microgreens were higher under 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Overall, 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was the optimal light intensity for enhancement of growth of broccoli microgreens, while 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was more feasible for improving the phytochemicals of broccoli microgreens in an artificial light plant factory.

Keywords: light intensity; broccoli microgreens; growth; phytochemicals



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1. Introduction

Microgreens are a kind of vegetable emerging in recent years for their higher contents of phytochemicals such as vitamin C, carotenoid, and flavonoid, than their mature stages [1]. Among these, broccoli (*Brassica oleracea* L. var. *italica*) microgreens are one of the most popular vegetables for their rich phytochemicals, especially glucosinolates [2,3]. Broccoli exhibited the highest total glucosinolates content in seeds (110.76 $\mu\text{mol}\cdot\text{g}^{-1}$) and sprouts (162.19 $\mu\text{mol}\cdot\text{g}^{-1}$) compared to some *Brassica* species, including cauliflower, cabbage, radish, baemuchae, pakchoi, Chinese cabbage, leaf mustard, and kale [3]. Glucosinolates are classified into aliphatic, indolic, and aromatic glucosinolates according to the side-chain structures. The defensive function of broccoli in many cancers is due to the hydrolysis of glucosinolates, isothiocyanates. Sulforaphane, a product of the hydrolysis of glucoraphanin (GRA), is known to be the most promising anticancer agent. Vitamin C, carotenoid, and flavonoid are also health-promoting phytochemicals with antioxidant, antibacterial, and anti-inflammatory effects [4].

Light intensity plays a crucial role in plant growth and development, including phytochemicals synthesis [5]. Artificial lighting conditions allow using varying light intensity to regulate plant growth and phytochemicals accumulation, which is a kind of environmentally friendly and effective method to produce high-quality products. Light-emitting diodes (LEDs) are one of the promising lighting technologies for plants, which can adjust light intensity to affect the growth and the phytochemical contents of the plant [6,7]. In tatsoi, 100 and 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were most beneficial to enhance total protein content [8]. In leaf lettuce, anthocyanin content was observed to be the highest under 290 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and the lowest under 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [9]. The effects of light intensity on the growth and phytochemicals of microgreens have been often reported. In hypocotyl length, red pak choi

and kohlrabi microgreens grown under $110 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had longer hypocotyl length than those grown under $220\text{--}545 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and hypocotyl length of two microgreens grown under $220\text{--}545 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had insignificant difference [10]. In phytochemical contents, higher vitamin C content of red pak choi and tatsoi was observed under lower light intensity ($110 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), while higher phenols content was found under higher light intensity ($440 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) [10]. Total carotenoid content in microgreens of red pak choi and tatsoi was higher grown under $330\text{--}440 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and in mustard microgreens under $110\text{--}220 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [11]. The ferric reducing antioxidant power (FRAP) in microgreens of red amaranth and leafy vegetable amaranth enhanced with the increased light intensity ($130, 180, 230, 280 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and anthocyanin content in red amaranth microgreens significantly increased under the highest light intensity of $280 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [12].

The light intensities from 100 to $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux density (PPFD) often used for microgreens production in the literature. Few studies on minimum PPFD for microgreens have been conducted [6]. Low light intensity can potentially decrease electrical energy cost of growing microgreens in a plant factory. With dimming option available for LED fixtures, power consumption can be lowered by decreasing the light intensity provided to microgreens. Lower electrical energy costs can increase profits. However, limited information is available on the effects of low light intensity on growth and phytochemical concentration of broccoli microgreens. The purpose of this study was to investigate the effects of light intensity (lower than $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD) on the growth and phytochemicals accumulation of broccoli microgreens and to provide information for the high-quality production of broccoli microgreens in the emerging microgreen industry.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The experiment was conducted in an artificial light plant factory, South China Agricultural University. Seeds of broccoli (*Brassica oleracea* var. *italica* cv. Lvhu) were sterilized with 5% sodium hypochlorite solution for 10 min and then washed with purified water ($\text{pH} = 7.0$, $\text{EC} = 20 \mu\text{S}\cdot\text{cm}^{-1}$, $\text{R.O} = 0.6 \mu\text{S}\cdot\text{cm}^{-1}$). Then seeds were soaked in purified water for 5 h. The seeds of broccoli were sprayed evenly into the seedling plate ($39 \text{ cm} \times 39 \text{ cm}$) with two layers of gauze, purified water was added to the plate, and the water at the bottom of the seedling plate was renewed every two days. About 20.0 g broccoli seeds were sowed for each treatment with three replications using a seeding density of $3 \text{ seeds}\cdot\text{cm}^{-2}$. The seedling plates were placed on the bench equipped with blue/red/green LED tubes. The microgreens were cultivated at $22 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ of relative humidity. The plates were arranged randomly and manually rotated every day to uniform illumination. The experimental bench is shown in Figure 1.



Figure 1. Photo of the experimental bench.

2.2. Experimental Treatments

After sowing, the seeds were placed in the dark for 4 days, then cultivated underneath the LED tubes for 4 days (12 h / 12 h, light / dark). Four different total light intensities (photosynthetic photon flux density, PPFD) were set: 30, 50, 70, 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Light spectra were measured by a spectral analyzer (Lighting Passport ALP-01, AsenseTek, Taiwan), and the light spectra of each treatment is shown in Figure 2. At 4 days after light treatment, samples were collected for the growth parameters determination, frozen rapidly with liquid nitrogen, and stored at $-40\text{ }^{\circ}\text{C}$ for the phytochemical determination.

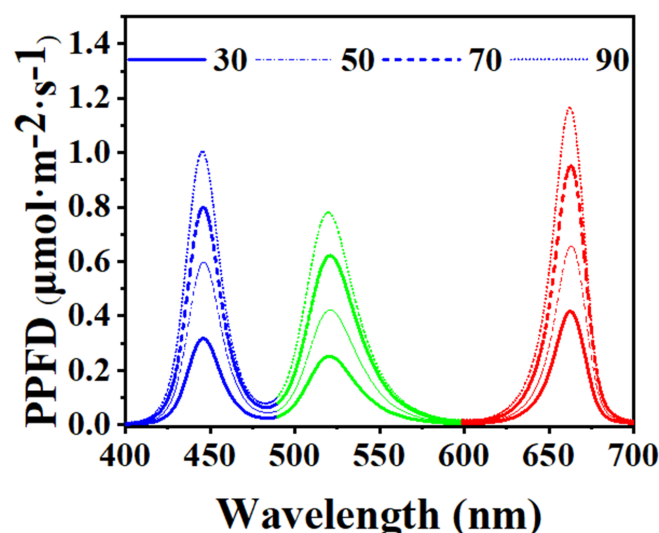


Figure 2. Light spectra of light treatment.

