Applying Blue Light Alone, or in Combination with Far-red Light, during Nighttime Increases Elongation without Compromising Yield and Quality of Indoor-grown Microgreens

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Abstract. To facilitate machine harvest for labor savings, the height of microgreens needs to reach ≈5 cm. Recent studies indicate that monochromatic blue light (B) can promote stem elongation similar to far-red light (FR). To examine whether nighttime B treatments can promote plant elongation without compromising yield and quality, mustard (*Brassica juncea*) and arugula (*Eruca sativa*) microgreens were grown under different light-emitting diode (LED) lighting regimes in a growth chamber. The 16-hour daytime lighting comprised 20% B and 80% red light (R), and had a total photosynthetic photon flux density (*PPFD*) of 300 μmol·m^{-2}·s^{-1} at canopy level. During the 8-hour nighttime, the plants were exposed to the following treatments: 1) dark (D) as one control; 2) 4 hours of B at 40 μmol·m^{-2}·s^{-1} followed by 4 hours of darkness (40B-D); 3) 4 hours of darkness followed by 4 hours of B at 40 μmol·m^{-2}·s^{-1} (D-40B); 4) 8 hours of B at 20 μmol·m^{-2}·s^{-1} (20B); 5) 8 hours of B + FR, and each of them at 20 μmol·m^{-2}·s^{-1} (20B20FR); and 6) 8 hours of FR at 20 μmol·m^{-2}·s^{-1} (20FR) as another control. The plants were harvested after 11 days of treatment. Nighttime B treatments (40B-D, D-40B, and 20B), compared with D, increased plant height by 34% and 18% for mustard and arugula, respectively, with no difference among the three B treatments. The combination of B and FR (20B20FR), compared with B alone, further increased plant height by 6% and 15% for mustard and arugula, respectively, and showed a similar promotion effect as 20FR. Plant height did not meet the machine harvest requirement for both species with the D treatment, but did so for mustard with the nighttime B treatments and for arugula with the 20B20FR treatment. There was no difference in biomass among all treatments except that 20B, compared with D, increased the fresh weight (FW) of arugula by 12%, showing a similar promotion effect as 20FR. Despite a greater promotion effect on elongation than B alone, 20FR reduced the leaf index compared with D. However, B alone or the 20B20FR treatment increased leaf thickness compared with D, and increased chlorophyll content index (CCI), leaf index, dry matter content, and leaf thickness to varying degree with species, compared with 20FR. Overall, nighttime B alone, or its combination with FR, promoted microgreen elongation without compromising yield and quality.

Microgreens are edible seedlings harvested when their cotyledons are fully expanded, with or without the appearance of true leaves, depending on species (Treadwell et al., 2016). Of the 80 to 100 plant species currently cultivated as microgreens, the most common are from the *Brassicaceae* family, including mustard and arugula (Björkman et al., 2011). As specialty vegetables, microgreens have various colors and flavors, a tender texture, and a high nutritional content (Kyriacou et al., 2016; Xiao et al., 2012). The growth periods of microgreens are relatively short, as they are normally harvested within 7 to 20 d after sowing (Treadwell et al., 2016).

Microgreens have been increasingly produced in controlled-environment facilities with electric lighting as the sole light source, including LEDs. LED lighting has made it possible to choose specific light intensities and spectral compositions (Morrow, 2008), which can be used to regulate growth and development according to plant species (Bergstrand, 2017). LED light with a combination of R (600–700 nm) and B (400–500 nm) (RB-LED lighting) has been popularly used for horticultural production in recent years (Davis and Burns, 2016). For indoor production of microgreens, RB-LED lighting with 15% to 20% B and 300 to 400 μmol·m^{-2}·s^{-1} *PPFD* for a 16-h photoperiod appears to be an optimal lighting regime in terms of crop yield and appearance quality (Jones-Baumgardt et al., 2019; Ying et al., 2020a). However, in the optimized lighting environment, microgreens are too short to be harvested by machine. Commercial production of microgreens is, increasingly, switching from hand- to machine-harvest to reduce labor costs. Microgreens with hypocotyls less than 5 cm are not feasible to be harvested by machine (Canadian microgreen growers, personal communications). Also, most microgreen species are harvested with a minimum height of around 5 cm, according to Kyriacou et al. (2016).

Although plant elongation can be promoted by creating a shade environment during daytime, there is normally a trade-off between stem elongation and plant yield under shade (Tang and Liesche, 2017). Also, our previous studies on microgreens indicated that adjusting the spectral quality of daytime lighting, such as adding small amount of FR or green light (G) to RB-LED lighting did not change plant elongation, and even had a negative effect on the appearance quality of four microgreen species (Ying et al., 2020b). Substituting G or FR for B in RB-LED lighting, or adding FR to RB-LED lighting reduced leaf greenness or redness in lettuce and kale, and reduced relative specific chlorophyll content in lettuce and basil seedlings despite the promoted elongation responses of leaves or hypocotyls (Meng and Runkle, 2019; Meng et al., 2019). Although lowering B percentage in daytime RB-LED lighting sometimes can also promote plant height, the effects vary with species, and also can compromise other growth metrics, such as reducing leaf thickness, relative dry weight (DW), shoot FW, leaf red color, (Meng and Runkle, 2019; Nanya et al., 2012; Wollaeger and Runkle, 2015; Ying et al., 2020a), and health-promoting phytochemical contents (our unpublished data).

