Improvement of the Growth and Nutritional Quality of Two-leaf-color Pak Choi by Supplemental Alternating Red and Blue Light

Jing Huang, Ya-liang Xu, Fa-min Duan, Xu Du, Qi-chang Yang, and Yin-jian Zheng

Institute of Urban Agriculture, Chinese Academy of Agricultural Sciences, Chengdu 610213, China; and National Chengdu Agricultural Science and Technology Center, Chengdu 610213, China

Additional index words. alternating red and blue light, growth, nutritional quality, pak choi, species specific, shade avoidance syndrome

Abstract. The aim of the present study was to evaluate the effects of alternating red (660 nm) and blue (460 nm) light on the growth and nutritional quality of two-leaf-color pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*). Four light treatments (supplemental alternating red and blue light with intervals of 0, 1, 2, and 4 hours, with a monochromatic light intensity of $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a cumulative lighting time of 16 hours per day) were conducted in a greenhouse under identical ambient light conditions (90 to $120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 12:00 AM) for 10 days before green- and red-leaf pak choi were harvested. The results showed that the two-leaf-color pak choi receiving alternating red and blue light exhibited more compact canopies and wider leaves than those under the control treatment, which was attributed to the shade avoidance syndrome of plants. The present study indicated that the biomass of green-leaf pak choi was much higher than that of red-leaf pak choi, but the nutritional quality of green-leaf pak choi was lower than that of red-leaf pak choi, and seemingly indicating that the regulation of metabolism for pak choi was species specific under light exposure. The trends of both biomass and the soluble sugar content were highest under the 1-hour treatment. The contents of chlorophyll a and total chlorophyll in both cultivars (green- and red-leaf pak choi) were significantly increased compared with control, without significant differences among the 1-, 2-, and 4-hour treatments, whereas chlorophyll b exhibited no significant difference in any treatment. Alternating red- and blue-light treatment significantly affected the carotenoid content, but different trends in green- and red-leaf pak choi were observed, with the highest contents being detected under the 1-hour and 4-hour treatments, respectively. With increasing time intervals, the highest soluble protein contents in two-leaf-color pak choi were observed in the 4-hour treatment, whereas nitrate contents were significantly decreased in the 4-hour treatment. Compared with 0 hours, the contents of vitamin C, phenolic compounds, flavonoids, and anthocyanins in two-leaf-color pak choi were significantly increased, but no significant differences were observed in vitamin C, phenolic compounds, and flavonoids among the 1-, 2-, and 4-hour treatments, similar to what was found for the anthocyanin content of green-leaf pak choi. However, the content of anthocyanins in red-leaf pak choi gradually increased with increasing time intervals, with the highest content being found in the 4-hour treatment. Supplemental alternating red and blue light slightly increased the antioxidant capacity [1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging rate and antioxidant power], but no significant differences were observed after 1, 2, and 4 hours of treatment. Taken together, treatment with an interval of 1 hour was the most effective for increasing the biomass of pak choi in this study, but treatment with a 4-hour interval should be considered to enhance the accumulation of health-promoting compounds.

sity or photoperiod (Chen et al., 2017). In addition, light quality induces a series of physiological and biochemical reactions that are mainly promoted through signal transduction pathways involving photoreceptors such as phytochrome, cryptochrome, and phototropin receptors, resulting in the upregulation or downregulation of related gene expression (Landi et al., 2020; Tissot and Ulm, 2020).

In research on monochromatic light spectra, the morphoanatomical, photosynthetic, and secondary metabolism characteristics of plants have been shown to be more significantly affected by monochromatic than multispectral light (Landi et al., 2020). Among various species, previous studies have suggested that red light results in the highest quantum yield of CO_2 fixation among the wavelengths in the photosynthetically active spectrum (Hogewoning et al., 2012; Wu et al., 2019). Plants exhibit some degree of undesirable growth characteristics (lower biomass, lower net photosynthetic rate, or downward leaf curling) under red light alone, but these symptoms are ameliorated when blue light is added (Kong et al., 2018; Miao et al., 2019). It has been reported that blue-light signaling triggers processes such as photomorphogenesis, stomatal opening, and phototropism, which broadly affect the level of photosynthesis (Horrer et al., 2016; Huché-Théliér et al., 2016). Blue light enhances the accumulation of carotenoids, flavonoids, and anthocyanins without substantially affecting plant morphoanatomical traits (Landi et al., 2020; Zhang et al., 2020), but long-term blue light exposure may affect plant growth and morphology, inducing changes in the photosynthetic apparatus (Huché-Théliér et al., 2016). However, red light strongly alters morphology and physiology without showing positive effects on secondary metabolites (Zhang et al., 2020). A large number of photophysiological studies have verified the importance of the combination of red and blue light for improving plant growth and nutritional quality compared with than monochromatic light in crops such as lettuce, cucumber, soybean seedlings, and pak choi (Chen and Yang, 2018; Ma et al., 2018; Song et al., 2020).

There are two lighting methods for exposing plants to red and blue light: the familiar method of simultaneous lighting and the Shigyo Method, the core concept of which is the alternation of red and blue light irradiation (Shimokawa et al., 2014). Alternating red and blue light was shown to significantly enhance lettuce growth when the total intensity was the same as that under the simultaneous irradiation with red and blue light each day (Shimokawa et al., 2014). In a study of different intervals of alternating red and blue light, treatment with an interval of 1 h was shown to be beneficial for the accumulation of biomass, sucrose, and starch in lettuce and promoted electric efficiency and light use efficiency (Chen et al., 2019). However, under alternating red and blue light with intervals of 2 and 4 h, soluble sugar and ascorbic acid levels were

Received for publication 27 May 2020. Accepted for publication 14 Aug. 2020.

Published online 23 December 2020.

This work was supported by Central Public-interest Scientific Institution Basal Research Fund (Y2020XK02), Local financial funds of National Agricultural Science & Technology Center, Chengdu (NASC2019AR01), the Agricultural Science and Technology Innovation Program (ASTIP-2020-001) and the Central Public-interest Scientific Institution Basal Research Fund (Y2020XK01). Y.-J.Z. is the corresponding author. E-mail: zhengyinjian@caas.cn.

This is an open access article distributed under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

Light is one of the most important environmental factors regulating plant growth, development, and photosynthesis (Claypool and Lieth, 2020; Ouzounis et al., 2015). Lighting-emitting diodes (LEDs) are regarded as the most effective light source with the highest potential and are being developed to provide powerful, effective, and environmental emission spectra covering the entire photosynthetically active radiation range to precisely regulate numerous types of light combinations (Avercheva et al., 2016). Light quality has more complex impacts on plant morphology and metabolism than light inten-

significantly increased, but the nitrate content was decreased (Chen et al., 2017). Various species exhibit different light-response modes; nevertheless, more experiments need to be conducted to ensure the application of optimal red and blue light with alternating intervals.

