Research Article

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Effects of Blue and Red Light On Growth And Nitrate Metabolism In Pakchoi

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Abstract: This study investigated the effects of blue and red light on metabolites of nitrate, key enzymes, and the gene expression of key enzymes in pakchoi plants (*Brassica campestris* L. var. Suzhouqing). Plants were grown under three light quality treatments, namely, white light (W), red light (R) and blue light (B), at the same photosynthetic photon flux density (PPFD) of approximately 150 μmol m^{-2} s^{-1} for 48 hours of continuous illumination, and W was set as the control. The dynamics of net photosynthetic rate in pakchoi subjected to different light treatments were the same as the total chlorophyll contents: blue light > white light > red light. The nitrate reductase (NR) activity, nitrite reductase (NiR) activity, glutamine synthetase (GS) activity and glutamate synthase (GOGAT) activity were highest under blue light. Further, the expression levels of NR, NiR and GS genes were significantly higher under blue light. Under continuous illumination, the auxin content (IAA) in pakchoi leaves was highest under blue light, whereas the abscisic acid (ABA) content was highest under red light. In contrast, there was no significant effect for gibberellin (GA) under any type of light treatment.

Keywords: Red and blue light; Nitrogen metabolism; Enzyme activity; Gene expression; Pakchoi.

1 Introduction

Nitrate is a main source of nitrogen, and its content reflects the nutrient status of plants. Nitrate positively influences the yield and quality of vegetables, and the accumulated level of nitrate is an important parameter that indicates the safety of vegetables. Many studies have demonstrated that light conditions are one of the main factors affecting plant nitrate levels. Among light conditions, light quality is a key factor in the regulation of nitrate concentration in plants [1].

Nitrate reductase (NR) is generally considered a key and rate-limiting enzyme for nitrate assimilation. Light markedly affects NR activity in plants by regulating the transcription, translation and post-translational activity of NR genes. Bian [1] proposed a model of NR regulation by light. The model suggested that light could regulate NR activity through two pathways, namely, the regulation of NR gene expression by photosynthetic products and the regulation of NR state by nicotinamide adenine dinucleotide phosphate (NADP).

Previous studies indicated that light quality has a significant effect on nitrate metabolism in plants. A five-minute red-blue light pulse can stimulate nitrate assimilation in red algae, however, this kind of effect can be neutralized by subsequent far-red light [2]. This pattern suggests that light-sensitive pigments participate in this process. A similar phenomenon is also found in green algae, in which low-intensity blue light facilitated nitrate absorption [3]. Another study in etiolated barley showed that the NR activity in red light-treated plants was two times higher than that in plants treated with white light, suggesting that light-sensitive pigment improved NR mRNA protein and enzyme activity [4]. The result is probably due to blue light stimulating NR absorption through photosynthesis [5], alternatively, the direct stimulation of NR because the chromophore of the blue-light receptor cryptochrome contains flavin adenine dinucleotide (FAD), and FAD is also present in the prosthetic group of NR [6].

At present, studies concerning the effects of light on nitrite reductase activity are relatively few. The NII gene is an important gene that encodes NIR [7]. It has been shown that red and blue light radiation can induce NII expression, which is probably pertinent to light-sensitive pigments and blue-light receptors [8,9]. GOGAT and GS are key enzymes for ammonia assimilation in plants. It was found that the light-sensitive pigments in red pine [10], lettuce [11], mustard [12] and *Arabidopsis thaliana* [13]

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significantly participated in the regulation of GS activity. The ferredoxin glutamate synthase (Fd-GOGAT) in plants is widely present in the photosynthetic tissue of plants, and its expression is affected by light. Both red and blue light can increase the Fd-GOGAT activity in etiolated duckweed seedlings, and two phytochromes and blue-light receptors were involved. Red light can stimulate Fd-GOGAT expression, while far-red light can reverse this effect, suggesting the involvement of phytochrome. Similarly, ultraviolet-A radiation promoted the expression of Fd-GOGAT by a special blue-light receptor, which implicated the blue-light receptor [10].

