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不同 LEDs 光质下普通白菜开花以及花期生理特性的动态变化

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摘　要:以普通白菜品种'苏州青'为试材,采用单因素随机区组设计的盆栽试验,将基质育苗后长至子叶展平时的幼苗转入荧光灯(FL,对照)、蓝光(B)发光二极管(LEDs)、蓝红复合光(BR)和红光(R)下进行照射处理至开花,考察不同光质对普通白菜开花以及花期光合色素含量、品质和碳代谢等的光效应,为利用人工光源调节普通白菜的育种周期提供理论指导。结果显示:(1)随着开花时间延长,普通白菜的开花数目均以 R 和 BR 处理显著高于 FL;花蕾数目在处理 100 d 时 R 和 BR 处理显著多于 FL,但是在 110 和 120 d 时 B 处理下明显多于 FL。(2)随着花期延长,白菜叶片中的光合色素含量呈降低趋势,其在处理 100 d 时表现为 BR 处理显著高于 FL,而在 110 d 时 B 处理最高,在 120 d 时 BR 处理最高。(3)随着花期延长,叶片中可溶性蛋白和抗坏血酸含量也呈逐渐降低的趋势;可溶性蛋白含量均在 B 处理下较大,而抗坏血酸含量在 100 d 时在 B 处理下最高,但是在 110 和 120 d 时 B 和 BR 处理下较高。(4)随着花期延长,叶片中碳水化合物含量也逐渐降低,其中可溶性糖、蔗糖和淀粉含量均以 R 处理下最高。研究表明,与荧光对照相比,LEDs 光源对普通白菜幼苗的营养生长和生殖生长更有效,其中蓝光有利于普通白菜的营养生长,而红光和蓝红复合光则有利于其生殖生长;可采用红光和蓝红复合光作为普通白菜育种的人工光源,有效促进其工厂化生产进程。

关键词:普通白菜;开花;光合色素;品质;碳代谢

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Effects of Light Quality on Flowering, Dynamic Variation in Physiological Characteristics of Pakchoi during Budding and Flowering Stage

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Abstract: With the purpose of examining the effects of light quality on flowering, the pigment content, quality and carbon metabolism of pakchoi (*Brassica campestris* L. ssp. *chinensis* (L) Makino var. *communis* Tsen et Lee) seedlings on the budding and flowering-age-types, the present study were carried out to use the cultivar 'Suzhouqing' as plant material, which were grown under four different light treatments including blue plus red light-emitting diodes (LEDs, B∶R=2∶7), blue LEDs (B), red LEDs (R) and fluorescent lamps (FL) for 120 days. Some indices such as flower buds, open flowers, pigments and ascorbic acid, soluble protein, sucrose, soluble sugar, and starch concentrations were determined. The results showed that. (1) with the extension of flowering time, the numbers of open flowers were significantly larger in seedlings under R and BR than that under FL. The number of

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flower buds was significantly larger under R and BR than that under FL at the 100th d. However, the number of flower buds was significantly larger under B than that under FL at the 110th d and 120th d; (2) the photosynthetic pigment content of leaves was gradually decreased with the extension of flowering period. The concentrations of pigments were significantly higher in seedlings under BR than that under FL at 100th d. However, the concentrations of pigments were significantly higher in seedlings under B than that under FL at 110th d. The concentrations of pigments were the highest in seedlings under BR at 120th d; (3) the soluble protein and ascorbic acid content in leaves decreased gradually as the flowering time was prolonged. The concentration of soluble protein was higher in seedlings under B than that under FL during the flowering periond. However, the concentration of ascorbic acid was higher in seedlings under B than that under FL at 100th d. The concentration of ascorbic acid was higher under B and BR in seedlings than that under FL at 110th d and 120th d; (4) as the extended flowering, leaf carbohydrate content also gradually reduced, which soluble sugar, sucrose and starch contents were significantly higher under R than that under the other lights. Obviously, compared with the FL, LEDs is more effective for the vegetative growth and reproductive growth of pakchoi seedlings, B is conducive to the vegetative growth of pakchoi, while R and BR are beneficial to their reproductive growth. The R LEDs and BR LEDs lights can be used as the artificial light source for the pakchoi breeding, and it might promote the factory production process of pakchoi.

