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Low R/FR Ratio Affects Pakchoi's Growth and Nitrate Content under Excess Nitrate Stress

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Abstract: Nitrate accumulation is one of the main factors of secondary soil salinization in protected horticulture in China. Previous studies have shown that a low red(R)/far-red (FR) ratio can improve the salt tolerance of plants under NaCl stress. However, the effect of a low R/FR ratio on plant growth under nitrate stress is not clear. In order to explore the effect of a low R/FR ratio on the adaptability of pakchoi under high nitrogen stress, the growth index, soluble protein content, soluble sugar content, nitrate content, nitrate reductase activity and *Nia2* gene expression of pakchoi's leaves were measured. The results showed that a high level of nitrogen (the addition of 80 mmol · L⁻¹ NO₃⁻ (N80) and 160 mmol · L⁻¹ NO₃⁻ (N160)) inhibited the growth of pakchoi and promoted the accumulation of osmoregulation substances and nitrate content, respectively. The reduction of the R/FR ratio under high nitrogen stress (L80) increased the fresh weight of the plants under it by 19.0%, reduced the nitrate content in the leaves by 22.7%, increased the NR activity by 29.9%, and made the *Nia2* gene expression more significant, compared with N80. There was a similar mitigation effect of a low R/FR ratio under 160 mmol · L⁻¹ excessive NO₃⁻ stress. Therefore, the reduction of the R/FR ratio can effectively control the nitrate content and improve the adaptability of pakchoi under high nitrogen stress. Thus, there is a practical application prospect for a low R/FR ratio for the production of pakchoi under a high level of nitrogen.

Keywords: pakchoi; nitrate content; nitrate stress



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1. Introduction

Protected vegetable cultivation in a greenhouse is considered to be a good method for a stable yield and high quality of vegetables. By using insect prevention nets, environmental control and other facilities to block the harm of pests and reduce the impact of disastrous weather, it plays an important role in the stabilization of urban vegetable supply. In recent years, the problem of soil secondary salinization in greenhouses caused by continuous cropping, a high multiple cropping index and excessive fertilization has become more and more serious in China. Reports have shown that the main salt of secondary saline soil is Ca(NO₃)₂, rather than NaCl [1,2]. Research has shown that the harm of excessive NO₃⁻ to plants is mainly manifested in the inhibition of seed germination [3], the disorder of the antioxidant enzyme system [4], the inhibition of growth, and the accumulation of osmotic regulatory substances.

Recently, more and more studies have shown that light environment regulation not only promotes crop growth but also plays an important role in improving plant stress resistance. In the natural environment, the green leaves of plants absorb red light, and increase the proportion of far-red light through reflection and transmission, resulting in the decrease of the ratio of R/FR around the leaves. Plants feel the change of the ratio of

R/FR in the environment through phytochromes, resulting in the increase of plant height and internode elongation. Studies have shown that, under the same light intensity, a low proportion of R/FR can improve the photosynthetic capacity and dry matter accumulation of plants [5–7]. The supplementation of far-red light to reduce the ratio of R/FR can improve the photochemical efficiency and carbon fixation abilities of soybean, so as to improve the growth quality of soybean [8]. In addition, studies have found that a low R/FR ratio can alleviate low-temperature [9], drought [10] and salt stress [11], and can participate in the regulation of signal pathways related to stress resistance. A low R/FR ratio allowed the infection of growth by salt stress in tomato seedlings, while the allowance was absent in the mutant *phyB* [11]. It has been reported that phytochromes were involved in controlling the hormone biosynthesis in plants exposed to different R/FR ratios, as well as being involved in their salt tolerance [12,13]. However, the research on the salt tolerance of the R/FR ratio mainly focuses on NaCl stress. The effect of a low R/FR ratio under NO_3^- stress remains unclear.

