Growth and Appearance Quality of Four Microgreen Species under Light-emitting Diode Lights with Different Spectral Combinations

Qinglu Ying, Yun Kong, and Youbin Zheng

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

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Abstract. To investigate plant growth and quality responses to different light spectral combinations, cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*), kale (*Brassica napus* L. ‘Red Russian’), arugula (*Eruca sativa* L.), and mustard (*Brassica juncea* L. ‘Ruby steak’) microgreens were grown in a controlled environment using sole-source light with six different spectra: 1) FL: cool white fluorescent light; 2) BR: 15% blue and 85% red light-emitting diode (LED); 3) BRFR_L: 15% blue, 85% red, and 15.5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ far-red (FR) LED; 4) BRFR_H: 15% blue, 85% red, and 155 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ FR LED; 5) BG_LR: 9% blue, 6% green, and 85% red LED; and 6) BG_HR: 5% blue, 10% green, and 85% red LED. For all the light treatments, the total photosynthetic photon flux density (PPFD) was set at $\approx 330 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under a 17-hour photoperiod, and the air temperature was $\approx 21^\circ\text{C}$ with 73% relative humidity (RH). At harvest, BR vs. FL increased plant height for all the tested species except arugula, and enlarged cotyledon area for kale and arugula. Adding high-intensity FR light to blue and red light (i.e., BRFR_H) further increased plant height for all species, and cotyledon area for mustard, but it did not affect the fresh or dry biomass for any species. Also, BRFR_H vs. BR increased cotyledon greenness for green-leafed species (i.e., arugula, cabbage, and kale), and reduced cotyledon redness for red-leafed mustard. However, BG_LR, BG_HR, and BRFR_L, compared with BR, did not affect plant height, cotyledon area, or fresh or dry biomass. These results suggest that the combination of 15% blue and 85% red LED light can potentially replace FL as the sole light source for indoor production of the tested microgreen species. Combining high-intensity FR light, rather than low-level ($\leq 10\%$) green light, with blue and red light could be taken into consideration for the optimization of LED light spectral quality in microgreen production under environmental conditions similar to this experiment.

Microgreens are gaining increasing attention and consumption in recent years because of their various colors, unique flavors and textures, and higher nutrition levels compared with their mature counterparts (Treadwell et al., 2016; Xiao et al., 2012). Typically, microgreens are harvested with two cotyledons fully expanded, and right before or after the first true leaf starts to

emerge. Within 80 to 100 plant species that are currently cultivated as microgreens, the most commonly cultivated species are from the Brassicaceae family, including, arugula, cabbage, kale, and mustard (Björkman et al., 2011).

Microgreens are increasingly grown in indoor production facilities with electrical lighting as the sole light source. Fluorescent lights (FLs), especially cool white FLs with enhanced blue and red spectra, were traditionally used for indoor production (Darko et al., 2014; Massa et al., 2006). Recently, LED lights have been developed as a new electrical light source in crop production, and LED light has many advantages over traditional lights (e.g., FLs) (Morrow, 2008). Among the advantages, the adjustable light spectral composition in LED lights enables researchers and growers to manipulate plant morphology and physiology based on production purposes (Davis and Burns, 2016).

LED light with blue light (B) and red light (R) combination (BR) has been popularly used for horticultural crop production in controlled environments (Goto, 2012). BR

(containing 5% to 15% B) at a PPFD of ≈ 300 to $400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a photoperiod of ≈ 16 h appears to be the optimal light environment for indoor production of microgreens based on crop yield and appearance quality (Jones-Baumgardt et al., 2019; Ying et al., 2020). Also, plants grown under BR vs. FL are generally better in terms of yield and quality. For example, BR, compared with FL with the PPFD ranging from 70 to $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, increases leaf area of chili pepper (*Capsicum annuum*) and hybrid moth orchid (*Phaenopsis* $\times$ *Doritis*); fresh weight (FW) and dry weight (DW) of chili pepper, lettuce (*Lactuca sativa* L.), and hybrid moth orchid; and FW of sprouting broccoli (*Brassica oleracea*) (Gangadhar et al., 2012; Johkan et al., 2010; Kopsell et al., 2014; Lin et al., 2013; Shin et al., 2008). However, there is a lack of study comparing the effects of BR relative to FL under a higher PPFD (e.g., $300\text{--}400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) on microgreen species.

