Overnight Supplemental Blue, Rather than Far-red, Light Improves Microgreen Yield and Appearance Quality without Compromising Nutritional Quality during Winter Greenhouse Production

Qinglu Ying, Yun Kong, and Youbin Zheng

School of Environmental Sciences, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada

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Abstract. To determine whether supplemental blue light (B) or far-red light (FR) overnight can promote microgreen elongation to facilitate machine harvesting and improve microgreen quality and yield, two common microgreen species, mustard (*Brassica juncea*) and arugula (*Eruca sativa*), were grown in a greenhouse in Guelph, Ontario, Canada, during January 2019. Low-intensity ($14 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) B or FR was applied to microgreens overnight from 1730 HR to 0630 HR, and no supplemental lighting (D) was used as a control. After 2 weeks of light treatments, B compared to D promoted stem elongation by 16% and 10%, respectively, and increased crop yield by 32% and 29%, respectively, in mustard and arugula. B compared to D also increased the cotyledon area in mustard and leaf mass per area in arugula and enhanced cotyledon color in both species despite having no effects on total chlorophyll, carotenoid, and phenolic contents. However, FR did not increase stem length or fresh weight compared with D, reduced plant height compared with B in both species, and reduced the cotyledon area in arugula. FR, compared with D and B, reduced the stem diameter and phytochemical contents of both species. Therefore, low-intensity B can be applied overnight for winter greenhouse microgreen production because of its beneficial effects on appearance quality and crop yield without negatively affecting nutritional quality.

Microgreens are tender leafy vegetables harvested after two cotyledons have fully developed, with or without the first true leaves, that are becoming popular in the worldwide markets due to their high nutritional value (Treadwell et al., 2016; Xiao et al., 2012). In some regions that have long and cold winters, like Canada, winter production of microgreens in local greenhouses has become an option. The profits of greenhouse production of fruits

and vegetables such as tomato and cucumber are decreasing due to the increased costs of greenhouse operation and intense price competition with imported produce. However, it is difficult to import or transport microgreens from other regions to Canada because they are highly perishable products (Mir et al., 2017). Moreover, microgreens have a short growth period (7–20 d), so they can be grown with many cropping cycles in greenhouses throughout the winter.

The low natural light level during winter months is one of the most limiting factors for greenhouse vegetable production in northern regions, such as Canada (Demers and Gosselin, 2002). During the winter months (November–January), the natural daily light integral (DLI) in the northern United States and southern Canada normally ranges between 5 and 15 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, resulting in a daily average photosynthetic photon flux density (PPFD) of 58 to 174 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Faust and Logan, 2018). The available light level in greenhouses could be further reduced by 30% to 60% due to the transmission losses through greenhouse construction and covering materials (Critten, 1993; Llewellyn et al., 2013). Therefore, in these regions, the DLI in greenhouses can be as low as 2 to 6 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ during the winter

months (daily average PPFD of 23–69 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ depending on the greenhouse structure). For microgreens, the recommended minimum DLI has been elusive in the literature. However, for greenhouse vegetable (including microgreens) production in southern Canada, the yield and most quality metrics increased with the increasing DLI within the range of 6.9 to 24 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ (Jones-Baumgardt et al., 2020; Kong et al., 2018a; Kong and Zheng, 2019). Consequently, winter greenhouse production under low natural light conditions is a great challenge for growers due to the decreased yield and quality of horticultural crops, including microgreens.

Supplemental lighting (SL) is a common practice for greenhouse production because it is a way to deal with low natural light issues. Hofstra et al. (1969) found that low-intensity supplemental light is efficient for carbon assimilation and plant growth, and $\approx 13 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ supplemental light is five-times more efficiently used during nighttime compared with daytime in terms of CO_2 fixation. Overnight SL can be economically beneficial in some regions, such as Ontario in Canada, where the electricity cost during nighttime is almost half that during the daytime. Therefore, overnight SL may benefit crop production more efficiently. Light-emitting diodes (LEDs) have been increasingly used as a SL source in greenhouses because of the many advantages over traditional lamps (Brandon et al., 2012; Gómez et al., 2013). Among the advantages, the adjustable spectral quality enables growers to control plant growth and development using LED lights based on their production purposes. However, the optimal spectral quality of LED is unclear for overnight low-intensity SL during winter greenhouse microgreen production in terms of yield and quality.