Pakchoi (*Brassica campestris* L. ssp. *chinensis*), known as non-heading Chinese cabbage, is one of the most widely cultivated and popular vegetables in China because of its rich nutritional value and short growth period [14]. However, high concentrations of nitrates seriously damage human health [15]. Excessive NO_3^- not only inhibits the growth of pakchoi but also causes a high nitrate content in pakchoi leaves. Nitrate accumulation in vegetables is a complex process which is affected by light quality, nitrate nitrogen absorption, reduction and assimilation. There is a significant correlation between nitrogen fertilizer use and nitrate accumulation in vegetables [16]. In addition, nitrate reductase (NR) is the first enzyme in the process of nitrogen metabolism in higher plants [17]. It is also the rate limiting enzyme in the whole reduction process, and plays a key role in plant nitrogen metabolism [18]. Liang Liang [19] pointed out that the accumulation of nitrate is inversely proportional to the activity of nitrate reductase. Curtis et al. [20] found that increasing the expression of the nitrate reductase gene reduced the nitrate content in lettuce leaves. Under high nitrogen stress, the NR activity and nitrogen metabolism-related enzymes of cucumber were inhibited, while exogenous humic acid treatment could increase NR activity and reduce the nitrate content [4]. Therefore, it is expected to reduce the nitrate accumulation under excessive NO_3^- stress by regulating the NR activity of pakchoi. In this experiment, the authors will further explore the effect of a low R/FR ratio on the expression of nitrate reductase and related genes in pakchoi under nitrogen stress, so as to preliminarily reveal the mechanism of low R/FR ratio on the regulation of nitrate content in pakchoi.

2. Materials and Methods

In this experiment, pakchoi (*Brassica campestris* L. ssp. *Chinensis* cv. Te Zhong Xiao Bai Cai) was used as the material. LED tubes were used as the light source. The experiment was conducted in Sichuan Agricultural University (Chengdu, China) from June 2020 to June 2021. The seeds were selected, soaked and planted in a seedling tray containing a vermiculite and perlite matrix. It was placed in a plant factory at 25 ± 1 °C during the day and 18 ± 1 °C at night. The photoperiod was 12 h/12 h (light/darkness), the relative humidity was 70%, and the light intensity was $200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. The seedlings were watered by 1/2 Hoagland nutrient solution every 4 days. The main elements of the nutrient solution were as follows: NO_3^- ($7.0 \text{ mmol} \cdot \text{L}^{-1}$), P ($0.5 \text{ mmol} \cdot \text{L}^{-1}$), K ($3.0 \text{ mmol} \cdot \text{L}^{-1}$), Ca ($2.0 \text{ mmol} \cdot \text{L}^{-1}$), Mg ($1.0 \text{ mmol} \cdot \text{L}^{-1}$) and S ($1.0 \text{ mmol} \cdot \text{L}^{-1}$). After 22 days of the seedling period, the seedlings were transplanted into the hydroponics box with 1/2 Hoagland nutrient solution.

After 10 days of the seedling transplant stage, the plants were stressed. A total of 6 treatments were set up: (1) N—normal nutrient supply condition, R/FR = 4.2; (2) L—normal nutrient supply condition, R/FR = 0.7; (3) N80—the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 4.2; (4) L80—the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 0.7; (5) N160—the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 4.2; (6) L160—added $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 0.7. $\text{Ca}(\text{NO}_3)_2$ was used as the nitrogen source for the stress treatment. The concen-

tration of NO_3^- in the normal nutrient supply was $7.0 \text{ mmol} \cdot \text{L}^{-1}$, and the total concentration of NO_3^- under N80 and L80 was $87 \text{ mmol} \cdot \text{L}^{-1}$, respectively; the total concentration of NO_3^- under N160 and L160 was $167 \text{ mmol} \cdot \text{L}^{-1}$. By the addition of far-red LED light and white LED light, the R/FR ratio can be reduced. The R/FR ratio was calculated as the red light quantum flux density from 655 to 665 nm divided by the far-red light quantum flux density from 725 to 735 nm. R/FR ratios of 4.2 and 0.7 were used for the control and low-R/FR ratio treatments, respectively. Regarding the lamp tubes, 8 white LED lamps were used as the control group (R/FR ratio = 4.2), and 4 far-red LED lamps and 6 white LED lamps were used as the experimental group (R/FR ratio = 0.7).