Pakchoi (*Brassica campestris* L.) is an important agricultural crop in China that can be produced in a plant factory and for which year-round production is feasible. The objectives of the present study were to investigate how blue light and red light affect nitrate metabolism and to provide a quick and effective way to reduce the nitrate content of pakchoi.

2 Experimental Section

2.1 Plant materials

The variety of pakchoi was *Suzhouqing*, which was supplied by the Jiangsu Academy of Agricultural Sciences of Vegetables. Seeds of Chinese cabbage were soaked for a period of 8 h in water and then were kept in a wet sponge seedling block. Environmental conditions were as follows: under natural light, the relative humidity (RH) was maintained at 80-90%, and the growth temperature was 20-22°C. After the two cotyledons of seedlings fully expanded, we placed the seedlings into a hydroponic box filled with Hoagland nutrient solution. When the Chinese cabbage had produced six true leaves, 60 seedlings were selected and were then cultured under three light-quality treatments for 48 h [14,15]. There were two fanners in the controlled environment, which maintained the CO_2 level to be the same as that of the atmosphere. The relative humidity (RH) was maintained at 70±10%, and the growth temperature was 18-20°C. Three replications were performed for each phytochemical measurement. All data are expressed on a fresh weight (FW) basis.

2.2 Light treatments

Nanjing Vegetation Spectrum Photoelectric Technology Co., Ltd designed all the lamps. The light treatments were designated with red LEDs (R), blue LEDs (B), and white LEDs (W). The white light treatment (W), with a 12 h photoperiod, was used as a control, and plants were grown under incandescent reflector lamps. The spectral distributions of the white (peak at 400-760 nm), red (peak at 658 nm) and blue (peak at 460 nm) lights were measured by using a spectroradiometer (OPT-2000, ABDPE Co., Beijing, China). All the treatments were irradiated with the same photosynthetic photon flux density (PPFD) of 150 μmol m^{-2} s^{-1}. PPFD was measured using a quantum sensor (LI-250, LI-COR, USA) and was separately controlled by adjusting both the electric currents. The parameters of the light in each treatment are shown in Table 1.

2.3 Methods

2.3.1 Determination of photosynthesis and chlorophyll

The chlorophyll content was determined according to Arnon's method with improvements [16]. A total of 0.1 g of leaf material was soaked in 10.0 mL (V) of 80% acetone for 2-6 h until the material turned white. Optical density was measured with a UV-1200 spectrophotometer (Jin Peng Inc., China) at 663 nm for chlorophyll a (Chl a) and at 645 nm for chlorophyll b (Chl b).

Net photosynthetic rate (Pn) was measured using a photosynthesis instrument (LI-6400, LI-COR, USA). PPFD was set to 800 μmol m^{-2} s^{-1}, and the experimental conditions of leaf temperature, CO_2 concentration and relative humidity (RH) were 23±1°C, 380±5 μL L^{-1} and 60%-70%, respectively. The Pn measurement was repeated 3 times with 3 plants in each treatment [17].

2.3.2 Determination of physiological metabolites

Soluble proteins were extracted from leaves with the improved method of Li et al. [18]. The proteins were precipitated with NH_4HSO_4 (45% saturation), collected by centrifugation and dissolved in 50 mmol L^{-1} sodium phosphate buffer (1 mmol L^{-1} Na_2EDTA, 1 mmol L^{-1} sodium molybdate, 5 mmol L^{-1} leupeptin, 10 mmol L^{-1} FAD, 5 mmol L^{-1} cysteine, 0.05 mmol L^{-1} NaH_2PO_4 $2H_2O$ and 0.05 mmol L^{-1} Na_2HPO_4 $2H_2O$ at a pH of 7.5). One milliliter of extract and 5.0 mL of Coomassie Brilliant Blue G-250 were mixed thoroughly. The absorbance was measured at 595 nm. Protein standard solution was prepared with bovine serum albumin. Then, 100 mg of bovine serum albumin was dissolved in 100 mL of deionized water to obtain a total volume of 1000 μg mL^{-1}, which was used as the standard fluid.