Microgreens with longer stems are normally more attractive to most consumers; therefore, plant height is one of the most important microgreen appearance qualities. In addition, plant height or stem length is an important technological quality trait. Most microgreens are harvested with a minimum height of 5 cm (Kyriacou et al., 2016), and inhibition of stem elongation would delay harvest time, thereby extending the crop cycle time. Also, commercial microgreen production has been increasingly switching from hand-harvesting to machine-harvesting to reduce labor costs. Microgreens with plant height less than 5 cm are difficult to harvest with machines (Kong et al., 2019a). Although daytime SL can increase the microgreen yield and some quality traits, it inhibits stem elongation and causes difficulty with machine harvesting (Jones-Baumgardt et al., 2019). Therefore, it would be interesting to investigate whether stem elongation can be promoted by overnight SL without compromising yield and quality during winter greenhouse production.

Recently, our laboratory found that monochromatic blue light (B, 400–500 nm) instead of red light (R, 600–700 nm) promoted stem elongation of indoor-grown microgreens under LED lighting as the sole light source with PPFD of ≈ 100 or 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and

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Y.Z. is the corresponding author. E-mail: yzheng@uoguelph.ca.

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photoperiods of both 24 and 16 h (Kong et al., 2019a, 2019b). In addition to promoting stem elongation, B compared to R also reduced cotyledon size, changed plant color, and increased biomass partitioning to the stem despite the similar fresh weight (FW) of the stems and leaves (Kong et al., 2019a). We concluded that the promoted stem elongation is a shade-avoidance response mediated by B associated with low phytochrome activity, as indicated by the low phytochrome photostationary state (PPS; <0.6) (Kong et al., 2018b), which may also involve a co-action among the three photoreceptors (phytochrome, cryptochrome, and phototropin) (Kong and Zheng, 2020). However, it is unclear whether a similar promotion effect on stem elongation associated with other responses can be found with overnight supplemental B in winter greenhouse microgreen production.

In a natural light environment, the enriched far-red light (FR) level can also promote stem elongation as a shade-avoidance response by lowering the phytochrome equilibrium (i.e., decreasing the phytochrome activity) (Demotes-Mainard et al., 2016). An increased FR level at the end of day has been shown to enhance stem elongation in many species, including chrysanthemum (*Chrysanthemum morifolium*) (Lund et al., 2007), poinsettia (*Euphorbia pulcherrima*) (Islam et al., 2014), and tomato (*Solanum lycopersicum*) rootstock (Chia and Kubota, 2010). Extending the photoperiod with supplemental FR light is extremely useful to promote shoot elongation of Japanese pear (*Pyrus pyrifolia*) during the first several months of the seedling stages (Ito et al., 2014). However, plants grown under a light environment with high FR levels might undergo some negative effects, such as decreased chlorophyll content and leaf thickness (Demotes-Mainard et al., 2016). These

negative effects may potentially compromise microgreen quality.

It is unknown whether B or FR is more effective as an overnight SL source for promoting elongation while having fewer negative effects on yield and other quality traits for winter greenhouse microgreen production. Therefore, the objective of this study was to evaluate the effects of overnight SL with low-intensity B or FR LED, using no SL as a control, on winter greenhouse production of arugula and mustard microgreens in terms of appearance quality (including stem elongation), crop yield, and phytochemical contents.

