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Effects of light intensity on leaf microstructure and growth of rape seedlings cultivated under a combination of red and blue LEDs

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Abstract

The aim of this study was to evaluate the growth of rape (*Brassica napus* L.) seedlings under different light intensities to select appropriate conditions for cultivation in an indoor system. Seedlings were grown under different light intensities of red and blue light provided by light-emitting diodes (LEDs) and their self-adjustment ability and changes in leaf microstructure were evaluated. Light was supplied by red LEDs with peak wavelengths of 630 (R_1) and 660 nm (R_2) and by blue LEDs (B) with a peak wavelength of 445 nm (the light intensity ratio of R_1:R_2:B was 3:3:2), at intensities of 400 (R_1R_2B400), 300 (R_1R_2B300), and 200 μmol m^{-2} s^{-1} (R_1R_2B200). Natural solar light served as the control (C). Plant height, stem diameter, root length, leaf area, and dry weight of rape seedlings gradually increased with increasing light intensity. The seedlings in the R_1R_2B400 treatment grew more vigorously, while those in the R_1R_2B200 treatment were weaker. The photosynthetic pigment contents did not differ significantly between the R_1R_2B400 treatment and C, but were significantly lower in the R_1R_2B300 and R_1R_2B200 treatments. The highest intercellular CO_2 concentration, stomatal conductance, and transpiration rate were in the R_1R_2B300 treatment. The highest photosynthetic rate was in the R_1R_2B400 treatment, and was related to more compact leaves, thicker and tidier palisade and spongy tissues, and well-developed chloroplasts. In contrast, the seedlings in the R_1R_2B200 treatment had disordered mesophyll cells, round chloroplasts, and fractured and fuzzy grana lamellae, all of which inhibited plant growth. In conclusion, the seedlings in the R_1R_2B400 treatment had well-developed leaves, which favored photosynthesis. Compared with the light intensities below 300 μmol m^{-2} s^{-1}, the light intensity of 400 μmol m^{-2} s^{-1} provided by a combination of red and blue LEDs was beneficial for cultivating strong and healthy rape seedlings in an artificial system.

Keywords: light intensity, rape seedlings, mesophyll cell, chloroplast, stomata, photosynthetic characteristics

1. Introduction

Rape (*Brassica napus* L.) is an important oil crop, but growing this crop on an industrial scale may affect food security in China (Wang and Feng 2013). Whereas plants cultivated in the natural environment can be exposed to unfavorable growth conditions, controllable artificial facilities can provide optimal conditions for cultivating plants, including rape. This is especially useful for breeding programs because it can accelerate the breeding process. In the future, knowledge

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of appropriate artificial cultivation conditions will be important for more applications, for example, space planting. To optimize plant growth in artificial facilities, it is important to establish suitable environmental conditions, expecially light intensity. Plants grown under low light have frequently been shown to be more susceptible to photoinhibition than those plants grown under high light intensity. Generally, the increases in net photosynthesis rate (P_n) correlates with increases in light intensity. However, over-strong light also resulted in decreases of P_n. Therefore, determining which light intensity conditions are optimal for the cultivation of plants, including rape seedlings in artificial facilities is an important research goal.

Leaves are the main organ of photosynthesis and transpiration. The level of development of stomata, mesophyll cells, and chloroplasts directly affects photosynthesis and transpiration, which affect plant growth (Li 2006). Leaf morphology shows plasticity, and the photosynthetic characteristics and structure of leaves differ markedly under different light intensities. In previous studies, weak light conditions resulted in a decrease in P_n of eggplant (Yu *et al.* 2004) and reduced dry weight of wheat (Chen 2012). Long-term weak-light conditions resulted in thinner leaves with a smaller leaf area in potato (Qin *et al.* 2014), and a decrease in the specific leaf area of *Datura* (Mao *et al.* 2012). High-light intensity is also not conducive to plant growth; high-light can lead to wilted leaves, reduced leaf area and chlorophyll content and photosynthetic efficiency (Shirke and Pathre 2003). Additionally, high-light intensity can destroy the photosynthetic system, and cause serious oxidative damage to leaf tissues (Farquhar and Sharkey 1982).

Only under an appropriate light intensity can plants fully self-regulate to achieve the best state to absorb and transform light energy. In tomato seedlings, the light saturation point (LSP) was much lower under artificial light provided by light-emitting diodes (LEDs) than under solar light, where the LSP was about 500 μmol m^{-2} s^{-1} (Liu *et al.* 2010). In lettuce, 100 μmol m^{-2} s^{-1} light provided by blue and red LEDs was optimal for growth (Wang *et al.* 2011). The best light intensity to cultivate non-heading Chinese cabbage in a plant factory was 200 μmol m^{-2} s^{-1} (Lu *et al.* 2015). Tomato plants grew well under a light intensity of 300 μmol m^{-2} s^{-1} provided by blue and red LEDs, and these light sources resulted in lower energy consumption than other light sources (Fan *et al.* 2013). The results of these studies illustrate that plants have different light intensity requirements under artificial lights than under natural light.