Five plants were taken randomly from each treatment, and the second and third fully expanded functional leaves from the top of the plant were taken, quick-frozen with liquid nitrogen, and stored at -80°C for the determination of the various indexes. Samples were taken at 0 h, 3 h, 6 h, 12 h, 24 h and 72 h after high nitrate stress for the determination of the *Nia2* gene. The growth and morphological indexes were measured on the 15th day of stress, while the other indexes were measured from fresh samples on the 0th, 5th and 10th days of stress, respectively. This experiment was repeated three times, and 420 seedlings were cultivated for experiment each time.

The root length, leaf width and leaf length were measured with a ruler. The fresh weight of the plants were measured with a balance. As for the content of soluble sugar and soluble protein, they were determined by the anthrone method and coomassie brilliant blue G250, respectively [21]. The fresh leaf nitrate content was determined by the nitrosylsalicylic acid method [21], i.e., 2 g (powdered) leaves was boiled in 10 mL deionized water for 30 min and then filtered; the filtrate was prepared to use after being adjusted to 25 mL by adding deionized water. In total, 0.5 g leaves were ground in 4 mL buffer, (25 mM sodium phosphate buffer, pH 8.7, 10 mM L-cysteine, 1 mM Na_2EDTA), and the centrifuged supernatant was used to determine the nitrate reductase activity using sulfanilamide colorimetry [21].

The total RNA of the leaves was isolated using the E.Z.N.A. Plant RNA Kit (Omega Bio-tek, Doraville, GA, USA), according to the manufacturer's instructions. The FastKing one-step method (Tiangen, Beijing, China) was used to remove DNA contamination, and to perform the reverse transcription. The real-time PCR assay was carried out on a CFX96TM real-time PCR cycler (Bio-Rad, Hercules, CA, USA). The fluorescence real-time quantitative dye used the SYBR Premix Ex TaqTM II (Tli RNaseH Plus) Kit (Takara, Kusatsu, Japan). The gene amplification primers specific to the desired reaction design were taken from Primer Premier 5.0.0 (Premier Biosoft, Palo Alto, CA, USA). *Nia2*'s primer sequences were F:5'-GTCGATGCGGGATATACAGT-3' and R:5'-CCATCGCCATCTCTTTCTTA-3'. Pakchoi's actin was used as an internal reference. The actin's primer sequences were F:5'-AAACGCCTACCACATCCA-3' and R:5'-CCATCGCCATCTCTTTCTTA-3'. The $2^{-\Delta\Delta\text{Ct}}$ method was used to calculate the relative expression of the genes [22].

SPSS 22.0 software was used to analyze the experimental data, and Origin 2019 software was used to create the graphs. The statistical analysis for the data was performed through analysis of variance (ANOVA) by using Duncan's multiple range test ($p < 0.05$).

3. Results

3.1. Under Different Nitrogen Levels, a Low R/FR Ratio Promoted the Growth of Pakchoi

From Table 1, under non-stress conditions, it can be seen that low R/FR can significantly enhance the fresh weight, root length, leaf width and leaf length of pakchoi compared with the treatment N (the control). When $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$ was added, the fresh weight, root length, leaf width and leaf length of the whole plant decreased by 42.1%, 22.2%, 23.4% and 4.2% after 15 days of treatment, respectively, in comparison with the control. The treatments with a low R/FR ratio (L80) provided better growth performance; the results showed that the fresh weight, root length, leaf width, and leaf length of the pakchoi were significantly greater than N80. With the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, the growth of the pakchoi was severely inhibited, while the growth index of plants treated with low

R/FR (L160) was significantly higher than that of N160. This showed that the reduction of the R/FR ratio can lead to a high accumulation of the fresh weight, root length, leaf width, and leaf length of pakchoi, and has a positive effect on their growth.

Table 1. Effects of the changes of the R/FR ratio on the fresh weight, root length, leaf width and leaf length of pakchoi under different nitrogen levels. N: normal nutrient supply condition, R/FR = 4.2; L: normal nutrient supply condition, R/FR = 0.7; N80: with the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 4.2; L80: with the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 0.7; N160: with the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 4.2; L160: with the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 0.7.