Materials and Methods

Greenhouse conditions and plant materials. The experiment was performed in the Edmund C. Bovey building research greenhouse at the University of Guelph, Guelph, ON (lat. 43° 33'N, long. 80° 15'W) during January of 2019. Three adjacent greenhouse compartments (6.2 m × 7.6 m) with three benches oriented east to west were used in the experiment (Fig. 1A). Each compartment was independently controlled using an Argus environmental control system (Argus Controls Systems Ltd., Surrey, BC, Canada) at day/night temperatures and relative humidity (RH) of 21/19 °C and 70%, respectively. The temperature and RH were also logged using the same system with 15-min intervals in each compartment. The natural light intensity in the greenhouse was logged by a sunlight-calibrated quantum sensor (SQ-110; Apogee Instruments, Logan, UT) tethered to the datalogger (HOBO U-12 Temp/RH/2 External Logger; Onset Computer Corp., Bourne, MA) and placed on the center bench in the middle compartment to measure natural PPFD data at the bench level in 5-min intervals throughout the trial. The

daily variations in air temperature, RH, natural PPFD, and DLI inside the experimental greenhouse are presented in Fig. 2.

Seeds of mustard (*Brassica juncea*; Johnny's Selected Seeds, Winslow, ME) and arugula (*Eruca sativa*; Suba Seeds Company S.P.A., Longiano, FC, Italy) microgreens were sown in fiber trays (48.5 cm × 23 cm × 3.5 cm) with pre-incorporated organic substrates from Greenbelt Microgreens Ltd. (Lynden, ON, Canada). The seeding rate was 36.4 g·m⁻² for both arugula (1.56 mg/seed) and mustard (1.63 mg/seed). A thin layer of coconut coir was used to cover the substrates to maintain moisture. On the same day of seeding, two sown trays (one species each) were placed at the center of each greenhouse bench to start treatments. Plants were monitored daily and top-irrigated until drainage was observed.

Experimental design and treatment setup. For each species, a randomized complete block design (3 treatments × 3 replicates) was used in the experiment (Fig. 1A). The three replicates (i.e., blocks) were allocated to the three greenhouse compartments. Each compartment had three benches. In each block (i.e., compartment), three treatments were randomly assigned to three benches: 1) D, no supplemental light (SL); 2) B, supplemental 14 μmol·m⁻²·s⁻¹ B LED light (400–500 nm, peak at 445 nm); and 3) FR, supplemental 14 μmol·m⁻²·s⁻¹ far-red LED light (700–800 nm, peak at 735 nm). The light spectral distributions under different SL light treatments are presented in Fig. 3. PPS values of nighttime light treatments were calculated based on the light spectral distribution using the protocol developed by our laboratory (Mah et al., 2019) according to Sager et al. (1988). For each bench, SL was provided by one programmable LED light fixture (LX601C; Heliospectra AB, Gothenburg, Sweden) mounted 1.35 m above the bench (measured from the bottom of the

Fig. 1. Schematic diagrams of the experimental design and spatial arrangement of the treatments (A) and the time windows of different light treatments during a day (B). B = supplemental blue LED light; D = no supplemental light; FR = supplemental far-red LED light.

Fig. 2. Daily variations in air temperature (A), relative humidity (B), natural photosynthetic photon flux density (C), and daily light integral (D) in the experimental greenhouse during the lighting treatment period from 1000 HR on 8 Jan. to 1000 HR on 21 Jan. 2019.

Fig. 3. Spectral distribution and phytochrome photostationary state (PPS) of overnight supplemental blue (B) and far-red (FR) light of $14 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

LED array). The lights were on 30 min after sunset (≈ 1730 HR) until 30 min before sunrise (≈ 0630 HR) throughout the experimental period (Fig. 1B). Each bench was isolated with automatic blackout when SL was on to avoid neighbor lighting effects. For each bench, light measurements were taken at the canopy level on a 4×4 square grid (i.e., at 16 different locations) centered below the light within an experimental area of $50 \text{ cm} \times 50 \text{ cm}$ using a radiometrically calibrated spectrometer (XR-Flame; Ocean Optics, Dunedin, FL) coupled to a $400\text{-nm} \times 1.9\text{-m}$ patch cord with a CC3 cosine corrector. The measured photon flux density (PFD) values of supplemental B and FR were $13.7 \pm 0.1 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and $14.1 \pm 0.4 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (mean $\pm$ SE; $n = 3$), respectively.