In previous studies on rape, 40 μmol m^{-2} s^{-1} red light was more advantageous than white light for seedling growth (Du *et al.* 2002), and compound light (35 μmol m^{-2} s^{-1}, with a ratio of three red to one blue LEDs) was better than monochromatic light (Chen *et al.* 2013). The LSP of rape under natural light was reported to be approximately 600 μmol m^{-2} s^{-1} (Li and Zhang 1997). However, Leng *et al.* (2002) found that the LSP of rape was about 20–30 Klux, which is equivalent to 360 μmol m^{-2} s^{-1} (1 Klux=18 μmol m^{-2} s^{-1}). Plantlets required a lower light intensity than seedlings did (Li *et al.* 2012). Clearly, the light intensity used by Chen *et al.* (2013) is too low to cultivate strong rape seedlings. In fact, there have been no reports to date on a suitable light intensity of LED lights for cultivating rape seedlings. In addition, photosynthetically active radiation and light conversion efficiency differ between solar and artificial light, and therefore the optimal light intensity of artificial light cannot be predicted from the optimal intensity of solar light. Photosynthetic pigments mainly absorb light in the red and blue regions of the spectrum (Pan 2000). Previous studies have shown that red and blue composite light significantly improve the photosynthetic rate and promote plant growth (Chang *et al.* 2010; Wang *et al.* 2011; Chen *et al.* 2013). Rape and non-heading Chinese cabbage both belong to the Cruciferae, and Chinese cabbage was reported to grow vigorously under composite lights at a ratio of three red to one blue. The addition of red light at 630 nm can increase the spectral width, which was shown to benefit rice seedling growth (Liu *et al.* 2015). In our previous research, we found that rape seedlings grew well under a combination of red LEDs with peak wavelengths of 630 and 660 nm and blue LEDs (B) with a peak wavelength of 445 nm (unpublished).

In this study, we evaluated the leaf development of rape seedlings grown under water-cooled LED lamps as described in our previous study (Liu *et al.* 2015), with natural light as the control. We investigated the effects of different light intensities on the leaf microstructure and growth of rape seedling grown under a combination of red and blue LEDs. We measured photosynthetic pigment contents and observed the structure and arrangement of mesophyll cells, chloroplasts, and stomata. Based on our results, we speculated about the self-adjustment ability and regulation mechanisms related to leaf microstructure of rape seedlings under different light intensities. These results allowed us to define a suitable light intensity to cultivate rape seedlings in an indoor system.

2. Materials and methods

2.1. Plant materials and growth condition

This experiment was conducted at the Nanjing Agricultural University, China in 2015. Seeds of rape (*Brassica napus* L.), provided by the oil crop research group of Nanjing Agricultural University, were soaked in distilled water at 30°C for 24 h, and then germinated in an incubator at 28°C. The seeds were cultivated in growth medium under natural light

after germinating. When the seedlings had two true leaves, they were transplanted into pots (12-cm diameter). After recovering for 1 day, 30 rape seedlings in each treatment were grown in a controlled indoor system under three light intensities; 400 (R_1R_2B400), 300 (R_1R_2B300), and 200 μmol m^{-2} s^{-1} (R_1R_2B200) provided by red and blue LEDs. Seedlings grown under natural light served as the control (C). The plants under C were placed an open fields with rainproof measure. The light intensity ratio of red light (peak wavelength 660 nm):red light (peak wavelength 630 nm):blue light (peak wavelength 445 nm) was 3:3:2. The spectrum distribution of red and blue light is shown in Fig. 1. The plants were grown under a 12-h light/12-h dark photoperiod ((25±2)/(17±2)°C day/night temperatures) and the CO_2 concentration and relative humidity were maintained at 400 μmol mol^{-1} and (70±10)%, respectively.

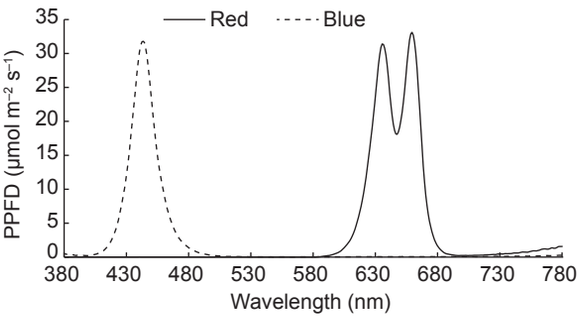

Fig. 1 Spectral distribution of red and blue light. PPFD, photosynthetic photon flux density.

2.2. Growth parameters and photosynthetic pigments

After irradiation for 15 days, three randomly selected seedlings were harvested for analysis. The number of leaves was counted, and the plant height and root length were measured with a ruler. Stem diameter was measured using Vernier calipers. To determine dry weight, the young plants were dried at 85°C to constant weight, and then weighed using an electronic balance. The leaves were photographed and then the pixel values counted with Photoshop software were used to calculate the leaf area (Xiao *et al.* 2005). Chlorophyll was extracted from leaves using the method of Harmutk (1987), and chlorophyll concentrations were calculated using the formula described by Holm (1954). The experiment was repeated three times.

2.3. Photosynthetic parameters

Photosynthetic parameters were measured using a LI-6400 portable photosynthesis measurement system (LI-COR, Lincoln, NE, USA). For measurements, the photosynthetic photon flux density was set to be 800 μmol m^{-2} s^{-1}, and the leaf temperature, CO_2 concentration, and relative humidity were (23±1)°C, (380±5) μL L^{-1}, and 60–70%, respectively. Measurements of P_n were repeated three times with three plants in each treatment. Stomatal limitation (L_s) was calculated as follows: $(C_a-C_i)/C_a\times100\%$ (where C_a is the atmospheric CO_2 concentration and C_i is the intercellular CO_2 concentration). The experiment was repeated three times with three plants in each treatment.

2.4. Morphological and physiological analyses

Anatomical features of leaf The anatomical analysis of the mesophyll cells in the leaves of the young plants was performed using the method of Clark (1981). The anatomical structure of the mesophyll cells was examined under an optical microscope (DP71; Olympus Inc., Tokyo, Japan). Leaf compactness was calculated using the following formula: Leaf compactness=Palisade tissue thickness/Leaf thickness (Song *et al.* 2015). This thickness ratio of palisade to spongy tissue (PT/ST) was calculated as follows: PT/ST=Palisade tissue thickness/Spongy tissue thickness (Yang *et al.* 2014). We analyzed 10 images per leaf, one leaf per plant, and three plants per treatment. The experiment was repeated three times.