The effects of supplementary light on growth and health-promoting compounds in *Brassica* vegetables have been reported; for example, the spectra and intensity of supplemental LED illumination were associated with the enhanced accumulation of lutein and β -carotene in *Brassicaceae* microgreens (Brazaitytė et al., 2015). Both plant growth and the accumulation of health-promoting compounds in Chinese kale and pak choi were increased in association with the supplemental blue light intensity (Li et al., 2019; Zheng et al., 2018). Epidemiological studies have shown that the consumption of *Brassicaceae* vegetables can effectively prevent cancer and cardiovascular diseases (Bjorkman et al., 2011). Pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*) is an annual leaf vegetable belonging to the *Brassicaceae* family that originated in China, whose leaves are rich in nutritional and functional health-related compounds (minerals, vitamins, flavonoids, glucosinolates, and anthocyanins) (Harbaum et al., 2008). However, there are fewer reports about growth and nutritional quality in pak choi, especially concerning the contents of functional compounds under supplemental alternating red and blue light.

Therefore, two cultivars (green- and red-leaf pak choi) were used in this study as test materials and were subjected to four supplemental light treatments, to study the effects on of alternating red and blue light the growth, nutritional quality, and antioxidant capacity of the plants and optimize the mode of supplemental red and blue light exposure.

Materials and Methods

Plant materials and growth conditions. The experiment was performed in an environmentally controlled greenhouse of Chengdu Academy of Agriculture and Forestry Sciences. The seeds of green and red pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*) were selected after soaking for 1 h and were then sown in a sponge block (2 × 2 × 2 cm). The seedlings were placed inside a growth chamber with white (Chenghui Equipment Co., Ltd, Guangzhou, China) LED at a photosynthetically active radiation density (PPFD) of 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Ten days later, seedlings with a consistent appearance with three true leaves were transplanted into hydroponic containers (25 L volume and 24 seedlings per container, three replicates per treatment, total of 12 containers). The environmental conditions were as follows: temperature 25/20 °C (day/night), 60% to 80% relative humidity, PPFD of ambient light in a greenhouse of 90 to 120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (12:00 AM), and watering with 1/2 Hoagland's nutrient solution with an electrical conductivity of 1.2 to 1.3 ds/m and pH of 5.8 to 6.0. The nutrient solution was refreshed every 5 d.

Experimental light treatment. Twenty-five days after transplantation, four supplemental light treatments were applied for 10 d with light sources of red and blue LEDs, in which red light (660 nm) and blue light (460 nm) were alternated at intervals of 0, 1, 2, and 4 h, with a cumulative supplemental light time of 16 h per day (from 0600 to 2200 HR) (Table 1). The treatments were applied in four compartments separated from each other with opaque tinfoil. By adjusting the distance between the plant canopy and LEDs, the PPFD of monochromatic light was maintained at 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. PPFD was measured with the spectrometer of a photometer (Apogee SS-110, Logan, UT).

Growth measurement and sampling. After 10 d of light treatment, six fully developed green- and red-leaf pak choi plants were harvested. The fresh weights of shoots and roots were measured on a balance with 0.001-g accuracy (JY2003, Shanghai, China). After drying at 105 °C for 1 h and 70 °C for 24 h in an oven (DHG-2200B, Zhengzhou, China), the dry weights were measured on a balance with a 0.0001-g accuracy (FA2004, Shanghai, China). The fresh leaf tissues of pak choi were collected for photosynthetic pigment measurement, and the samples of fresh leaf tissue used for physiological and biochemical measurements were refrigerated at -80 °C in an ultra-low-temperature freezer (DW-86L338, Qingdao, China).

Photosynthetic pigment measurement. Photosynthetic pigments were measured via the extraction method with ethanol and acetone (Gratani, 1992). A total of 0.5 g of leaf tissue was soaked in 25 mL of absolute ethanol and acetone (v/v 1:1) until the leaf tissue turned white, followed by filtration into 25-mL test tubes. The filtration solution was measured at 663, 645, and 440 nm with a ultraviolet spectrophotometer (MAPADA, ultraviolet-1200, Shanghai, China).

$$\begin{aligned}\text{Chlorophyll a (mg/L)} &= 12.7 \times \text{OD}_{663} \\ &- 2.69 \times \text{OD}_{645}\end{aligned}$$

$$\begin{aligned}\text{Chlorophyll b (mg/L)} &= 22.9 \times \text{OD}_{645} \\ &- 4.86 \times \text{OD}_{663}\end{aligned}$$

$$\begin{aligned}\text{Total Chlorophyll (mg/L)} &= \text{chlorophyll a} \\ &+ \text{chlorophyll b}\end{aligned}$$

$$\begin{aligned}\text{Carotenoids (mg/L)} &= 4.7 \times \text{OD}_{440} \\ &- 0.27 \times \text{total} \\ &\quad \text{chlorophyll}\end{aligned}$$

$$\begin{aligned}\text{Photosynthetic pigment content (mg/g)} &= \text{photosynthetic pigment concentration} \\ &\times \text{extraction solution volume}/0.5\text{g}\end{aligned}$$

Soluble sugar measurement. The contents of soluble sugars were measured by the method of anthrone colorimetry (Kohyama and Nishinari, 1991). An accurately weighed

0.5-g frozen tissue sample was placed in a test tube, and 10 mL of distilled water was added, followed by thorough mixing. The test tubes were sealed with plastic film and placed in boiling water for 30 min (repeated twice for extraction). The solution was filtered into 25-mL volumetric flasks, the test tubes were rinsed repeatedly, and the final volume was brought to 25 mL with distilled water. Then, 0.5 mL of the sample solution was placed in a 20-mL graduated test tube and mixed with 1.5 mL distilled water. Next, 0.5 mL ethyl anthrone ethyl acetate and 5 mL concentrated sulfuric acid were added to the test tube, which was thoroughly shaken and allowed to cool to ambient temperature. The solution was measured at 630 nm with a ultraviolet spectrophotometer.

Soluble protein measurement. The soluble protein content was measured via the Coomassie brilliant blue staining method (Blakesley and Boezi, 1977). Fresh-frozen tissue (0.5 g) was mixed with 5 mL distilled water and fully ground into a homogenate. The extraction solution together with the slurry was transferred to a 10-mL centrifuge tube. After centrifugation at 10,000 rpm for 10 min, 0.5 mL of the sample solution was pipetted into a test tube with the same volume of distilled water, followed by thorough mixing with 5 mL of Coomassie brilliant blue G-250 staining solution. Two minutes later, the solution was measured at 595 nm in a ultraviolet spectrophotometer.