Growth and appearance quality measurements. Both mustard and arugula microgreens were harvested 14 d after sowing immediately after the first true leaf started to emerge. In each tray, three subsamples (with a substrate surface area of 76.4 cm^2 each, including plants and the substrate) of microgreens were randomly sampled from each tray using a cylindrical core sampler, and all plants within each core were cut from the substrate level and weighed to determine the FW. Then, the harvested plants were placed in paper bags and dried at 65°C until a constant weight was reached for DW determination. Another 15 plants were randomly sampled for measurements of plant heights with a ruler. The plant height was

measured from the base of the stem to the top of the cotyledon in the life position. From the remainder of the plants left in each tray, three subsets of five plants were sampled randomly for measurements of stem length and diameter, cotyledon area and color, and leaf mass unit area (LMA). The cotyledons were cut from the stems, and the cotyledons and stems were imaged together with a standard reference using a scanner (Canoscan LiDE 25; Canon Inc., Tokyo, Japan) at 600 dpi. The averaged cotyledon area, stem length, and diameter of each subset (five plants) were measured by ImageJ (version 1.42; National Institutes of Health, Bethesda, MD). The stem diameter was measured at 1 cm above the bottom of the stem. The R, G, and B values of the cotyledon color were also obtained by ImageJ; then, the hue angle of cotyledon was calculated based on method of Karcher and Richardson (2003) after calibration between the scanned and actual color using Munsell color chips (Munsell color system, 2018). After scanning, the cotyledons and stems of five plants were weighed separately and also placed in paper bags and dried at 65°C until they reached a constant weight for DW measurements. The LMA was calculated according to Eq. [1].

$$\text{LMA} (\text{g} \cdot \text{m}^{-2}) = \text{Cotyledon DW} / \text{Cotyledon area} \quad [1]$$

Phytochemical measurements. Three subsamples ($\approx 5 \text{ g}$ FW each) of fresh plant tissue,

including cotyledons, stems, and first true leaves, were randomly taken from each tray, quickly frozen in liquid N_2 , pulverized into fine powder with an ice-cold mortar and pestle, and collected in a 50-mL conical tube. These flash-frozen microgreen tissues were stored at -80°C before the measurements. Unless otherwise mentioned, all chemicals were purchased from Sigma-Aldrich Inc. (Oakville, ON, Canada).

To measure the total chlorophyll and carotenoid contents, $\approx 20 \text{ mg}$ of each frozen sample was used. The samples were re-suspended with 1 mL ice-cold 100% methanol in 1.7 mL pre-chilled Eppendorf tubes. The samples were stored on ice under darkness after they were vortexed for 1 min twice; then, they were centrifuged at $13,000 g_n$ for 5 min at 4°C . The supernatants were collected in new 1.7-mL pre-cooled Eppendorf tubes and serial dilutions (up to three times) in a final volume of 200 μL and prepared in 100% ice cold methanol. The methanolic extracts were transferred to a 96-well microplate reader (BioTek, Winooski, VT) to measure absorbance. The absorbance readings were obtained at wavelengths of 665 nm, 652 nm, and 476 nm for chlorophyll *a*, chlorophyll *b*, and carotenoid, respectively. The total chlorophyll and total carotenoid concentrations were calculated using the equations of Lichtenthaler and Buschmann (2001). Each subsample was measured six times (i.e., supernatant twice and its 1:2 and 1:3 dilutions twice).

