Effects of Light and Phytohormone Treatments on the Expression of ζ-Carotene Desaturase Gene (BoaZDS) in Chinese Kale

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Abstract. Chinese kale variety 'Sijicuitiao' was used as the plant material. Chinese kale seedlings were treated by different light qualities, light intensities and phytohormones, and the expression of ζ-carotene desaturase gene (BoaZDS) was analyzed. The results showed that red & blue light significantly promoted the expression of BoaZDS gene, peaked at 6 h and the expression level was 2.33 folds than that of the control. Blue light also induced the expression of BoaZDS gene in the late stage (72 h). Strong light had a significant effect on BoaZDS gene expression, peaked at 24 h and the expression level was 5.64 folds than that of the control, whereas weak light inhibited the BoaZDS gene expression. Abscisic acid and salicylic acid induced the expression of BoaZDS gene, and expression patterns were bimodal. Both methyl jasmonate and gibberellin acid inhibited the expression of the BoaZDS gene. The results provided a basis for further study on the regulation mechanism of BoaZDS gene.

1. Introduction

Chinese kale (Brassica oleracea var. alboglabra) is a member of the Brassicaceae, with the tender bolting stem and young leaves as the main edible organs. Chinese kale has rich nutrients including vitamin C, carotenoids, glucosinolates and polyphenols [1], it is one of the original Chinese vegetables. Carotenoids are light-harvesting pigments and photoprotective pigments for photosynthesis in plants [2]. Carotenoids are also as precursors to phytohormone abscisic acid [3]. ζ-carotene desaturase (ZDS) is one of the key enzymes in the carotenoid biosynthesis pathway, it catalyzes the conversion of ζ-carotene into neurosporene and then further convert the neurosporene to lycopene [4]. ZDS gene is closely related to carotenoid biosynthesis, numerous studies have shown that the expression level of ZDS gene can affect carotenoid accumulation in plants. Rodrigo et al. cloned the cDNA sequence of ZDS from citrus fruits and found that ZDS gene positively regulates carotenoid accumulation in citrus fruits [5]. Wu et al. overexpressed ZDS gene and found that β-carotene content respectively increased by 49% in flowers and 91% in leaves in tobacco [6]. Shi et al. found that carotenoid content was significantly reduced in tobacco NtzDS gene silencing plants [7]. At present, there are limited reports on ZDS gene regulation mechanism. We cloned the BoaZDS gene from Chinese kale in our previous study and found its promoter sequence has a large number of cis-acting elements that are related to light and phytohormone responses [8]. Therefore, to clarify the effects of light and phytohormone treatments on the expression...
of BoaZDS gene, this study used different light qualities, light intensities and phytohormones to treat Chinese kale seedlings and BoaZDS gene expression was analyzed by qRT-PCR. The results provided a basis for further study on the regulation mechanism of BoaZDS gene.

2. Materials and methods

2.1. Plant materials
Chinese kale variety 'Sijicutiao' was used as the plant material. Chinese kale seedlings were grown on culture medium V (peat): V (vermiculite): V (perlite) = 3: 1: 1, and they were placed on tissue culture chambers with temperature of 25/20°C (day/night), light intensity of 160 μmol/m²·s, light cycle of 12/12 h (day/night), and air relative humidity of 75%.

2.2. Reagents
Reverse transcription kit (PrimeScript™ RT reagent Kit with gDNA Eraser) and fluorescence quantification kit (TB Green™ Premix Ex Taq™ II) were purchased from TaKaRa. Salicylic acid (SA), Gibberellin acid (GA₃), Abscisic acid (ABA), etc. were purchased from Sangon Biotech. Methyl jasmonate (MeJA) was purchased from Sigma.

2.3. Light quality, light intensity and phytohormone treatments
After 30 days of cultivation, uniform seedlings were chosen. Red, blue, and red & blue (1: 1) lights were used for treatments, and white light was set as a control. Dark, weak (80μmol m⁻² s⁻¹) and strong (240 μmol m⁻² s⁻¹) lights were used for treatments, and 160 μmol m⁻² s⁻¹ was set as a control. 1 mM SA, 5 μM GA₃, and 0.5 μM ABA were used for leaf spray treatments and 100 μM MeJA were used for fumigation in transparent and closed containers, and distilled water as a control. Each treatment and control were respectively sampled after 0, 1, 3, 6, 12, 24, 48, and 72 h, with 3 repetitions per time, 4 plants for each repetition, and the 5th leaf as the sampling object. After sampling, the samples were quick-frozen with liquid nitrogen and stored at -80°C.

2.4. Realtime fluorescence quantitative PCR (qRT-PCR)
Total RNA was extracted using the improved CTAB method [9]. RNA integrity was detected using agarose gel electrophoresis, RNA concentration and purity was detected using nucleic acid protein analyzer. cDNA was synthesized from 1 μg of total RNA using the PrimeScript™ RT reagent Kit with gDNA Eraser. According to our previously obtained BoaZDS gene sequence (GenBank Accession No. KY662297) [9], the gene-specific primer was designed and the internal control gene β-actin is quoted from the primers of Büchert et al. [10] (Supplementary Table 1). Each PCR was performed in a 20 μl reaction mix containing 10 μl of TB Green Premix Ex Taq II (2×), 0.8 μl of each primer (10 μM), 2.0 μl of template cDNA and 6.4 μl of ddH₂O. PCR was performed as follows: 95°C for 30 s; then 40 cycles of 95°C for 5 s, 60°C for 30 s for the dissociation stage. The target gene BoaZDS and the internal control gene β-actin were amplified. The relative amounts of target gene expression for each sample were calculated using the formula 2⁻ΔΔCT [11].