Promoted plant growth has been found when adding FR to BR by eliciting shade-avoidance responses through phytochromes (Park and Runkle, 2017). Under a background lighting with BR (20% B and 80% R; B₂₀R₈₀) at a PPFD of $130 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, adding FR with increased levels from ≈ 12 to $149 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (i.e., R:FR ratio decreased from 8.6 to 0.7) increases the aboveground FW and DW, and leaf area of lettuce accordingly (Lee et al., 2016). When FR ($16\text{--}64 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) is added to B₂₀R₈₀ (PPFD of $160 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), total leaf area and shoot DW are increased in geranium (*Pelargonium hortorum*) and snapdragon (*Antirrhinum majus*) transplants (Park and Runkle 2017). However, little relevant information is available under higher PPFD (e.g., $300\text{--}400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for microgreens, which have a much shorter growth period than lettuce and flower transplants. For microgreens, B₉R₈₄ FR₇ (i.e., 9% B, 84% R, and 7% FR), compared with B₁₃R₈₇, increases hypocotyl length and FW in mustard (*Brassica juncea* L.) under a total photon flux of $315 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Gerovac et al., 2016). However, in this study, FR was not added to BR, but partially replaced R and B in BR. In this case, the BRFR, compared with the BR, had a lower B percentage (9.7% vs. 13%) in the total PPFD, which can also affect microgreen growth (Ying et al., 2020). Therefore, it is difficult to conclude whether the effects are attributed to higher FR or lower B. Furthermore, only low-level FR (i.e., high R:FR ratio of 12:1) was used for the previous microgreen study. If higher-level FR is included in BR (i.e., lower R:FR ratio), it is unknown whether there will be differences in responses of growth and yield of microgreens under a higher PPFD (e.g., $300\text{--}400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in indoor production.

Partially replacing B in BR with green light (G) has also been shown to promote plant growth by inducing shade-avoidance responses through G-B-interaction-inhibited cryptochrome activity (Meng et al., 2019), as well as by the better ability of G to penetrate

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

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Table 1. The four experimental microgreen species, seeding density, and the start and end dates of each replicate.

Plant materials	Scientific name	Plant color	Seeding density (g·m ⁻²)	Seed source	Seeding date	Harvest date
Cabbage	<i>Brassica oleracea</i> L. var. <i>capitata</i> f. <i>rubra</i>	Purple stem and leaf margin with green leaf	68.6	Mumm's Sprouting Seeds, Parkside, SK, Canada	Rep. 1: 6 Dec. 2016 Rep. 2: 6 Jan. 2017 Rep. 3: 24 Jan. 2017	Rep. 1: 16 Dec. 2016 Rep. 2: 16 Jan. 2017 Rep. 3: 4 Feb. 2017
Kale	<i>Brassica napus</i> L. 'Red Russian'	Purple stem with green leaf	54.9	High Mowing Organic Seeds, Wolcott, VT	Rep. 1: 6 Dec. 2016 Rep. 2: 6 Jan. 2017 Rep. 3: 24 Jan. 2017	Rep. 1: 17 Dec. 2016 Rep. 2: 17 Jan. 2017 Rep. 3: 5 Feb. 2017
Arugula	<i>Eruca sativa</i> L.	Green leaf	27.4	Suba Seeds Company S.P.A., Longiano, FC, Italy	Rep. 1: 6 Dec. 2016 Rep. 2: 6 Jan. 2017 Rep. 3: 24 Jan. 2017	Rep. 1: 17 Dec. 2016 Rep. 2: 17 Jan. 2017 Rep. 3: 5 Feb. 2016
Mustard	<i>Brassica juncea</i> L. 'Ruby Steak'	Purple leaf vein or whole leaf	27.4	Johnny's Selected Seeds, Winslow, ME	Rep. 1: 6 Dec. 2016 Rep. 2: 6 Jan. 2017 Rep. 3: 24 Jan. 2017	Rep. 1: 16 Dec. 2016 Rep. 2: 16 Jan. 2017 Rep. 3: 4 Feb. 2017