Nitrate content measurement. The nitrate content was measured colorimetrically (Chang et al., 2013). A mixture of 0.5 g of fresh-frozen tissue with 10 mL deionized water was transferred to a test tube, which was placed in boiling water for 30 min. The extraction solution was filtered through a funnel into a 25-mL volumetric flask, and deionized water was added to a volume of 25 mL, after which 0.5 mL of the sample solution was mixed with 0.4 mL of 5% (w/v) salicylic acid (in pure H₂SO₄) and 9.5 mL of an 8% (w/v) NaOH solution, followed by cooling to ambient temperature. Finally, the absorbance of the extraction solution at 410 nm was measured with a ultraviolet spectrophotometer.

Vitamin C content measurement. Vitamin C content was measured colorimetrically with 2,6-dichlorophenol indophenol (Song et al., 2020). Two grams of fresh-frozen tissue was ground to homogenate with 3 mL of 2% (w/v) oxalic acid solution and placed in a 100-mL volumetric flask. Subsequently, 1 mL of a 30% (w/v) ZnSO₄ solution was added, followed by mixing; 1 mL of 15% (w/v) potassium ferrocyanide was added to remove fat-soluble pigments; the solution was then diluted to scale with a 1% (w/v) oxalic acid solution. The sample solution was filtered into a 100-mL volumetric flask. Four milliliters of extraction solution were mixed with 2 mL of 2,6-dichlorophenol indophenol dye solution and 5 mL of xylene in the test tube, and the tube was quickly shaken for $\approx$ 0.5 min. The tube was left to sit, and xylene

Table 1. The modes of alternating red and blue light exposure under different treatments at 0-, 1-, 2-, and 4-h intervals.

Treatment	Time intervals of alternating light exposure	
	Red	Blue
0 h	—	—
1 h	0600–0700; 0800–0900; 2000–2100 HR	0700–0800; 0900–1000; 2100–2200 HR
2 h	0600–0800; 1000–1200; 1400–1600; 1800–2000 HR	0800–1000; 1200–1400; 1600–1800; 2000–2200 HR
4 h	0600–1000; 1400–1800 HR	1000–1400; 1800–2200 HR

was separated from the water layer, carefully pouring out the supernatant in a 1-mL cuvette. The absorbance of the sample solution was measured at 500 nm with an ultraviolet spectrophotometer.

Total phenolic, total flavonoid, and total anthocyanin content measurements. The contents of total phenolics (TP), total flavonoids (TF), and total anthocyanins (TA) were measured according to the method of Pirie and Mullins (1976). A pool of 0.5-g tissue samples were ground together with 1% (v/v) hydrochloric acid and methanol in an ice-water bath, and the homogenate was then transferred to a 20-mL graduated test tube, diluted to 20 mL with 1% hydrochloric acid and methanol, fully mixed, extracted for 20 min in the dark, and filtered into a 20-mL test tube. The absorbance of TP and TF was measured at 280 nm and 325 nm, respectively, via ultraviolet spectrophotometry, and TA was measured at 530 nm and 600 nm.

Measurement of the DPPH radical scavenging rate. The method of DPPH radical scavenging was described by Liu et al. (2020). Fresh-frozen tissue (0.5 g) was fully ground with absolute ethanol, the homogenate was placed in a 10-mL centrifuge tube, and the washing liquid was poured into the centrifuge tube. The final extraction solution was diluted to 9 mL with absolute ethanol. After standing for 30 min, the solution was centrifuged at 3000 rpm for 15 min, and the supernatant was collected and stored in the dark until use.

Ai: 2 mL 0.2 mmol/L DPPH solution + 0.5 mL extract + 1.5 mL absolute ethanol; after 30 min, the absorbance at 517 nm was measured as Ai.

Aj: 0.5 mL extract + 3.5 mL absolute ethanol, the absorbance at 517 nm was measured as Aj.

Ac: 2 mL 0.2 mmol/L DPPH solution + 2 mL ethanol; after being shaken well, the absorbance at 517 nm was measured as Ac. DPPH free radical scavenging rate = $[1 - (A_i - A_j)/A_c] \times 100\%$.

Antioxidant power measurement. The determination of the ferric-reducing antioxidant power was conducted according to Dinis et al. (1994). A 0.1-mL aliquot of the sample solution (obtained via the same extraction method used in determining the DPPH free radical scavenging rate) was collected with a pipette and mixed with 0.3 mL of absolute ethanol, after which 3.6 mL of a tripyridyl-triazine (TPTZ) working solution was added (0.3 mol/L acetate buffer, 10 mmol/L TPTZ solution, 20 mmol/L FeCl₃ solution, v:v:v,

Fig. 1. Effects on the plant canopy of green- and red-leaf pak choi under supplemental alternating red and blue light. 0 h indicates no supplemental light exposure. 1 h, 2 h, and 4 h indicate alternating red and blue light at intervals of 1, 2, and 4 h, respectively.

10:1:1); after mixing, reaction was allowed to occur in a water bath at 37 °C for 10 min, and the absorbance was measured at 593 nm. Taking 1.0 mmol/L FeSO₄ as the standard, the ferric reducing-antioxidant power (FRAP) value was expressed in millimoles of FeSO₄ required to achieve the same absorbance.

Statistical analysis. All measurements conducted in this experiment were performed in three biological replicates. Significant differences among the treatments were assessed with SPSS 25.0 via one-way analysis of variance followed by Duncan's multiple test ($P < 0.05$), and tables and figures were generated with Microsoft Word 2010 and Origin 2018.

Results

Morphology of pak choi. Significant differences in plant morphology were observed between green- and red-leaf pak choi (Figs. 1 and 2), and smaller plants were found in the control group (0 h) than under the 1-, 2-, and 4-h treatments. As the time of alternating red and blue light increased, the plant canopy of two-leaf-color pak choi became much more compact. The maximum leaf size was observed in the 1-h treatment, and the minimum leaf size was observed in the 0-h treatment. In contrast to the 0-h time point, the color of both cultivars was much darker under supplemental light treatment, especially for red-leaf pak choi.