To promote stem elongation without compromising other growth metrics, it may be feasible to extend the daily lighting period during nighttime (e.g., longer photoperiod). A longer photoperiod is normally associated with an increased daily light integral (DLI), which has been found to induce taller plants and greater biomass in some plant species, such as vinca (*Vinca minor*) and zinnia (*Zinnia elegans*) (Faust, 2002), and pea shoots (*Pisum sativum* L.) (Kong et al., 2018a). For indoor microgreen production, a 24-h photoperiod (i.e., 0 h of darkness) did not cause an obvious negative effect on plant quality, and it induced a greater promotion effect on stem elongation compared with a 16-h photoperiod (Kong et al., 2019a). Nighttime lighting could be particularly useful in some regions, such as Ontario, Canada, where the price of

electricity is much lower during the nighttime compared with the daytime. Also, the crop cycles can be potentially shortened by applying nighttime lighting, which may speed up the cash flow to some degree. However, the optimal spectral quality and lighting regimens at night for indoor-grown microgreens are unknown.

Promotion of stem elongation has been widely documented in environments containing high-level FR (Demotes-Mainard et al., 2016). The elongation response to FR is a typical shade-avoiding response mediated by phytochromes, which are photoreceptors activated mainly by R but deactivated by FR (Sager et al., 1988). End-of-day FR treatments were reported to enhance stem elongation in tomato (*Solanum lycopersicum*) (Kubota et al., 2012), chrysanthemum (*Chrysanthemum morifolium*) (Lund et al., 2007), and poinsettia (*Euphorbia pulcherrima*) (Islam et al., 2014). Nighttime FR was also extremely useful in promoting shoot elongation during the early stage for Japanese pear seedlings (*Pyrus pyrifolia*) (Ito et al., 2014). However, plants grown in high-level FR environments might undergo some undesirable responses, such as decreased chlorophyll content and leaf thickness (Demotes-Mainard et al., 2016). Furthermore, FR LEDs have not been popularly available for horticultural production until recent years, as a result of their higher price in the past (Kubota et al., 2012), and lower photosynthetic photon efficiency relative to R and B (Nelson and Bugbee, 2014).

Apart from FR, monochromatic B LED lighting at modest intensities (range, 40–250 μmol·m^{-2}·s^{-1}) has been also reported to promote stem elongation in some plant species such as cherry tomato (*Solanum lycopersicum* L. 'Cuty') (Kim et al., 2014), cucumber (*Cucumis sativus*) (Hernández and Kubota, 2016), sesame (*Sesamum indicum*) (Hata et al., 2013), and sunflower (*Helianthus annuus*) (Schwend et al., 2015). Recently, based on a series of 24-h LED lighting experiments on bedding plants, microgreens, and *Arabidopsis* mutants, our laboratory has concluded that the promotion effect of monochromatic B is related to low phytochrome activity, which may modify the activity of B receptors (e.g., reduced cryptochrome activity and increased phototropin activity) (Kong and Zheng, 2020; Kong et al., 2018b, 2019b). Also, for arugula (*Eruca sativa*) and mustard (*Brassica juncea*) microgreens exposed to 24-h lighting, the promoted elongation by monochromatic B increased, with *PPFD* decreasing from 650 to 20 μmol·m^{-2}·s^{-1} (Johnson et al., 2020). However, the stem elongation promoted by 24-h lighting of modest-intensity B is a shade avoidance response in plants, as reduced cotyledon size and side branch number, and increased biomass partitioning to stems have been observed at the same time (Kong et al., 2018a, 2019b). It is unknown whether whole-night lighting (i.e., total 24-h photoperiod) with a low intensity (e.g., 20–40 μmol·m^{-2}·s^{-1}) of

monochromatic B can promote elongation without compromising yield and quality for indoor microgreen production with 16-h daytime RB-LED lighting.

Although both B and FR can promote stem elongation, there is not enough information comparing the promotion effect among B, FR, and B + FR. For microgreens (i.e., arugula, cabbage, and kale) exposed to 24-h lighting, B plus low-level FR slightly promoted plant elongation compared with B, but the promotion effect was far less than that of B relative to R (Kong et al., 2019c). It appears that B has a similar or greater promotion effect on microgreen elongation compared with FR, because more than one photoreceptor system (i.e., phytochromes) is involved in the promotion effect of B rather than FR (Kong and Zheng, 2020). However, in the aforementioned microgreen study, the intensity of FR was only 10% of the B intensity. A recent study indicates that, although FR alone affects plant photosynthesis minimally, it is equally efficient at driving canopy photosynthesis when it is coacting with photosynthetically active radiation (Zhen and Bugbee, 2020). Possibly, the combination of B and FR at the same levels could increase plant biomass. Therefore, the promotion effects on plant elongation as well as biomass need to be compared among B, FR, and B + FR as whole-night lighting (i.e., total 24-h photoperiod) for indoor microgreen production under 16-h daytime RB-LED lighting.