Table 1: Major technique parameters of different light spectral energy distribution.

Light treatment	Peak wavelength λp(nm)	Halfwave width Δλ(nm)	PPFD (μmol m^{-2} s^{-1})	Power (W)
W(CK)	580	—	150	400
R	658	±12	150	54
B	460	±11	150	54

W, white light; R, red light; B, blue light.

The soluble sugar contents were measured by the modified anthrone method of Li et al. [18]. Leaves (0.1 g dry weight) were placed in a test tube, to which 10 mL of distilled water was added. After 30 min in a water bath at 85°C, the supernatant was collected for determination. Soluble sugar content was measured at 620 nm.

Nitrate content was determined according to Li [19]. Leaf tissue (0.2 g) was added to 10 mL of deionized water and placed in a boiling water bath for 30 min. The supernatant was adjusted to 25 mL, then, 0.4 mL of salicylic acid-sulfuric acid mixture (5%) was pipetted into 0.1 mL of the supernatant. The mixture was shaken, allowed to rest for 20 minutes, and finally mixed with 9.5 mL of NaOH. The absorption spectrum was at 410 nm.

Nitrite content was measured according to Qin [20]. A leaf sample (1.0 g) was homogenized in 2 mL of buffer solution. The homogenate was transferred to a 50 mL volumetric flask. One mL of ferrocyanide and 1 mL of $ZnSO_4$ were then added to the volumetric flask, and the volume was adjusted to 50 mL. The mixture was shaken and allowed to rest for 10 minutes before filtering. The reaction mixture containing 5 mL of filtrate, 5 mL of sulfanilamide and 5 mL of 1-naphthylamine was incubated in a 30°C water bath for 30 min. The absorbance was read at 530 nm.

Concentrations of glutamic acid and glutamine were determined using a kit made by the Nanjing Institute of Biological Engineering and following the principles of Lund [21]. The test steps are shown in the supplementary materials section.

The glutamic acid assay kit is based on glutamic acid catalyzed by glutamate dehydrogenase to produce 2-oxoglutarate, NH_3 and NADH. With the ultraviolet spectrum, NADH was measured at 340 nm to calculate the glutamic acid content.

The glutamine assay kit is based on hydrolysis of glutamine to glutamate and colorimetric determination of the product. The intensity of the product color measured at 630 nm is proportional to the glutamine concentration in the sample.

2.3.3 Nitrate reductase and nitrite reductase activities assay

NR was measured according to Bian et al. [15]. Leaf tissue (0.1 g) was homogenized with 4 mL of phosphate buffer (the same as in 2.3.2) and then centrifuged at 17,000 r for 15 min at 4 °C. The supernatant contained crude enzyme extract. The assay mixture contained 100 μL of the extraction, 200 μmol KNO_3 and 0.2 μmol nicotinamide adenine dinucleotide. After reacting at 30 °C for 20 min, 50 μL of 1 M zinc acetate was added to stop the reaction. The mixture was centrifuged at 7600 r for 5 min, and the absorbance was read at 530 nm. The amount of formed NO_2^- was calculated using a standard curve prepared with $NaNO_2$. The activity of nitrate reductase is expressed by the produced nitrite nitrogen content per gram of fresh sample per hour (μg g^{-1} h^{-1}).