The total phenolic content was measured according to the methods of Ainsworth and Gillespie (2007) with some modifications. Approximately 20 mg of frozen microgreen tissues were transferred in a 1.7-mL Eppendorf tube and resuspended with 1 mL of ice-cold 100% methanol, and vortexed twice for 1 min. Then, the samples were centrifuged at $13,000 g_n$ for 5 min at 4°C . Then, 25 μL of each sample supernatant, 1:2 dilution of the supernatants, standards, and blanks were dispensed in different wells in a 96-well microplate reader. Thereafter, 125 μL 10% Folin-Ciocalteu reagent was added to each well, and the plate was incubated at room temperature for 10 min. Thereafter, 125 μL of 7.5% (w/v) Na_2CO_3 was added, and the A was measured at 765 nm. The total phenolic concentrations were calculated against the gallic acid standard curve ranging from 0.018 to $0.6 \text{ mg} \cdot \text{L}^{-1}$.

Statistical analyses. Data were analyzed by SPSS statistical software (version 25.0; IBM, New York, NY). Treatment effects were determined by a one-way analysis of variance, and mean separations were performed using Tukey's honestly significant difference test at $P \leq 0.05$.

Results

Appearance quality. B compared to D increased plant height by 16% and 10% in mustard and arugula, respectively (Fig. 4A). FR compared to D increased plant height by 7% in mustard, but there was no effect on arugula. For both mustard and arugula, plants

grown under B were 8% taller than those under FR. Stem length was increased by 12% and 20% under B compared to D for mustard and arugula, respectively. However, stem length was not different between FR and D for both species (Fig. 4B). Also, B compared to FR increased stem length for arugula by 14%, but not for mustard.

Compared with D, B increased the cotyledon area of mustard by 22%, but it did not affect the cotyledon area of arugula (Table 1). In contrast, FR reduced the cotyledon area of arugula by 24% compared with D. For mustard and arugula, B compared to FR increased the cotyledon area by 44% and 32%, respectively. There was no difference in LMA among the three treatments (B, FR, and D for mustard (Table 1). However, for arugula, B increased LMA by 21% and 18% compared with D and FR, respectively, and there was no difference between D and FR. Regarding the stem diameter, stems were thicker under B and D than under FR for both species, although no difference was observed in stem diameter under B and D for both species. The cotyledon hue angle was smaller for mustard and larger for arugula under B compared with D and FR; however, there was no difference in the cotyledon hue angle with D and FR (Table 1). Changes in the hue angle under B relative to D and FR indicated that the cotyledon color increased redness and greenness in mustard and arugula, respectively (Fig. 5).

Plant biomass. Compared with D, B increased the FW ($\text{kg}\cdot\text{m}^{-2}$) by 36% and 28% for mustard and arugula, respectively, whereas FR did not affect the FW of either species (Fig. 6A). Under B, the FW was increased by 55% and 26% compared to FR for mustard and arugula, respectively. There was no difference in DW among D, B, and FR for mustard (Fig. 6B). However, the DW of arugula was increased by 36% under B compared with D and by 56% under B compared with FR, and it was not changed by FR or D.

Phytochemical contents. For both species, B compared to D did not affect the total chlorophyll content (Fig. 7A). However, FR compared to D reduced the total chlorophyll content by 30% and 31% for mustard and arugula, respectively. Also, B increased this trait by 52% compared to FR for arugula but showed no difference for mustard.

The total carotenoid content was unaffected by B compared to D for both species, but it was reduced by 23% under FR compared to D for arugula (Fig. 7B). B compared to FR increased the total carotenoid content by 42% and 35% for mustard and arugula, respectively.

For both species, B compared to D did not change the total phenolic content, but FR compared to D reduced this trait by 23% and 33% for mustard and arugula, respectively (Fig. 7C). Under B compared to FR, the total phenolic content was 39% higher for arugula, but it was not different for mustard.

Discussion

Overnight supplemental B improves appearance quality compared with D,

Fig. 4. Plant height (A) and stem length (B) of mustard and arugula microgreens grown in the greenhouse with no supplemental light (D) and $\approx 14 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ supplemental blue (B) or far-red (FR) light overnight. Data are means $\pm$ SE ($n = 3$). For each species, bars bearing the same letter are not significantly different according to Tukey's honestly significant difference test at $P \leq 0.05$.