Treatments	Fresh Weight (g)	Root Length (cm)	Leaf Width (cm)	Leaf Length (cm)
N	$122.60 \pm 0.88^{\alpha\beta}$	$39.00 \pm 1.00^{\text{b}}$	$14.10 \pm 0.78^{\text{b}}$	$19.20 \pm 0.46^{\text{b}}$
L	$215.60 \pm 0.26^{\text{a}}$	$45.50 \pm 1.63^{\text{a}}$	$17.20 \pm 0.46^{\text{a}}$	$22.00 \pm 0.61^{\text{a}}$
N80	$71.00 \pm 2.65^{\text{d}}$	$30.33 \pm 5.41^{\text{c}}$	$10.80 \pm 1.31^{\text{c}}$	$18.40 \pm 1.45^{\text{b}}$
L80	$84.47 \pm 0.40^{\text{c}}$	$37.50 \pm 5.72^{\text{b}}$	$14.50 \pm 0.17^{\text{b}}$	$20.80 \pm 0.36^{\text{a}}$
N160	$15.36 \pm 0.49^{\text{f}}$	$15.86 \pm 1.05^{\text{e}}$	$8.40 \pm 0.62^{\text{d}}$	$8.30 \pm 0.78^{\text{d}}$
L160	$21.07 \pm 0.60^{\text{e}}$	$19.93 \pm 0.96^{\text{d}}$	$9.76 \pm 0.75^{\text{cd}}$	$12.16 \pm 0.80^{\text{c}}$

^α Values shown are means \pm standard deviation (n = 3). ^β Values followed by different letter(s) within a column for each species are significantly different ($p < 0.05$).

3.2. The R/FR Ratio Affects the Content of Osmotic Regulatory Substances in Pakchoi under Different Nitrogen Levels

It can be seen from Figure 1 that without the addition of nitrogen, the soluble sugar content of pakchoi showed an upward trend (0–10 d). After 10 days of treatment with low R/FR conditions, there was no significance in the soluble sugar content of the L treatment or the N treatment. After the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, the soluble sugar content of the N80 treatment increased 1.07 times compared with the N treatment on the tenth day. After the addition of far-red light, the soluble sugar content of the L80 treatment was increased. However, after the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, the soluble sugar content of N160 was slightly higher than that of N on the tenth day, and the difference was not significant. After the addition of far-red light, the L160 treatment increased by 27% compared with the N160 treatment on day 10 of the treatment. The soluble protein content was consistent with the soluble sugar content trend. The soluble protein content of the pakchoi increased continuously within 10 days. After the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, the N80 treatment increased significantly on the tenth day compared with the N treatment. After the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, the soluble protein content of the N160 treatment decreased, but a low R/FR ratio can increase the content of osmotic regulatory substances, reduce the harm of nitrogen stress, and promote the growth of pakchoi.

3.3. Nitrate Content in Pakchoi under Different Nitrogen Levels of the R/FR Ratio

It is shown in Figure 2 that the nitrate contents in the pakchoi leaves in N80 and N160 were 1.34 times and 1.81 times that in the control (N) on day 10 of treatment, respectively. Consequently, the pakchoi leaves exposed to calcium nitrate stress contained a higher level of nitrate, which reduced their quality. However, as a result of the low R/FR treatment, the nitrate content of the L treatment was reduced by 14.0% compared to the N treatment, the L80 treatment was reduced by 22.7% compared with the N80 treatment, and the L160 treatment was reduced by 16.3% compared with the N160 treatment after 10 days of exposure to stress from an excess of NO_3^- . The above shows that the reduction of the R/FR ratio can effectively reduce the nitrate content in the leaves of pakchoi.