Stomatal traits The imprint method was used to visualize stomata (Liu *et al.* 2005). Microscopy images were processed using an optical microscope (DP71; Olympus Inc.) connected to an image analysis system. The number of stomata per field of view in the leaf epidermis was used to calculate stomatal density (stomata per mm^2). We analyzed 20 images per leaf, one leaf per plant, and three plants per treatment. The stomatal aperture, length and width were measured for at least 32 randomly sampled stomata.The experiment was carried out three times.

Chloroplast ultrastucture On day 15 of irradiation, leaf samples were collected after illumination for approximately 3 h, placed in 0.2 mol L^{-1} phosphate buffer (pH 7.2) containing 2.5% glutaraldehyde, and then placed under vacuum. Then, the samples were incubated at 4°C for 8 h before being washed three times with 0.2 mol L^{-1} phosphate buffer (pH 7.0). The washed samples were fixed for 2 h in 1% osmic acid prepared with 0.2 mol L^{-1} phosphate buffer (pH 7.2) and washed again with the same buffer (pH 7.0) three times. The resulting samples were finally washed twice with double-distilled water at an interval of 10 min. Samples were washed with double-distilled water, then dehydrated in an ethanol series (twice each at 30, 50, 70, 90, and 100%) and soaked in epoxy propane. The samples were embedded in Epon-812 epoxy resin, which was polymerized at 30°C for 24 h, 40°C for 24 h, and 60°C for 48 h. The resulting samples were sectioned with an ultra-microtome (Powertome XL, RMC Products; Boeckeler Instruments Inc., Tucson,

AZ, USA) and treated with uranyl acetate, followed by lead citrate. The treated samples were observed and photographed with an electron microscope (H-7650; Hitachi Ltd, Tokyo, Japan). We analyzed 18 images per leaf, one leaf per plant, and two plants per treatment.

2.5. Statistical analyses

Statistical analyses were conducted using Statistical Product and Service Solutions for Windows, ver. 19.0 (SPSS Inc., Chicago, IL, USA). Data were subjected to analysis of variance, and differences between means were tested using Duncan's multiple range test (P<0.05).

3. Results

3.1. Morphology and biomass

We evaluated the morphology and biomass of plants grown under composite red and blue light at intensities of 400, 300, and 200 μmol m^{-2} s^{-1} (R_1R_2B400, R_1R_2B300, and R_1R_2B200 treatments, respectively). Plants grown under natural light served as the control (C). The plant height, stem diameter, root length, leaf area, and dry weight of rape seedlings gradually increased with increasing light intensity. The plant height, stem diameter, root length, leaf area, and shoot, root and plant dry weights were significantly lower in the R_1R_2B200 treatment than in the R_1R_2B300 and R_1R_2B400 treatments. The shoot, root and plant dry weight was significantly lower in C than in the R_1R_2B300 and R_1R_2B400 treatments, respectively. There were no significant differences between R_1R_2B300 and R_1R_2B400 treatments in plant height, stem diameter, and root length, but leaf area and shoot, root and plant dry weight were significantly higher in the R_1R_2B400 treatment than in the R_1R_2B300 treatment (Fig. 2, Table 1).

3.2. Photosynthetic characteristics

Under increasing light intensities, the P_n increased, while the intercellular CO_2 concentration (C_i), transpiration rate (T_r), and stomatal conductance (G_s) first increased and then decreased. The P_n was significantly higher in the R_1R_2B400 treatment than in the other treatments. The C_i, T_r, and G_s were significantly higher in the R_1R_2B300 treatment than in the other treatments. The lowest values for P_n, G_s, C_i, and T_r were in C. Stomatal limitation (L_s) was significantly higher in R_1R_2B400 and C than in the other two treatments, and did not differ significantly between the R_1R_2B300 and R_1R_2B200 treatments (Table 2).

3.3. Photosynthetic pigments

The seedling chlorophyll (Chl) content did not differ significantly between the R_1R_2B200 and R_1R_2B300 treatments, but their Chl *b* and (*a*+*b*) contents were significantly higher than those in the R_1R_2B400 treatment. The Chl *a*/*b* was higher in the R_1R_2B400 treatment and C than in the other two treatments. The Chl *a*, *b*, and (*a*+*b*) contents did not differ significantly between the R_1R_2B400 treatment and C. The carotenoid contents were higher in the three LED light treatments than in C (Table 3).

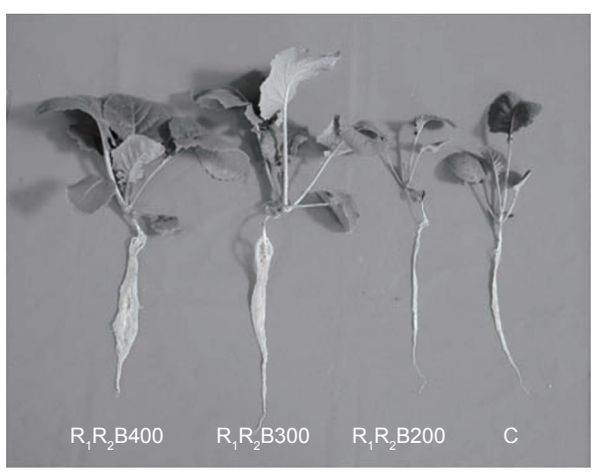

Fig. 2 Morphology of rape seedlings in different light treatments. R_1R_2B400, R_1R_2B300, and R_1R_2B200 represent composite light supplied by red and blue light-emitting diodes (LEDs) at light intensities of 400, 300, and 200 μmol m^{-2} s^{-1}, respectively. C, control (natural light). The same as below.