Biomass of pak choi. The biomass of two-leaf-color pak choi was significantly affected

Fig. 2. Effects on the morphological characteristics of green- and red-leaf pak choi under supplemental alternating red and blue light. 0 h indicates no supplemental light exposure. 1 h, 2 h, and 4 h indicate alternating red and blue light at intervals of 1, 2, and 4 h, respectively.

by the different modes of light exposure (Table 2), resulting in a higher biomass of green-leaf pak choi than red-leaf pak choi. Compared with the control, the fresh and dry weights of green- and red-leaf pak choi were significantly increased. Under the 1-h treatment interval, the highest fresh weights of the shoots and total plants of green-leaf pak choi increased by 211.75% and 210.54%, respectively, compared with 0 h. Moreover, compared with 0 h, the highest fresh weights of the shoots, roots, and total plants increased by 202.23%, 236.76%, and 204.35%, respectively, in red-leaf pak choi in the 1-h treatment. However, the highest fresh weight of roots in green-leaf pak choi under the 2-h treatment increased by 239.78% compared with 0 h. In the 1-h treatment, the dry weights of the shoots and total plants were highest in green-leaf pak choi among all treatments, increasing by 207.35% and 202.67%, respectively, compared with 0 h. Additionally, the highest dry weights of the shoots and total plants in red-leaf pak choi under the 1-h treatment were increased by 204.08% and 201.85%, respectively, compared with 0 h. The roots of green-leaf pak choi exhibited the highest dry weight in the 4-h treatment, increasing by 250.00% compared with 0 h, whereas those of red-leaf pak choi showed the highest dry weight under the 2-h treatment, increasing by 220.00% compared with 0 h.

Chlorophyll and carotenoids. The highest contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were observed in green-leaf pak choi under the 1-h treatment (Table 3). In contrast to the 0-h treatment, the chlorophyll a and carotenoid contents under the 1-h treatment were increased by 24.14% and 36.84%, respectively. However, there

Table 2. The biomass of two-leaf-color pak choi under supplemental alternating red and blue light.

Plant type	Treatment ^z	Fresh wt/g			Dry wt/g		
		Shoot	Root	Plant	Shoot	Root	Plant
Green-leaf	0 h	15.49 ± 0.76 c ^y	0.93 ± 0.10 b	16.42 ± 0.80 c	0.68 ± 0.03 c	0.06 ± 0.006 c	0.75 ± 0.04 b
	1 h	48.29 ± 2.11 a	2.70 ± 0.24 a	50.99 ± 2.21 a	2.09 ± 0.06 a	0.18 ± 0.005 b	2.27 ± 0.07 a
	2 h	46.37 ± 1.83 a	3.16 ± 0.22 a	49.53 ± 1.74 a	2.05 ± 0.11 ab	0.17 ± 0.011 b	2.22 ± 0.11 a
	4 h	40.59 ± 1.30 b	2.67 ± 0.25 a	43.25 ± 1.30 b	1.88 ± 0.05 b	0.21 ± 0.013 a	2.09 ± 0.06 a
Red-leaf	0 h	8.97 ± 0.42 c	0.68 ± 0.03 c	9.66 ± 0.44 c	0.49 ± 0.02 c	0.05 ± 0.002 c	0.54 ± 0.02 c
	1 h	27.11 ± 2.25 a	2.29 ± 0.17 a	29.40 ± 2.40 a	1.49 ± 0.11 a	0.14 ± 0.013 a	1.63 ± 0.12 a
	2 h	23.55 ± 1.04 a	2.08 ± 0.15 ab	25.63 ± 1.08 a	1.30 ± 0.07 a	0.16 ± 0.012 a	1.46 ± 0.08 a
	4 h	18.21 ± 0.78 b	1.72 ± 0.15 b	19.93 ± 0.91 b	1.00 ± 0.06 b	0.11 ± 0.008 b	1.11 ± 0.06 b

^zThe light treatments are as follows: 0 h indicates no supplemental light exposure. 1 h, 2 h, and 4 h indicate supplemental alternating red and blue light at 1-, 2-, and 4-h intervals, respectively.

^yValues presented are mean ± SD (n = 6). Different letters among treatments represent statistically significant differences according to Duncan's multiple test ($P < 0.05$).

Table 3. Effects of supplemental alternating red and blue light on chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents.

Plant type	Treatment ^z	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Carotenoids (mg/g)
Green-leaf pak choi	0 h	0.58 ± 0.05 b ^y	0.21 ± 0.02 a	0.79 ± 0.07 a	0.19 ± 0.02 b
	1 h	0.72 ± 0.03 a	0.23 ± 0.01 a	0.95 ± 0.04 a	0.26 ± 0.02 a
	2 h	0.62 ± 0.03 ab	0.20 ± 0.01 a	0.82 ± 0.04 a	0.22 ± 0.01 ab
	4 h	0.65 ± 0.04 ab	0.21 ± 0.01 a	0.86 ± 0.06 a	0.24 ± 0.01 ab
Red-leaf pak choi	0 h	0.76 ± 0.05 b	0.34 ± 0.04 a	1.10 ± 0.09 b	0.23 ± 0.02 c
	1 h	1.09 ± 0.05 a	0.40 ± 0.02 a	1.49 ± 0.07 a	0.49 ± 0.05 a
	2 h	0.97 ± 0.04 a	0.36 ± 0.02 a	1.33 ± 0.06 a	0.35 ± 0.01 b
	4 h	1.07 ± 0.03 a	0.41 ± 0.01 a	1.48 ± 0.04 a	0.57 ± 0.02 a

^zThe light treatments are as follows: 0 h indicates no supplemental light exposure; 1 h, 2 h, and 4 h indicate supplemental alternating red and blue light at 1-, 2-, and 4-h intervals, respectively.

^yValues presented are mean ± SD (n = 3). Different letters among treatments represent statistically significant differences according to Duncan's multiple test ($P < 0.05$).

were no significant differences in the chlorophyll b and total chlorophyll contents of green-leaf pak choi among all treatments. Generally, the chlorophyll a, chlorophyll b, and total chlorophyll contents of red-leaf pak choi were higher than those of green-leaf pak choi. Compared with 0 h, the contents of chlorophyll a and total chlorophyll in red-leaf pak choi were the highest under the 1-h treatment, increasing by 43.42% and 35.45%, respectively, but no significant difference in chlorophyll b was observed among the treatments. In addition, the highest carotenoid content of red-leaf pak choi was observed in the 4-h treatment, under which it was increased by 147.83% compared with that under the 0-h treatment.

Soluble sugar, soluble protein, nitrate, and vitamin C. As shown in Fig. 3, there were significant effects on the soluble sugar (Fig. 3A), soluble protein (Fig. 3B), nitrate (Fig. 3C), and vitamin C (Fig. 3D) contents of both cultivars among all treatments. The highest contents of soluble sugars in green- and red-leaf pak choi were observed under the 1-h treatment, increasing by 100% and 57.14%, respectively, compared with those at 0 h. The soluble sugar contents of green-leaf pak choi were markedly higher than those of red-leaf pak choi. With an increasing interval time, the contents of soluble protein in two-leaf-color pak choi gradually increased compared with that at 0 h, increasing by 74.36% and 26.98%, respectively, in the 4-h treatment. The content of nitrate was progressively reduced with increasing interval time, and green-leaf pak choi exhibited a lower content of nitrate than red-leaf pak choi. The nitrate contents of green-leaf pak choi under the 2- and 4-h treatments were decreased by 27.37% and 16.05%, respectively, compared

with 0 h. At the same time, the nitrate content of red-leaf pak choi was lowest under the 4-h treatment, which was reduced by 12.23% compared with 0 h. The same increasing trend of the vitamin C content was observed with an increasing time interval in two-leaf-color pak choi, and the highest contents were observed under the 4-h treatment, which increased by 12% and 90.9%, respectively, relative to 0 h. In general, green-leaf pak choi presented a higher vitamin C content than red-leaf pak choi.