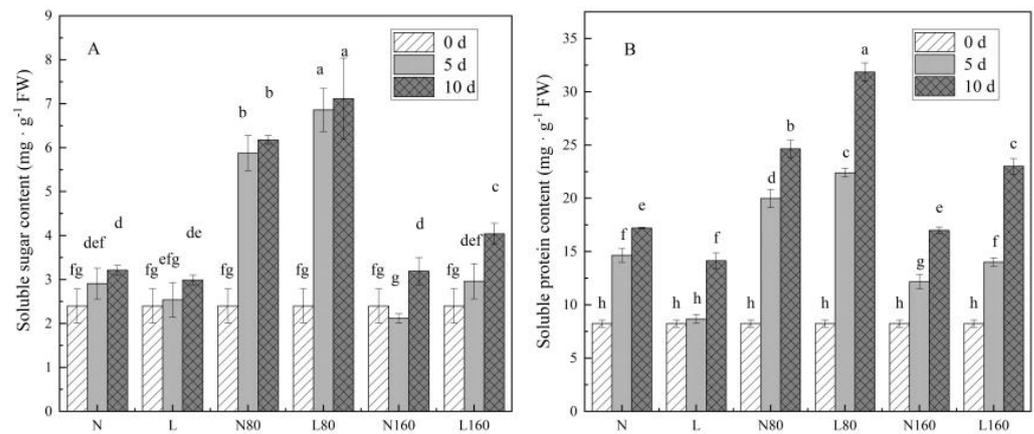


Figure 1. Effects of the changes of the R/FR ratio on the soluble sugar content (A) and soluble protein content (B) of pakchoi under different nitrogen levels. The values shown are means \pm standard deviation ($n = 3$). Different letters (a–h) indicate significant differences at $p < 0.05$ according to Duncan's multiple range test. 0 d: on day 0 after treatment; 5 d: on the fifth day after treatment; 10 d: on the tenth after treatment. N: normal nutrient supply condition, R/FR = 4.2; L: normal nutrient supply condition, R/FR = 0.7; N80: with the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 4.2; L80: with the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 0.7; N160: with the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 4.2; L160: added $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 0.7.

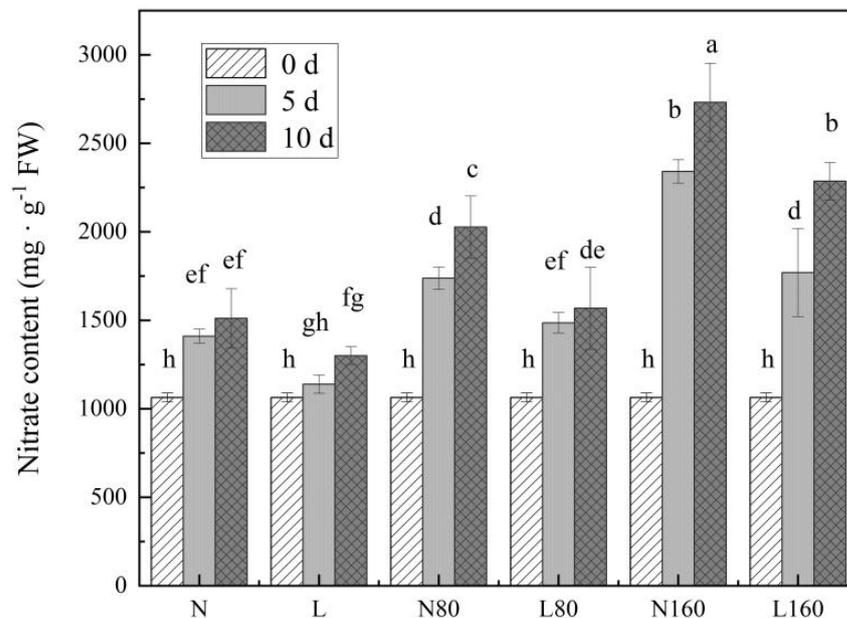


Figure 2. Effects of the changes of the R/FR ratio on the nitrate content of pakchoi under different nitrogen levels. Values shown are means \pm standard deviation ($n = 3$). Different letters (a–h) indicate significant differences at $p < 0.05$ according to Duncan's multiple range test. 0 d: on day 0 after treatment; 5 d: on the fifth day after treatment; 10 d: on the tenth after treatment. N: normal nutrient supply condition, R/FR = 4.2; L: normal nutrient supply condition, R/FR = 0.7; N80: with the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 4.2; L80: with the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 0.7; N160: with the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 4.2; L160: added $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 0.7.