Table 1 Effect of different light intensities on morphological indexes and dry weight of rape seedlings

Light treatment[1)]	Plant height (cm)	Stem diameter (cm)	Root length (cm)	Leaf area (cm^2)	Shoot dry weight (g)	Root dry weight (g)	Plant dry weight (g)
R_1R_2B400	10.23 a	0.38 a	21.24 a	73.94 a	1.05 a	0.18 a	1.22 a
R_1R_2B300	9.70 ab	0.35 a	20.13 a	44.74 b	0.83 b	0.14 b	0.93 b
R_1R_2B200	8.13 b	0.17 b	14.66 c	18.46 d	0.12 c	0.02 c	0.13 c
C	11.23 a	0.20 b	16.14 b	23.26 c	0.25 c	0.03 c	0.27 c

[1)] R_1R_2B400, R_1R_2B300, and R_1R_2B200 represent composite light supplied by red and blue light-emitting diodes (LEDs) at light intensities of 400, 300, and 200 μmol m^{-2} s^{-1}, respectively; C, control (natural light).
Different lowercase letters in the same column indicated significant differences among treatments (P≤0.05; n=3).
The same as below.

3.4. Palisade and spongy tissues

In terms of leaf microstructure, the mesophyll cell structures were clear in the R_1R_2B400 and R_1R_2B300 treatments, and there were three layers of palisade cells with a compact and tidy arrangement. The spongy tissue cells were also distributed in an orderly and compact manner. In the R_1R_2B200 treatment, the palisade tissue also had three layers, but the cells were arranged loosely and irregularly, and interlaced between layers. The spongy tissue cells were sparse and loosely arranged. The mesophyll cells in C were similar to those in R_1R_2B200, and the mesophyll layer had a loose and poorly defined structure in both of these treatments (Fig. 3). The highest values for leaf thickness, palisade tissue thickness, spongy tissue thickness, leaf compactness, and PT/ST were in the R_1R_2B400 treatment, and were significantly higher than their respective values in the R_1R_2B300 and R_1R_2B200 treatments. The lowest values for leaf thickness, palisade tissue thickness, and spongy tissue thickness were in C. The PT/ST and leaf compactness did not differ significantly between C and the R_1R_2B400 treatments (Table 4).

3.5. Chloroplast unltrastructure

The chloroplasts in the R_1R_2B400 and R_1R_2B300 treatments were spindle shaped (Fig. 4-A and B), while those in the

Table 2 Effect of different light intensities on photosynthetic characteristics of rape seedlings[1)]

Light treatment	P_n (μmol m^{-2} s^{-1})	G_s (mmol m^{-2} s^{-1})	C_i (μmol mol^{-1})	T_r (mmol m^{-2} s^{-1})	L_s
R_1R_2B400	25.93 a	0.54 b	288.08 c	3.90 b	0.43 a
R_1R_2B300	21.21 b	0.86 a	407.90 a	5.00 a	0.12 b
R_1R_2B200	20.42 b	0.55 b	390.45 b	3.95 b	0.14 b
C	13.14 c	0.09 c	287.62 c	1.17 c	0.38 a

[1)] P_n, net photosynthesis rate; G_s, stomatal conductance; C_i, intercellular CO_2 concentration; T_r, transpiration rate; L_s, stomatal limitation.

Table 3 Effect of different light intensities on photosynthetic pigment contents in rape seedling leaves

Light treatment	Chl *a* (mg g^{-1})	Chl *b* (mg g^{-1})	Chl (*a*+*b*)(mg g^{-1})	Chl *a*/Chl *b*	Carotenoid (mg g^{-1})
R_1R_2B400	1.11 b	0.88 b	1.99 b	1.27 a	0.53 a
R_1R_2B300	1.20 ab	1.27 a	2.46 a	0.95 b	0.57 a
R_1R_2B200	1.22 a	1.19 a	2.41 a	1.02 b	0.53 a
C	1.13 ab	0.83 b	1.95 b	1.36 a	0.46 b

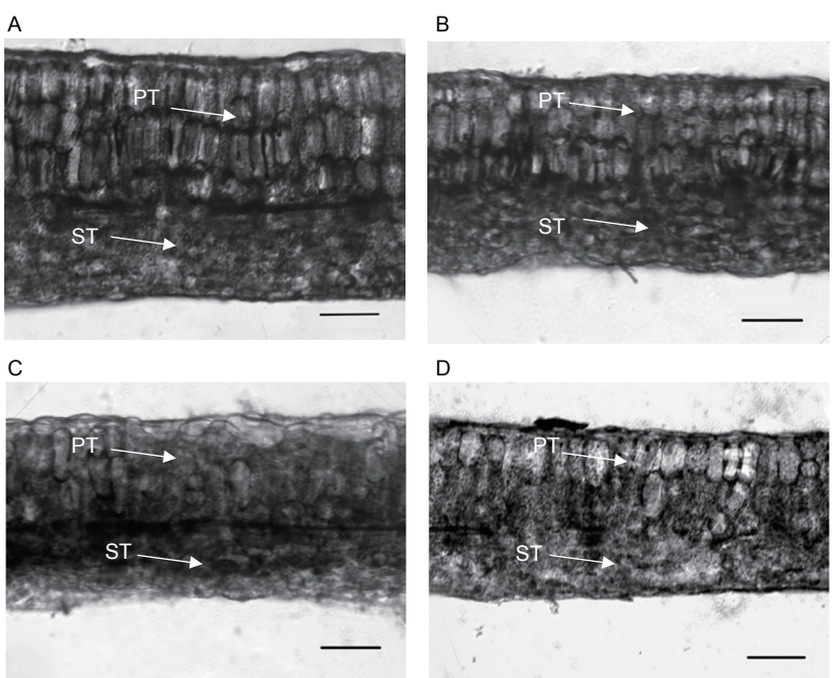

Fig. 3 Effect of different light intensities on leaf anatomical structure of *Brassica napus* L. seedlings. A–D, anatomy of leaf in R_1R_2B400, R_1R_2B300, R_1R_2B200, and C, respectively. PT, palisade tissue; ST, spongy tissue. Scale bar=100 μm.