Key words: pakchoi; flowering; pigments; quality; carbon metablism

Brassica campestris ssp. *chinensis* Makino var. *communis* is a typical biennial vegetables, the advantages of crossbreeding often need eight to nine generations of breeding, so as to accelerate the breeding process, shrinking the short breeding period, breeders often use the greenhouse artificially created to promote green vegetables transition from vegetative growth to reproductive growth, plus generation of breeding[1]. The timing of flowering is primarily influenced by environmental factors, which serve to communicate the time of year and/or growth conditions favorable for sexual reproduction and seed maturation, including light quality, photoperiod, light quantity, and verbalization[2]. The sun emits the most of its radiation in the visible range, it covers the range of wavelength from 400－700nm[3]. The integration, quality, duration and intensity of red, far-red, blue, UV-A and UV-B light have a profound influence on plants by triggering physiological reactions to control their growth and development[4-6]. The quality and quantity of light affect plant development mainly through two types of photoreceptors-the red/far red light receptors phytochromes and blue/UV-A light receptors cryptochromes[7]. LEDs are solid-state, long-lasting and durable sources of narrow-band light that can be implemented in dynamic lighting strategies to control plant growth, development, physiological responses and production, it is important to learn more about the influence of light quality on these processes [8-11].

Various studies have shown that LEDs have been successfully used for cultivation in several horticultural plant species such as lettuce, tomato, cucumber, Chinese cabbage, pepper, rapeseed *etc*[8-10,12-20]. Although previous studies have identified various physiological and morphological effects of light quality in many plant species, few reports have addressed the effect of LED light sources and fluorescent lamps on flowering, the sugar metabolism and quality of pakchoi (*Brassica campestris* L. ssp. *chinensis* (L) Makino var. *communis* Tsen et Lee) during the budding and flowering stages. The objective of the present study was to examine the effects of blue LEDs, red LEDs, blue plus red LEDs (BR)[18] and fluorescent lampson flowering, sugar metabolism and quality in leaves of pakchoi seedlings during the budding and flowering stages and to select the best lights for the cultivation of pakchoi seedlings under a controlled environment.

1　Materials and methods

1.1　Plant materials

The experiments were conducted in RXZ-1 Phytotron (Ningbo Jiangnan Instrument Factory CO., Ningbo, China) at Anhui Science and Technology University. Pakchoi cultivar 'Suzhouqing' seeds with a similar size were selected for sowing. Seeds were sown in cells filled with vermiculite and peat (1 ∶ 1 by volume) for cultivation, with one seed per cell. After seven days, seedlings with two expanded cotyledons were transferred to the different lights.

1.2 Light treatments

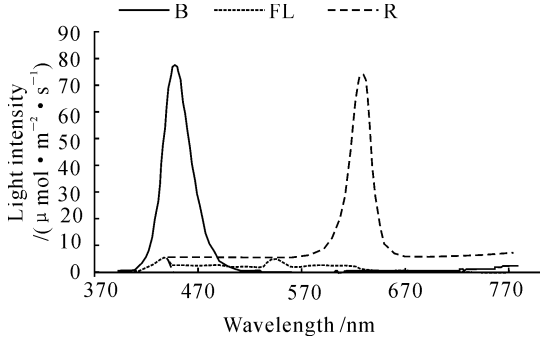
Seedlings were grown under a mixture of blue plus red light-emitting diodes (LEDs) (BR, B∶R=2∶7), blue LEDs, red LEDs (OPTORUN LTD. CO., Shanghai, China) at a photosynthetic photo flux density (PPFD) of 140 μmol·m^{-2}·s^{-1} and fluorescent lamps (FL, the control, T5-28 W, PHILIPS CO., Yangzhou, China) PPFD of 85 μmol·m^{-2}·s^{-1} (Fig. 1). The growth temperature was set at 24－26 ℃, and the relative humidity fluctuated between 55% and 60%. The photoperiod was 12 hours. Seedlings were randomly assigned to each light treatment, and LEDs arrays were randomly assigned positions in the greenhouse. Seedlings were cultured under the four lights for samples at the budding stage (100th days), at the 10th (110th days) and 20th (120th days) of flowering stages.

1.3 Flower buds and open flowers measurements

When seedlings were cultured under the four lights for samples at the budding stage (100th days), at the 10th (110th days) and 20th (120th days) of flowering stages, and recorded the number of flowering and the number of buds on that day.