NiR was measured by the modified method of Mendez and Vega [22]. Leaf tissue (1 g) was kept at 0°C for 2 h and then homogenized in 4 mL of buffer solution. The homogenate was centrifuged at 12 000 r at 4°C for 15 min. The supernatant contained crude enzyme extract. Three test tubes were numbered, and 2 mL of PBS was added to each tube. Then, 1 mL of distilled water was added to tube No. 1, 1 mL of KNO_2 (1 mmol) and 2 mL of color-developing reagent were added to tube No. 2, and 1 mL of KNO_2 (1 mmol) was added to tube No. 3. All three tubes were incubated at 30°C for 10 min. Subsequently, 0.1 mL of toluene and 2 mL of crude enzyme extract were added to these tubes and incubated at 30°C for 2 h. In tubes No. 1 and No. 3, 2 mL of color-developing reagent was added, and after sufficient color developed, 3 mL of trichloroacetic acid (TCA) was added. The mixture was centrifuged at 12 000 r for 10 min. The absorbance was read at 530 nm. The NO_2^- content of the solution was calculated from a standard curve. The amount of NO_2^- reduction was calculated as follows: content in tube No. 1 + content in tube No. 2 - content in tube No. 3. The consumption of nitrite (tube No. 1 + tube No. 2 - tube No. 3) was used to calculate enzyme activity.

The NiR activity was expressed by the reduction in NO_2^- content per gram of fresh sample per hour (μg g^{-1} h^{-1}).

2.3.4 Glutamate synthase and glutamine synthetase activities assay

The leaf tissues (0.5 g) were homogenized in an ice bath with 50 mM buffer solution containing 1% (w/v) insoluble polyvinylpyrrolidone, 1.5% (w/v) soluble casein, 2 mM EDTA and 2 mM dithiothreitol. The homogenate was centrifuged at 4°C and 12 000 r for 15 min. The supernatant contained crude enzyme extract, which was used for the glutamine synthetase and glutamate synthase enzyme activity assay.

GS activity was determined by the method of Cánovas et al. [23]. One mL of crude enzyme extract was added to 3 mL of reaction solution containing 50 mmol·L^{-1} L-sodium glutamate, 4 mmol·L^{-1} ATP-2Na, 40 mmol·L^{-1} hydroxylamine, 20 mmol·L^{-1} $MgSO_4$, 10 mmol·L^{-1} L-cysteine and 40 mmol·L^{-1} PBS. The resulting mixture was allowed to react at 30°C for 15 min; then, 1 mL of reaction solution containing 30% trichloroacetic acid (g/v), 8% $FeCl_3$ (g/v) and 5.5 mol L^{-1} HCl was added, and these three solutions were mixed at 1:1:1 (v:v:v). After 10 min of rest, the absorbance was read at 540 nm. The activity of GS was expressed as μmol γ-glutamyl hydroxamate formed per gram of fresh sample per minute (μmol g^{-1} min^{-1}).

GOGAT activity was measured by the method of Migge et al. [24]. The reaction mixture was incubated at 30 °C for 30 min. In total, 40 μL of N-methylmaleimide (pH of 8.0) was added to the mixture, incubated in a 95 °C water bath for 10 min, and then centrifuged at 12 000 r for 8 min. Then, 70 μL of supernatant was added to 100 μL of solution to be tested, and the absorbance was read at 540 nm. The reaction mixture contained 20 μL of crude enzyme extract, 5 μL of methyl viologen (100 mmol·L^{-1}), 40 μL of HEPES (150 mmol·L^{-1}), 10 μL of L-glutamine (100 mmol·L^{-1}), 5 μL of α-ketoglutaric acid (60 mmol·L^{-1}), 10 μL of aminooxyacetic acid (100 mmol·L^{-1}), 20 g·L^{-1} of NaS_2O and 20 g·L^{-1} of $NaHCO_3$. The methyl viologen-dependent activity of GOGAT was calculated from the increase in glutamate per gram fresh sample per minute (μmol g^{-1} min^{-1}).

2.3.5 RNA extraction and RT-PCR for gene expression analysis

Leaf samples were fixed and stored in liquid nitrogen. Total RNA content was extracted with Trizol according to the manufacturer's instructions. A fluorescence quantitative reverse PCR kit was used for reverse transcription (Zhongding, PC04-5OT). To guarantee reverse transcription efficiency, oligo (dT) and random primers were used. Specific gene primers of 3 nitrogen metabolism genes, as shown in Table 2, were used for RT-PCR.

Table 2: The primers for RT-qPCR were as follows.