3.4. R/FR Ratio Affects the Activity of Nitrate Reductase in Pakchoi at Different Levels of Nitrogen

As shown in Figure 3, without the addition of nitrogen, the nitrate reductase activity of pakchoi showed an upward trend within 10 days. On day 10 of treatment, with the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{N}$ (N80), the activity of nitrate reductase in the leaves of pakchoi

was significantly increased, and was 49.0% higher than that of the N treatment. After the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{ N}$ (N160), the nitrate reductase activity in pakchoi leaves dramatically decreased, declining by 59.0% from that in the N treatment. This indicated that a lower level ($80 \text{ mmol} \cdot \text{L}^{-1}$) of nitrogen increased the activity of nitrate reductase in the leaves of pakchoi, but a higher level ($160 \text{ mmol} \cdot \text{L}^{-1}$) of nitrogen weakened its activity. When treated with $\text{R/FR} = 0.7$, the activity of nitrate reductase significantly increased. The L treatment increased the nitrate reductase activity by 51.8% compared with the N treatment after 10 days of exposure to stress from an excess of NO_3^- . In addition, the L80 treatment increased by 29.9% compared with the N80 treatment, and the L160 treatment increased by 242.7% compared with the N160 treatment at 10d. This indicates that the low R/FR treatment would increase the nitrate reductase activity in pakchoi.

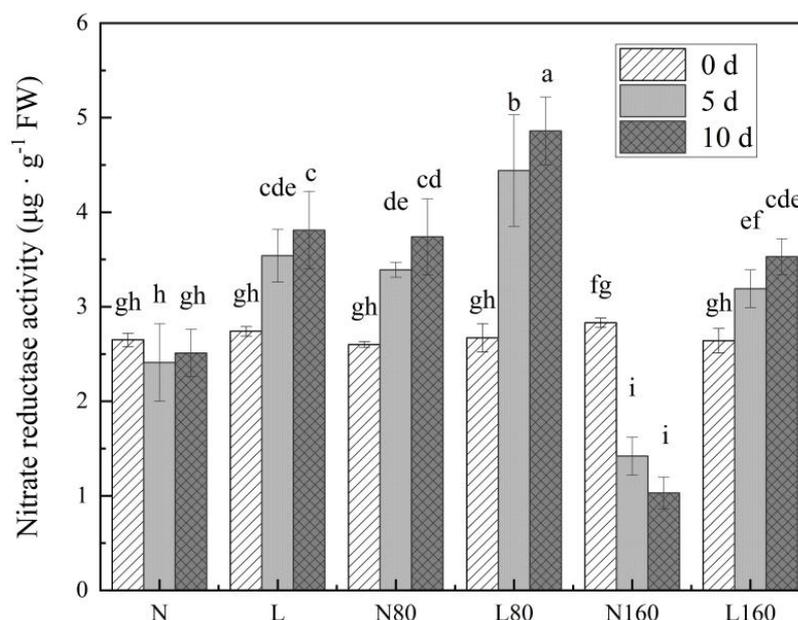


Figure 3. Effects of the changes of R/FR ratio on the nitrate reductase activity of pakchoi under different nitrogen levels. The values shown are means \pm standard deviation ($n = 3$). Different letters (a–i) indicate significant differences at $p < 0.05$ according to Duncan’s multiple range test. 0 d: on day 0 after treatment; 5 d: on the fifth day after treatment; 10 d: on the tenth after treatment. N: normal nutrient supply condition, $\text{R/FR} = 4.2$; L: normal nutrient supply condition, $\text{R/FR} = 0.7$; N80: with the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{ NO}_3^-$, $\text{R/FR} = 4.2$; L80: with the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{ NO}_3^-$, $\text{R/FR} = 0.7$; N160: with the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{ NO}_3^-$, $\text{R/FR} = 4.2$; L160: with the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{ NO}_3^-$, $\text{R/FR} = 0.7$.

3.5. Levels of R/FR Have Different Effects on *Nia2* Gene Expression under Different Nitrogen Levels in Pakchoi

According to the results (Figure 4), the expression of the *Nia2* gene increased initially within 72 h and then declined under non-stress conditions. The peak occurs at 12 h. On the other hand, the expression of the *Nia2* gene under $\text{R/FR} = 0.7$ is always higher than that with white light treatment. Under nitrogen stress, N80 treatment first inhibited pakchoi *Nia2* gene expression, and then promoted it over time. By contrast, the L80 treatment increased the expression of the *Nia2* gene. At 72 h, the expression of the *Nia2* gene was 9.5 times that at 0 h, and was significantly higher than that under N80 treatment. Under $160 \text{ mmol} \cdot \text{L}^{-1}$ conditions, the *Nia2* gene expression in pakchoi showed a trend of first increasing and then decreasing, reaching a peak at 24 h. Moreover, the expression of the *Nia2* gene under light quality treatment with $\text{R/FR} = 0.7$ is better than that under white-light treatment. There were also significances at the different time points (3 h–72 h) in N and L, respectively (Table S1).