R_1R_2B200 treatment were slightly round (Fig. 4-C), and those in C were relatively flat (Fig. 4-D). The grana lamella were more clearly defined in the R_1R_2B400 and R_1R_2B300 treatments than in the R_1R_2B200 treatment, in which the grana were poorly developed, damaged, and disorderly. There were many starch grains and osmiophilic particles in the chloroplasts in the R_1R_2B200 treatment (Fig. 4-G). The largest chloroplasts were in the R_1R_2B400 treatment, and their area was significantly greater than that in the R_1R_2B300 and C treatments, but not significantly different from that in the R_1R_2B200 treatment. The grana thickness was smaller in R_1R_2B400 than in the R_1R_2B300 and C treatments. However, there were more grana in chloroplasts in the R_1R_2B400 treatment than in the other treatments. There were significantly fewer grana in the R_1R_2B300 and C treatments than in the R_1R_2B200 and R_1R_2B400 treatments (Table 5). Therefore, the highest value for total grana thickness in chloroplasts was in the R_1R_2B400 treatment. The grana thickness increased with increasing light intensity (Table 5).

3.6. Stomatal traits

Stomata length did not differ significantly among treatments. In the control, stomata width was smaller than in the other treatments, but the ratio of stomatal length:width was significantly greater, and stomata were longer. The ratio of stomata length:width was significantly higher in the R_1R_2B400 treatment than in the R_1R_2B300 and R_1R_2B200 treatments, while did not differ significantly between the R_1R_2B300 and R_1R_2B200 treatments. The stomatal frequency/density was higher in the R_1R_2B300 and R_1R_2B200 treatments than in the other treatments, but did not differ significantly between the R_1R_2B300 and R_1R_2B200 treatments. The lowest stomatal density was in C treatment. The stomatal aperture was greater in the R_1R_2B200 and C treatments than in the other two treatments (Table 6).

4. Discussion

The light intensity demands of plants vary according to the

Table 4 Effect of different light intensities on leaf anatomical structure of rape seedlings[1)]

Light treatment	Leaf thickness (μm)	PT thickness (μm)	ST thickness (μm)	PT/ST	Leaf compactness
R_1R_2B400	425.54 a	221.70 a	120.41 a	1.84 a	0.52 a
R_1R_2B300	333.12 b	159.24 b	92.67 b	1.72 b	0.48 bc
R_1R_2B200	332.29 b	154.95 b	89.40 b	1.73 ab	0.47 c
C	301.79 c	153.34 b	86.34 b	1.77 ab	0.51 ab

[1)] PT, palisade tissue; ST, spongy tissue.

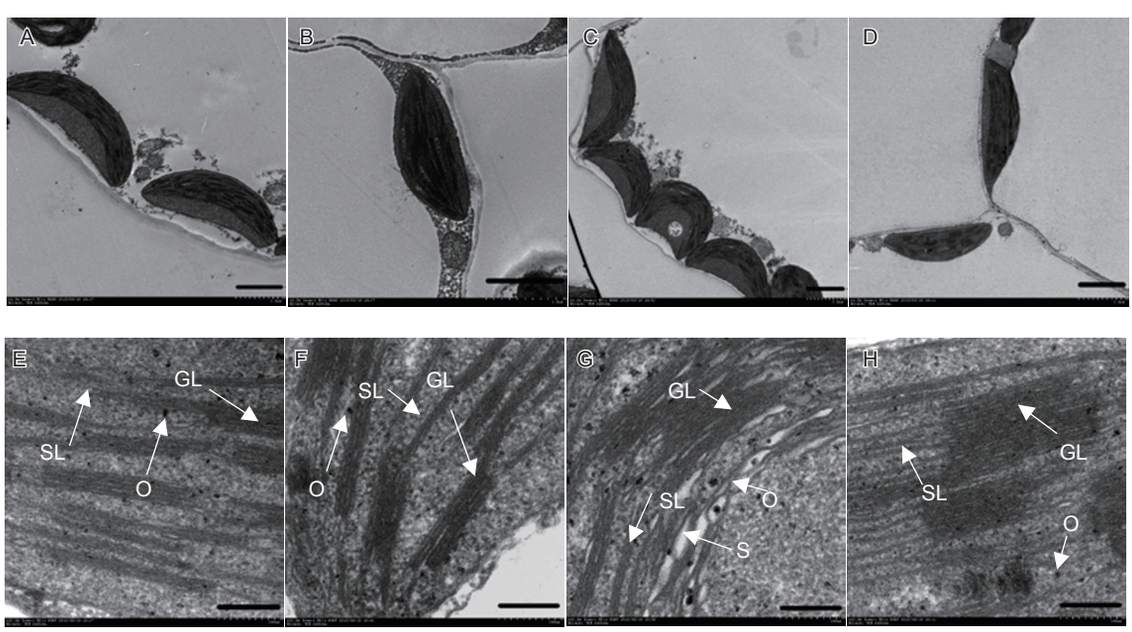

Fig. 4 Effect of different light intensities on chloroplast ultrastructure in leaves of *B. napus* L. A and E, chloroplast ultrastructure of R_1R_2B400 treatment. B and F, chloroplast ultrastructure of R_1R_2B300 treatment. C and G, chloroplast ultrastructure of R_1R_2B200 treatment. D and H, chloroplast ultrastructure of control. GL, grana lamellae; SL, stroma lamella; O, osmiophilic particles; S, starch grains. A–D, scale bar=2 μm; E–F, scale bar=200 nm.