Name of primer	Primer sequence	Fragment length
F3675BcNR-F	GCCTCAAGAATCCGACAG	233
F3675BcNR-R	CTCCTCCAGAGTAAGCATAGC	
4239-BcNiR-F	CATCGCAGGAAGCATCAA 185	185
4239-BcNiR-R	TCAGGCACATCAGGCAAC	
4239-BCGS2-F	GTCTTCTTCAAGTTCCAGG 119	119
4239-BCGS2-R	CACAACCGAGTTCCATAA	
ACTIN-F	GAATCCACGAAACAACTTACAACTC	131
ACTIN-R	CTCTTTGCTCATACGGTCAGC	

The relative amount of gene expression was calculated by $2^{-\ ct}$.

PCRs included 10 μL of SYBR Green qPCR Mix, 2 μL of diluted cDNA and 0.4 μL of each primer. ddH_2O was added to a final volume of 20 μL. The conditions for RT-qPCR were 30 s at 94°C, 10 s at 94 °C for denaturing, and 30 s at 72°C for annealing. The RT-qPCR included 45 cycles. The fluorescence data were collected at 72°C. Relevant gene expression was calculated according to Livak and Schmittgen [25].

2.4 Statistical analyses

Statistical analyses were conducted using Statistical Product and Service Solutions for Windows version 16.0 (SPSS Inc., Japan), and the differences between the means were tested using Duncan's multiple range test ($P = 0.05$).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Effects of 48 h of continuous light on the contents of nitrate in pakchoi

As shown in Figure 1, the nitrate content in pakchoi decreased rapidly in the initial 24 h in the W, R and B treatments. The content was lowest at 24 h and increased thereafter. However, the effect of B was the

Table 3: Effects of 24 h continuous light on content of nitrogen metabolites in pakchoi.

Light treatment	Soluble sugar (mg g^{-1})	Soluble protein (g L^{-1})	Glutamic acid (μmol $gprot^{-1}$)	Glutamine (μmol $gprot^{-1}$)
W(CK)	30.62a	7.13b	6.64b	0.10a
R	38.08a	7.10b	8.04ab	0.08a
B	33.59a	7.25a	11.99a	0.07a

W, white light; R, red light; B, blue light. Mean separation within a row by Duncan's multiple range test, values followed by different letters are significantly different at 5% level.

most significant. At 24 h, the nitrate content under the B treatment was approximately 23.9% lower than that of W-grown plants.

3.2 Effects of 24 h of continuous light on nitrogen metabolites in pakchoi

After 24 h of continuous light, the glutamate content was not significantly different among the three light treatments. The glutamic acid and soluble protein contents were significantly higher in the B treatment than in the W and R treatments. In particular, the glutamic acid content under the B treatment was 80.5% and 49.1% higher than that in the W and R treatments, respectively. The soluble sugar content under the R treatment was 13% and 24% higher than that under the B and W treatments (Table 3).

3.3 Effects of 24 h of continuous light on chlorophyll content and net photosynthetic rate in pakchoi

As shown in Figure 2, after treatment with different sources of light, the total chlorophyll pigment under the W, R and B treatments was 33.9, 31.7 and 41.7 $mg \cdot g^{-1}$, respectively. The dynamics of net photosynthetic rate in pakchoi were the same trend as those of the total chlorophyll content, i.e., blue light > white light > red light. The B treatment increased the Pn by 29.4% and 88.4% compared to the W and R treatments.