Table 1. Primers used in this experiment.

| Primer name | Primer sequence (5’-3’) |
|-------------|------------------------|
| BoaZDS F    | CCTCGGAGGTTTCTATGGTGCTTC |
| BoaZDS R    | CAGTTGACATGCCAGCAAGTCC |
| β-actin F   | CCAGAGGTCTTGGTCCAGCCATC |
| β-actin R   | GTTCCACCAGAGCAAATGTTAC |
3. Results

3.1. BoaZDS gene expression analysis under different light quality treatments

The relative expression levels of BoaZDS in Chinese kale were shown in Figure 1 after treatments with red, blue, and red & blue lights. Under the red & blue light treatment, the relative expression of BoaZDS gene was significantly higher than that of the control (except 1 h). The result showed a trend that increased first and then decreased, peaked at 6 h and was 2.33 folds than that of the control. Under the blue light treatment, the relative expression of BoaZDS gene was slightly lower than that of the control in the early stage (3 h and 6 h), but it was gradually up-regulated and significantly higher than the control after 24 h, peaked at 72 h and was 4.08 folds than that of the control. However, the result under the treatment of red light was similar to the control except for the early stage (3 h and 6 h).

![Figure 1. Effect of different light qualities on relative expression levels of BoaZDS in Chinese kale](image)

3.2. BoaZDS gene expression analysis under different light intensity treatments

The relative expression levels of BoaZDS in Chinese kale were shown in Figure 2 after treatments with dark, weak, and strong lights. Under the strong light treatment, the relative expression of BoaZDS gene was not significantly different from that of the control in the early stage, but it was significantly up-regulated after 12 h, peaked at 24 h and was 5.64 folds than that of the control. In contrast, under the weak light treatment, the relative expression of BoaZDS gene was significantly lower than that of the control (except 24 h and 48 h) and was only 30.56% of the control at 6 h. Under the dark light treatment, the result showed that there was no obvious regular pattern. The two peaks respectively appeared at 3 h and 12 h, which were 1.76 times and 2.10 times that of the control, but decreased at 6 h and was only 56.67% of the control. The rest of the time period was no significant difference with the control.

![Figure 2. Effect of different light intensities on relative expression levels of BoaZDS in Chinese kale](image)
3.3. *BoaZDS* gene expression analysis under different phytohormone treatments

The relative expression levels of *BoaZDS* in Chinese kale were shown in Figure 3, all the relative expression levels were significantly lower than the control at 1 h treated by phytohormones. Both under the treatment of ABA and SA, the relative expression levels of *BoaZDS* gene were lower first and then higher than that of the control, and expression patterns were bimodal in the middle and late stages. Specifically, the result showed two peaks at 6 h and 48 h treated by ABA, which were 1.55 times and 2.76 times that of the control. But the result showed two peaks at 12 h and 72 h treated by SA, which were 1.54 times and 2.48 times the control. In contrast, both under the treatment of MeJA and GA3, the relative expressions of *BoaZDS* gene were significantly lower than that of the control at most time. At 12 h and 24 h, the relative expression of *BoaZDS* gene was only 19.33% and 30.81% of the control treated by MeJA. At 24 h, the relative expression of *BoaZDS* gene was only 36.49% of the control treated by GA3.

![Figure 3. Effect of different phytohormones on relative expression levels of *BoaZDS* in Chinese kale](image)

4. Discussion

Light quality is an important environmental factor in plant growth and development, studies have shown that light quality has regulatory effects on plant photosynthesis and gene expression [12]. Xu et al. found that red and blue lights reduced carotenoid content in leaf lettuce [13]. In our study, the results showed that red & blue light significantly promoted the expression of *BoaZDS* gene, while red light only had a certain inhibitory effect in the early stage. There are certain differences with the findings of predecessors may be due to differences in the proportion of light absorption by different plants.

Zhang et al. used strong light to treat *Eucommia ulmoides* Calli and found that the nine enzyme genes involved in carotenoid synthesis were up-regulated [14]. In our study, strong light had a significant effect on *BoaZDS* gene expression in the middle and late stages, whereas weak light inhibited the *BoaZDS* gene expression, dark treatment had no obvious regular pattern, which are consistent with the results of previous studies. Combining the production experience that Chinese kale growth requires good light, it is indicated that light intensity has an important influence on the growth of Chinese kale and carotenoid synthesis.

In our study, the results shown that both SA and ABA induced the expression of *BoaZDS* gene, while GA3 inhibited *BoaZDS* gene expression. It is indicated that *BoaZDS* gene and carotenoids may focus on plant resistance direction in the balance between plant growth and development and plant resistance, and participate in SA and ABA-mediated plant resistance. MeJA as signalling molecules to stimulate the expression of plant resistance genes and induce chemical defence in plants [15]. Zhang et al. found that the relative expression of *PtZDS* increased first and then decreased with the enhancement of MeJA concentration in *phaeodactylum tricornutum*, and the expression level of *PtZDS* reached a peak value under the treatment of 50 μM MeJA [16]. In our study, 100 μM MeJA inhibited the expression of the *BoaZDS* gene. Different results among the previous studies may be due to distinct plant species and MeJA concentrations.
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