For microgreens, when the photoperiod was reduced from 24 to 16 h, hypocotyl elongation was still promoted by monochromatic B, and met the requirement of machine harvest (i.e., ≥5 cm), although the promotion effect was reduced to some extent (Kong et al., 2019a). As for nighttime B treatments, it remains unclear—when the total DLI is the same—whether partial vs. whole nighttime lighting (e.g., 20- vs. 24-h photoperiod) would affect the promotion effect on elongation. Also, for partial nighttime lighting (e.g., 20-h photoperiod), when to apply B (i.e., early or late nighttime) so that it would have a greater promotion effect is unknown. It has been found that B reduces the promotion effect and gradually switches to an inhibitory effect on plant elongation relative to R when the phytochrome photostationary state (PPS), an indicator of phytochrome activity, increases from 0.50 to 0.69 (Kong et al., 2018b, 2019c). Apparently, the activity of cryptochrome, B photoreceptor, is increased by activated phytochrome, possibly resulting from a crosstalk between the two photoreceptor systems (Liu et al., 2016). A previous study of cucumber (*Cucumis sativus* L.) seedlings indicated that phytochrome still remained activated after "lights off" and might last for several hours in the dark, although the phytochrome activity gradually decreased over time (Gaba and Black, 1979). Consequently, the effect of nighttime B on plant elongation may differ between early and later hours during the nighttime as a result of the difference in

phytochrome activity. This speculation needs to be confirmed for indoor microgreen production with 16-h daytime RB-LED lighting.

Although many previous studies have reported the promotion effect of B as the sole light source on plant elongation, little information is available on the application of B as a supplemental lighting source to indoor microgreen production. Therefore, the objective of this study was 1) to evaluate the effect of nighttime B, or its combination with FR treatments, relative to dark and FR, on hypocotyl elongation as well as crop yield and appearance quality; and 2) with equal DLI, to determine the optimum nighttime lighting strategy (e.g., initiation time and duration) using B for indoor microgreen production with 16-h RB-LED lighting during the daytime.

Materials and Methods

Plant materials and growing conditions. This experiment was performed in a walk-in growth chamber (7.3 × 4.0 × 2.5 m), which was divided into six compartments by opaque curtains, from Oct. to Nov. 2018. Seeds of arugula (*Eruca sativa*; 'unknown name', Suba Seeds Company S.P.A., Longiano, FC, Italy) and mustard (*Brassica juncea*; 'mizuna', Johnny's Selected Seeds, Winslow, ME) were sown in fiber trays (48.5 × 23 × 3.5 cm) were filled with substrate consisting of 30% compost, 30% peat, 30% coir, and 10% perlite (v/v). The seeding rate was 36 g·m^{-2} for both arugula and mustard, and each tray had one species. After seeding, the sown trays were placed on a bench in each compartment for lighting treatments. The substrate was prepared by Greenbelt Microgreens Ltd. (Lynden, Ontario, Canada), and had a pH of 6.9 and an electrical conductivity (EC) of 1.9 dS·m^{-1}. The nutrient content (in milligrams per kilogram) in the substrate was measured by a commercial analytical laboratory (A&L Canada Laboratories Inc., London, Ontario, Canada) using test methods for composting and compost, and the result was as follows: N, 9400; P, 329; K, 1410; S, 220; Mg, 295; Ca, 1390; Zn, 9.4; Mn, 10; Fe, 47; Cu, 0.6; Na, 218; and B, 0.7 mg·kg^{-1}. All trays were top irrigated at least daily using well water (pH = 7.5, EC = 0.8 dS·m^{-1}) until drainage was visible. The temperature was set at 20 °C during the daytime (10:00 AM–2:00 AM) and 17 °C during the nighttime, and the relative humidity (RH) was set at 70%. Temperature and RH were controlled by an environmental control system (Titan, Argus Controls Systems Ltd., Surrey, British Columbia, Canada).

Experimental design and treatments. The experiment was a randomized complete block design with six treatments and three consecutive replicates. The six treatments differed only during the 8-h nighttime, but had the same 16-h daytime lighting, which was provided by RB-LED with a photon flux ratio of 20% B and 80% R at a *PPFD* of 300 μmol·m^{-2}·s^{-1} from 10:00 AM to 2:00 AM.