2.3. Growth Parameters Determination

Fresh weight (FW) and hypocotyl length, the growth parameters of broccoli microgreens, were determined by an electronic analytical balance and a ruler, respectively. Fresh microgreens dried at $75\text{ }^{\circ}\text{C}$ for 72 h to obtain the dry weight (DW). Fifteen broccoli microgreens were randomly sampled from each treatment. The moisture content of fresh broccoli microgreens was calculated: $\text{Moisture content (\%)} = (\text{FW} - \text{DW}) / \text{FW} \times 100\%$.

2.4. Phytochemical Determination

2.4.1. Chlorophylls (Chls) and Carotenoid Contents

Fresh samples of cotyledon of broccoli microgreens (0.2 g) were soaked in 6.0 mL of acetone ethanol mixture (acetone: ethanol = 1:1, v: v) and incubated at $25\text{ }^{\circ}\text{C}$ in dark for 24 h. The extract solution absorbance was determined with UV-spectrophotometer (Shimadzu UV-16A, Shimadzu, Corporation, Kyoto, Japan) at 663 nm (A_{663}), 645 nm (A_{645}) and 440 nm (A_{440}). The pigments contents were calculated according to Gratani [13] as follows: Chl a content (mg/g FW) = $(12.70 \times A_{663} - 2.69 \times A_{645}) \times 6\text{ mL} / (1000 \times 0.2\text{ g})$; Chl b content (mg/g FW) = $(22.90 \times A_{645} - 4.86 \times A_{663}) \times 6\text{ mL} / (1000 \times 0.2\text{ g})$; Chl a + Chl b content (mg/g FW) = $(8.02 \times A_{663} + 20.20 \times A_{645}) \times 6\text{ mL} / (1000 \times 0.2\text{ g})$; carotenoid content (mg/g FW) = $(4.70 \times A_{440} - 2.17 \times A_{663} - 5.45 \times A_{645}) \times 6\text{ mL} / (1000 \times 0.2\text{ g})$.

2.4.2. Soluble Protein Content

Soluble protein content was determined by Coomassie blue staining [14]. Fresh frozen tissue (0.5 g) was mixed with 4.0 mL deionized water and then centrifuged at 3000 rpm for 10 min. The supernatant (0.2 mL) was diluted in the 0.8 mL deionized water and well mixed with 5.0 mL Coomassie brilliant blue G-250 (Solarbio, Beijing, China) solution. 5 min later, the absorbance of the mixture was measured at 595 nm by UV-spectrophotometer (Shimadzu UV-16A, Shimadzu, Corporation, Kyoto, Japan), using deionized water as a

blank. Bovine serum albumin (Solarbio, Beijing, China) was used as a reference substance and the results were expressed in mg/g FW.

2.4.3. Soluble Sugar Content

Soluble sugar content was determined by anthrone colorimetry [15]. Fresh frozen tissue (1.0 g) was mixed with 10.0 mL deionized water, sealed with a plastic film, and boiled in a 100 °C-water bath for 30 min. Again, deionized water (10.0 mL) was added, boiled in a 100 °C-water bath for 30 min, and filtered by a funnel with double filter papers. The filtered solution was collected into a 25-mL measuring flask and cooled to 25 °C, then added deionized water to 25 mL. Later, the filtered solution (0.2 mL) and deionized water (0.8 mL) were mixed in a 20-mL test tube. 0.5 mL anthrone ethyl acetate reagent (Sinophaem, Beijing, China) and 5.0 mL concentrated sulfuric acid were added, mixed with vortex, and then placed in a boiling water bath for 10 min. After cooling to ambient temperature, the solution was measured at 625 nm by UV-spectrophotometer (Shimadzu UV-16A, Shimadzu, Corporation, Kyoto, Japan), using deionized water as a blank. Sucrose (Guangzhou Chemical Reagent Factory, Guangzhou, China) was used as a reference substance and the results were expressed in mg/g FW.

2.4.4. Free Amino Acid Content

Free amino acid content was determined according to the method described by Yao et al. [10]. The ground samples (0.5 g) were extracted by 50 mL hot deionized water for 10 min. Then, the solution was filtered, and whose supernatant (1.0 mL) was mixed with 0.5 mL phosphate buffer solution (pH 8.0) and 0.5 mL 2% ninhydrin solutions containing 0.8 mg/mL of tin chloride in 25-mL volumetric flask. The mixture was heated for 15 min in the boiling water bath and cooled to ambient temperature. It was then diluted to 25 mL with deionized water and laid aside for 15 min. The absorbance of the solution was determined at 570 nm by UV-spectrophotometer (Shimadzu UV-16A, Shimadzu, Corporation, Kyoto, Japan), using deionized water as a blank. Leucine (Biosharp, Guangzhou, China) was used as a reference substance and the results were expressed in mg/g FW.

2.4.5. Flavonoid Content

The flavonoid content was determined by an aluminum nitrate method [16]. Fresh frozen tissue (0.5 g) was extracted by 8.0 mL absolute ethanol. 1.0 mL broccoli microgreens extract was mixed with 0.7 mL 5% sodium nitrite solution in a 10 mL test tube for 5 min. Then, 0.7 mL 5% aluminum nitrate was added to the mixture for 6 min. 5.0 mL 5% sodium hydroxide solution was added and reacted at 25 °C. The absorption at 510 nm was measured by UV-spectrophotometer (Shimadzu UV-16A, Shimadzu, Corporation, Kyoto, Japan), absolute ethyl alcohol as a blank. Rutin (Sinophaem, Beijing, China) was used as a reference substance and the results were expressed in mg/g FW.