plants compared with B and R (Sun et al., 1998). At a *PPFD* of $\approx 173 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the shoot FW of red leaf lettuce under B₁₀G₁₀R₈₀ (i.e., 10% B, 10% G, and 80% R) is $\approx 61\%$ higher than those under B₂₀R₈₀ (Son and Oh, 2015). Also, at a *PPFD* of $160 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, B₂₅G₂₅R₅₀ vs. B₅₀R₅₀ increases shoot biomass, plant height, or leaf area in tomato (*Solanum lycopersicum*), petunia (*Petunia ×hybrida*), impatiens (*Impatiens walleriana*), and salvia (*Salvia splendens*) (Wollaeger and Runkle, 2015). Similarly, at a *PPFD* of $180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, B₂₂G₁₁R₆₇ or B₁₁G₂₂R₆₇, comparing to B₃₃R₆₇, promotes leaf expansion and increases shoot FW in lettuce and kale (*Brassica oleracea*) (Meng et al., 2019). However, the preceding studies were carried out on plants other than microgreens, and under a *PPFD* of $<200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. For microgreens, B₈G₁₈R₇₄, comparing to B₁₃R₈₇, increases hypocotyl length and shoot FW in mustard under *PPFD* of $315 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Gerovac et al., 2016). However, in this study, both B and R were replaced by G, which can complicate the G effects interacting with B. Also, only one G level (i.e., G% > B%) was designed for BGR light, which made it impossible to compare the effects with lower G level (i.e., G% < B%). Furthermore, the comparison between BGR and BRFR effects on microgreens under a higher *PPFD* (e.g., $300\text{--}400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) needs further study.

Although some previous studies have explored the optimization of LED lighting spectral quality for microgreen production, the optimal "light recipe" seems to vary with lighting intensity, growth stage, and plant species. The objective of this study was to investigate the plant responses to 1) BR vs. FL, 2) adding different levels of FR to BR, and 3) partial replacement of B in BR by different levels of G, in terms of growth and quality of four microgreen species under sole-source LED lighting at a *PPFD* of $\approx 330 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Materials and Methods

Plant material and growing conditions. The experiment was conducted on four microgreen species (Table 1) in a walk-in

growth chamber ($7.3 \times 4.0 \times 2.5$ m, model W-IN FLUSH 00VT 34; Foster Refrigerator of Canada, Calgary, Alberta, Canada). There were six compartments within the chamber, separated by an opaque curtain to prevent neighboring effects. Black plastic trays ($54 \times 27 \times 4$ cm) were filled with an organic growing substrate that consisting of 30% compost, 30% peat, 30% coir, and 10% perlite (v/v). Four microgreen species were seeded in the growing substrate (one species per tray) and covered with coconut coir to maintain moisture during germination. Eight trays (two for each species) were placed inside each compartment under different lighting treatments with the same *PPFD* of $330 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 17-h photoperiod. All plants were top irrigated at least once daily using well water (pH = 7.5; electrical conductivity = $0.8 \text{ dS}\cdot\text{m}^{-1}$) until visible drainage. The air temperature was set at 20°C and the relative humidity (RH) at 75%. The measured temperature in the growth chamber was $20.7 \pm 0.4^\circ\text{C}$ and RH was $72.5\% \pm 1.8\%$ throughout the study ($n = 3$).

Light treatments. Randomized complete block design was used for this experiment, with one factor (light quality) and three replicates over time. Light quality treatments included the following: 1) FL: cool white FL; 2) BR: 15% B and 85% R; 3) BRFR_L: 15% B and 85% R with additional $15.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ FR; 4) BRFR_H: 15% B and 85% R with additional $155 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ FR; 5) BG_LR: 9% B, 6% G, and 85% R; 6) BG_HR: 5% B, 10% G, and 85% R. Sole-source LED lights (Heliospectra LX602C; Heliospectra AB, Gothenburg, Sweden) or cool white linear FLs (F96T12/CW/VHO; Osram Sylvania Ltd., Markham, Ontario, Canada) were used for the preceding treatments. For each replicate, the six light quality treatments were randomly allocated to six compartments. After each replicate, the six light quality treatments were switched to different compartments within the chamber.

Within the treatment area (54×54 cm), the LED and FL fixtures were placed 75 cm and 140 cm, respectively, above the plant canopy to achieve the average target *PPFD* of $330 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The light intensity and spectrum of the LED arrays were set up by

Heliospectra System Assistant (Version 1.3.0; Heliospectra AB). To check light uniformity, light intensity and spectrum were measured at 25 spots at the canopy level within the treatment area under each treatment using a Flame-S spectrometer with a 25- μm slit, coupled to a $1.89 \text{ m} \times 400 \mu\text{m}$ solarization-resistant fiberoptic patch chord with a CC-3 Cosine Corrector with spectralon diffuser (Ocean Optics, Inc., Dunedin, FL). The spectrometer was calibrated over 350 to 800 nm before light measurements. Light intensity and spectra were also checked and confirmed at the end of each replicate. The light intensity and spectral distribution under different light treatments are presented in Fig. 1 and Table 2.