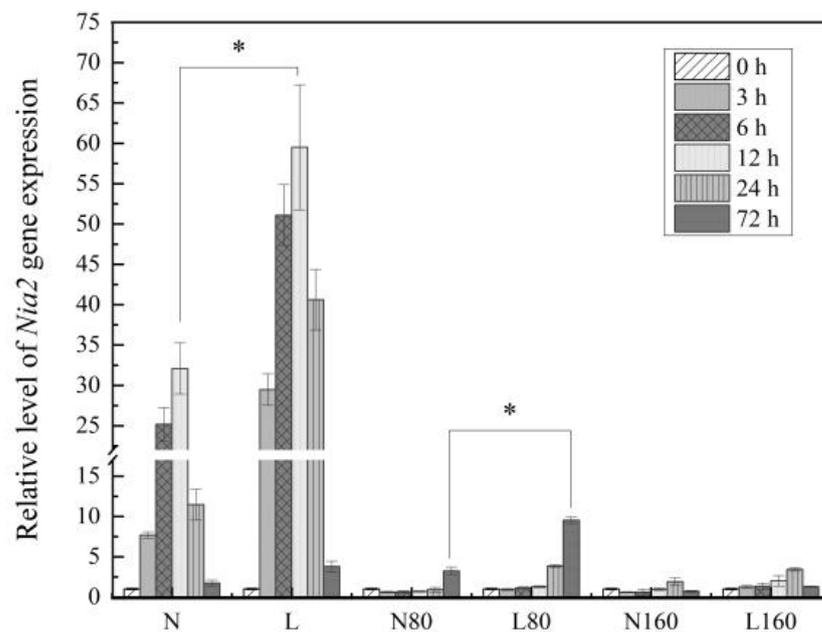


Figure 4. Expression levels of the *Nia2* gene in leaves of pakchoi under different R/FR ratio and N levels at 0, 3, 6, 12, 24, 72 h. The relative gene expression levels were detected by quantitative reverse transcription polymerase chain reaction (RT-qPCR) using actin as the reference gene. * $p < 0.05$. 0 h: 0 h after treatment; 3 h: at the 3rd hour after treatment; 6 h: at the 6th hour after treatment; 12 h: at the 12th hour after treatment; 24 h: at the 24th hour after treatment; 72 h: at the 72nd hour after treatment. N: normal nutrient supply condition, R/FR = 4.2; L: normal nutrient supply condition, R/FR = 0.7; N80: with the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 4.2; L80: with the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 0.7; N160: with the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 4.2; L160: with the addition of $160 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$, R/FR = 0.7.

4. Discussion

Red light can reduce the length of internodes and plant height, while far-red light can increase the length of internodes and plant height [23]. The reduction of the R/FR ratio can promote plant stem growth and adjust the phytochrome to adapt to the external environment [24,25]. In this study, we showed that low R/FR could significantly enhance the fresh weight, root length, leaf width and leaf length of pakchoi under non-stress conditions. Similarly, Yang et al. [26] revealed that the reductions in the R/FR ratio increased the height and stem mass fractions of soybean seedlings. Yang et al. [7] also found that a low R/FR ratio significantly increased the total biomass and leaf area of soybean seedlings.

When plants are under salt stress, their normal life activities are blocked, and the growth of plant organs and tissues are inhibited [27]. Recent studies have shown that a low R/FR value can significantly reduce the oxidative damage induced by salt stress, and can reduce the inhibition of PSII electron transfer and promote seedling growth [11]. In this study, we found that the growth of pakchoi was inhibited under high nitrogen stress, with a decrease in the fresh weight, leaf width, and leaf length (Table 1). Similar observations were found in tomato seedlings under high nitrogen stress by Zhou et al. [28]. However, a higher proportion of far-red light in the environment can significantly improve the accumulation of the fresh weight of pakchoi under high nitrogen concentrations (Table 1). This is consistent with the literature reported by Zhu et al. [29] and Cao et al. [11]. In addition, the reduction of the R/FR ratio accelerated the photosynthesis capacity in pakchoi under excess nitrate stress [30]. Another study found that a low R/FR ratio promoted the net photosynthetic rate by increasing the expression of a Rubisco gene (RBCS) of tomato seedlings under calcium nitrate stress [28]. Thus, the enhancement of a low R/FR ratio on photosynthesis may provide the plants with the potential for growth under excess nitrate stress.