2.4.6. Vitamin C Content

Vitamin C content was determined by molybdenum blue spectrophotometry [17]. Fresh frozen tissue (0.5 g) was homogenized with 25 mL of oxalic acid ethylene diamine tetraacetic acid solution (*w/v*) in a volumetric flask. Then, the solution was filtered by a funnel with double filter papers. 10.0 mL supernatant was mixed with 1.0 mL partial phosphoric acid-acetic acid solution (*w/v*) and 2.0 mL 5% sulfuric acid solution (*v/v*) and 4.0 mL 5% ammonium molybdate solution (*w/v*). The supernatants were mixed well and set still for 15 min, then measured at 705 nm by a UV-spectrophotometer (Shimadzu UV-16A, Shimadzu, Corporation, Kyoto, Japan), using oxalic acid-ethylene diamine tetraacetic acid as a blank. L-ascorbic acid (Guangzhou Chemical Reagent Factory, Guangzhou, China) was used as a reference substance and the results were expressed in mg/g FW.

2.4.7. Glucosinolates Contents Determination

Glucosinolates were extracted and analyzed as previously described [18]. The frozen-dried sample was extracted with methanol and then the extracts were purified and desulfurized with the ion-exchange method. The glucosinolates were separated and identified by high-performance liquid chromatography (HPLC, Waters Alliance e2695). A 5 μm C18 column (Waters, 250 mm length, 4.6 mm diameter) was used for glucosinolate separation. Elution was performed with mobile phase A (water, 18.2 M Ω ·cm resistance) and mobile phase B (acetonitrile). The optimum column temperature was 30 °C. At a flow rate of 1.0 mL/min, the gradient conditions were set as follows: solvent A volume at 100% for 0 to 32 min, 80% for 32 to 38 min, and solvent B volume at 100% for 38 to 40 min. 42–50 min was the time for elution. The detector monitored glucosinolates at 229 nm. The individual glucosinolates were identified according to their HPLC retention times and our database, and quantified with sinigrin (Sigma-Aldrich, St. Louis, MO, USA) was used as an internal reference substance with their HPLC area and relative response factors (ISO 9167-1,1992) [19]. The results were expressed as $\mu\text{mol/g}$ DW.

2.5. Statistical Analysis

Unless mentioned otherwise, all data were showed as means $\pm$ standard error (SE) of three replications, and they were assessed for analysis of variance (ANOVA) using Duncan's test at $p < 0.05$ with SPSS 26.0 software (Chicago, IL, USA). Line charts were constructed with Origin 2018 software (Origin Lab, Northampton, MA, USA). The heatmap function of TBtools software [20] was used to study the overall data. We calculated the Euclidean distance among samples and used the complete clustering method.

3. Results

3.1. Effects of Light Intensity on Growth of Broccoli Microgreens

Light intensity markedly affected the growth of broccoli microgreens (Figure 3). At harvest, the highest FW and DW of broccoli microgreens were observed under 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (54.33 mg/plant and 2.97 mg/plant, respectively), while moisture content and hypocotyl length were markedly reduced as light intensity increased (from 30 to 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), the lowest moisture content and hypocotyl length were found under 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the reductions were up to 0.82% and 24%, respectively, compared to those under 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. These results implied that 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was the suitable light intensity for broccoli microgreens growth.

3.2. Effects of Light Intensity on Chls Contents in Broccoli Microgreens

The contents changes of Chl a, Chl b, and Chl a + Chl b were consistent among all light treatments. The contents of Chl a, Chl b, and Chl a + Chl b in broccoli microgreens increased as light intensity increased from 30 to 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figure 4A–C). As the light intensity increased from 30 to 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the contents of Chl a, Chl b, and Chl a + Chl b increased by 15.3%, 24.0%, and 17.3% respectively, while Chl a / Chl b ratio was not affected by light intensity (Figure 4D). Hence, higher light intensity was beneficial to the accumulation of Chl a, Chl b, and Chl a + Chl b.

3.3. Effects of Light Intensity on Phytochemical Contents in Broccoli Microgreens

Light intensity affected the contents of soluble protein, soluble sugar, free amino acid, flavonoid, vitamin C, as well as carotenoid in broccoli microgreens (Figure 5). The soluble protein content in broccoli microgreens significantly responded to the changes in light intensity. The soluble protein content in broccoli microgreens grown under 30, 50 and 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was significantly higher than those under the irradiance of 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figure 5A). The lowest soluble sugar content (5.44 mg/g) was observed in broccoli microgreens grown under the irradiance of 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, which was significantly lower than that in broccoli microgreens under the irradiance of 70 and 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figure 5B). The content of free amino acids in broccoli microgreens

grown under the irradiance of $30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was significantly higher than those grown under the irradiance of 50 and $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figure 5C). The content of flavonoid decreased, then increased with the increase of light intensity (Figure 5D). The lowest and the highest content of flavonoid were respectively found in broccoli microgreens grown under the irradiance of 50 and $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and they were 0.79 mg/g and 1.13 mg/g . The vitamin C content in broccoli microgreens grown under $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was significantly lower (-6% to -14%) than those in broccoli microgreens grown under other light intensities (Figure 5E). With increasing light intensity (from 30 to $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), the carotenoid content decreased (from 0.09 to 0.07 mg/g FW) (Figure 5F). These results indicated that $70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was better for the accumulation of phytochemicals in broccoli microgreens.

