

# Effects of different light quality on growth, chlorophyll concentration and chlorophyll biosynthesis precursors of non-heading Chinese cabbage (*Brassica campestris* L.)

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**Abstract** The aim of this study was to evaluate the effects of different light quality of light emitting diode (LED) on the growth, concentration of chlorophyll and chlorophyll biosynthesis precursors of non-heading Chinese cabbage (*Brassica campestris* L.). Seedlings of the cultivar Te Ai Qing were cultured for 28 days under 6 treatments: red light (R), blue light (B), green light (G), yellow light (Y), red plus blue light (RB) and dysprosium lamp (CK). Lighting experiments were performed under controlled conditions (photon flux density  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; 12 h photoperiod; 18–20 °C). The fresh and dry mass were the greatest under RB, which were significantly higher than other light treatments. The fresh mass under RB was almost twice higher compared to other light treatments. Plant height was highest

under R treatment and was lowest under B. RB treatment also lowered the plant height significantly. The highest soluble sugar concentration was observed under B. The soluble protein concentration was the greatest under RB. The R treatment was adverse to pigment accumulation. The concentration of photosynthetic pigments and chlorophyll biosynthesis precursors were higher under RB. The RB treatment was beneficial to pigment accumulation.

**Keywords** 5-Aminolevulinic acid · Chlorophyll biosynthesis precursors · LEDs · Light quality · Non-heading Chinese cabbage · Photosynthetic pigments

## Abbreviations

ALA	5-Aminolevulinic acid
B	Blue LED
CK	Dysprosium lamp
G	Green LED
LED	Light emitting diode
Mg-Proto IX	Mg-proporphyrin IX
Pchlde	Protochlorophyllide
PFD	Photon flux density
Proto IX	Protoporphyrin IX
R	Red LED
RB	Red plus blue LED
Y	Yellow LED

## Introduction

Among various environmental factors, light is one of the most important variables affecting the photo-morphogenesis and photosynthesis of plants (Avercheva et al. 2009). Light is also an essential energy source for plant photosynthesis and an important signal for plant growth and

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development. Changes in light quality have a profound effect on plant growth, particularly on photosynthesis (Neff et al. 2000; Fukuda et al. 2008).

Chlorophyll is the material base of plant photosynthesis. Its concentration and composition directly influence the photosynthetic rate of the leaves. Chlorophyll is synthesized via a complex biosynthetic pathway: glutamate is converted to 5-aminolevulinic acid (ALA), protoporphyrin IX (Proto IX) is biosynthesized, magnesium is inserted into Proto IX to form Mg-proto IX, proto chlorophyll (Pchl<sub>id</sub>) is turned into Pchl<sub>id</sub> a, and chlorophyll<sub>id</sub> a is esterified into chlorophyll *a* (Porra 1997; Tanaka and Tanaka 2007).

Chlorophyll biosynthesis requires light (Jilani et al. 1996; Hooper and Eggink 1999). The formation of photosynthetic pigment is controlled by different light qualities. At the same time, different photosynthetic pigments absorb different light spectrum. Blue light is generally considered to be beneficial for the formation of Chl *a*. Higher Chl *a/b* ratios are observed under blue light and lower Chl *a/b* ratios under red light (Rivkin et al. 1989; Marsac and Houmard 1993). Blue light improves gene expression of MgCH, GluTR and FeCH which regulates synthesis of chlorophyll (Wang et al. 2009) and promotes chlorophyll synthesis (Poudel et al. 2008; Kurilcik et al. 2008). Red light is not conducive to the formation of chlorophyll, because of the reduction in tetrapyrrole precursor 5-aminolevulinic acid (Tanaka et al. 1998; Sood et al. 2005).

However, previous reports have scarcely described the effects of LED light sources on the biosynthesis of chlorophyll in plant. In this paper, the chlorophyll biosynthesis of non-heading Chinese cabbage cultured under different light treatments was investigated to examine how different light qualities affect chlorophyll biosynthesis in the plant.

## Materials and methods

### Light treatments

All the LEDs were designed by College of Agriculture, Nanjing Agricultural University, China. The light treatments were designated as red LEDs, blue LEDs, green LEDs, yellow LEDs, a mixture of red plus blue LEDs (R:B = 6:1) and dysprosium lamp(CK). The spectral distributions of the yellow (peak at 590 nm), green (peak at 520 nm), red (peak at 658 nm) and blue (peak at 460 nm) lights were measured using a spectroradiometer (OPT-2000, ABDPE CO., Beijing, China). All the treatments were irradiated with the same photon flux density (PFD) of 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . PFD was measured using a quantum sensor (LI-250, LI-COR, USA) and was separately controlled by adjusting both the electric currents and numbers of light bulbs. The parameters of the light in each treatment are shown in Table 1.