Growth and quality measurements. Plants were harvested 11 d after seeding for cabbage and kale, and 12 d after seeding for mustard and arugula (Table 1). Before harvest, for each replicate, four plants from each species were sampled randomly from each tray (treatment) to measure the heights using a ruler. At harvest, 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. All the microgreens within each core were cut right above the substrate level and collected to determine FW. These plants were then put in paper bags and dried at 65°C until they reached a constant weight to determine DW. The fresh and dry biomass ($\text{kg}\cdot\text{m}^{-2}$) were estimated based on average FW and DW of collected microgreens as well as surface areas of sampled cores. From the remaining microgreens in each tray, five plants from each species were selected randomly to analyze the cotyledon area and color. The cotyledons were cut off from the stem and scanned to save as color digital images using a scanner (CanoScan F910111; Canon Inc., Tokyo, Japan) with 300 dpi resolution. The cotyledon area, as well as R, G, and B values (i.e., the amount of R, G, and B light emitted from each pixel in the image on a scale of 0 to 255) were obtained from the digital images using ImageJ 1.42 software (National Institutes of Health, Bethesda, MD). Subsequently, hue angle of the cotyledon color

Fig. 1. Spectral distribution of the six light treatments. FL = fluorescent light; BR = 15% blue and 85% red; BRFR_L = 15% blue and 85% red with 15.5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ far-red light; BRFR_H = 15% blue and 85% red with additional 155 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ far-red light; BG_LR = 9% blue, 6% green, and 85% red; BG_HR = 5% blue, 10% green, and 85% red. The photosynthetic photon flux density of each lighting treatment is 330 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

was calculated from these RGB values using formulas according to Karcher and Richardson (2003). The hue angle of the cotyledon color was calibrated with the actual hue angle of Munsell color chips using Munsell Conversion software for 2018 (Munsell color, 2018).

Statistical analysis. Data were subjected to one-way analysis of variance using the SPSS software (Version 25.0; IBM, New York, NY) and were presented as mean $\pm$ SE. Separation of means was performed using Tukey's honestly significance difference test at the $P \leq 0.05$ level.

Results

Cotyledon color. Different light treatments affected cotyledon color differently,

based on the variation of hue angle in the tested microgreen species (Fig. 2). Under FL, the hue angles of cotyledons were $\approx 55^\circ$ (greenness), 65° (greenness), 66° (greenness), and 349° (redness) for cabbage, kale, arugula, and mustard, respectively. BR vs. FL reduced the hue angle (i.e., increased redness) of cotyledons for mustard, but showed no differences for the other three species. The cotyledon hue angles of all four tested species were increased under BRFR_H compared with BR, indicating increased greenness for cabbage, kale, and arugula, and reduced redness for mustard. There was no difference in cotyledon hue angle among BR, BG_LR, and BG_HR for all the tested species; however, BG_LR reduced cotyledon hue angle from 72° to 68° for arugula com-

pared with BRFR_L, showing reduced greenness. Also, BG_HR reduced this trait from 356° to 347° for mustard compared with BRFR_H, showing increased redness.

Plant size. The plant heights of all tested microgreen species were affected by different light spectral combinations (Fig. 3). BR, compared with FL, increased plant height by 31%, 42%, and 27% for cabbage, kale, and mustard, respectively. BRFR_L increased plant height of arugula by 36% compared with BR. BRFR_H, compared with BR, increased the plant heights by 36%, 31%, 68%, and 57% for cabbage, kale, arugula, and mustard, respectively. There was no difference in this trait among BR, BG_LR, and BG_HR for all the tested species. However, plant height was reduced for cabbage, kale,

Table 2. Mean photon flux density from blue, green, red, far-red, and photosynthetically active radiation (*PAR*), red:far-red ratios, and phytochrome photostationary state (PPS) of the different light quality treatments.