TP, TF, and TA contents. Compared with the control, there was a significant impact on the TP, TF, and TA contents of green- and red-leaf pak choi (Fig. 4), which showed a consistent change trend, increasing with increasing interval time. The highest TP, TF, and TA contents of green-leaf pak choi were observed under the 4-h treatment, compared with 0 h, which increased by 36.71%, 42.45%, and 17.17%, respectively. As shown in Fig. 4, the TP, TF, and TA contents of red-leaf pak choi were higher than those of green-leaf pak choi. The TP content of red-leaf pak choi with highest in the 2-h treatment, under which it was increased by 22.45% compared with 0 h; however, no significant differences in TPs were found among the 1-, 2-, and 4-h treatments. The contents of TF and TA in the 4-h treatment increased by 21.62% and 115.86%, respectively.

DPPH and FRAP. In contrast to the 0-h treatment, promotion effects on the DPPH radical scavenging rate and FRAP of green- and red-leaf pak choi were observed under the 1-, 2-, and 4-h treatments (Fig. 5). With an increasing interval time, the DPPH radical scavenging rate (Fig. 5A) and FRAP (Fig. 5B) were increased. The largest changes

in both cultivars of pak choi were observed under the 4-h treatment, where the DPPH radical scavenging rate was increased by 18.12% and 25.79%, respectively, compared with 0 h, and FRAP was increased by 24.03% and 46.21%, respectively, compared with 0 h.

Discussion

Light signaling mediates the synthetic pathways of auxin, cytokinin, and gibberellin through photoreceptors, thereby regulating plant growth, development, and leaf morphology (Kobayashi et al., 2012; Kurepin and Pharis, 2014). Compared with the 0-h treatment, the canopy of two-leaf-color pak choi was more compact under supplemental light exposure (Figs. 1 and 2). It has been reported that blue light can make the plant morphology of cucumber, arugula, and mustard more compact (Johnson et al., 2020; Song et al., 2019), which is closely associated with shade avoidance syndrome in plants. In this shade reaction, auxin plays an important role in controlling the angle of the leaves and is transported from the leaf tip to the petiole (Gao et al., 2020). Moreover, cryptochromes (CRYs) and phototropins (PHOTs) mediate the movement of auxin in a blue light-dependent manner (Kong and Zheng, 2020; Pedmale et al., 2016). Recent research has indicated that light synergistically regulates plant branching by coupling the phytochrome signaling pathway with the strigolactone pathway to inhibit branching (Xie et al., 2020). In this experiment, under treatment with alternating red and blue light, it was observed that the plant morphology became increasingly compact with an increasing interval between red and blue light exposure, which was consistent with a study by

Fig. 3. Effects on the (A) soluble sugar, (B) soluble protein, (C) nitrate, and (D) vitamin C contents of two-leaf-color pak choi under supplemental alternating red and blue light. The values indicated by different letters among treatments represent statistically significant differences according to Duncan's multiple test at $P < 0.05$ ($n = 3$).

Fig. 4. Effects on the (A) total phenolic, (B) total flavonoid, and (C) total anthocyanin contents of two-leaf-color pak choi under alternating supplemental red and blue light. The values indicated by different letters among treatments represent statistically significant differences according to Duncan's multiple test at $P < 0.05$ ($n = 3$).

Chen et al. (2017) and may be related to the duration of plant exposure to alternating red and blue light. Both red and blue light promote the elongation of stems and petioles, but blue light has a stronger influence than red light (Johnson et al., 2020). The length of the petiole and the stem length of lettuce reached their maximum levels under intermittent red and

blue light exposure with intervals of 6 and 1 h (Shimokawa et al., 2014). Red and blue light promote leaf expansion, and alternating red and blue light exposure contributes to elongating the leaves of lettuce (Ohtake et al., 2018). The results showed that the leaves of both cultivars of pak choi were larger under supplemental alternating red and blue light than

they were at 0 h (Fig. 2), and the largest leaves were observed under the 1-h treatment; larger leaves capture more light energy to increase the net photosynthetic rate, resulting in the highest biomass of two-leaf-color pak choi under the 1-h treatment (Table 2). According to previous research, compared with simultaneous irradiation with red and blue light,

Fig. 5. Effects on (A) 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging rate and (B) ferric reducing-antioxidant power (FRAP) in two-leaf-color pak choi under alternating supplemental red and blue light. The values indicated by different letters among treatments represent statistically significant differences according to Duncan's multiple test at $P < 0.05$ ($n = 3$).

alternating red and blue light promotes the growth of lettuce, especially at 22 to 31 d (Ohtake et al., 2018), suggesting a significant effect of alternating irradiation on plants in the later cultivation period. In addition, it was shown that with the exception of consistent irradiation with red and blue light, the fresh and dry weights of lettuce under alternating red and blue light exposure were highest with an interval of 1 h vs. 2, 4, and 8 h (Chen et al., 2019), which was consistent with the results of the present study.

Plants mainly absorb and use visible light with wavelengths of 400 to 700 nm (Landi et al., 2020). Light signaling controls a variety of physiological and biochemical processes by driving photosynthesis. The peak absorption values of photosynthetic pigments in plant leaves under illumination with red and blue light have been reported (Zheng et al., 2018). Red and blue light are associated with differences in the synthesis of chlorophyll. Red light is beneficial to the synthesis of chlorophyll, but blue light is not conducive to the accumulation of chlorophyll and is instead beneficial for increasing the ratio of chlorophyll a to chlorophyll b (Landi et al., 2020). The chlorophyll a and total chlorophyll contents of lettuce under alternating red and blue light are higher with a 1-h interval than a 4-h interval (Chen et al., 2019). Our experiment showed that the contents of chlorophyll a, chlorophyll b, and total chlorophyll in green- and red-leaf pak choi under supplemental alternating red and blue light were significantly higher than those at 0 h, with no significant differences among the 1-, 2-, and 4-h treatments, but the total chlorophyll content was slightly higher in the 1-h treatment (Table 3). Studies have reported that continuous exposure to monochromatic blue light might have negative effects; for example, photosynthetic pigments may be reduced due to chloroplast escape reactions and damage to mesophyll cells (Landi et al., 2020). Therefore, under exposure to light sources of different quality after an appropriate interval (alternating red and blue light), it is possible that exposure to monochromatic red and blue light maximizes light utilization without negative impacts. Carotenoids are active antiox-

idant substances that not only are beneficial phytochemicals for human health (Britton, 2020; Watkins and Pogson, 2020) but also take part in photosynthetic system II as auxiliary pigments by absorbing excess light energy and transferring to the photosynthetic reflection center (Hoffmann et al., 2016). In addition, the xanthophyll cycle reduces damage in the plant light protection system (Llorente et al., 2017). In this study, the carotenoid contents of two-leaf-color pak choi were higher under irradiation with alternating red and blue light than at 0 h of treatment. The carotenoid content of green-leaf pak choi was not significantly different after 1, 2, or 4 h of treatment, whereas the carotenoid content of red-leaf pak choi was highest after 4 h of treatment (but was not significantly different from that after 1 h), seemingly indicating that the regulation of carotenoids was species specific in response to light.