### Plant materials

Seedlings of non-heading Chinese cabbage (*Brassica campestris* L. *Te'aiqing*) were germinated and were planted in plastic pots containing a mixture of peat, vermiculite and perlite (1:1:1, v/v/v). When the second leaf was fully expanded, 120 seedlings were selected, randomized into six groups and were cultured under six light quality treatments for 28 days. Plants were fertigated using Garden Test Standard nutrient solution. Since there was ventilation in the controlled environment, the CO<sub>2</sub> level was the same as the CO<sub>2</sub> level of atmosphere. The relative humidity (RH) was maintained at 70 ± 10 %, with a 12-h photoperiod, and the growth temperature was at 18–20 °C.

### Biomass and growth parameters analysis

A total of 20 seedlings for each treatment were selected for biomass analysis. To determine the dry mass, the seedlings were dried at 85 °C until a constant mass was reached. The fresh mass and dry mass of the seedlings were measured using an electronic balance. Plant height was measured from main stem base to top of the plant by a ruler.

### Soluble protein measurement

Leaves (1.0 g of fresh weight, *W*) were ground in a mortar with liquid nitrogen, to which 5 ml (*V*<sub>1</sub>) of 0.067 mol l<sup>-1</sup> potassium phosphate buffer (PBS) was added, and were then filtered through filter paper. The extract was centrifuged at 12,000g for 10 min, and the supernatant was removed. The extract (1 ml, *V*<sub>2</sub>) and Coomassie brilliant blue G-250 (5 ml) was thoroughly mixed. The optical density was measured using a UV-1200 spectrophotometer (Jin Peng, Shanghai, China) at 595 nm. The optical density was measured by a UV-1200 spectrophotometer at 595 nm ( $\rho$ ). The concentration of soluble protein was determined using the following equation: soluble protein ( $\text{mg g}^{-1}$ ) =  $\rho V_1/W V_2$  (Li et al. 2010).

**Table 1** Major technique parameters of light spectral energy distribution under LED lights

Light treatment	Peak wavelength, $\lambda_p$ (nm)	Halfwave width, $\Delta\lambda$ (nm)	Photon flux density ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
CK	400–760	–	150
Y	590	±12	150
G	520	±15	150
R	658	±12	150
B	460	±11	150
R:B = 6:1	658 + 460	±12 and ±11	150

### Soluble sugar measurement

The sampling timing was at 10 a.m. after the plants were irradiated for 3 h. Leaves (0.5 g, dry weight) were ground in a mortar with liquid nitrogen. Then 1 ml of 80 % ethanol was added, and the mixture was filtered through filter paper. The filtrates were recovered, and the residues were washed again with 70 % ethanol and filtered. Both filtrates were mixed, and 3 ml of distilled water was added. The extract was centrifuged at 12,000g for 15 min, and 1 ml of supernatant was collected. Soluble sugar concentration was determined by the sulfuric acid anthrone method and measured at 620 nm (Fairbairn 1953).

### Pigment measurement

0.1 g of leaves (fresh mass,  $W$ ) was submerged in 10 ml ( $V$ ) of 80 % acetone. The pigment was extracted until the leaf turned white. Optical density (OD) was measured with a UV-1200 spectrophotometer at 470 nm for carotenoid (OD<sub>470</sub>), at 663 nm for chlorophyll  $a$  (OD<sub>663</sub>), and at 645 nm for chlorophyll  $b$  (OD<sub>645</sub>), and calculated by the following equations by Holm (1954):

$$\text{Chl } a \text{ (mg g}^{-1}\text{)} = (12.72 \text{ OD}_{663} - 2.59 \text{ OD}_{645}) / V / 1,000 W$$

$$\text{Chl } b \text{ (mg g}^{-1}\text{)} = (22.88 \text{ OD}_{645} - 4.67 \text{ OD}_{663}) / V / 1,000 W$$

$$\text{Chl } (a + b) \text{ (mg g}^{-1}\text{)} = (8.05 \text{ OD}_{663} + 20.29 \text{ OD}_{645}) / V / 1,000 W$$

$$\text{Carotenoid (mg g}^{-1}\text{)} = (1,000 \text{ OD}_{470} - 3.27 \text{ Chl } a - 104 \text{ Chl } b) / (229 \times 1,000 W)$$

where  $V$  is the total volume of acetone extract (ml), and  $W$  is the fresh weight (g) of the sample.