Soluble protein and soluble sugar are important osmotic adjustment substances in plants; they can alleviate the water stress caused by salt damage and other adversities, maintain a certain turgor pressure in cells, and ensure the normal operation of plant metabolic activities [31]. In this study, the addition of $80 \text{ mmol} \cdot \text{L}^{-1} \text{NO}_3^-$ significantly promoted the nitrogen metabolism of pakchoi, causing the soluble protein content and soluble sugar content to increase significantly. However, it showed a downward trend under a higher nitrogen treatment (N160). The study of Sun et al. [32] also showed that high nitrogen application was not conducive to sugar accumulation in the later period of beet root tubers. The reduction of the R/FR ratio can increase the osmotic adjustment substances of pakchoi, indicating that a low R/FR ratio can alleviate excessive NO_3^- stress.

NR is the key enzyme in regulation of the nitrate content in higher plants [17,18]. In this study, a low R/FR ratio enhanced the activities of NR under both excessive NO_3^- stress and non-stress. Under the excessive NO_3^- stress, the content of nitrate was increased, while it was inhibited by the low R/FR ratio. These results indicated that a low R/FR ratio can reduce nitrate accumulation under excessive NO_3^- stress by regulating the NR activity of pakchoi. The *Nia* gene encodes the NR protein, which plays an important role in nitrogen metabolism. Previous studies have reported that the transcriptional abundance of *Nia* mRNA was related to the nitrogen source, concentration and induction time of the induction substrate [33]. In our study, there was a significant decrease 3 h after high nitrate stress ($80 \text{ mmol} \cdot \text{L}^{-1}$ and $160 \text{ mmol} \cdot \text{L}^{-1}$). This indicated that the *Nia* gene can quickly respond to the change of the nitrogen level. Mesnard and Ratcliffe [34] thought that there is a self-regulation mechanism in the plant nitrogen metabolism system. They pointed out that the assimilation amount of NO_3^- of plants balances with their metabolism, i.e., the amino acids produced per unit of time should not exceed the metabolic requirements. Otherwise, the accumulation of NO_2^- will cause toxic effects to the cells. Thus, the reduction of *Nia* gene expression could be an adaptation mechanism under high nitrate stress. In this study, with the low R/FR ratio treatment, the *Nia2* gene expression in L80 was always higher than that of N80. Similar results were also found in the concentration of 160 mM of nitrate stress. This might be attributed to the interaction of nitrogen and light. Studies have shown that the reduction of the red light to blue light ratio promotes the nitrogen metabolism [35–37], but there are few reports related to the effect of a low R/FR ratio on nitrogen metabolism, especially regarding *Nia* gene expression [38]. According to the study reported by Jonassen et al. [39], HY5 (ELONGATED HYPOCOTYL5) positively regulates nitrate reductase in plants. The author also found direct evidence of the positive regulation of *Nia* by HY5 in *Arabidopsis thaliana*. In addition, HY5 can sense and transmit light signals, including the change of the R/FR ratio [40]. The question of whether reducing the R/FR ratio enhances *Nia2* gene expression via HY5 needs further research.

5. Conclusions

In conclusion, the growth of pakchoi was inhibited under high nitrogen levels, but the reduction of the ratio of red light to far-red light in the environment can significantly improve its soluble protein content, soluble sugar content and nitrate reductase activity, and can regulate the expression of the nitrate reductase gene of pakchoi positively, so as to reduce the nitrate content and alleviate the negative effects of a high nitrogen level in pakchoi.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae8030186/s1>, Table S1: Expression levels of *Nia2* gene in leaves of pakchoi under different LED light quality and N levels at 0, 3, 6, 12, 24, 72 h.

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