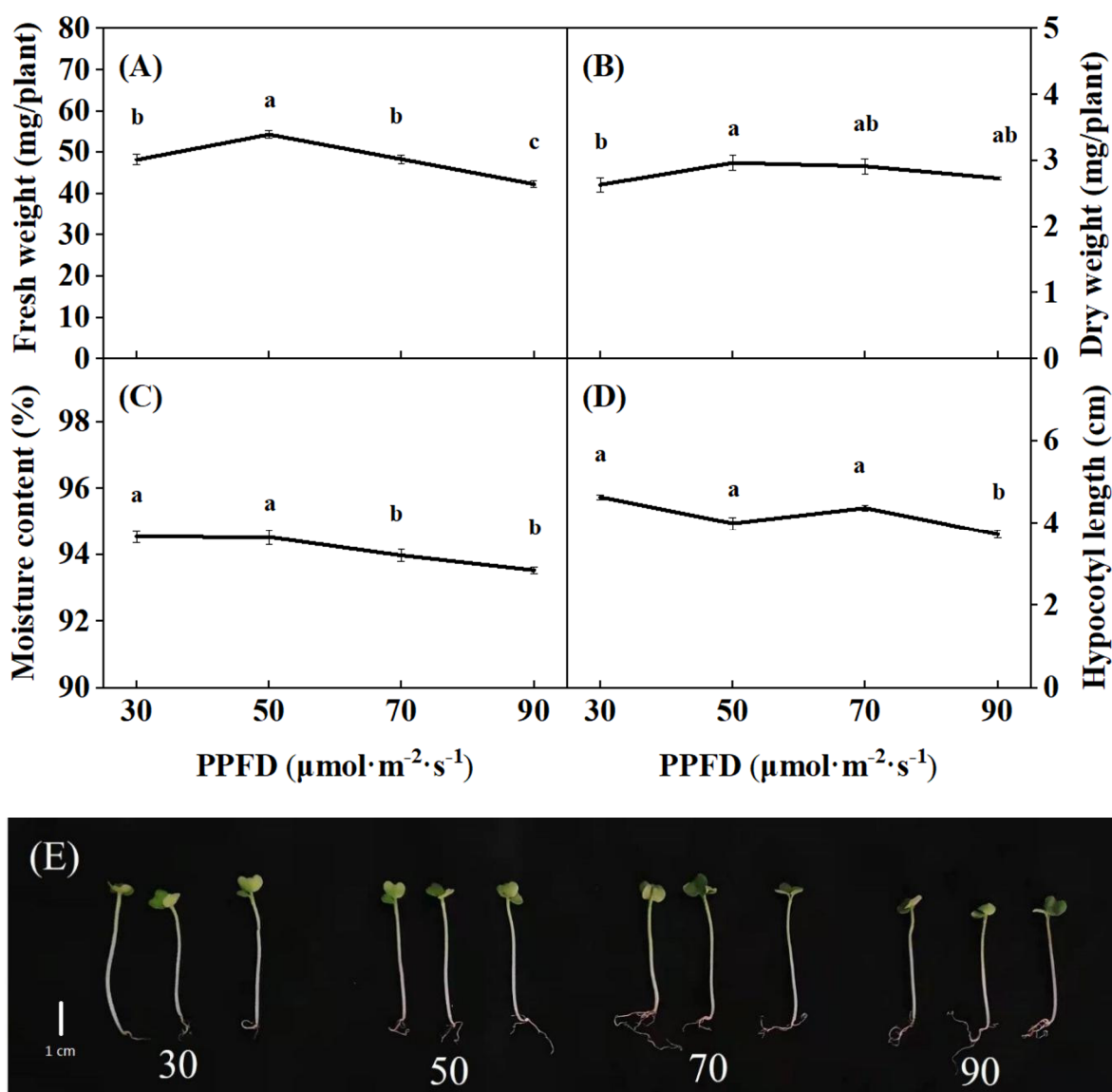


Figure 3. Fresh weight (A), dry weight (B), moisture content (C), and hypocotyl length (D) of broccoli microgreens response to different light intensity treatments. The lowercase letters in all figures represent the significant difference ($p < 0.05$). Vertical bars indicate the means $\pm$ SE ($n = 15$). Photo of broccoli microgreens (E) was taken at 8 days after sowing, where the bar represents 1 cm.

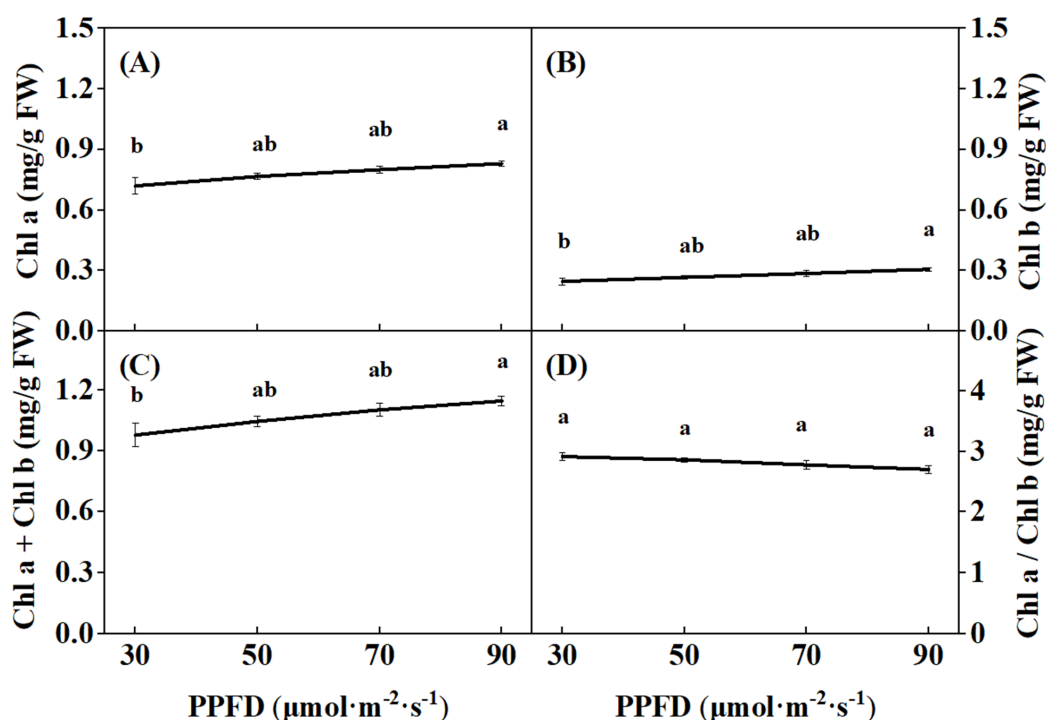


Figure 4. Effects of different light intensity on the contents of Chl a (A), Chl b (B), Chl a + Chl b (C), and Chl a / Chl b (D) in broccoli microgreens. The lowercase letters in all figures represent the significant difference ($p < 0.05$). Vertical bars indicate the means $\pm$ SE ($n = 3$).