Treatments ^z	Light intensity ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)				<i>PAR</i>	Red:far-red ratio	PPS ^y
	Blue (400–500 nm)	Green (500–600 nm)	Red (600–700 nm)	Far-red (700–800 nm)			
FL	59.3 $\pm$ 1.3 ^x	181 $\pm$ 3	90 $\pm$ 2	7.9 $\pm$ 6.2	330 $\pm$ 5	18.0 $\pm$ 8.1	0.86 $\pm$ 0.00
BR	47.4 $\pm$ 0.6	—	280 $\pm$ 5	3.5 $\pm$ 2.7	329 $\pm$ 5	—	0.88 $\pm$ 0.00
BRFR _L	47.1 $\pm$ 0.7	—	278 $\pm$ 5	15.0 $\pm$ 1.2	327 $\pm$ 6	18.5 $\pm$ 0.6	0.87 $\pm$ 0.00
BRFR _H	47.7 $\pm$ 0.8	—	281 $\pm$ 3	155 $\pm$ 12	331 $\pm$ 4	1.8 $\pm$ 0.1	0.78 $\pm$ 0.00
BG _L R	30.7 $\pm$ 0.1	16.6 $\pm$ 0.6	279 $\pm$ 4	—	326 $\pm$ 4	—	0.88 $\pm$ 0.00
BG _H R	18.8 $\pm$ 1.2	28.5 $\pm$ 0.8	278 $\pm$ 3	—	325 $\pm$ 4	—	0.88 $\pm$ 0.00

^zFL = fluorescent light; BR = 15% blue and 85% red; BRFR_L = 15% blue and 85% red with 15.5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ far-red light; BRFR_H = 15% blue and 85% red with additional 155 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ far-red light; BG_LR = 9% blue, 6% green and 85% red; BG_HR = 5% blue, 10% green and 85% red.

^yPPS is calculated according Sager et al. (1988).

^xData are means $\pm$ SE (n = 3).

Fig. 2. Hue angle of cotyledon color from four microgreen species grown under different light treatments. Data are the means $\pm$ SE (n = 3). Bars bearing the same letter are not significantly different at $P \leq 0.05$. FL = fluorescent light; BR = 15% blue and 85% red; BRFR_L = 15% blue and 85% red with 15.5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ far-red light; BRFR_H = 15% blue and 85% red with additional 155 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ far-red light; BG_LR = 9% blue, 6% green, and 85% red; BG_HR = 5% blue, 10% green, and 85% red. The photosynthetic photon flux density of each lighting treatment is 330 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

and mustard under BG_HR vs. BRFR_H, and for arugula under both BG_HR vs. BRFR_H and BG_LR vs. BRFR_L.

As shown in Fig. 4, light treatments affected cotyledon area of the tested microgreen species except cabbage. Under BR vs. FL, this trait was increased by 32% and 23% for kale and arugula, respectively. Compared with BR, the cotyledon area was increased by 31% for mustard under BRFR_H and was reduced by 22% for kale under BRFR_L; however, there was no difference among BR, BG_LR, and BG_HR for all the tested species. Also, cotyledon areas were similar between BG_HR and BRFR_H or between BG_LR and BRFR_L for all the tested species except kale.

Plant biomass. Either fresh biomass or dry biomass was unaffected by the different light treatments for the tested microgreen species (data not shown). The average fresh and dry biomass were 1.60 and 0.13 $\text{kg}\cdot\text{m}^{-2}$ for cabbage, 1.54 and 0.12 $\text{kg}\cdot\text{m}^{-2}$ for kale,

1.48 and 0.14 $\text{kg}\cdot\text{m}^{-2}$ for arugula, and 1.50 and 0.13 $\text{kg}\cdot\text{m}^{-2}$ for mustard.

Discussion

BR light vs. FL improves appearance quality for some microgreen species without compromising yields. BR, compared with FL, increased the redness of cotyledon color for red-leafed mustard; however, it did not affect cotyledon color for the other three green-leafed species in the present study. The results suggest that red- vs. green-leafed plant species are more sensitive to BR compared with FL. The differences in responses to BR for plants with different leaf color were supported by a previous study on 18 vegetable genotypes in our laboratory (Kong and Zheng, 2019). In that study, red- vs. green-leafed plant genotypes showed higher phenotypic plasticity indices (i.e., higher sensitivity) in response to BR relative to dark. The increase in red coloration is also observed in

lettuce leaves under prior-to-harvest supplemental BR (Owen and Lopez, 2015). The increased leaf redness under BR might be due to an increased anthocyanin content, because a significant positive correlation is reported between plant redness and anthocyanin content (Manetas, 2006).