environment and the species. In this study, a light intensity of 400 μmol m^{-2} s^{-1} provided by red and blue LEDs boosted photosynthesis and growth of rape seedlings (Tables 1 and 2). Photosynthetic pigments are the material basis of photosynthesis, and their composition and content directly affect the photosynthetic rate of leaves (Stenbaek and Jensen 2010). In this study, both Chl *a* and *b* contents in leaves increased under weak light, and the larger increase in Chl *b* content led to a decrease in the ratio of Chl *a*/*b*, similar to the results reported elsewhere (Lakshmi *et al.* 2011). Under low light, plants accumulate more photosynthetic pigments to absorb more light energy (Chen *et al.* 2007). However, owing to the limited supply of light energy, the P_n of seedlings was lower in the R_1R_2B300 and R_1R_2B200 treatments (in which seedlings had higher photosynthetic pigments contents) than in the R_1R_2B400 treatment (Table 2). The chlorophyll contents in seedlings and the P_n did not differ significantly between the R_1R_2B300 and R_1R_2B200 treatments (Table 3). However, the seedling biomass was greater in the R_1R_2B300 treatment than in the R_1R_2B200 treatment (Table 1). When the leaf thickness is the same, larger leaves can capture more light energy, leading to a significant increase in biomass (Li and Kubota 2009). The larger leaves of seedlings in the R_1R_2B300 treatment than in the R_1R_2B200 treatment explains why the seedling dry weight was greater in the former than in the latter.

Leaves show plasticity and adapt to their environment by changing their structure (Wright *et al.* 2004). In *Radix bupleuri*, the PT/ST decreased and the thickness of the leaf, palisade tissue, and spongy tissue were reduced under shade conditions (Liu *et al.* 2015). These adaptive characteristics were also reported for *Datura stramonium* (Qin *et al.* 2014), *Zingiber officinale* Rosc (Zhang *et al.* 1999), and *Patrinia villosa* (Thunb.) Juss (Zheng *et al.* 2014). Larger mesophyll cells can increase the internal surface area of the leaf, and more robust aerenchyma can improve photosynthetic efficiency (Wang and Liu 2015). Approximately 70% of chloroplasts are located in palisade cells, and most of the remainder are located in spongy mesophyll cells (Nobel *et al.* 2011). Consequently, the main site of photosynthesis is the palisade tissue. In another study, rape plants with thicker palisade tissue showed a higher net photosynthetic rate (Yang *et al.* 2014). The seedlings in R_1R_2B400 treatment had the thickest leaves and palisade tissues, and orderly, compact, and evenly distributed mesophyll cells (Fig. 3). These physiological characteristics explain the high P_n of seedlings in the R_1R_2B400 treatment. There were no significant differences in the thickness of leaves, palisade tissue, and spongy tissue between the R_1R_2B300 and R_1R_2B200 treatments (Table 4). However, compared with seedlings in the R_1R_2B300 treatment, those in the R_1R_2B200 treatment had disordered and fuzzy mesophyll cells and poorly developed chloroplasts that were not conducive to photosynthesis (Fig. 3 and Fig. 4-C). The poorly developed chloroplasts in these seedlings are one explanation for the lower photochemical conversion efficiency, which was not beneficial for seedling growth.

Under suitable light conditions, chloroplasts are oval or spindle shaped, and grana lamellae are arranged in an orderly pattern, parallel to the long axis of the chloroplast (Zhen and Zhang 2000; Tang *et al.* 2011). Seedlings in the R_1R_2B400 treatment had chloroplasts with this shape and lamellae arrangement. Under weak light, chloroplasts change in shape and structure to adapt to their conditions. They become longer and larger, and form more grana and grana lamellae to increase their ability to absorb light under low-light conditions (Niinemets *et al.* 2010; Wu *et al.* 2014). If light is very weak, chloroplasts become round, and the number of chloroplasts, grana, and grana lamellae decrease, and grana lamellae can become damaged at last (Yamazaki *et al.* 2013). In the present study, the number of chloroplasts increased, but the chloroplasts did not become longer as the light intensity decreased. There were fewer

Table 5 Effect of different light intensities on chloroplast (CP) ultrastructure of rape seedlings

Light treatment	CP length (μm)	CP width (μm)	CP area ($μm^2$)	Grana thickness (nm)	Grana number CP^{-1}	Grana total thickness (nm CP^{-1})	CP number (mm^{-2})
R_1R_2B400	5.90 a	2.22 ab	11.06 a	50.29 b	195.21 a	9 817.11 a	5 631 c
R_1R_2B300	4.04 c	1.93 ab	5.80 b	97.35 a	73.12 c	7 118.23 b	10 828 b
R_1R_2B200	4.22 bc	2.51 a	9.45 a	50.69 b	130.99 b	6 639.88 c	15 597 a
C	5.41 ab	1.55 b	6.69 b	71.60 ab	92.79 c	6 643.76 c	9 864 b

Table 6 Effect of different light intensities on leaf stomata of rape seedlings

Light treatment	Stomatal length (μm)	Stomatal width (μm)	Ratio of stomatal length:width	Stomatal density mm^{-2}	Stomatal aperture $μm^2$
R_1R_2B400	21.94 a	14.21 a	1.54 b	258.00 b	37.04 b
R_1R_2B300	20.08 a	14.85 a	1.35 c	297.69 a	40.06 b
R_1R_2B200	19.84 a	14.96 a	1.32 c	304.30 a	50.09 a
C	21.49 a	12.29 b	1.75 a	211.69 c	50.87 a

grana in chloroplasts in the R_1R_2B300 treatment than in the R_1R_2B400 treatment. However, the plants compensated for the decrease in total irradiation by increasing grana thickness and the number of chloroplasts. In the R_1R_2B200 treatment, the chloroplasts became almost round and the grana lamellae showed signs of damage as a result of the low-light intensity. As a mechanism to capture more light, the number of chloroplasts and grana increased and the grana thickness decreased in the R_1R_2B200 treatment. Additionally, the chloroplasts in the R_1R_2B200 treatment accumulated many osmiophilic granules, which form *via* a chemical reaction between osmiophilic acid and phenolic compounds (Jiang *et al.* 2012). Osmiophilic granules form as a protective mechanism against chloroplast dysplasia. Plant polyphenols can clear the free radicals produced by light, thereby protecting plants from damage (Shi and Di 2000; Xu *et al.* 2011). These adaptations allowed the photosynthetic rate in the R_1R_2B200 treatment to remain equal to that in the R_1R_2B300 treatment.