The six nighttime treatments were: 1) D, dark for 8 h as one control; 2) 40B-D, 40 μmol·m^{-2}·s^{-1} B for the first 4 h and dark for last 4 h; 3) D-40B, dark for the first 4 h and 40 μmol·m^{-2}·s^{-1} B for last 4 h; 4) 20B, 20 μmol·m^{-2}·s^{-1} B for 8 h; 5) 20B20FR: 20 μmol·m^{-2}·s^{-1} B and 20 μmol·m^{-2}·s^{-1} FR together for 8 h; and 6) 20FR: 20 μmol·m^{-2}·s^{-1} FR for 8 h as another control. Both daytime and nighttime light treatments were provided solely by LEDs (LX602C; Heliospectra AB, Gothenburg, Sweden). For each replicate, the six treatments were allocated randomly to the six compartments in the growth chamber, and there were two trays of microgreens (one species per tray) for each treatment. The trial was replicated three times over time, and the treatment locations were randomized within the growth chamber for each replication.

In each compartment, two LED lamps were hung 56.5 cm above the top of the substrate (measured from the bottom of the lamps) and separated by 36 cm (on center). The individual B and R channels were set to achieve an average *PPFD* of 300 μmol·m^{-2}·s^{-1} during the 16-h daytime. In addition, the B and FR channels were set up to provide the various nighttime spectrum treatments, all of which had an average photon flux density of 20 or 40 μmol·m^{-2}·s^{-1}. The actual lighting conditions are summarized in Table 1. The PPS values for the daytime lighting and the different nighttime lighting were calculated using the protocol (Mah et al., 2019) developed by our laboratory according to Sager et al. (1988). The DLI and daily photon integral were calculated using the total photons from 400 to 700 nm and 400 to 800 nm, respectively. Photon flux density and spectra of the LED arrays were controlled by using software (System Assistant Version 1.3.0; Heliospectra, AB). Photon flux density and spectra were measured at 16 spots (a 4 × 4 grid) within a square treatment area of 0.25 m^2, centered between the LED fixtures, using a radiometrically calibrated spectrometer (Flame-S; Ocean Optics, Inc., Dunedin, FL) with a 25-μm slit, coupled to a 1.89 m × 400 μm fiber optic patch chord with a CC-3 cosine corrector. Light intensity and spectra were also checked at the end of each replicate.

Data collection and analyses. Both arugula and mustard microgreens were harvested 11 d after seeding. Plants were harvested with two fully expanded cotyledons and visual observation of the first-emerged true leaves. For each replicate and each species, three subsamples (with a substrate surface area of 76.4 cm^2 each, including plants and the substrate) of microgreens were sampled randomly from each tray using a cylindrical core sampler. All the microgreens in each subsample were then cut at substrate level and measured for FW. These plants were then dried at 65 °C until a constant weight was reached to determine DW. Twenty-five plants from each genotype were selected randomly from the remainder of the microgreens in each tray to evaluate plant height. Another 15 plants (5 plants × 3 sets) were selected randomly from the plants left in each tray, and were separated into cotyledons and hypocotyls for quality evaluation. After measuring FW of cotyledons and stems separately, they were imaged using a flat-bed digital scanner (Canoscan LiDE 25; Canon Inc., Tokyo, Japan). The images were saved in JPG format with 600-dpi resolution, and they were later processed using ImageJ 1.42 software (National Institutes of Health, Bethesda, MD) to determine cotyledon area. After scanning, the cotyledons and hypocotyls were dried separately at 65 °C until a constant weight and DW were recorded. The leaf index, dry matter content, and specific leaf area (SLA) were determined for the three sets of five plants from each replicate and each treatment, and were calculated as

$$\text{Leaf index (g·g}^{-1}\text{)} =$$

$$\text{DW of cotyledons/DW of hypocotyls} \quad [1]$$

$$\text{Dry matter content(\%)} = \text{DW/FW} \times 100 \quad [2]$$

$$\text{SLA (cm}^2\text{·g}^{-1}\text{)}$$

$$= \text{Cotyledon area/DW of cotyledons} \quad [3]$$

Another 15 plants from each tray were selected randomly to measure the CCI of

the cotyledons using a chlorophyll meter (SPAD 502; Spectrum Technologies, Inc., Aurora, IL).

Data were subjected to analysis of variance using SPSS software (version 25.0; IBM, Anorak, NY) followed by multiple comparisons using Tukey's honestly significant difference test at $P \leq 0.05$. Data are presented as mean ± SE (n = 3).

Results

Compared with D, all nighttime B treatments increased plant height, with similar trends in both microgreen species (Fig. 1). The average plant heights for the nighttime B treatments (i.e., 40B-D, D-40B, and 20B) were 6.4 cm and 4.2 cm for mustard and arugula, respectively. The three nighttime B treatments showed a similar promotion effect on plant height in both species, and increased plant height by 34% for mustard (Fig. 1A) and 18% for arugula (Fig. 1B) compared with D. The combination of B and FR treatment (20B20FR), compared with B alone further increased plant height by 6% and 15% for mustard and arugula, respectively, and showed a similar promotion effect as 20FR. The plant height for the 20B20FR treatment was 6.8 cm and 5.1 cm for mustard and arugula, respectively.