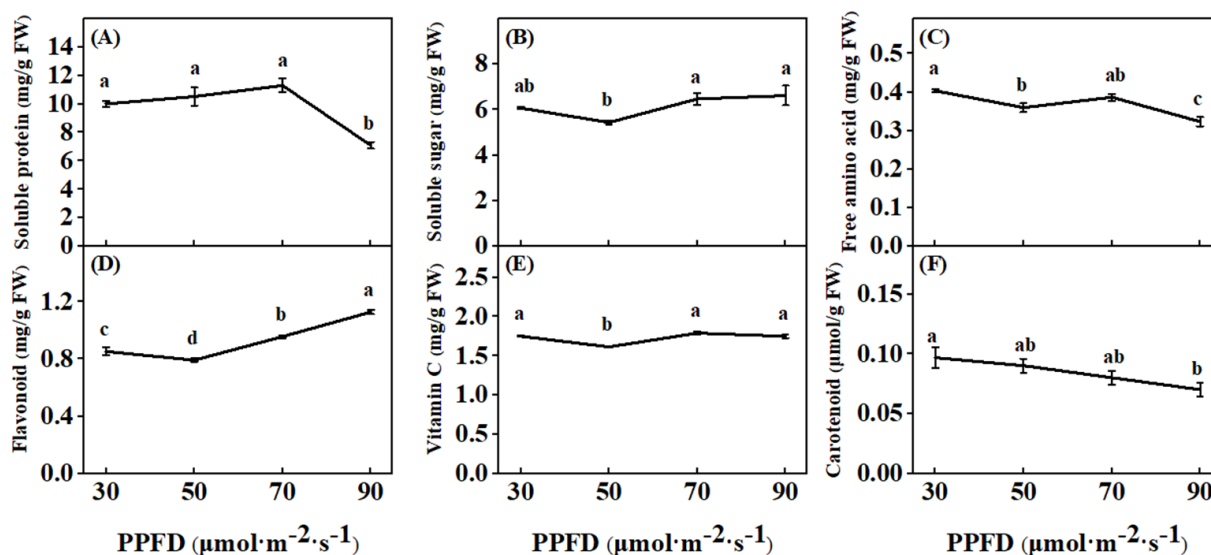


Figure 5. Effects of different light intensity on the contents of soluble protein (A), soluble sugar (B), free amino acid (C), flavonoid (D), vitamin C (E), and carotenoid (F) in broccoli microgreens. The lowercase letters in all figures represent the significant difference ($p < 0.05$). Vertical bars indicate the means $\pm$ SE ($n = 3$).

3.4. Effects of Light Intensity on Glucosinolates Profile and Contents in Broccoli Microgreens

Glucosinolates in broccoli microgreens were analyzed using HPLC. 9 glucosinolates were identified in broccoli microgreens (Figure 6), including 5 aliphatic glucosinolates (progoitrin, glucoraphanin, sinigrin, glucobrassicinapin, and glucoerucin) and 4 indolic glucosinolates (4-hydroxy-glucobrassicin, glucobrassicin, 4-methoxy-glucobrassicin, and neoglucobrassicin). The contents of glucobrassicin (GBS), total aliphatic glucosinolates, and total glucosinolates all increased with the enhancement of light intensity (Table 1), but

there were no significant differences in the contents of them in broccoli microgreens under between $70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. However, 4-hydroxy-glucobrassicin (4-HGBS) content decreased significantly with increasing light intensity. Progoitrin (PRO) content was lower in broccoli microgreens under the irradiation of 50 and $70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, while glucoraphanin (GRA) content in broccoli microgreens at the irradiation of 50, 70 and $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was significantly higher than that at the irradiation of $30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Glucobrassicinapin (GBN) content was the highest in broccoli microgreens grown under $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and was significantly higher than that grown under other light intensities. Meanwhile, the contents of glucoerucin (GER), 4-methoxy-glucobrassicin (4-MGBS), and neoglucobrassicin (NGBS) were the lowest in broccoli microgreens grown at the irradiation of $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. These results showed that different light intensity affected glucosinolates contents to various extent.