More stomata on the leaf promote CO_2 absorption (Lichtenthaler *et al.* 1981). In general, P_n and C_i are negatively correlated when light irradiation is less than the saturated light intensity of photosynthesis (Chen *et al.* 2010). Our results were consistent with this trend. Under the high-light intensity in R_1R_2B400, the lower C_i resulted from the high photosynthetic activity of mesophyll cells and high P_n. However, it does not always follow that lower C_i accompanies higher P_n. The lowest P_n (in C) resulted from a decrease in G_s (Chen *et al.* 2010), which was mainly owing to stomatal limitation (Farquhar and Sharkey 1982). Higher G_s was more beneficial to photosynthesis in the R_1R_2B300 treatment than in the R_1R_2B200 treatment.

5. Conclusion

The leaf microstructure of rape seedlings changed to adapt to different light intensities. Compared with rape seedlings grown under 400 μmol m^{-2} s^{-1}, those grown under 200 and 300 μmol m^{-2} s^{-1} accumulated less biomass, had a lower photosynthetic rate, and their leaf microstructure was unfavorable for accumulation of photosynthetic products. The seedlings grown under a light intensity of 400 μmol m^{-2} s^{-1} had a higher photosynthetic rate, well-developed leaves, and stronger growth. These results indicate that a light intensity of 400 μmol m^{-2} s^{-1} is suitable for cultivating rape in artificial systems.

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References

Chang T T, Liu X Y, Xu Z G, Yang Y. 2010. Effects of light spectral energy distribution on growth and development of tomato seedlings. *Scientia Agricultura Sinica*, **43**, 1748–1756. (in Chinese)

Chen E Y. 2012. Grain yield and protein quality in response to shading after anthesis and nitrogen regulation in wheat. Ph D thesis, Shandong Agricultural University, China. (in Chinese)

Chen G Y, Chen J, Xu D Q. 2010. Thinking about the relationship between net photosynthetic rate and intercellular CO_2 concentration. *Plant Physiology Bulletin*, **46**, 64–66. (in Chinese)

Chen X H. 2007. Exploring the adaptation mechanism of the shade plant to shading environment. *Bulletin Biology*, **71**, 853–859. (in Chinese)

Chen Z, Sun Q L, Wang Y T, Lv Y P, Mou H J, Ji H F, Chen J P. 2013. Influence of light quality on growth of oil rape seedlings. *Agricultural Engineering*, **3**, 143–149. (in Chinese)

Clark G. 1981. *Staining Procedures*. 4th ed. Williams and Wilkins, London. pp. 325–326.

Du J F, Liao X R, Ye B Q, Li M. 2002. Effect of light quality on the growth and antioxidant enzyme activities of rape seedings. *Chinese Bulletin of Botany*, **19**, 743–745. (in Chinese)

Fan X X, Xu Z G, Liu X Y, Tang C M, Wang L W, Han X L. 2013. Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. *Scientia Horticulturae*, **153**, 50–55.

Farquhar G D, Sharkey T D. 1982. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*, **33**, 317–345.

Harmutk L. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzimology*, **148**, 350–382.

Holm G. 1954. Chlorophyll mutation in barley. *Acta Agric Scandinavica*, **1**, 457–471.

Jiang J F, Zhang Y, Tian Z S, Fan X C, Liu C H. 2012. Observation on microstructure and ultrastructure of leaves in *Vitis* L. *Acta Botanica Boreali-Occidentalia Sinica*, **32**, 1365–1371. (in Chinese)

Lakshmi P M, Vanangamudi M, Thandapani V. 2011. Effects of low light on yield and physiological attributes of rice. *International Rice Research Notes*, **29**, 71–73.

Leng S H, Xia J F, Hu Z Z, Chen X L. 2002. Research of photosynthetic characteristic of rape seeding. *Chinese Journal of Oil Crop Sciences*, **24**, 10–18. (in Chinese)

Lichtenthaler H K, Buschmann C, Doli M, Fietz H J, Bach T, Kozel U, Meier D, Rahmsdorf U. 1981. Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynthesis Research*, **2**, 115–141.

Li H M, Tang C M, Xu Z G, Liu X Y, Han X L. 2012. Effects of different light sources on the growth of non-heading Chinese cabbage (*Brassica campestris* L.). *Journal of Agricultural Science*, **4**, 262–273.