Table 1. Cotyledon area, leaf mass per unit area (LMA), stem diameter, and hue angle of mustard and arugula microgreens grown in the greenhouse with no supplemental light [dark (D)] and $\approx 14 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ supplemental blue (B) or far-red (FR) light overnight.

Treatment	Cotyledon area (cm^2/plant)	LMA ($\text{g}\cdot\text{m}^{-2}$)	Stem diam (mm)	Cotyledon hue angle ($^\circ$)
<i>Mustard</i>				
D	1.1 ± 0.1 b ^z	76.1 ± 5.5 a	0.8 ± 0.0 a	44.2 ± 4.8 a
B	1.4 ± 0.1 a	78.3 ± 5.9 a	0.8 ± 0.0 a	28.5 ± 5.2 b
FR	0.9 ± 0.1 b	84.3 ± 14.0 a	0.7 ± 0.0 b	36.9 ± 3.5 a
<i>Arugula</i>				
D	1.6 ± 0.1 a	85.7 ± 3.0 b	0.8 ± 0.0 a	78.3 ± 0.5 b
B	1.6 ± 0.1 a	104.0 ± 10.2 a	0.8 ± 0.0 a	80.0 ± 0.1 a
FR	1.2 ± 0.1 b	81.6 ± 7.1 b	0.7 ± 0.0 b	78.8 ± 0.5 b

^zData are means $\pm$ SE ($n = 3$). For each species, means in the same column followed by the same letter are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$.

Fig. 5. Cotyledons of mustard (left) and arugula (right) microgreens grown in the greenhouse with no supplemental light (D), $\approx 14 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ supplemental blue (B) or far-red (FR) light overnight.

Fig. 6. Fresh (A) and dry (B) weights of mustard and arugula microgreens grown in the greenhouse with no supplemental light (D) and $\approx 14 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ supplemental blue (B) or far-red (FR) light overnight. Data are means $\pm$ SE ($n = 3$). For each species, bars bearing the same letter are not significantly different according to Tukey's honestly significant difference test at $P \leq 0.05$.

showing better effects than supplemental FR. For indoor microgreen production using LED lighting as the sole light source, 50 to $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ B can promote stem elon-

gation to meet the demands of machine harvesting (Kong et al., 2019a). For controlled-environment chrysanthemum production, nighttime supplemental B LED of 20

Fig. 7. Total chlorophyll, carotenoid, and phenolic contents of mustard and arugula microgreens grown in the greenhouse with no supplemental light (D) and $\approx 14 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ supplemental blue (B) or far-red (FR) light overnight. Data are means $\pm$ SE ($n = 3$). For each species, bars bearing the same letter are not significantly different according to Tukey's honestly significant difference test at $P \leq 0.05$.

or $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ promoted stem length (Jeong et al., 2014; Nissim-Levi et al., 2019). Similarly, in the present study, for winter greenhouse microgreen production, overnight supplemental $14 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ B LED, compared with D, increased plant height to taller than that required for machine harvesting (i.e., 5 cm) in both species, which was associated with the promoted stem elongation. Obviously, B LED can be used as either sole-source or supplemental light to promote microgreen elongation, thus facilitating machine harvesting. Although the detailed pathway involved in B-induced stem elongation is still unclear, recent studies indicate that deactivated cryptochrome and activated phototropin (i.e., two blue light photoreceptors) in addition to low-activity phytochrome may also contribute to this process (Huché-Thélier et al., 2016; Kong et al., 2018b; Kong and Zheng, 2020; Pashkovskiy et al., 2016). Interestingly, in the present study, overnight supplemental FR, compared to D, did not promote stem elongation for both species and increased plant height only for mustard, possibly due to the promoted petiole elongation. It appears that overnight supplemental B compared to FR has a greater promotion effect on microgreen elongation, which is also supported by the increased plant height for both species.