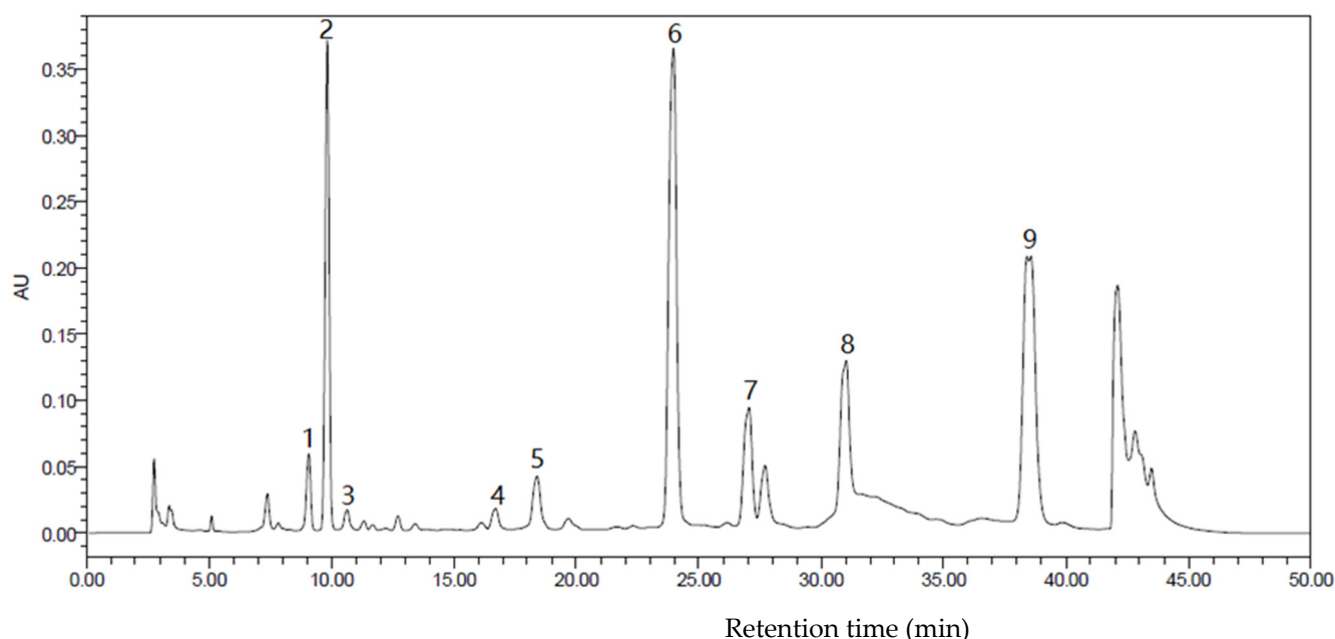


Figure 6. HPLC chromatogram of glucosinolates. 1: progoitrin (PRO); 2: glucoraphanin (GRA); 3: sinigrin (SIN); 4: 4-hydroxy-glucobrassicin (4-HGBS); 5: glucobrassicinapin (GBN); 6: glucoerucin (GER); 7: glucobrassicin (GBS); 8: 4-methoxy-glucobrassicin (4-MGBS); 9: neoglucobrassicin (NGBS).

Table 1. Effects of different light intensity on glucosinolates contents in broccoli microgreens.

Glucosinolates Content ($\mu\text{mol}\cdot\text{g}^{-1}$ DW)	Light Intensity ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)			
	30	50	70	90
PRO	3.69 ± 0.11 a	2.82 ± 0.03 b	2.94 ± 0.03 b	3.46 ± 0.14 a
GRA	18.70 ± 0.57 b	24.42 ± 0.16 a	25.22 ± 0.46 a	24.20 ± 0.68 a
SIN	0.88 ± 0.04 a	0.79 ± 0.01 a	0.87 ± 0.06 a	0.98 ± 0.10 a
GBN	5.45 ± 0.08 b	6.12 ± 0.18 a	5.46 ± 0.19 b	5.24 ± 0.19 b
GER	36.72 ± 1.12 b	34.72 ± 0.63 b	37.91 ± 0.75 ab	40.14 ± 1.19 a
TAGSL	65.44 ± 1.02 c	68.87 ± 0.73 b	72.40 ± 0.67 a	74.01 ± 0.86 a
4-HGBS	0.81 ± 0.01 a	0.67 ± 0.00 b	0.61 ± 0.03 b	0.42 ± 0.02 c
GBS	1.18 ± 0.06 c	2.10 ± 0.04 b	2.82 ± 0.08 a	2.96 ± 0.05 a
4-MGBS	2.64 ± 0.13 bc	2.41 ± 0.07 c	3.91 ± 0.20 a	3.01 ± 0.17 b
NGBS	2.04 ± 0.06 c	1.21 ± 0.04 d	2.40 ± 0.14 b	3.05 ± 0.13 a
TIGSL	6.66 ± 0.15 b	6.39 ± 0.06 b	9.75 ± 0.19 a	9.45 ± 0.33 a
TGSL	72.10 ± 1.16 b	75.25 ± 0.80 b	82.15 ± 0.83 a	83.46 ± 1.08 a

Note: Different lowercase letters in the same row show significant differences ($p < 0.05$). The data were showed as means $\pm$ SE ($n = 3$). TAGSL, total aliphatic glucosinolate; TIGSL, total indolic glucosinolate; TGSL, total glucosinolate.

3.5. Heatmap Analysis

A heatmap integrating the response of the measured parameters showed a comprehensive view of the effects of light intensity on the growth and the contents of phytochemicals in broccoli microgreens (Figure 7). In terms of measured parameters responses, the 30 and the 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ clusters are the closest to each other, while the 70 and the 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ clusters are the closest to each other. Lower light intensities (30 and 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and higher ones (70 and 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) showed opposite responses in most of the measured parameters. The 30 and the 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ clusters both showed higher fresh and DW, moisture content as well as hypocotyl length than the 70 and the 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ clusters. Meanwhile, the 70 and the 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ clusters showed a similar response of most of the phytochemical parameters, such as the contents of flavonoid, TGSL, 4-MGBS, TIGSL, and so on. Clusters 70 and 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ led to higher contents of Chls, soluble sugar, flavonoid, vitamin C, GER, GBS, 4-MGBS, and NGBS in broccoli microgreens. Besides, cluster 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ elicited more phytochemicals than the other three clusters. Compared to those under 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, under the irradiation of 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the content of PRO was lower, and the contents of soluble protein, free amino acid, 4-HGBS as well as hypocotyl length were higher.