Li H S. 2006. *Modern Plant Physiology*. Higher Education Press,

China. pp. 54–151. (in Chinese)
Li Q, Kubota C. 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environmental and Experimental Botany*, **67**, 59–64.
Li W F, Zhang M N. 1997. Structure and photosynthetic characteristics of rape leaf. *Journal of Anhui Agricultural Sciences*, **25**, 213–215. (in Chinese)
Liu M Z, Nuerbayi A, Pan X L. 2005. The methods for making stomata of leaf according to tearing membrane by painting nail polish on leaf. *Bulletin of Biology*, **40**, 40–44. (in Chinese)
Liu S Y, Zhang M S, Gao X F, Liu G Q, Wei B H, Lv C. 2015. Effect of sunlight intensity on leaf morphology and structure of *Premna puberula*. *Guizhou Agricultural Sciences*, **43**, 175–178. (in Chinese)
Liu X Y, Xu Z G, Chang T T, Guo S R. 2010. Growth and photosynthesis of cherry tomato seedling exposed to different low light of LED light quality. *Acta Botanica Boreali-Occidentalia Sinica*, **30**, 645–651. (in Chinese)
Lu H Y, Liu X Y, Si C C, Jiao X L, Xu Z G. 2015. Effects of different PPFD on growth and quality of non-heading Chinese cabbages. *Plant Physiology Journal*, **51**, 909–915. (in Chinese)
Mao L Y, Mu X Q, Dong G G, Cui H A, Ran J X. 2012. Influence of light intensity on growth of *Datura stramonium* and *D. stramonium* var. *tatual*. *Chinese Journal of Plant Ecology*, **36**, 243–252. (in Chinese)
Niinemets Ü. 2010. A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. *Ecological Research*, **25**, 693–714.
Nobel P S. 2011. *Physicochemical and Environmental: Plant Physiology*. 4th ed. Authorized to be Published by Elsevier (Singapore) Pte., Science Press, China. pp. 5–7, 371–373.
Pan R C. 2000. *Phytophysiology*. Higher Education Press, Beijing. p. 63. (in Chinese)
Qin Y Z, Xing Z, Zou J F, He C Z, Li Y L, Xiong X Y. 2014. Effects of sustained weak light on seedling growth and photosynthetic characteristics of potato seedlings. *Scientia Agricultura Sinica*, **47**, 537–545.
Shi B, Di Y. 2000. *Plant Polyphenol*. Science Press, China. pp. 134–135. (in Chinese)
Shirke P A, Pathre U V. 2003. Diurnal and seasonal changes in photosynthesis and photosystem 2 photochemical efficiency in *Prosopis juliflora* leaves subjected to natural environmental stress. *Photosynthetica*, **41**, 83–89.
Song B, Wang D X, Zhang Y, Liu H R. 2015. Leaf structures and relationship with SO_2-absorption capacity of 13 ornamental trees. *Acta Botanica Boreali-Occidentalia Sinica*, **35**, 1206–1214. (in Chinese)
Stenbaek A, Jensen P E. 2010. Redox regulation of chlorophyll biosynthesis. *Phytochemistry*, **71**, 853–859.
Tang H W, Qu Y T, Zhang X. 2011. Effect of shading on chlorophyll content and chloroplast ultrastructure in leaves of *Tulipa gesneriana*. *Journal of Northeast Agricultural University*, **42**, 77–82. (in Chinese)
Wang C D, Liu M Z. 2015. Morphology, structure and physiological characteristics of alpine plant. *Bulletin Biology*, **50**, 4–6. (in Chinese)
Wang L, Feng Z C. 2013. Evaluation of rape industry chain and the security situation estimation. *Industrial Economy*, **12**, 58–67. (in Chinese)
Wang T, Li W L, Gong F E, Yu J H. 2011. Effect of different LED light qualities on growth and physiological characteristics of non-heading Chinese cabbage. *Journal of Gansu Agricultural University*, **46**, 69–73. (in Chinese)
Wang Z M, Song F F, Xu Z G, Liu X Y, Yang Y. 2011. Effect of red and blue LED light intensity on growth and quality of lettuce. *China Vegetables*, **16**, 44–49. (in Chinese)
Wright I J, Reich P B, Westoby M, Ackerly D D, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen J H C, Diemer M, Flexas J, Garnier E, Groom P K, Gulias J, Hikosaka K, Lamont B B, Lee T, Lee W, Lusk C, Midgley J J, *et al.* 2004. The worldwide leaf economics spectrum. *Nature*, **428**, 821–827.
Wu Z F, Sun X W, Wang C B, Zheng Y P, Wan S B, Liu J H, Zheng Y M, Wu J X, Feng H, Yu T Y. 2014. Effects of low light stress on rubisco activity and the ultrastructure of chloroplast in functional leaves of peanut. *Chinese Journal of Plant Ecology*, **38**, 740–748. (in Chinese)
Xiao Q, Ye W J, Zhu Z, Chen Y, Zheng H L. 2005. A simple non -destructive method to measure leaf area using digital camera and Photoshop software. *Chinese Journal of Ecology*, **24**, 711–714. (in Chinese)
Xu G Q, Zhang Z W, Guo A Q, Luan L Y. 2011. Progress on the stress-resistant ecological function of plant polyphenols. *Acta Botanica Boreali-Occidentalia Sinica*, **31**, 423–430. (in Chinese)
Yamazaki J, Shinomiya Y. 2013. Effect of partial shading on the photosynthetic apparatus and photosystem stoichiometry in sunflower leaves. *Photosynthetica*, **51**, 3–12.
Yang N N, Sun W C, Liu Z G, Shi P H, Fang Y, Wu J Y, Zeng X C, Kong D J, Lu M H, Wang Y. 2014. Morphological characters and physiological mechanisms of cold resistance of winter rapeseed in northern China. *Scientia Agricultura Sinica*, **47**, 452–461. (in Chinese)
Yu J H, Shu Y J, Lv J F, Zhang G B. 2004. Influences of low temperature and poor light on photosynthetic characteristics in eggplant seedlings. *Acta Botanica Boreali-Occidentalia Sinica*, **24**, 831–836. (in Chinese)
Zhang Z X, Guo Y K, Zou Q. 1999. Effect of shading on microstructure and chloroplast ultrastructure of Ginger leaves. *Acta Horticulturae Sinica*, **26**, 96–100. (in Chinese)
Zheng B, Chen H G, Yan Z Q, Luo X Z, Duan L J, He H G. 2014. Effects of shade on morphological, anatomical and physiological properties of *Patrinia villosa* (Thunb.) Juss. leaves. *Hubei Agricultural Sciences*, **53**, 3111–3115. (in Chinese)
Zhen W, Zhang M F. 2000. Effect of low light on photosynthetic characteristics and functional ultrastructure of cucumber leaf. *Acta Horticulturae Sinica*, **27**, 290–292. (in Chinese)

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