1.4 Pigment measurements

Leaves were weighed to 0.1 g (fresh weight, W), and 10 mL (V) of 80% acetone was added to



FL. Fluorescent lamp(control); B: Blue light emitting diodes; R: Red light emitting diodes

Fig. 1 The light energy distribution of different lights

0.1 g of leaf samples placed into a mortar with quartz sand. The chlorophyll was extracted until the leaf turned white. The optical density (OD) was measured with a UV-1200 spectrophotometer (Jinpeng, Shanghai, China) at 470 nm for carotenoid (OD_{470}), at 663 nm for chlorophyll a (OD_{663}), and at 645 nm for chlorophyll b (OD_{645})[21].

1.5 Soluble protein measurements

Leaves (1.0 g of fresh weight, W) were ground in a mortar with liquid nitrogen, to which 5 mL (V_1) of 0.067 mol·L^{-1} potassium phosphate buffer (PBS) was added, and were then filtered through filter paper. The extract was centrifuged at 12000 g for 10 min, and the supernatant was removed. The extract (1 mL, V_2) and Coomassie brilliant blue G-250 (5 mL) was thoroughly mixed. The optical density was measured using a UV-1200 spectrophotometer at 595 nm. To determine a standard curve, 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of 100 μg·L^{-1} bovine serum albumin was added to 6 volumetric flasks, and distilled water was added to reach a volume of 1 mL. The optical density was measured by a UV-1200 spectrophotometer at 595 nm (ρ). The concentration of soluble protein was determined using the following equation: soluble protein (mg·g^{-1}) $=\rho V_1/WV_2$[22]. Where ρ is optical density, V_1 is total volume of extract, V_2 is volume of reactions, and W is fresh weight (g) of the samples.

1.6 Ascorbic acid measurements

Leaves (1.0 g, fresh weight, W) were ground in a mortar with liquid nitrogen. Next, 5 mL (V_1) of 5% trichloroacetic acid (TCA) was added and the mixture was filtered through filter paper. The extract was centrifuged at 10000 g for 10 min, and the supernatant was removed. The extract (1.0 mL, V_2) and 1.0 mL of ethanol were thoroughly mixed. Next, 0.5 mL of 0.4% phosphoric acid-ethanol, 1 mL of 0.5% 1, 10- phenanthroline-ethanol and 0.5 mL of 0.03 g·L^{-1} ferric chloride were added for a total volume of 5 mL. The optical density was measured using a UV-1200 spectrophotometer (Jinpeng, Shanghai, China) at 534 nm. To obtain a standard curve, 0, 0.2, 0.4, 0.6, 0.8, or 1.0 mL of 100 mg·L^{-1} bovine serum albumin was added to 6 volumetric flasks, and distilled water was added to reach a volume of 1 mL. The optical density was measured by a UV-1200 spectrophotometer at 534 nm (ρ). The concentration of ascorbic acid was determined using the following equation: ascorbic acid concentration (mg·g^{-1}) $=\rho V_1/WV_2$[22]. Where ρ is optical density, V_1 is total volume of extract, V_2 is volume of reactions, and W is fresh weight (g) of the samples.

1.7 Sugar and starch measurements

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 12 000 g 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. Sucrose concentration was determined using the phloroglucinol method and measured at 480 nm[21]. Takahashi's method was used for starch extraction[23]. The residue obtained after ethanol extraction was re-suspended with 0.1 mol · L^{-1} sodium acetate buffer (pH 4.8) and boiled for 20 min. The gelatinized starch was digested with amyloglucosidase for 4 h at 37 ℃ and boiled again to stop the enzymatic reaction. After cooling, the mixture was centrifuged, and the amount of soluble sugar in the supernatant was determined by anthrone colorimetry[22]. The starch concentration was estimated by converting glucose to starch equivalents using a factor of 0.9.

1.8 Statistical analysis

Statistical analyses were conducted with Statistical Product and Service Solutions (SPSS) for Windows, Version 16.0 (SPSS Inc. 2007). Data were analyzed using analysis of variance (ANOVA), and the differences between means were tested using Tukey's Test ($P<0.05$).

2 Results

2.1 The number of flower buds and open flowers

Different light sources had variable effects on the development of flowers in pakchoi seedlings from 100 to 120 days (Table 1). The numbers of open flowers were significantly higher in seedlings under R and BR than in those under FL during the flowering stage. The number of flower buds was significantly higher at the 100th day under R and BR than FL and B. However, The number of flower buds was significantly higher at the 110th day and 120th day under B than under FL. The present results showed that R and BR LEDs promoted the flowering process.