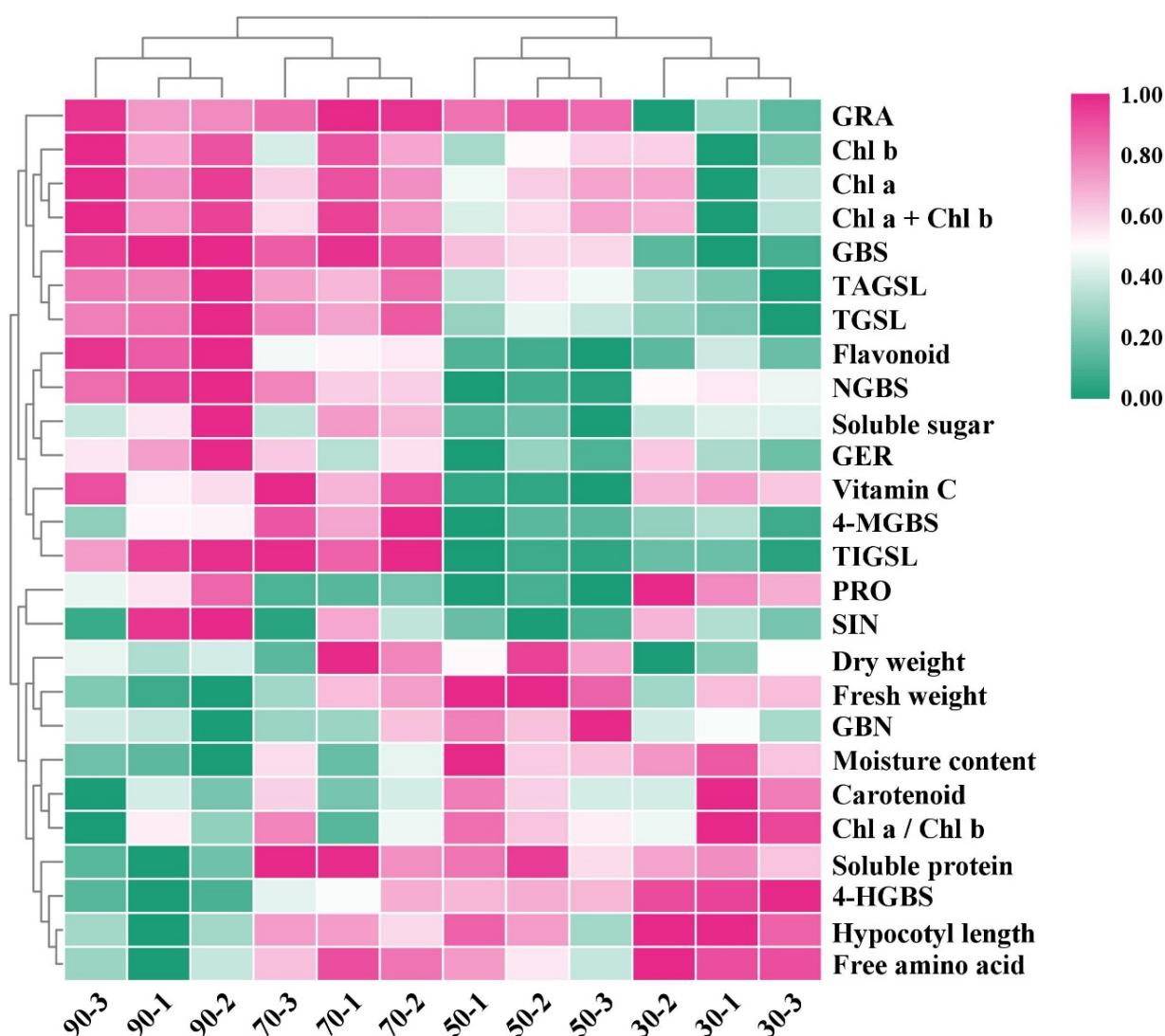


Figure 7. Cluster heatmap analysis summarizing broccoli microgreens responses to light intensity (30, 50, 70, and 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Pink and green indicate an increase and a decrease of the response parameters, respectively.

4. Discussion

4.1. Effects of Light Intensity on Growth of Broccoli Microgreens

Less than $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ is good enough for microgreens growth in an artificial light environment [6]. In this study, under irradiation of lower than $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (30, 50 and $70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) treatment had a promoting effect on the growth of broccoli microgreens, increasing in FW and hypocotyl length, compared to those under $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figure 3A,D). Increasing blue light intensity suppressed hypocotyl elongation in six vegetable sprouts included broccoli sprouts [21]. Although no direct comparisons could be made owing to different growing conditions, it was worth mentioning that the inhibition of hypocotyl elongation might be mainly due to the increase of blue light intensity in this study (Figure 3D). However, lower light intensity ($<90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) treatment led to reducing Chls contents (Figure 4A–C). The light intensity of $130\text{--}280 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ could not affect chlorophylls contents of amaranth microgreens, which might be due to the intensity was too low for amaranth microgreens [12]. However, for broccoli microgreens, $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ might be high enough to enhance the biosynthesis of the contents of Chls, which might vary with plant species. Previous studies indicated that at the de-yellowing stage, low light intensity promoted hypocotyl growth and inhibited chlorophyll accumulation for the sake of reaching more light [22,23]. It appears that low light intensity promotion on elongation growth might be a common phenomenon, which could be beneficial to microgreens production. The lowest light intensity ($30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) had the lowest DW (2.63 mg/plant). These were in accordance with the report that low irradiance decreased the leaf DW of lettuce [24]. Perhaps, under lower light intensity, photosynthesis produced fewer carbohydrates, which therefore produces less DW.

4.2. Effects of Light Intensity on Phytochemical Contents in Broccoli Microgreens

Light intensity plays a vital role in the accumulation of soluble protein, soluble sugar and free amino acid. In broccoli microgreens, the soluble protein content was higher under the irradiation of 30, 50 and $70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ but decreased sharply under the irradiation of $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figure 5A). Similarly, the lowest protein content in tatsoi was observed under the higher light intensities (400 and $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) [8]. These suggested that higher light intensity could reduce soluble protein content, and the mechanism needs further research. The highest FW and the lowest soluble sugar content were found under $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in this study (Figures 3A and 5B). This might be due to broccoli microgreens under $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had the highest FW (54.33 mg/plant) diluting soluble sugar content. In tatsoi, higher contents of free amino acids including glutamate, asparagine, and aspartate were determined under low ($100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or high light ($500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), which might be due to the stressful light conditions [8]. It might be due to stress from high light that the lowest free amino acid content in broccoli microgreens was found under the highest irradiation ($90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (Figure 5C).

Flavonoid, vitamin C, and carotenoid are antioxidants that could reduce or clear reactive oxygen species (ROS) formed under high light intensity in some plant species [11,25–27]. Higher light intensity seemed to promote antioxidants contents, which were similar to those found in *Brassica* microgreens [10] and lettuce [26]. The total flavonoid in ferns species *Matteuccia struthiopteris* (L.) Tod. and *Brassica campestris* L. increased with the increasing light intensity [28,29]. This might be that light energy of high light intensity was more than which could be used up for the fixation of CO_2 in the Calvin cycle [25], and this excess energy could induce ROS which could damage photosystems [30]. In broccoli microgreens, over the light intensity of $70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ irradiation, flavonoid content increased while carotenoid content reduced (Figure 5D,F). The flavonoid as an antioxidant might increase in leaves to retain the survival of plants exposed to high light intensity when other ROS anti-toxifying systems had already been damaged [31]. The photoprotective action of some antioxidants was coordinated with their capacity of interacting with the polar phospholipid head at the water-lipid interface of the membranes, reducing the risk of oxidant-induced damage [32]. The content of vitamin C in broccoli microgreens under the irradiation of 30,

70 and 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were higher than those under the irradiation of 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figure 5E). This might be a “diluted effect” due to the highest FW in broccoli microgreens under the irradiation of 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figure 3A).