The FW of mustard was unaffected by any of the nighttime treatments (Fig. 2A). The FW of arugula was 12% greater for both the 20B and 20FR treatments compared with D, with 20B showing a similar promotion effect as 20FR (Fig. 2B). There were no treatment effects on DW for mustard or arugula (Fig. 2C and D).

Cotyledon area of arugula was increased by 21% with the D-40B treatment compared with D, whereas other treatments had no effect on this trait in either species (Table 2). Leaf indices were greater in mustard for the D, D-40B, and 20B20FR treatments than for 20FR, and in arugula for 20B and 20B20FR than for D. The dry matter content was less with the 20FR treatments compared with all other treatments for mustard, but was not different among all the treatments for arugula. The CCI of the cotyledons was increased by 12%, 13%, and 10% for the D-40B, 20B, and

Table 1. Photon flux density (PFD) from blue (400–500 nm) and red (600–700 nm) light, photosynthetically active radiation (*PAR*), and phytochrome photostationary state (PPS) during daytime; average PFD from blue and far-red (700–800 nm) light, and PPS during nighttime; and the daily photon integral (DPI) and daily light integral (DLI) under six light spectral treatments.

Nighttime treatment	Daytime PFD (μmol·m^{-2}·s^{-1})[z] and PPS				Nighttime PFD (μmol·m^{-2}·s^{-1}) and PPS			DPI[y] (mol·m^{-2}·d^{-1})	DLI (mol·m^{-2}·d^{-1})
	Blue	Red	*PAR*[y]	PPS	Blue	Far-red	PPS		
D[x]	60 ± 0.5	239 ± 2.1	299 ± 2.6	0.88 ± 0.0	—	—	—	17.3	17.3
40B-D	60 ± 0.5	239 ± 1.3	299 ± 1.4	0.88 ± 0.0	40 ± 0.3	—	0.53 ± 0.0	17.9	17.9
D-40B	60 ± 0.3	239 ± 1.2	300 ± 1.3	0.88 ± 0.0	41 ± 0.2	—	0.53 ± 0.0	17.9	17.9
20B	60 ± 0.8	237 ± 0.4	298 ± 1.3	0.88 ± 0.0	20 ± 0.4	—	0.52 ± 0.0	17.9	17.9
20B20FR	61 ± 0.7	238 ± 1.1	300 ± 2.2	0.88 ± 0.0	20 ± 0.5	20.3 ± 0.5	0.23 ± 0.0	18.5	17.9
20FR	61 ± 0.9	239 ± 2.5	300 ± 1.9	0.88 ± 0.0	—	19.7 ± 0.4	0.18 ± 0.0	17.9	17.3