Soluble sugars, soluble protein, nitrate, and vitamin C are important indicators for evaluating the taste, safety, and nutritional value of vegetables. Compared with the control, this study showed that the contents of soluble sugars in both cultivars were significantly increased under alternating red and blue light (Fig. 3A). Red light promotes the process of carbohydrate synthesis and upregulates the expression of related genes (Yuan et al., 2020). Blue light promotes the degradation of guard cell starch into soluble sugars, which depends on the *PHOT1/PHOT2* signaling pathway cascade (Horrer et al., 2016). The accumulation of sucrose and starch in lettuce is enhanced by alternating red and blue light with an interval of 1 h (Chen et al., 2019). The results of the present study also show that the soluble sugar contents of green- and red-leaf pak choi in the 1-h treatment were higher than those in the other treatments, whereas the contents of soluble protein (Fig. 3B) and vitamin C (Fig. 3D) increased gradually with an increasing time interval, whereas nitrate contents decreased (Fig. 3C). The level of nitrate is mainly affected by two factors: light and nitrogen fertilization (Nicole et al., 2018). Red light and blue light can alter the activities of nitrate reductase and nitrite reductase, thereby reducing the concentration of nitrate (Nicole

et al., 2018). Nitrate is transported, assimilated, and subsequently reduced to amino acids under catalysis by enzymes (Wang et al., 2018), providing substrates for the synthesis of soluble proteins. In addition, the precursor of the phenylpropane metabolic pathway is phenylalanine, which may provide a nitrogen source for the synthesis of TP, TF, and TA, leading to increases in their respective contents. The results showed that an increase in soluble sugars was accompanied by a decrease in nitrate content because the absorption and transportation of nitrate require energy from adenosine triphosphate produced by photosynthesis and respiration (Wang et al., 2018). In summary, it is possible to provide substrates and energy for the synthesis of these metabolites via the consumption of soluble sugars and nitrate, contributing to increases in soluble protein, vitamin C, TP, TF, and TA.

TP, TF, and TA are active antioxidant compounds with health benefits to humans that act as defensive phytochemicals in plants (Harbaum et al., 2008), reducing the damage caused by abiotic stress and increasing ultraviolet tolerance under ultraviolet radiation (Moreira-Rodríguez et al., 2017; Taulavuori et al., 2018). The accumulation of TP, TF, and TA is affected by light quality, intensity, and exposure duration, and the contents of TP, TF, and TA in two-leaf-color pak choi were found to be significantly higher than those at 0 h in our experiment (Fig. 4). Within the range of visible light, blue light has the greatest impact on the metabolism of phenylpropane (Landi et al., 2020), and a large number of studies have reported that blue light promotes the synthesis of TP, TF, and TA in crops such as lettuce (Kitazaki et al., 2018), Chinese kale (Li et al., 2019), and pak choi (Zheng et al., 2018). In *Arabidopsis hy4* mutants (*cry1*), it was found that the accumulation of anthocyanins decreased and that the mRNA expression of the core *CHS* (chalcone synthase), *CHI* (chalcone isomerase), and *DFR* (dihydroflavonol reductase) genes of the phenylpropane metabolism pathway mediated by blue light was downregulated (Jackson and Jenkins, 1995). Although red light has no positive effect on the metabolism of phenylpropane in most cases, some phenolic compounds are dependent on red light in some

special cases. For example, red light increases the synthesis of polyphenols in green basil (Landi et al., 2020), and the contents of phenolic compounds and total flavonoids are increased by continuous red light exposure in tomato fruits (Panjai et al., 2019). In this study, green- and red-leaf pak choi showed no significant differences in TP and TF among the 1-, 2-, and 4-h treatments, but the highest contents were observed under the 4-h treatment. The TA content of green-leaf pak choi exhibited no significant differences among the 1-, 2-, and 4-h treatments, whereas red-leaf pak choi showed a higher TA content in the 4-h treatment than in the other treatments, which was possibly related to the species-specific response and the longer interval between red light and blue light. The longer the duration of alternating red light and blue light irradiation, the more beneficial it was to the synthesis of TA.

Antioxidant compounds at low concentrations protect biomolecules (soluble sugars, proteins, nucleic acids, and polyunsaturated lipids) from oxidative damage through free radical-scavenging reactions (Bendary et al., 2013). Because the metabolic pathways of various phytochemicals in plants are relatively complicated, the DPPH free radical scavenging rate and FRAP are important reference indicators for measuring the antioxidant capacity of plants (Zheng et al., 2018). Both the DPPH free radical scavenging rate and the FRAP antioxidant capacity were the highest after 4 h of treatment (Fig. 5), which was consistent with the trends observed for vitamin C, TP, and TF. The antioxidant capacity of two-leaf pak choi was closely associated with health compounds, resulting in the highest antioxidant capacity under the 4-h treatment in this work.

Conclusion

Significant effects on the morphology, biomass, and nutritional quality of two-leaf-color pak choi were observed under supplemental alternating red and blue light. The production of green-leaf pak choi was higher than that of red-leaf pak choi, but its nutrition quality was lower than that of red-leaf pak choi. A 1-h interval of alternating red and blue light was most effective for improving morphological regulation and the accumulation of biomass and soluble sugars in pak choi. Soluble protein and TA contents were strongly increased by alternating red and blue light with a 4-h interval, and a decrease in nitrate was simultaneously observed. As a result, this study suggests a strategy of supplemental illumination to improve the growth and nutritional quality of plants.