3.4 Effects of 24 h of continuous light on activity of nitrogen metabolism enzymes in pakchoi

As shown in Table 4, the NR activity in pakchoi was 19.3% higher under the B treatment than that under the W treatment, while the difference between the W and R

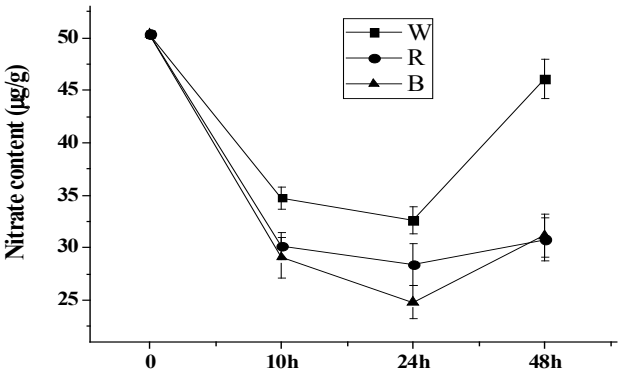

Figure 1: Effects of continuous light on the contents of nitrate in pakchoi.

treatments was not significant. The NIR activity was not significantly different among the three light treatments. The B treatment increased the GS activity by 29.4% compared to the W treatment, however, there was no significant difference between the R and W treatments. The GOGAT activity was significantly increased by 86.8% and 45% after exposure to B and R compared with that of W-grown plants.

3.5 Effects of 24 h of continuous light on nitrogen metabolism gene expression in pakchoi

RT-PCR was used to analyze the relative transcript levels of 3 genes involved in nitrogen metabolism in plants after irradiation with different light treatments. As shown in Figure 3, the transcriptional levels of 3 genes were not significantly different after exposure to the three light treatments. The expression levels of NR, NIR and GS genes were greatest under the B treatment, followed by the R and W treatments (Figure 3).

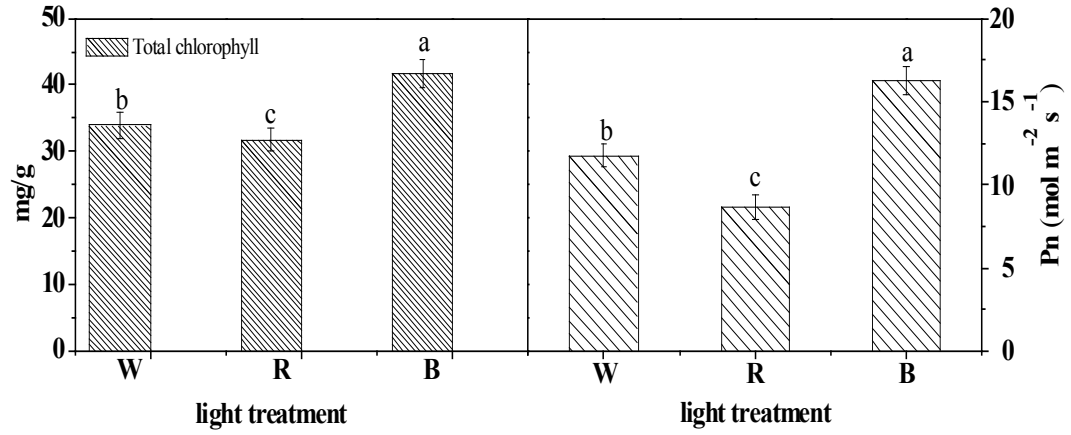

Figure 2: Effects of 24 h continuous light on net photosynthetic rate and chlorophyll content.

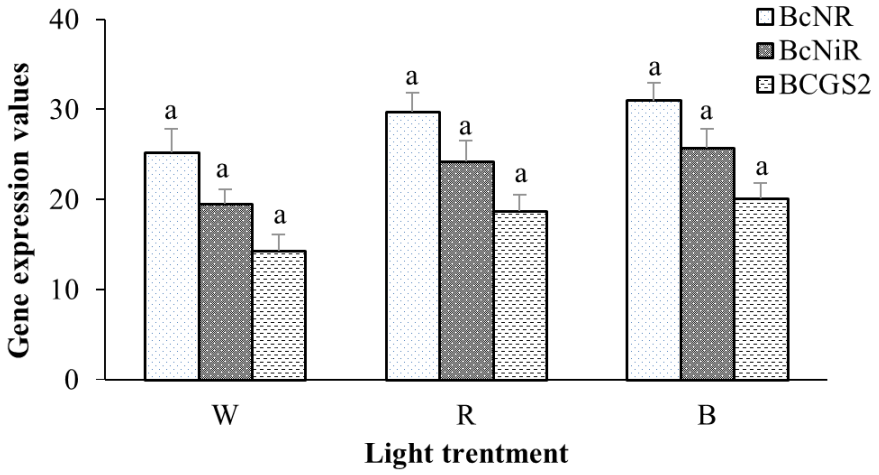

Figure 3: Effects of 24 h continuous light on key nitrogen metabolism gene gene expression in pakchoi.