The results from the present study are somehow in contrast to another one of our microgreen studies performed in a growth chamber using an LED combination of R and B (20% R and 80% B) as the daytime light source (Ying et al., 2020); in that study,

nighttime FR compared to B has a greater promotion effect on elongation. The difference between the two studies might have resulted from the different phytochrome activities before starting supplemental B or FR. In the growth chamber, a combination of R and B LEDs used for daytime lighting had a higher PPS value (i.e., ≈ 0.88) and, consequently, was supposed to induce high phytochrome activity that could be maintained for several hours even when the light was off (Gaba and Black, 1979). In the greenhouse, the natural light at the end of day normally causes low phytochrome equilibrium due to a low R:FR ratio (Smith, 1982) (e.g., R:FR = 0.6, and PPS = 0.48 in our experimental greenhouse, unpublished data). It has been proven that the effect of B on plant elongation varies with changes in phytochrome equilibrium induced by mixing with other wavelengths (e.g., R/FR) at low levels. High PPS (e.g., >0.6) leads to an inhibition effect of B on plant elongation, whereas low PPS (e.g., <0.6) leads to a promotion effect of B on plant elongation (Kong et al., 2019c, 2018b).

Supplemental B, compared with D, also increased the cotyledon area and redness for mustard and the cotyledon thickness (i.e., LMA) and greenness for arugula. The changes in these traits would be more appealing to the customers and could potentially increase the microgreens appearance quality based on our communication with commercial growers. In contrast, supplemental FR reduced the cotyledon area and color and stem diameter for both species compared with D, and it reduced the LMA for arugula

and the cotyledon area and stem diameter for both species compared with B. Obviously, supplemental FR also induced some typical responses to shade in other traits (Smith and Whitelam, 1997) despite showing little promotion effects on plant elongation. Pure FR indicates a deep shade signal, which may cause plants to switch to other responses (e.g., decreased leaf greenness through the reduced chlorophyll content) to adapt to the deep shade other than elongation to capture light, in which phytochrome A is known to have a role (Barnes et al., 1996; Gommers et al., 2013; Yang et al., 2018). Considering the negative effects of FR on these plant traits, supplemental B seems to have better effects on the microgreens appearance quality.

Overnight supplemental B, rather than FR, promotes plant biomass accumulation in microgreens. Compared with D, supplemental B ($\approx 32\%$ of natural DLI; average, $2.2 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) increased the FW per unit area (i.e., crop yield) for both species, but supplemental FR did not. Also, supplemental B compared to FR increased the crop yield for both species. It appears that overnight supplemental B, rather than FR, can promote fresh biomass accumulation in microgreens during winter greenhouse production. Similar promotion effects have been found by other growth chamber studies. Under daytime fluorescent light of $3.6 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, overnight supplemental B of $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for 14 h increases the shoot FW by 26% to 54% in chrysanthemum, mustard, and onion (*Allium cepa*) compared with no SL, whereas supplemental FR does not affect or reduces the FW (Sase et al., 2012). In the growth chamber under R and B LED light of $17.3 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, supplemental B of $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased the FW of arugula but not of mustard microgreens (Ying et al., 2020). These studies show the potential of using supplemental B to increase FW, although the efficiency of the promotion is affected by daytime light conditions, supplemental light intensity, and plant species. The higher yield of microgreens under supplemental B, in the present study, was also supported by the modified appearance quality traits such as longer and thicker stems or larger and thicker cotyledons. The increased yield under overnight supplemental B will potentially benefit microgreen production because these crops are sold on a FW basis.