[z]Data are means ±SE (n = 3).
[y]PPS = phytochrome photostationary state, which is the estimated phytochrome photoequilibrium according to the method by Sager et al. (1988). *PAR* = photosynthetically active radiation ranging from 400 to 700 nm. DPI = daily photon integral (mol·m^{-2}·d^{-1}), ranging from 400 to 800 nm.
[x]D = dark for 8 h; 40B-D = 40 μmol·m^{-2}·s^{-1} blue light for the first 4 h and dark for last 4 h; D-40B = dark for the first 4 h and 40 μmol·m^{-2}·s^{-1} blue light for the last 4 h; 20B = 20 μmol·m^{-2}·s^{-1} blue light for 8 h; 20B20FR = 20 μmol·m^{-2}·s^{-1} blue light and 20 μmol·m^{-2}·s^{-1} far-red light together for 8 h; 20FR = 20 μmol·m^{-2}·s^{-1} far-red light for 8 h. The six nighttime treatments had the same 16-h daytime lighting, which was provided by a combination of 20% blue and 80% red light-emitting diodes at a photosynthetic photon flux density of 300 μmol·m^{-2}·s^{-1} from 10:00 AM to 2:00 AM.
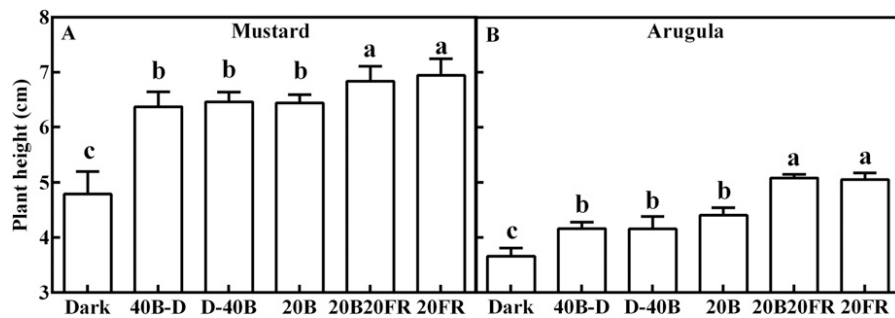
Fig. 1. Effect of nighttime spectrum treatments on height of mustard (**A**) and arugula (**B**) microgreens. Data are means ± SE (n = 3). Bars bearing the same letter are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$. D, dark for 8 h; 40B-D, 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for the first 4 h and dark for last 4 h; D-40B, dark for the first 4 h and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for the last 4 h; 20B, 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for 8 h; 20B20FR, 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light and 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red light together for 8 h; 20FR, 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red light for 8 h. The six nighttime treatments had the same 16-h daytime lighting, which was provided by a combination of 20% blue and 80% red light-emitting diodes at a photosynthetic photon flux density of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from 10:00 AM to 2:00 AM.
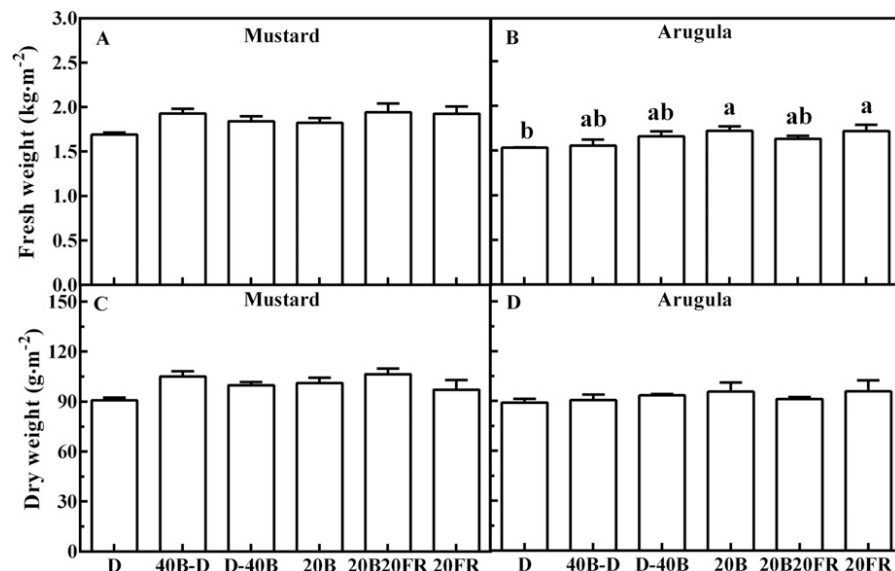

Fig. 2. Effect of nighttime spectrum treatments on fresh weight (**A, B**) and dry weight (**C, D**) of mustard and arugula microgreens. Data are means ± SE (n = 3). Bars bearing the same letter are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$. D, dark for 8 h; 40B-D, 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for the first 4 h and dark for last 4 h; D-40B, dark for the first 4 h and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for the last 4 h; 20B, 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light for 8 h; 20B20FR, 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ blue light and 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red light together for 8 h; 20FR, 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red light for 8 h. The six nighttime treatments had the same 16-h daytime lighting, which was provided by a combination of 20% blue and 80% red light-emitting diodes at a photosynthetic photon flux density of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from 10:00 AM to 2:00 AM.

20B20FR treatments, respectively, for mustard, and by 9% for the 20B20FR treatment for arugula compared with 20FR. The SLA of arugula decreased by 17% with the D-40B, 20B, and 20B20FR treatments compared with D, and by 15% with the 20B20FR treatment compared with 20FR, but this trait showed no difference in all treatments for mustard.

Discussion

Nighttime B increases elongation growth, with a promotion effect comparable to FR, for indoor microgreen production. Compared with D (i.e., no lighting at night), nighttime B treatments (i.e., 40B-D, D-40B, and 20B) promoted elongation by 34% and 18% for mustard and arugula, respectively, in our study. Apparently, the extended lighting time

contributed to elongation growth. Normally, stem elongation increased by extended lighting time could be caused by increased photoassimilation for growth, and/or the production of light-dependent elongation-promoting hormones (Bergstrand, 2017; Warrington and Norton, 1991; Yamaguchi, 2008). In our study, there was a lesser possibility of increased assimilation supply by nighttime lighting with B, which was also supported by a similar dry biomass for nighttime B treatments and darkness. During the relatively short growth period (from seeding to cotyledon fully unfolding), it seems that microgreens mainly experience a transition from heterotrophic to autotrophic growth, and photosynthesis contributes little to elongation growth (Jones-Baumgardt et al., 2019). In a previous study on petunias, high levels of bioactive gibberellins were

found under B, which was related to B-enhanced elongation growth (Fukuda et al., 2016). Therefore, gibberellin production could have been increased by the extended lighting with monochromatic B at night for the tested microgreen species in our study, although this needs further confirmation.