### Assessment of chlorophyll biosynthetic precursors

The concentration of 5-aminolevulinic acid (ALA) was determined according to Dei (1985). Plant culture solution (200 ml) was centrifuged at 1,000×g for 5 min. The plant pellet was collected. 200 ml of buffer solution with 10 mmol l<sup>-1</sup> of levulinic acid was used to suspend plant cells, which were then cultured in an incubator under the original culture conditions but under light for 12 h. ALA was extracted using 4 % trichloroacetic acid. The extracted solution (5 ml) was added with 2.35 ml of 1 mol l<sup>-1</sup> sodium acetate, 0.15 ml of acetylacetonate and 2.5 ml of 1 mol l<sup>-1</sup> acetate buffer (pH 4.6), and was heated in boiling water for 10 min. Finally, the solution was cooled down and added with Ehrlich reagent. The concentration of ALA was analyzed by UV-1200 spectrophotometer at 553 nm.

The concentration of protoporphyrin IX (Proto IX), Mg-protoporphyrin IX (Mg-Proto IX) and protochlorophyllide

(Pchl<sub>id</sub>) were measured according to Rebeiz et al. (1975) and Lee et al. (1992). The plant was cultured in the dark for 12 h, and was then homogenized by ultrasonication with 8 ml of extraction solution (acetone: 0.1 mol l<sup>-1</sup> NH<sub>3</sub>.H<sub>2</sub>O = 9:1, V/V) in an ice bath. The extracted solutions were then re-extracted by equal volume and 1/3 volume *n*-hexane in turn. Finally, the acetone phase was taken to measure fluorescence intensities at 633, 622, 640 and 595 nm with 400 nm of excitation light, by utilizing the LS 55 fluorescence/phosphorescence/luminescence spectrophotometer (PerkinElmer USA). The relative concentration of Mg-Proto IX was directly represented by 595 nm (Lee et al. 1992). The relative concentration of Proto IX and Pchl<sub>id</sub> was calculated according to following equations by Rebeiz et al. (1975):

$$\text{Proto IX} = (F_{633} - 0.25 \times F_{622} - 0.24 \times F_{640}) / 0.95$$

$$\text{Pchl}_{id} = (F_{640} - 0.03 \times F_{633}) / 0.99$$

### Statistical analyses

Statistical analyses were conducted using SPSS 16.0 (SPSS, version 16.0 Inc., USA). The experiment was repeated six times with five plants in each treatment. The data were analyzed by ANOVA and the differences between the means were tested using Duncan's multiple range test ( $P < 0.05$ ).

## Results

### Growth, biomass and concentration of soluble sugar and soluble protein

Different light quality had variable effects on the growth of non-heading Chinese cabbage (Table 2). In general, the fresh and dry mass under LEDs were greater than those under CK treatment. There were no significant differences in fresh mass between CK and Y treatment. Under RB, the fresh and dry mass were the greatest in the seedlings, meanwhile the fresh mass was almost twice higher compared to other light treatments. The fresh mass was the lowest under CK and the dry mass was the lowest under Y. Plant height of non-heading Chinese cabbage was highest under R treatment and was lowest under B. RB treatment also inhibited plant height significantly.

Under B, the highest concentration of the soluble sugar was observed, and the lowest under Y. Under RB, the soluble protein concentration was the greatest in seedlings and the lowest under Y. These results showed that the B treatment might be suitable for accumulation of soluble sugars, and the RB treatment seemed to promote the accumulation of soluble protein.