2.2 The concentrations of pigments

The leaf pigments of pakchoi seedlings varied in response to the different lights. The photosynthetic pigment content of leaves was gradually decreased with the extension of flowering period. The concentrations of chlorophyll a, b and total chlorophyll were greatest in seedlings under BR at 100th day, followed by B, which were significantly higher than FL and lowest under FL (Fig. 2, A—C). The concentrations of carotenoid was highest under BR and B, which were significantly higher than FL, and lowest under FL (Fig. 2, D). The concentrations of pigments were greatest in seedlings under B at 110th day, which were significantly higher than BR, R and FL (Fig. 2). The concentrations of pigments were greatest in seedlings under BR at 120th day, followed by B and FL and lowest under R (Fig. 2). The present results demonstrated that BR and B LEDs were beneficial to pigments accumulation of pakchoi seedlings.

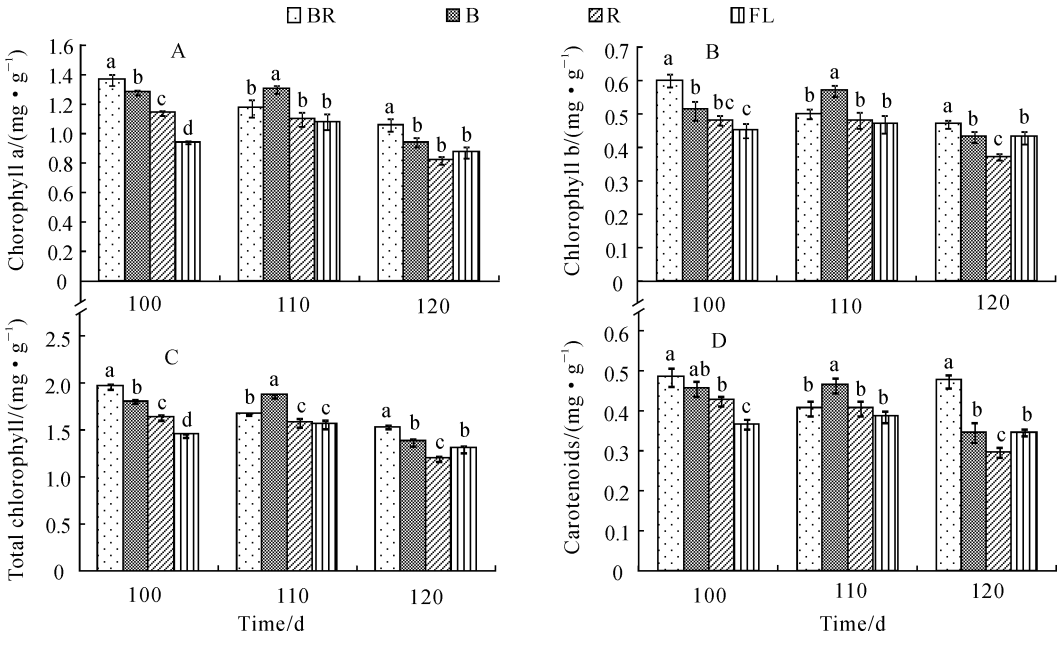
2.3 Soluble protein concentration

The soluble protein content in leaves decreased gradually as the flowering time was prolonged. The concentration of soluble protein was highest in pakchoi seedlings under B LEDs at 100th day, followed by BR and lowest under FL (Fig. 3).

Table 1　The number of flower buds and open flowers per plant in pakchoi grew under different lights until flowering (100 -120 days)

Light treatment	Number of flower buds			Number of open flowers		
	100th day	110th day	120th day	100th day	110th day	120th day
BR	2.67a	5.33b	11.67bc	2.33a	5.01a	10.33ab
B	1.33b	7.33a	17.33a	0.33b	1.33b	9.33b
R	2.67a	5.33b	12.33b	2.67a	5.67a	11.33a
FL	1.33b	3.33c	10.33c	0.33b	0.67b	7.05 c

Note: BR. Blue plus red light-emitting diodes; B: Blue light-emitting diodes; R. Red light-emitting diodes; FL. Fluorescent lamp. Values are the mean ± standard deviation. Different letters within the column indicate significant differences among light treatments at 0.05 level according to Tukey's test (n=3). The same as below.



Different letters within the same stage indicate significant differences among light treatments at 0.05 level according to Tukey's test (n=3). The bars represent the standard error. The same as below.