The promotion effect of nighttime B on elongation growth was comparable to FR in our study. Under nighttime B vs. FR treatments, the microgreen plants were only 8% and 17% shorter for mustard and arugula, respectively. Also, the plant height of mustard under nighttime B treatments reached the greater than 5-cm requirement for machine harvest, suggesting that nighttime B can be potentially used to promote plant elongation for microgreen production. Recent studies in our laboratory have found that the promoted elongation growth by B is related to low phytochrome activity, indicated by a lower PPS value (Kong et al., 2018b, 2019c). The PPS threshold value to induce activated phytochrome responses is controversial, but a PPS value of more than 0.6 could generally induce these responses (Stutte, 2009). In our study, the PPS values were less than 0.6, indicating a potentially deactivated phytochrome response for all nighttime B and FR treatments. However, B treatments had a relatively greater PPS value than FR (0.52 vs. 0.18), which may explain the slightly reduced promotion effect on elongation.

Nighttime B vs. FR is better for indoor microgreen production in terms of yield and quality. In our study, 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B applied throughout the nighttime for 8 h increased microgreen FW by 12% for arugula compared with D, showing a biomass promotion effect similar to FR. Similarly, FW of Chinese kale (*Brassica alboglabra* 'Bailey') was increased by 41% when 50 or 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B was supplemented for 12 h in a greenhouse (Li et al., 2019). The increased FW of arugula under 20B would potentially promote yield and benefit production, because microgreens are sold on an FW basis. Furthermore, B (as opposed to FR) has been more popular for a long time because of the lower price of the LED lighting fixture, and its greater photosynthetic photon efficiency (Kubota et al., 2012; Nelson and Bugbee, 2014). However, in recent years the price of FR LED has decreased rapidly, so the comparison of economic effects between the two types of LED lighting needs further study.

Extended lighting using B alone at night also increased microgreen quality compared with D or FR in many cases. For example, cotyledon area and thickness (i.e., reciprocal of SLA) were increased for arugula with B treatments compared with D, dry matter content and CCI were increased for mustard, and leaf index was enhanced for both species with B alone compared with FR. These quality improvements under nighttime B treatments would potentially benefit microgreen production, because larger and greener cotyledons are more appealing in appearance to most customers. Also, a greater dry matter content and thicker leaves can potentially increase microgreen shelf life, because

Table 2. Effect of nighttime spectrum treatments on cotyledon area, leaf index, dry matter content, chlorophyll content index (CCI), and specific leaf area (SLA) of arugula and mustard microgreens.

Nighttime treatment	Cotyledon area (cm²/plant)	Leaf index[z] (g·g⁻¹)	Dry matter content (%)	CCI	SLA (cm²·g⁻¹)
Mustard					
D[y]	2.3 ± 0.2[x]	2.6 ± 0.5 a	5.4 ± 0.0 ab	32.8 ± 1.3 ab	78.6 ± 0.2
40B-D	2.5 ± 0.1	2.3 ± 0.5 ab	5.5 ± 0.1 a	32.6 ± 0.6 ab	74.3 ± 7.5
D-40B	2.3 ± 0.1	2.3 ± 0.6 a	5.4 ± 0.1 a	34.5 ± 0.6 a	77.5 ± 1.7
20B	2.3 ± 0.2	2.2 ± 0.6 ab	5.6 ± 0.1 a	34.8 ± 0.6 a	74.4 ± 1.6
20B20FR	2.5 ± 0.2	2.4 ± 0.6 a	5.5 ± 0.1 a	33.9 ± 0.6 a	68.8 ± 4.7
20FR	2.3 ± 0.2	1.8 ± 0.2 b	5.1 ± 0.2 b	30.9 ± 0.3 b	77.6 ± 3.1
Arugula					
D	2.6 ± 0.2 b	3.0 ± 0.3 a	6.2 ± 0.5	33.6 ± 1.6 ab	70.5 ± 0.9 a
40B-D	2.9 ± 1.9 ab	2.7 ± 0.3 ab	5.9 ± 0.3	33.4 ± 0.8 ab	65.2 ± 2.8 ab
D-40B	3.1 ± 0.3 a	2.8 ± 0.4 ab	5.7 ± 0.2	34.1 ± 1.1 ab	61.7 ± 3.9 bc
20B	2.8 ± 0.2 bc	2.2 ± 0.1 bc	5.7 ± 0.1	35.0 ± 0.0 ab	59.3 ± 3.5 bc
20B20FR	2.9 ± 0.2 ab	2.2 ± 0.3 bc	5.8 ± 0.1	35.3 ± 0.3 a	55.4 ± 2.6 c
20FR	2.8 ± 0.2 ab	1.9 ± 0.0 c	5.7 ± 0.2	32.5 ± 0.3 b	65.3 ± 7.5 ab