**Table 2** Effects of different light quality on biomass mass, soluble sugar and soluble protein concentration of non-heading Chinese cabbage

Light treatment	Fresh mass (g)	Dry mass (g)	Plant height (cm)	Soluble sugar (mg g <sup>-1</sup> )	Soluble protein (mg g <sup>-1</sup> )
CK	19.25d	1.72c	9.98b	9.86c	4.64a
Y	20.57cd	1.53d	9.64b	5.51d	3.20c
G	21.22c	1.59d	9.77b	9.31c	3.88b
R	21.94bc	1.84b	10.86a	18.39b	4.27ab
B	22.17b	1.78bc	8.56d	25.38a	4.54ab
R:B = 6:1	39.15a	2.11a	9.36c	13.04b	4.89a

Different letters in columns indicate statistically significant differences ( $P < 0.05$ ) according to Duncan's multiple range test

**Table 3** Effect of different light quality on photosynthetic pigments concentration of non-heading Chinese cabbage

Light treatment	Chl <i>a</i> (mg g <sup>-1</sup> )	Chl <i>b</i> (mg g <sup>-1</sup> )	Carotenoids (mg g <sup>-1</sup> )	Chl <i>a/b</i> (mg g <sup>-1</sup> )	Chl ( <i>a + b</i> ) (mg g <sup>-1</sup> )
CK	0.85a	0.38a	0.14a	2.38a	1.23a
Y	0.48b	0.18c	0.08b	1.39b	0.82b
G	0.42b	0.24b	0.07bc	1.21b	0.76b
R	0.32c	0.16c	0.05c	2.05a	0.48c
B	0.65b	0.23b	0.08b	2.82a	0.88b
R:B = 6:1	0.83a	0.36a	0.13a	2.18a	1.19a

Different letters in columns indicate statistically significant differences ( $P < 0.05$ ) according to Duncan's multiple range test

### Concentration of photosynthetic pigments

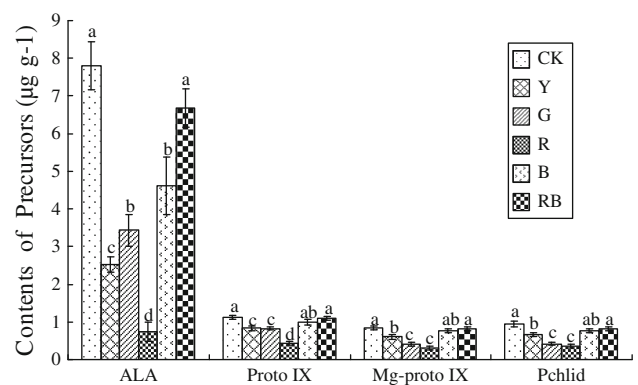
The concentration of photosynthetic pigments of non-heading Chinese cabbage varied in response to different light quality (Table 3). The concentration of chlorophyll *a* and chlorophyll *b* and the total concentration of chlorophyll (*a + b*) were higher in seedlings under RB and the lowest under R light. The concentration of carotenoid presented the same trend. The largest value of chlorophyll *a/b* was found in the B treatment, and the lowest in the G treatment. There were no significant differences between RB and CK in the concentration of pigments, and RB was the same effective as CK.

### Biosynthesis precursors of chlorophyll

The variation in the chlorophyll concentration detected in different light treatments prompted us to further test changes in the concentration of chlorophyll biosynthesis precursors including ALA, Proto IX, Mg-proto IX and Pchlid. We found that all the chlorophyll biosynthesis precursors in non-heading Chinese cabbage were the lowest under R treatment. There were no significant differences between RB and CK treatments in the concentration of chlorophyll biosynthesis precursors in seedlings (Fig. 1).

### Discussion

The results of the present study demonstrated that under LEDs, the fresh mass and dry mass in seedlings were significantly higher than under CK, which agreed with



**Fig. 1** Effect of different light quality on concentration of chlorophyll biosynthesis precursors of non-heading Chinese cabbage. Different letters in columns indicate statistically significant differences ( $P < 0.05$ ) according to Duncan's multiple range test

those observed by Went (1957) and Li et al. (2010). Under the RB treatment, the fresh mass and dry mass were the greatest in the seedlings, since blue and red lights promote development and drive photosynthesis. In our study, the fresh mass and dry mass of plants under RB were the greatest in seedlings, and the fresh mass was almost twice higher comparing to other light treatments. Previous study also found that the combination of red and blue light might promote fresh weight and dry weight in many plant species such as chrysanthemum, upland cotton and tomato (Kim et al. 2004; Li et al. 2010; Liu et al. 2011).