Fig. 2 The pigment concentrations of pakchoi seedlings under different light qualities for 100th, 110th and 120th day

The concentration of soluble protein was greatest in seedlings under B LEDs at 110th day, and the other light treatments showed no significant differences. The concentration of soluble protein was highest in seedlings under B and FL at 120th day and lowest under R. The present results showed that B LEDs was responsible for the accumulation of soluble protein in pakchoi seedlings.

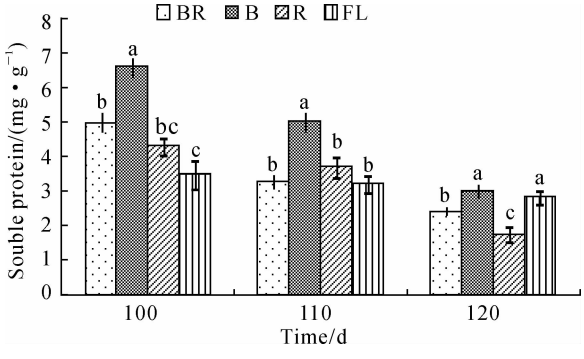


Fig. 3 The soluble protein concentration of pakchoi seedlings under different lights treatments for 100th, 110th and 120th day.

2.4 Ascorbic acid concentration

The ascorbic acid content in leaves decreased gradually as the flowering time was prolonged. The The concentration of ascorbic acid was highest in pakchoi seedlings under B LEDs at 100th day, followed by R LEDs and lowest under FL (Fig. 4). The concentration of ascorbic acid was significantly higher under B and BR in seedlings than under FL at 110th day and 120th day (Fig. 4). The results showed that B and BR LEDs was responsible for the accumulation of ascorbic acid in pakchoi seedlings.

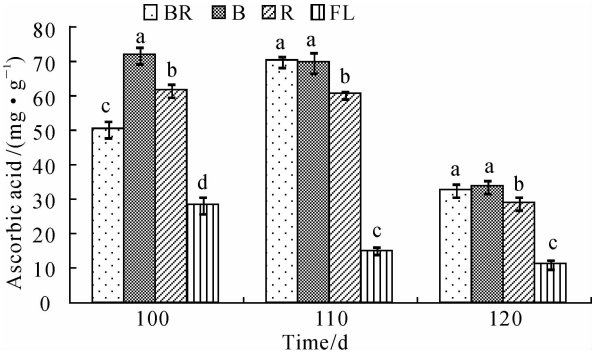


Fig. 4 The ascorbic acid concentration of pakchoi seedlings under different light treatments for 100th, 110th and 120th day

2.5 Sugar and starch concentrations

The sugar and starch concentrations of pakchoi seedlings varied in response to different lights treatments. As the extended flowering, leaf carbo hydrate content is also gradually reduced. The

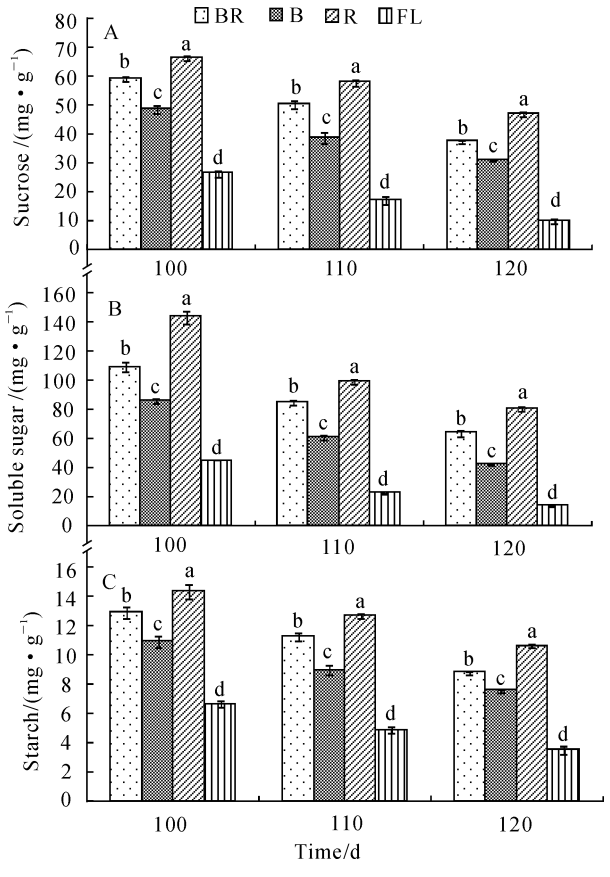


Fig. 5　The photosynthesis production of pakchoi seedlings under different light treatments for 100th, 110th and 120th day