3.6 Effects of 24 h of continuous light on phytohormone content in pakchoi leaves

For the different light treatments, the IAA content under the W and R treatments was markedly lower than that under the B treatment. At 24 h, the IAA content under the B treatment was significantly increased to 59.65 ng·g^{-1} compared with that under the W (53.65 ng·g^{-1}) and R (54.58 ng·g^{-1}) treatments. However, the difference between the R and W treatments was not significant. The GA content was not significantly different among the three treatments. With continuous light treatment, the differences in ABA content between the R and other treatments became increasingly greater. After 24 h, the ABA content under the R treatment was significantly increased by 8.5% and 23.4% compared with that under the W and B treatments, respectively.

4 Discussion

Light plays very important roles in nitrate metabolism in plants. It is also one of the main factors determining nitrate content. Among light conditions, light quality is a key factor in regulating the nitrate concentration in plants. Our results show that pakchoi grown under B and R treatments had a lower nitrate content than that grown under a W treatment (Figure 1). The change in nitrate in plants is closely linked to internal physiological activities, including the concentration of soluble protein, carbon and photosynthetic capacity [1].

In the present study, nitrate content decreased sharply under the three light treatments between 0 and 24 h, and the effect of the B treatment was the most significant. The reason for this result may be that the B treatment promoted the synthesis of soluble protein and caused an increase

Table 4: Effects of 24 h continuous light on nitrogen metabolism enzyme activity in pakchoi.

Light treatment	NR ($\mu g\ g^{-1}\ h^{-1}$)	NIR ($\mu g\ g^{-1}\ h^{-1}$)	GS ($\mu mol\ g^{-1}\ min^{-1}$)	GOGAT ($\mu mol\ g^{-1}\ min^{-1}$)
W(CK)	62.92b	57.17a	40.80b	11.40c
R	71.44ab	65.33a	42.41b	16.53b
B	75.05a	69.42a	52.86a	21.30a

W, white light; R, red light; B, blue light. Mean separation within a row by Duncan's multiple range test, values followed by different letters are significantly different at 5% level.

Table 5: Effects of 24 h continuous light on phytohormone content in pakchoi.

Light treatment	IAA ($ng\ g^{-1}\ FW^{-1}$)	GA ($ng\ g^{-1}\ FW^{-1}$)	ABA ($ng\ g^{-1}\ FW^{-1}$)
W(CK)	53.65b	6.34a	64.34b
R	54.58b	6.38a	69.83a
B	59.65a	6.17a	58.02b

W, white light; R, red light; B, blue light. Mean separation within a row by Duncan's multiple range test, values followed by different letters are significantly different at 5% level.

in the supply of enzymes and NADPH, which are used for the reduction of nitrate in leaves. The result is similar to the previous discovery of Lillo and Appenroth [26]. Another reason light regulates nitrate metabolism is that the expression of NR is controlled by light. NR is the most important and most limiting step in nitrogen metabolism [27]. The results revealed that the B and R treatments had positive effects on NR activity in pakchoi, and the effect of the B treatment was more pronounced. Under the B treatment, the nitrate content was significantly decreased, which might be partly attributable to the higher activity of NR. This result was similar to that reported by Qi et al. [27].