In addition to changing cotyledon color, BR vs. FL also increased plant height for the tested species except arugula, and increased cotyledon area for arugula and kale. Similar species-specific light responses were reported in previous studies. Chili pepper plants are taller with larger leaves under BR vs. FL, but no difference is found in leaf area of red-leafed lettuce under the same light condition (Johkan et al., 2010). Plant heights of all four tested ornamental species (i.e., petunia, calibrachoa, geranium, and marigold) are increased by BR vs. FL (Mah et al., 2018). The lower B photon flux under BR vs. FL could partly explain the promoted elongation in microgreens in our experiment. Previous studies on tomato and cucumber seedlings indicate that hypocotyl elongation and leaf expansion are inhibited with increased B light photon flux in BR (Nanya et al., 2012; Hernández and Kubota, 2016). Also, FL contains a small proportion of UVA light, which is not present in BR and may also inhibit plant elongation (Kong et al., 2019b).

There was no difference in fresh or dry biomass between BR and FL treatments for all the tested microgreen species in the present study. Similar results are reported on the DW of broccoli microgreens under BR vs. FL (Kopsell et al., 2014). However, BR vs. FL increases fresh biomass in chili pepper leaves and fruits (Gangadhar et al., 2012). The different responses to BR vs. FL treatment between microgreens and chili pepper might have resulted from their different growth periods under lighting treatments. Microgreens were grown for only 11 to 12 d, during which plant photosynthesis and biomass accumulation might be less affected by light spectra than mature plants.

Adding high FR to BR light promotes elongation for some microgreens species and modifies leaf color without affecting yields. With additional high FR added to BR, the cotyledon redness was reduced for red-leafed mustard in the present study. In this case, leaf anthocyanin content might

Fig. 3. Individual plant height of four microgreen species grown under different light treatments. Data are the means $\pm$ SE ($n = 3$). Bars bearing the same letter are not significantly different at $P \leq 0.05$. FL = fluorescent light; BR = 15% blue and 85% red; BRFR_L = 15% blue and 85% red with 15.5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ far-red light; BRFR_H = 15% blue and 85% red with additional 155 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ far-red light; BG_{LR} = 9% blue, 6% green and 85% red; BG_{HR} = 5% blue, 10% green, and 85% red. The photosynthetic photon flux density of each lighting treatment is 330 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

have decreased when high FR was added to BR and resulted in a decreased R:FR ratio, which is also reported in long-stalk starwort (*Stellaria longipes*) (Alokam et al., 2002). In the present study, the greenness of cotyledons was increased for the green-leafed microgreens (i.e., cabbage, kale, and arugula). This seemed to be contradictory with the common

opinion that increased FR level can reduce leaf chlorophyll content (Demotes-Mainard et al., 2016). However, increased leaf greenness normally resulted from increased relative, rather than absolute, chlorophyll content. In other words, the other pigments, such as anthocyanins and/or carotenoids, might have reduced more than chlorophyll.

Fig. 4. Cotyledon area of four microgreen species grown under different light treatments. Data are the means $\pm$ SE ($n = 3$). Bars bearing the same letter are not significantly different at $P \leq 0.05$. FL = fluorescent light; BR = 15% blue and 85% red; BRFR_L = 15% blue and 85% red with 15.5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ far-red light; BRFR_H = 15% blue and 85% red with additional 155 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ far-red light; BG_{LR} = 9% blue, 6% green, and 85% red; BG_{HR} = 5% blue, 10% green, and 85% red. The photosynthetic photon flux density of each lighting treatment is 330 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Similarly, increased relative chlorophyll content is reported in kohlrabi microgreens under BRFR relative to BR (Gerovac et al., 2016).