The spectra of red and blue light are in accordance with absorption spectrum peak area of chlorophyll, and the percentage absorption of blue and red light by plant leaves is about 90 % (Terashima et al. 2009). Therefore, the red and blue light strongly influence the development and

physiology of plant and have the best effective photosynthesis rate (Hogewoning et al. 2010). While white light contains a green component which consumes part of the red and blue lights (Went 1957), and the effects of green light may possibly be reconciled as the negative cry-dependent or cry-independent effects on seedling development and growth (Folta and Maruhnich 2007). Consequently, the RB treatment had better effect on biomass accumulation of non-heading Chinese cabbage.

Our study showed that plant height was highest under R, and was inhibited significantly under B and RB treatment. Previous study also indicated that red light promoted stem elongation while blue light had inhibition effect on stem elongation (Nhut et al. 2003; Li et al. 2013). The photoinhibitions of elongation by blue light are achieved through changes in cell wall properties. Blue light reduces relaxation mainly by increasing in the yield threshold (Kigel and Cosgrove 1991). The current results suggested blue light seemed to be an important factor for inducing the compact shape of plant; therefore, under RB treatment the plant height was lower. Generally speaking, plants with restricted growth or even more compact shape have higher level of chlorophyll. This may one of the reasons why RB treatment caused the highest concentration of chlorophyll.

Light quality regulates the carbohydrate metabolism of plants. Compared to the control, the R, B and RB treatments contributed to the accumulation of soluble sugar in leaf, with the B treatment being the most effective in increasing the soluble sugar concentration (Table 1). Blue light effectively promoted soluble sugar accumulation and was considered a means of studying the regulation of photosynthetic carbon metabolism in relation to plant growth (Britz and Sager 1990; Shin et al. 2008; Chang et al. 2010). Saebo et al. (1995) and Li et al. (2013) found that photosynthetic capacity was highest when the plants were exposed to blue light, which enhanced carbohydrate metabolism of higher plants, while single yellow light caused weak photosynthesis of plants, resulting in reduction of carbohydrate synthesis (Liu et al. 2011). Changes in carbohydrate concentration under different light qualities are also controlled by key enzyme activities related to soluble sugar metabolism in several species (Heo et al. 2006). Under Y treatment, the concentration of soluble protein was the lowest; hence, we speculated that the concentration and activity of enzyme are relatively low.

Chlorophyll plays an important role in photosynthesis, and light is the essential condition for chlorophyll formation. Light quality also plays an important role in chlorophyll biosynthesis (Jilani et al. 1996). Recent studies show that red light was not conducive to the formation of chlorophyll, because of the reduction in tetrapyrrole precursor 5-aminolevulinic acid (Tanaka et al. 1998; Sood et al. 2005). We made the same observation that the chlorophyll

concentration was the lowest under the R treatment, and so were the concentrations of ALA, Proto IX, Mg-proto IX and Pchlide which are important biosynthetic intermediates of chlorophyll. We found that red light reduced chlorophyll biosynthesis because less ALA was available for further synthesis.

Blue light was reported to be beneficial to pigment accumulation (Tanaka et al. 1998; Poudel et al. 2008; Kurilcik et al. 2008). It could reverse red light-induced inhibition response (Sood et al. 2005). Long-term exposure to blue light enhanced the ALA-synthesizing activity (Kamiya et al. 1981). The present results were consistent with previous studies. However, added with blue light, the RB treatment promoted chlorophyll biosynthesis even more significantly in this experiment.

We found that, under the G treatment chlorophyll biosynthesis was decreased with less ALA. The Y treatment induced higher concentration of chlorophyll biosynthesis precursors, but lower concentration of chlorophyll. The biosynthesis of chlorophyll could be blocked at any step. We speculated that low protein concentration led to low availability of enzymes which were responsible for the metabolism of chlorophyllide esterification to chlorophyll *a* (Stobart et al. 1985). This phenomenon requires further research.

Our study showed that red plus blue LEDs (R:B = 6:1) increased the biomass of non-heading Chinese cabbage. Red light alone was unprofitable for chlorophyll biosynthesis. There were no significant differences between RB and CK treatment in the concentration of photosynthetic pigments and of chlorophyll biosynthesis precursors. The RB light might be propitious to non-heading Chinese cabbage cultivation.

**Author contribution** XiaoXue Fan and Jie Zang designed and conducted the experiments, collected and analyzed data, and wrote the manuscript. ZhiGang Xu and ShiRong Guo designed, conducted the experiments, and assisted in writing the manuscript. Xuelei Jiao participated in the LED devices. XiaoYing Liu and Gao Ying helped in the biochemical analysis.

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