Previous studies found that NR activity is subject to changes in NR gene expression. The qRT-PCR analysis in our study showed that the expression of NR, NiR and GS genes was upregulated in response to the B treatment, suggesting the induction of nitrogen metabolism enzymes possibly (Figure 3). Our result of the effect of B treatment was similar to the results in spinach, tobacco and rice [27,29,30]. This result could also be caused by action through a signal transduction chain involving phosphorylation since the blue-light effect may be sensitive to protein kinase inhibitors and regulation of the absorption of nitrate [31,32]. The significant nitrate reduction in pakchoi under continuous light at 24 h may also lie in the fact that the higher NR activity stimulated by the increase in NR expression leads to enhanced nitrate reduction [33].

After the reduction of nitrate to nitrite in the cytosol, nitrite is translocated into the chloroplasts with the help of NiR. In our study, the NiR activity under the B and R treatments was higher than that under the W treatment (Table 4). However, there was no significant difference among the three treatments. It was found that NII is a gene encoding NiR and red and blue irradiation induced NII expression, consistent with the involvement of phytochrome and possibly a blue-light receptor [8,9]. Blue light especially stimulated the accumulation of one of the NiR proteins (NiR2). In research on lettuce, Bian et al. [35] reported that supplementation with green light had a positive effect on NR and NiR gene expression levels under short-term continuous light. Therefore, further studies using supplementation with green light may be able to promote nutritional value.

The GS-GOGAT cycle is the main pathway for ammonia assimilation in plants. Light directly participates in the expression of the GS2 gene and in its activity [34]. In the present study, we found that light quality had similar effects on GS, GOGAT and NR, i.e., B > R > W (Table 4). The result is similar to the previous discovery of Bian et al. [35], who reported significant positive correlations between activities of GS and GOGAT and the activity of NR. As was reported by Teller et al. [36], Fd-GOGAT was induced by red and blue light in etiolated turions of Spirodela, and both phytochrome and a blue-light receptor were involved.

However, we still do not know the synaptic mechanisms, hence, more research on this phenomenon is needed.

It is well known that photosynthesis plays an important role in nitrate metabolism, and products of photosynthesis are known to stimulate NR activation. In our present study, we found that the B treatment increased soluble protein content in pakchoi by 1.68% compared to the W treatment, which can provide more protein for both photosynthesis and nitrate metabolism. However, there is a strong relationship between photosynthesis and chlorophyll content. Our study found that the B treatment dramatically increased the net photosynthetic rate, which also contributed to a high chlorophyll content. In a study on rapeseed, Li et al. [20] also found that blue light at a higher percentage can increase chlorophyll content. The result is consistent with an early observation that higher NR was positively correlated with photosynthesis.

Light up regulates genes that are involved in the assimilation of ammonium into glutamine and glutamate. There were negative correlations between nitrogen assimilation and nitrate content. The products of nitrogen assimilation, especially glutamine, are known to exert negative feedback on NIA expression, which is a gene coding for NADH:NR in Arabidopsis [37,38]. In our present study, a decrease in glutamine content under the B treatment was observed, which may have reduced the inhibition of enzyme activity.

In plants, light is an important environmental signal that induces photomorphogenesis and the regulation of hormone activity [39]. We found that different light qualities can affect the phytohormone levels in pakchoi (Table 5). The changes in IAA contents under different light treatments were similar to those reported by Su [40], who showed that red light can reduce the activity of IAA synthesis enzymes, while blue light can increase the activity of IAA oxidase. In this study, we also found that blue light increased IAA content and NR activity in pakchoi, which was similar to the results observed in Arabidopsis [41]. There is little research on the regulatory mechanism of nitrate metabolism and hormones, and the question of how light participates in this process remains. These mechanisms still need further study.

5 Conclusion

The results of this study showed that the metabolites of pakchoi could be changed by exposure to continuous blue light. First, continuous blue light reduced nitrate content significantly, which is beneficial for promoting the safety and quality of pakchoi. The reduction in nitrate content under B treatment is mostly attributed to the higher activity of NR, GS and GOGAT enzymes. Second, the photosynthetic ability of pakchoi was also influenced by different light sources. However, there is still a need for further detailed study of changes in plant metabolites under different light treatments.

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Conflict of interest: Authors declare no conflict of interest.

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