Glucosinolate profile is largely affected by genetic and environmental factor [33], and vary from species and cultivars [3]. 7 glucosinolates were identified in broccoli sprouts (cv. Waltham 29) [34]. In this study, 9 glucosinolates were identified in broccoli microgreens (cv. Lvhu) (Figure 6). In one-leaved seedlings of choy sum, more than 80% of the total glucosinolates were aliphatic glucosinolates [33]. Aliphatic glucosinolates were the major glucosinolates in broccoli microgreens (about 90% of the total glucosinolates) even under different light intensity, followed by indolic glucosinolates (Table 1), which was similar to *Brassica oleracea* leaves grown at 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [35]. Glucosinolates content in broccoli microgreens varied under different light intensity (Table 1). It was reported that light could regulate genes of glucosinolates biosynthesis controlled by the LONG HYPOCOTYL5 (HY5) transcription regulator [36]. In broccoli microgreens, the contents of total glucosinolates, aliphatic glucosinolates, and indolic glucosinolates increased (16%, 13%, and 53%, respectively) when light intensity increased from 30 to 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Table 1). However, total glucosinolates content in *Brassica oleracea* decreased with increasing light intensity (from 200 to 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) [35]. Among 9 glucosinolates in broccoli microgreens, the content of glucorucin (GER, an aliphatic glucosinolate) was the highest, followed by glucoraphanin (GRA, an aliphatic glucosinolate), which accounted for about 50% and 30% of total glucosinolate, respectively (Table 1). Side-chain modification of GER led to GRA synthesis [37]. Significantly higher GER content in all treatments might lead to the relatively inefficient conversion of GER to GRA [35]. Interestingly, in broccoli microgreens, the accumulation of different glucosinolates was induced by different light intensity ranges. The accumulations of GRA and GBS were probably stimulated by higher light intensity (over 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The highest content of GRA occurred under 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The contents of PRO, SIN, GBN, GER, and NGBS decreased significantly when light intensity increased from 30 to 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ but increased as light intensity increased from 50 to 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Table 1). It is known that GRA and GBS are beneficial glucosinolates [38,39], while SIN and PRO are not only detrimental to human health but also contribute to bitter flavor [40–42]. It has been previously reported that 194 μmol of goitrin (PRO degradation product) could inhibit radioiodine uptake to the thyroid, but 77 μmol of goitrin could not [43]. Therefore, excessive consumption of *Brassica* crops (broccoli, Chinese cabbage, kale, etc.) should be avoided. Therefore, from a human health standpoint, it might be desirable to enhance the contents of GRA and GBS, and to reduce the contents of SIN and PRO in broccoli microgreens. Blue light promoted the biosynthesis of glucosinolates in broccoli sprouts [44], which indicated the light intensity of blue light might be the main reason for affecting glucosinolates accumulation in this study. Above, 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was feasible for increasing desired glucosinolates content and decreasing undesired glucosinolates content.

4.3. Relationship between Growth and Phytochemicals of Broccoli Microgreens at Different Light Intensity

The heatmap carried out in this study exhibits a broad view of growth parameters and phytochemicals of broccoli microgreens under different light intensity (Figure 7). The heatmap analysis showed a net separation between the high light intensity (70 and 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and low light intensity (30 and 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) clusters, suggesting an opposite response of them in terms of growth characteristics and phytochemical contents. The light intensity of 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was considered to be low light stress for some field crops (i.e., rice, maize, wheat) and some horticultural crops (i.e., cucumber, pepper, tomato, leafy vegetables) [45–47]. However, the microgreens could suffer lower light intensity. It might be that the light intensity between 50 and 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had a critical light intensity that significantly changed the biosynthesis and accumulation of some phytochemicals or their precursor in broccoli microgreens. In *Chrysanthemum*, higher light intensity (>100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was beneficial for biomass accumulation, including fresh

and dry weight [48]. However, for broccoli microgreens growth without supplying nutrient solution in this study, the highest fresh and dry weight were found under $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the biomass gradually reduced over $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, these might suffer high light intensity over $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Moreover, the higher light intensity ($> 90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) may reduce biomass of and contents of soluble protein, free amino acid, GBN, 4-HGBS, and 4-MGBS in broccoli microgreens. However, the contents of PRO and NGBS may increase, which needs further research. It seemed that more biomass tended to relate to lower phytochemical contents, including glucosinolates (PRO and NGBS) in this study. One possible reason might be that they were mediated by growth-induced dilution effects [49]. Another possible reason might be that plants regulate the balance between growth and defense via a change in the allocation of resources and would allocate the limited resources to growth processes over differentiation [50]. Since glucosinolates use a kind of soluble sugar—glucose as a carbon skeleton, the glucosinolate (GBN and 4-MGBS) synthesis might have depleted the carbon source (e.g., soluble sugar) allocation in this study. Transcriptomic analysis showed that plant hormone signal transduction, starch and sucrose metabolism, phenylpropanoid biosynthesis, peroxisome, and photosynthesis—antenna proteins pathways potentially related to response to the low light intensity of $80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for zucchini [51]. Furthermore, these results indicate the complex relationships between light intensity and glucosinolates biosynthesis.

5. Conclusions

The effects of various light intensities ($30, 50, 70$, and $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) on the growth and phytochemical contents of broccoli microgreens were investigated. The broccoli microgreens under $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had the largest fresh and dry weight. The contents of soluble protein, soluble sugar, free amino acid, flavonoid, and vitamin C were higher under irradiations of $70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The beneficial glucosinolate (GRA) content was the highest and detrimental glucosinolate (PRO) content was lower under $70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Therefore, $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD LED (red: green: blue = 1:1:1) might be the optimal light intensity for growth and $70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was good for the accumulation of phytochemicals in broccoli microgreens production. The light intensity between $50\text{--}70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ might take account of growth and phytochemicals concentration.

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