In addition to FW, compared with D, supplemental B increased DW per unit growing area for arugula, but supplemental FR did not for either species. Also, supplemental B compared to FR increased DW for arugula. It appears that overnight supplemental B, rather than FR, can promote dry biomass accumulation in some microgreen species (i.e., arugula). The increased DW under supplemental B, rather than FR in the present study might have resulted from the SL-increased photo-assimilate accumulation because B falls in the range of *PAR*, but FR does not. However, the contribution of photosynthetic assimilates to microgreen biomass may vary among species with different seed sizes. During the short growth period of microgreens, plants

mainly experience a transition from heterotrophic to autotrophic growth, and photosynthesis contributes less to plant biomass than seed reservation for larger compared to smaller species (Jones-Baumgardt et al., 2019, 2020). In the present study, mustard had a larger seed size than arugula (1.63 vs. 1.56 mg/seed), which may partly explain why supplemental B, compared with D or supplemental FR, did not increase DW in mustard but did in arugula.

Overnight supplemental B does not reduce phytochemical contents compared with D, but supplemental FR does. It has been documented that higher B levels can increase phytochemical contents in plants. For example, when up to 50% B is added to R, the chlorophyll content increases in cucumber seedlings (Hogewoning et al., 2010). The total carotenoid content increases in beet (*Beta vulgaris* L.) microgreens when 0% up to 47% B is added to R (Samuolienė et al., 2017). The total phenolic concentration in lettuce increases when adding 0% up to 47% B to R (Son and Oh, 2013). However, in the present study, supplemental B did not affect the total chlorophyll, carotenoid, or phenolic contents (mg·g⁻¹ FW) in either species compared with D. Possibly, in the previous studies, B acted together with the other wavelength (e.g., R); however, in the present study, B was applied alone during the nighttime and at very low levels. It has been proven that B mediates phytochemical (e.g., anthocyanin) synthesis mainly through cryptochrome, which requires active phytochrome for full expression, and this requirement can be supplied by low levels of R (Ahmad and Cashmore, 1997). Another explanation for the inconsistency between the previous studies and ours is that the responses to B might vary among species or even cultivars. A previous greenhouse study of pak choi (*Brassica oleracea*) indicated that daytime supplemental 50 μmol·m⁻²·s⁻¹ of B, compared with no SL, does not increase the total chlorophyll, carotenoid, or phenolic contents in green-leafed cultivars, but it does increase those phytochemical contents in red-leafed cultivars (Zheng et al., 2018).

In the present study, supplemental FR reduced the contents of phytochemicals (i.e., total chlorophyll, carotenoid, and phenolic contents) in microgreens compared with D or supplemental B in most cases. Similarly, lower chlorophyll content (either unit leaf area or unit FW) was found in petunia (*Petunia axillaris*) plants treated with FR compared with R at the end of the photoperiod (Casal et al., 1987). Supplementing FR to cool white fluorescent light causes plants to express diverse shade-avoidance syndrome, such as decreasing the contents of chlorophyll and carotenoid, which corresponds with decreasing phytochrome equilibrium (Kalaitzoglou et al., 2019). The biosynthesis of chlorophyll is negatively regulated by phytochrome interacting factor 1 (PIF 1), and its activity is greatly affected by phytochrome equilibrium (Huq et al., 2004). Also, FR radiation, as a light competition

signal, can elicit down-regulation of plant chemical defenses (e.g., reduced biosynthesis of phenolic) (Moreno et al., 2009). This may partly explain the decrease in the total phenolic content under supplemental FR in the present study.

In summary, during winter greenhouse production (with a natural DLI of ≈2.2 mol·m⁻²·d⁻¹ at the canopy level), overnight supplemental 14 μmol·m⁻²·s⁻¹ B, compared with D or FR, improved the microgreen appearance quality demonstrated by the increased plant height (essential for machine harvesting), stem length, cotyledon area, LMA, or cotyledon coloring, which varied with the species. Also, B compared to D, increased the microgreen yield, but FR compared to D did not. Furthermore, FR compared to D or B reduced the contents of phytochemicals (i.e., total chlorophyll, carotenoid, and phenolic contents). Therefore, overnight supplemental low-intensity B, rather than FR, is beneficial to winter greenhouse production of microgreens.

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