Adding high FR to BR resulted in taller plants of all four microgreen species. This would potentially benefit machine harvesting, which requires a minimum height of 5 cm (Kong et al., 2019a). However, adding low FR did not affect plant height except for arugula. The difference between high and low FR might be due to their reduction on phytochrome activity to different degree. The phytochrome photostationary state (PPS) value, an indicator of phytochrome activity (Sager et al., 1988), was reduced from 0.88 to 0.78, when high FR was added to BR (Table 2). Normally, plant height increases as a shade-avoidance response with decreasing phytochrome activity (Demotes-Mainard et al., 2016; Hendricks and Borthwick, 1967); however, when low FR was added to BR, the decrease in PPS was too minuscule (from 0.88 to 0.87; Table 2) to affect most microgreen species except arugula. This species-specific response was also found on cotyledon area, which was reduced in kale and increased in mustard after adding low and high FR, respectively, to BR in the present study. Similarly, when adding FR to BR, leaf area decreases in geranium and snapdragon, but not in petunia and impatiens (Park and Runkle, 2017).

Although plant height and cotyledon area were increased in some species when high FR was added to BR, the morphological changes did not lead to a significant yield increase. This may be due to the thinner leaves developed under decreased R:FR ratios, as supported by previous studies on bean (*Phaseolus vulgaris* L.) leaves (Barreiro et al., 1992), and cucumber seedlings (Shibuya et al., 2011). Moreover, the FR LED has had a lower energy efficiency, and a higher price than R or B LED in the past decade (Kubota et al., 2012; Nelson and Bugbee, 2014), which might not be cost-effective for microgreen production currently. However, with the increasing energy efficacy and decreasing price due to the rapid development of LED technology, the potential application of FR LED may become popular in the future (Kusuma et al., 2020).

Combining G with BR light causes no changes in microgreens yield and appearance quality, showing smaller effects than addition of FR. G has a better ability to penetrate plant canopy than R or B, thus BGR (i.e., combining G with BR) would potentially promote whole-canopy photosynthesis, and benefit plant growth (Kim et al., 2004; Klein, 1992). For example, BGR increases lettuce FW, DW, and leaf area, compared with BR (Son and Oh, 2015); however, in the present study, BGR vs. BR did not change microgreen growth (e.g., biomass). Similar result is also reported in sprouting broccoli (Kopsell et al., 2014). Possibly, for plants without leaves at lower canopy, such as microgreens, combining G with BR has little promotion effects on canopy photosynthesis, and thereby plant growth. Nevertheless,

BGR would benefit growers in checking plant health, because BR produces a purple light that causes plants to look gray/black rather than green (Smith et al., 2017).

Increased G level in BGR can also induce shade-avoidance response to promote plant growth. For example, in lettuce and kale under a *PPFD* of 180 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, when G proportion in total *PPFD* increased from 11% to 22% (i.e., B decreased from 22% to 11%), leaf expansion and biomass increased accordingly (Meng et al., 2019). However, in the present study, under a *PPFD* of 330 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, when G percentage increased from 6% to 10% (i.e., B decreased from 9% to 5%), no change was found in microgreen growth and appearance quality. Possibly, in the present study, the increase of G percentage was too small to induce shade-avoidance response especially under a higher *PPFD*. As well-known, under the natural vegetative shade, the increased G proportion is normally accompanied by decreased *PPFD*. The effect of greater variation of G proportions in BGR on microgreens growth and quality needs further studies under a high *PPFD* of ≈ 300 to 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Although both G and FR can induce shade-avoidance response to promote plant elongation growth, G seems to have less pronounced effects than FR does in a previous study on lettuce and kale (Meng et al., 2019). Similar results were achieved on the plant height of microgreens under BGR vs. BRFR in the present study. Possibly, FR is a more important shade signal than G, because increase of both G and FR intensity indicates actual shading, whereas the increase of FR intensity also warns potential light competition (Keuskamp et al., 2012).

Conclusions

In summary, at a *PPFD* of 300 to 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, BR vs. FL increased plant height for cabbage, kale, and mustard; enlarged cotyledon area for arugula and kale; and enhanced cotyledon redness for mustard. Adding high-intensity FR (155 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to BR further increased plant height for all the species, cotyledon area for mustard, and cotyledon greenness for green-leafed species; however, it reduced cotyledon redness for red-leafed mustard. Substituting G partially for B in BR showed little beneficial effect on crop yield and appearance quality, possibly due to a low proportion of G ($\leq 10\%$) presented. Therefore, BR without G can potentially replace FL as the sole light source for future indoor production of the tested microgreen species under a higher *PPFD*. Although adding FR to BR benefits microgreen growth, the relatively high energy consumption of FR needs to be taken into consideration when growers are optimizing their light spectral quality.

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