Literature Cited

- Avercheva, O.V., Y.A. Berkovich, I.O. Konova-lova, S.G. Radchenko, S.N. Lapach, E.M. Bassarskaya, G.V. Kochetova, T.V. Zhigalova, O.S. Yakovleva, and I.G. Tarakanov. 2016. Optimizing LED lighting for space plant growth unit: Joint effects of photon flux density, red to white ratios and intermittent light pulses. *Life Sci. Space Res. (Amst.)* 11:29–42.
- Bendary, E., R.R. Francis, H.M.G. Ali, M.I. Sarwat, and S. El Hady. 2013. Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds. *Ann. Agr. Sci.* 58:173–181.
- Bjorkman, M., I. Klingen, A.N. Birch, A.M. Bones, T.J. Bruce, T.J. Johansen, R. Meadow, J. Molmann, R. Seljasen, L.E. Smart, and D. Stewart. 2011. Phytochemicals of Brassicaceae in plant protection and human health—influences of climate, environment and agronomic practice. *Phytochemistry* 72:538–556.
- Blakesley, R.W. and J.A. Boezi. 1977. A new staining technique for proteins in polyacrylamide gels using Coomassie brilliant blue G250. *Anal. Biochem.* 82:580–582.
- Brazaitytė, A., S. Sakalauskienė, G. Samuolienė, J. Jankauskienė, A. Viršilė, A. Novičkovas, R. Sirtautas, J. Miliauskienė, V. Vaštakaitė, L. Dabašinskis, and P. Duchovskis. 2015. The effects of LED illumination spectra and intensity on carotenoid content in Brassicaceae microgreens. *Food Chem.* 173:600–606.
- Britton, G. 2020. Carotenoid research: History and new perspectives for chemistry in biological systems. *Biochim. Biophys. Acta (BBA)—Mol. Cell Biol. Lipids* 1865:158699.
- Chang, A.C., T.Y. Yang, and G.L. Riskowski. 2013. Ascorbic acid, nitrate, and nitrite concentration relationship to the 24-hour light/dark cycle for spinach grown in different conditions. *Food Chem.* 138:382–388.
- Chen, X.-L., L.-c. Wang, T. Li, Q.-c. Yang, and W.-z. Guo. 2019. Sugar accumulation and growth of lettuce exposed to different lighting modes of red and blue LED light. *Sci. Rpt.* 9:6926.
- Chen, X.-L. and Q.-c. Yang. 2018. Effects of intermittent light exposure with red and blue light emitting diodes on growth and carbohydrate accumulation of lettuce. *Scientia Hort.* 234:220–226.
- Chen, X.-L., Q.-c. Yang, W.-p. Song, L.-c. Wang, W.-z. Guo, and X.-z. Xue. 2017. Growth and nutritional properties of lettuce affected by different alternating intervals of red and blue LED irradiation. *Scientia Hort.* 223:44–52.
- Claypool, N.B. and J.H. Lieth. 2020. Physiological responses of pepper seedlings to various ratios of blue, green, and red light using LED lamps. *Scientia Hort.* 268:109371.
- Dinis, T.C.P., V.M.C. Madeira, and L.M. Almeida. 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation as peroxyl radical scavengers. *Arch. Biochem. Biophys.* 315:161–169.
- Gao, C., X. Liu, N. De Storme, K.H. Jensen, Q. Xu, J. Yang, X. Liu, S. Chen, H.J. Martens, A. Schulz, and J. Liesche. 2020. Directionality of plasmodesmata-mediated transport in *Arabidopsis* leaves supports auxin channeling. *Curr. Biol.* 30:1970–1977.e4.
- Gratani, L. 1992. A non-destructive method to determine chlorophyll content of leaves. *Photosynthetica* 26:469–473.
- Harbaum, B., E.M. Hubbermann, Z. Zhu, and K. Schwarz. 2008. Free and bound phenolic compounds in leaves of pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*) and Chinese leaf mustard (*Brassica juncea* Coss.). *Food Chem.* 110:838–846.
- Hoffmann, A.M., G. Noga, and M. Hunsche. 2016. Alternating high and low intensity of blue light affects PSII photochemistry and raises the contents of carotenoids and anthocyanins in pepper leaves. *Plant Growth Regulat.* 79:275–285.
- Hogewoning, S.W., E. Wientjes, P. Douwstra, G. Trouwborst, W. van Ieperen, R. Croce, and J. Harbinson. 2012. Photosynthetic quantum yield dynamics: From photosystems to leaves. *Plant Cell* 24:1921.
- Horner, D., S. Flutsch, D. Pazmino, J.S.A. Matthews, M. Thalmann, A. Nigro, N. Leonhardt, T. Lawson, and D. Santelia. 2016. Blue light induces a distinct starch degradation pathway in guard cells for stomatal opening. *Curr. Biol.* 26:362–370.
- Huché-Thélier, L., L. Crespel, J.L. Gourrierc, P. Morel, S. Sakr, and N. Leduc. 2016. Light signaling and plant responses to blue and UV radiations—Perspectives for applications in horticulture. *Environ. Exp. Bot.* 121:22–38.
- Jackson, J.A. and G.I. Jenkins. 1995. Extension-growth responses and expression of flavonoid biosynthesis genes in the *Arabidopsis hy4* mutant. *Planta* 197:233–239.
- Johnson, R.E., Y. Kong, and Y. Zheng. 2020. Elongation growth mediated by blue light varies with light intensities and plant species: A comparison with red light in arugula and mustard seedlings. *Environ. Exp. Bot.* 169: 103898.
- Kitazaki, K., A. Fukushima, R. Nakabayashi, Y. Okazaki, M. Kobayashi, T. Mori, T. Nishizawa, S. Reyes-Chin-Wo, R.W. Michelmore, K. Saito, K. Shoji, and M. Kusano. 2018. Metabolic reprogramming in leaf lettuce grown under different light quality and intensity conditions using narrow-band LEDs. *Sci. Rpt.* 8:7914.
- Kobayashi, K., S. Baba, T. Obayashi, M. Sato, K. Toyooka, M. Keränen, E.M. Aro, H. Fukaki, H. Ohta, K. Sugimoto, and T. Masuda. 2012. Regulation of root greening by light and auxin/cytokinin signaling in *Arabidopsis*. *Plant Cell* 24:1081–1095.
- Kohyama, K. and K. Nishinari. 1991. Effect of soluble sugars on gelatinization and retrogradation of sweet potato starch. *J. Agr. Food Chem.* 39:1406–1410.
- Kong, Y., M. Stasiak, M.A. Dixon, and Y. Zheng. 2018. Blue light associated with low phytochrome activity can promote elongation growth as shade-avoidance response: A comparison with red light in four bedding plant species. *Environ. Exp. Bot.* 155:345–359.
- Kong, Y. and Y. Zheng. 2020. Phototropin is partly involved in blue-light-mediated stem elongation, flower initiation, and leaf expansion: A comparison of phenotypic responses between wild *Arabidopsis* and its phototropin mutants. *Environ. Exp. Bot.* 171:103967.
- Kurepin, L.V. and R.P. Pharis. 2014. Light signaling and the phytohormonal regulation of shoot growth. *Plant Sci.* 229:280–289.
- Landi, M., M. Zivcak, O. Sytar, M. Brestic, and S.I. Allakhverdiev. 2020. Plasticity of photosynthetic processes and the accumulation of secondary metabolites in plants in response to monochromatic light environments: A review. *Biochim. Biophys. Acta (BBA)—Bioenergetics* 1861:48131.
- Li, Y., Y. Zheng, H. Liu, Y. Zhang, Y. Hao, S. Song, and B. Lei. 2019. Effect of supplemental blue light intensity on the growth and quality of Chinese kale. *Hort. Environ. Biotechnol.* 60:49–57.
- Liu, D., Y. Guo, P. Wu, Y. Wang, M. Kwaku Golly, and H. Ma. 2020. The necessity of walnut proteolysis based on evaluation after in vitro simulated digestion: ACE inhibition and DPPH radical-scavenging activities. *Food Chem.* 311:125960.
- Llorente, B., J.F. Martinez-Garcia, C. Stange, and M. Rodriguez-Concepcion. 2017. Illuminating colors: Regulation of carotenoid biosynthesis