[z]Leaf index (g·g⁻¹) = dry weight of cotyledons ÷ dry weight of hypocotyls.
[y]D = dark for 8 h; 40B-D = 40 µmol·m⁻²·s⁻¹ blue light for the first 4 h and dark for last 4 h; D-40B = dark for the first 4 h and 40 µmol·m⁻²·s⁻¹ blue light for the last 4 h; 20B = 20 µmol·m⁻²·s⁻¹ blue light for 8 h; 20B20FR = 20 µmol·m⁻²·s⁻¹ blue light and 20 µmol·m⁻²·s⁻¹ far-red light together for 8 h; 20FR = 20 µmol·m⁻²·s⁻¹ far-red light for 8 h. The six nighttime treatments had the same 16-h daytime lighting, which was provided by a combination of 20% blue and 80% red light-emitting diodes at a photosynthetic photon flux density of 300 µmol·m⁻²·s⁻¹ from 10:00 AM to 2:00 AM.
[x]Data are means ± SE (n = 3). Letters are shown only when there is significant treatment effect at $P \leq 0.05$. Means sharing the same letter are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$.

microgreens are highly perishable products (Di Gioia et al., 2015). Taking into account the negative effects of FR on some quality traits, monochromatic B appears to be a better nighttime spectrum treatment for increasing plant height of indoor-grown microgreens.

Strategies of using nighttime B for indoor microgreen production. In our study, the shorter time (4 h) of B treatments (D-40B and 40B-D) during the nighttime did not affect microgreen stem elongation, yield, or quality compared with a longer 8-h B treatment (20B). This differed from a recent study in our laboratory, where shortening the daily lighting time from 24 h to 16 h reduced the promotion effects of monochromatic B on stem elongation, cotyledon size, and plant color in microgreens to some degree (Kong et al., 2019a). Also, in natural light conditions in the greenhouse, the shortened photoperiod inhibited plant elongation in some species through reduced DLI (Bergstrand, 2017). In our study, although the duration of nighttime B treatment was shortened from 8 h to 4 h, the total DLI did not change because a twofold light intensity (40 vs. 20 µmol·m⁻²·s⁻¹) was used for 4-h B lighting. However, in previous studies, the shortened photoperiods were also accompanied by lower DLIs. This may explain in part the inconsistency between the results of our study and those of others.

For partial nighttime lighting (4 h rather than 8 h), B treatments at earlier vs. later night hours (40B-D vs. D-40B) had similar effects on microgreen elongation, yield, and quality in our study. The sole-source RB-LEDs used for daytime lighting in our study should induce a relatively high phytochrome activity (PPS = 0.88; Table 1) immediately after the light is turned off. However, monochromatic B may need some time to reduce the PPS. For white mustard (*Sinapsis alba*) seedlings, around 30 min was needed for monochromatic B to reduce the PPS value to initiate inactive phytochrome responses

(Jabben et al., 1982). Possibly, at the beginning (e.g., 30 min) of the 4-h nighttime B treatments, plant elongation was inhibited to a larger degree with the 40B-D treatment compared with D-40B as a result of the gradually decreasing phytochrome activity at night. However, this greater inhibition at the beginning with the 40B-D vs. the D-40B treatment might be eliminated by the elongation promoted by later 4-h dark with the 40B-D treatment. The underlying mechanism still needs further study. Nevertheless, the flexible time window to apply B during the nighttime provides an opportunity to lower lighting costs, because the price of electricity is much less during the off-peak period in some regions.

The combination of B and FR further promoted plant elongation compared with B alone. and showed a promotion effect similar to FR, but exerted no additive effect on yield or other quality metrics. Similarly, after adding low-level (10% total *PPFD*) FR to B, plant height increased slightly in arugula and mustard microgreens under 24-h sole-source LED lighting compared with B (Kong et al., 2019c). The increases in plant height under B + FR, compared with B, may have resulted from further reduced phytochrome activity, as indicated by a lower PPS value (0.23 vs. 0.52). However, the sensitivity of the elongation response to the decreased phytochrome activity seems to vary among species. In our study, the plants grown under monochromatic B alone treatments met the 5-cm height requirement for machine harvest for mustard, but not for arugula. For arugula, only B together with FR could promote elongation sufficient to meet the height requirement. It appears that, for indoor microgreen production, B alone—or together with FR—can be used as nighttime lighting for promoting elongation, depending on plant species sensitivity to decreased phytochrome activity.

In summary, for indoor production of mustard and arugula microgreens under 16-h day-

time RB-LED lighting, 40B-D, D-40B, and 20B increased plant height for both species compared with D, and had no differences among the three B treatments. 20B20FR further increased plant height for both species compared with B alone, and showed a promotion effect similar to 20FR. 20B vs. D increased FW for arugula, showing a similar promotion effect as 20FR. Compared with D, B alone or 20B20FR increased leaf thickness, but 20FR reduced the leaf index. Furthermore, B alone or 20B20FR, compared with 20FR, increased the CCI, leaf index, dry matter content, and leaf thickness to varying degrees with species. Apparently, although 20FR, compared with B alone, had a greater promotion effect on elongation, it showed some negative effects on microgreen quality. Plant height met the machine harvest requirement for mustard under nighttime B treatments and for arugula under 20B20FR, but not for both species under D. Overall, there is the potential to use monochromatic B with or without FR, depending on plant species, during the nighttime to promote stem elongation without compromising crop yield quality appearance.

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