concentrations of sucrose, soluble sugar and starch were greatest in seedlings under R LEDs, which showed significant higher than the other lights, followed by BR and B LEDs, which showed significant higher than FL and lowest in seedlings under FL (Fig. 5, A—C). These results revealed that R LEDs are the best lights for accumulation of sucrose, starch and soluble sugar in pakchoi seedlings.

3　Discussion

3.1　Blue plus red LEDs and red LEDs may profit for the flowering of plants

Flowering is one of the morphogenic events in plants, which is affected by light irradiance and / or wavelength[24]. The number of flowers was highest in non-heading Chinese cabbage seedlings grown under R LEDs and B plus R LEDs (1 ∶ 8)[18]. The numbers of *Cyclamen* flower buds and open flowers were highest in plants grown under a mixture of B plus R LEDs (B ∶ R＝10 ∶ 1) compared with FL and other light sources[25]. However, the development of visible flower buds in marigolds was about five times greater in FL than in B or R LEDs[26]. Monochromatic B light delayed flowering in *Arabidopsis* possibly by influencing cryptochromes[7]. The present study showed that the number of flowers was highest in pakchoi seedlings grown under R LEDs and B plus R LEDs (2 ∶ 7), and the number of flower buds was higher in seedlings grown under LEDs than FL. The findings from the present study are consistent with those of Li *et al.*[18], Heo *et al.*[25] and Mockler *et al.*[7], but inconsistent with a report from Heo[26]. The shift in plants from vegetative growth to floral development is regulated by red-far-red light receptors (phytochromes) and blue-ultraviolet A light receptors (cryptochromes)[27]. The number of flower buds and open flowers and the duration of flowering may correlate with the different plant species, which reactions to the light receptors were variable[18]. Spectral quality has a major influence on induction rate of flower budding and subsequent development. The present study showed that R LEDs and BR LEDs were benefit for the flowering process.

3.2　Blue LEDs benefited the leaf quality of plants

The present results indicated that B LEDs benefits ascorbic acid and soluble protein accumulation of pakchoi seedlings, which are consistent with reports by Li *et al.*[18], Yang *et al.*[28] and Zhang *et al.*[29]. However, the concentrations of soluble protein in lettuce leaves showed no significant differences among treatments[10]. B LEDs might benefit the accumulation of nutritional substances, and these effects may correlate with plant species or cultivars[18]. The present study also showed that the flowering was delayed under B LEDs, this might relate with the high nutritional substances, which the substances was accumulated in leaves offered the material security to vegetative growth of green vegetables. In summary, for the purpose of improving the nutritional quality of veg-

etables, B LEDs could be chosen as the preferred lights in artificial cultivation of pakchoi.

3.3 Which light was the best light for accumulation of photosynthates in plants

Variations in light conditions will affect the metabolic processes[30]. Light quality regulates the carbohydrate metabolism of higher plants, and carbohydrate content is increased under red light[31]. Red light may inhibit the translocation process of photosynthates[32]. Red light enhances starch accumulation in glycine and sorghum species[33]. The present study revealed that the starch concentration was greatest in seedlings grown under R LEDs, and this light was advantageous to accumulation of starch in pakchoi which was consistent with the previous studies. R LEDs may promote the accumulation of the photosynthetic products but inhibit the translocation of photosynthetic products out of leaves. Thus, the starch ultimately accumulated in leaves[18]. The present study also showed that the concentrations of sucrose and soluble sugar were greatest in seedlings under R LEDs during the budding and flowering stages and the R LEDs and B plus R LEDs promoted the flowering process. The flowering might relate with the high sugar and starch concentrations, which advanced the transition from vegetative growth to reproductive growth and early flowering. R LEDs may be used as the main lights for reproductive growth of pakchoi seedlings.

In conclusion, R LEDs and B plus R (2 ∶ 7) LEDs should be selected as the preferred lights in the artificial cultivation of pakchoi seedlings to get more flowers and early flowering. By contrast, B LEDs should be used as the preferred lights for higher nutritional quality to improve the growth and development of pakchoi seedlings.

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