- and accumulation by light. *Curr. Opin. Plant Biol.* 37:49–55.
- Ma, Z., H. Nian, S. Luo, Q. Ma, Y. Cheng, and Y. Mu. 2018. Growth responses of soybean (*Glycine max* L.) seedlings as affected by monochromatic or mixture radiation provided by light-emitting diode. *IFAC-PapersOnLine* 51:770–777.
- Miao, Y., Q. Chen, M. Qu, L. Gao, and L. Hou. 2019. Blue light alleviates 'red light syndrome' by regulating chloroplast ultrastructure, photosynthetic traits and nutrient accumulation in cucumber plants. *Scientia Hort.* 257:108680.
- Moreira-Rodríguez, L., V. Nair, J. Benavides, L. Cisneros-Zevallos, and D. Jacobo-Velázquez. 2017. UVA, UVB light, and methyl jasmonate, alone or combined, redirect the biosynthesis of glucosinolates, phenolics, carotenoids, and chlorophylls in broccoli sprouts. *Intl. J. Mol. Sci.* 18:2330.
- Nicole, C.C.s., J. Mooren, A. Stuks, and M.P.C.M. Krijn. 2018. Nitrate control using LED lights. *Acta Hort.* 1227:661–668.
- Ohtake, N., M. Ishikura, H. Suzuki, W. Yamori, and E. Goto. 2018. Continuous irradiation with alternating red and blue light enhances plant growth while keeping nutritional quality in lettuce. *HortScience* 53:1804–1809.
- Ouzounis, T., E. Rosenqvist, and C.-O. Ottosen. 2015. Spectral effects of artificial light on plant physiology and secondary metabolism: A review. *HortScience*. 50:1128–1135.
- Panjai, L., G. Noga, M. Hunsche, and A. Fiebig. 2019. Optimal red light irradiation time to increase health-promoting compounds in tomato fruit postharvest. *Scientia Hort.* 251:189–196.
- Pedmale, Ullas V., S.-S. C. Huang, M. Zander, B.J. Cole, J. Hetzel, K. Ljung, P.A.B. Reis, P. Sridevi, K. Nito, J.R. Nery, J.R. Ecker, and J. Chory. 2016. Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. *Cell* 164:233–245.
- Pirie, A. and M.G. Mullins. 1976. Changes in anthocyanin and phenolics content of grapevine leaf and fruit tissues treated with sucrose, nitrate, and abscisic acid. *Plant Physiol.* 58:468–472.
- Shimokawa, A., Y. Tonooka, M. Matsumoto, H. Ara, H. Suzuki, N. Yamauchi, and M. Shigyo. 2014. Effect of alternating red and blue light irradiation generated by light emitting diodes on the growth of leaf lettuce. *bioRxiv*. 8 Mar. 2020. <<https://www.biorxiv.org/content/10.1101/003103v1.full.pdf>>.
- Song, J., K. Cao, Y. Hao, S. Song, W. Su, and H. Liu. 2019. Hypocotyl elongation is regulated by supplemental blue and red light in cucumber seedling. *Gene* 707:117–125.
- Song, Y., K. Qiu, J. Gao, and B. Kuai. 2020. Molecular and physiological analyses of the effects of red and blue LED light irradiation on postharvest senescence of pak choi. *Postharvest Biol. Technol.* 164:111155.
- Taulavuori, K., A. Pyysalo, E. Taulavuori, and R. Julkunen-Tiitto. 2018. Responses of phenolic acid and flavonoid synthesis to blue and blue-violet light depends on plant species. *Environ. Exp. Bot.* 150:183–187.
- Tissot, N. and R. Ulm. 2020. Cryptochrome-mediated blue-light signalling modulates UVR8 photoreceptor activity and contributes to UV-B tolerance in *Arabidopsis*. *Nat. Commun.* 11:1323.
- Wang, X.-L., X. Cai, C. Xu, S. Wang, S. Dai, and Q. Wang. 2018. Nitrate accumulation and expression patterns of genes involved in nitrate transport and assimilation in spinach. *Molecules* 23:2231.
- Watkins, J.L. and B.J. Pogson. 2020. Prospects for carotenoid biofortification targeting retention and catabolism. *Trends Plant Sci.* 25:501–512.
- Wu, B.-S., A.-S. Rufyikiri, V. Orsat, and M.G. Lefsrud. 2019. Re-interpreting the photosynthetically action radiation (PAR) curve in plants. *Plant Sci.* 289:110272.
- Xie, Y., Y. Liu, M. Ma, Q. Zhou, Y. Zhao, B. Zhao, B. Wang, H. Wei, and H. Wang. 2020. *Arabidopsis* FHY3 and FAR1 integrate light and strigolactone signaling to regulate branching. *Nat. Commun.* 11:1955.
- Yuan, H., X. Zhang, Z. Jiang, X. Wang, Y. Wang, L. Cao, and X. Zhang. 2020. Effect of light spectra on microalgal biofilm: Cell growth, photosynthetic property, and main organic composition. *Renew. Energy* 157:83–89.
- Zhang, X., Z. Bian, X. Yuan, X. Chen, and C. Lu. 2020. A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. *Trends Food Sci. Technol.* 99:203–216.
- Zheng, Y.-j., Y.-t. Zhang, H.-c. Liu, Y.-m. Li, Y.-l. Liu, Y.-w. Hao, and B.-f. Lei. 2018. Supplemental blue light increases growth and quality of greenhouse pak choi depending on cultivar and supplemental light intensity. *J. Integr. Agr.* 17:2245–2256.