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Early-stage Dark Treatment Promotes Hypocotyl Elongation Associated with Varying Effects on Yield and Quality in Sunflower and Arugula Microgreens

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Abstract: Microgreens growing under electric (aka artificial) lighting in controlled environments often have short hypocotyls, which can be difficult for machine harvest. To investigate whether early-stage dark treatment can promote hypocotyl elongation without compromising microgreen yield and quality, two different seed-size species, sunflower (*Helianthus annuus* ‘Black oil’) and arugula (*Eruca sativa* ‘Rocket’), were tested. Seeds of sunflower and arugula were sown in pots, and the pots were placed inside support trays. During the first 5 days after seeding, half of the pots per species within each tray were covered with an upside-down black tray as dark treatment, and another half were kept under light. The light treatment was provided by continuous (24-h) lighting with a combination of red (85%) and blue (15%) light-emitting diodes at a photosynthetic photo flux density of around $100 \mu\text{mol m}^{-2} \text{s}^{-1}$. After 5 days of dark treatment, the black covers were removed and the plants were grown under the above lighting treatment till harvesting. The microgreens were harvested at 7 d and 12 d after seeding for sunflower and arugula, respectively.

Early-stage dark treatment promoted hypocotyl elongation by 26% and 28% for sunflower and arugula, respectively. Microgreen yield was increased by 13% for sunflower and reduced by 24% for arugula under dark treatment. Dark treatment increased cotyledon succulence by 14% for sunflower, but reduced cotyledon size by about 25% for arugula despite increases in red color and succulence of arugula hypocotyls. For both species, cotyledon color and soluble solids content were not affected by dark treatment.

Keywords: *Eruca sativa*, *Helianthus annuus*, morphology, quality, yield.

Introduction

Microgreen consumption has increased in recent years because of their various attractive colors, different flavours, unique textures, and high nutritional values (Treadwell et al. 2015; Kyriacou et al. 2016; Turner et al. 2020). Light-emitting diode (LED) lighting has been increasingly used as sole light source for indoor production of vegetables such as microgreens (Kozai et al. 2015; Alrifai et al. 2019; Jones-Baumgardt et al. 2019; Rajan et al. 2019; Ying et al. 2020a; Zhang et al. 2020). However, electric lighting in controlled environments often results in microgreens with short hypocotyls. This can cause difficulty when machine harvest is employed. Although hypocotyl elongation of microgreens can be promoted by night supplemental lighting, prolonged photoperiod, or certain light recipes (Kong et al. 2019; Ying et al. 2020a; Ying et al. 2020b, 2020c; Gao et al. 2021), there are associated costs for the increased energy use and lighting fixture purchase and installation.

Previous studies on *Arabidopsis* indicate that after germination, seedlings typically grow

with long hypocotyls in the absence of light, but with short hypocotyls in the presence of sufficient natural light or non-LED lighting (Von Arnim and Deng 1996; Fankhauser and Chory 1997; Kami et al. 2016; Seluzicki et al. 2017). Similar promotion effect on hypocotyl elongation by dark treatment, relative to Red and Blue-LED (RB-LED) lighting, were found in the seedlings of 18 vegetable genotypes during de-etiolation stage, i.e., from seed germination to cotyledon unfolding (Kong and Zheng 2019b). In addition to promoting plant elongation, dark, compared to RB-LED light, also increased individual shoot fresh biomass, inhibited cotyledon opening, reduced cotyledon size, and inhibited inherent color formation due to skotomorphogenesis (Kong and Zheng 2019b). The change of shoot biomass and plant morphology under dark treatment could potentially affect yield positively and quality negatively for microgreens, since microgreens also experience the de-etiolation process. However, this study was performed in Petri dishes with filter papers, which had different growth environments (e.g., much higher humidity) from commercial microgreen production practices. Also, the seedlings in this study had a much lower density than those used in commercial microgreen productions. More importantly, the dark treatment was applied to the seedlings during the whole experimental (or growth) period. How dark treatment at the early stage only (i.e., dark treatment first, then exposure to lighting before harvest) affects final plant elongation, yield and quality of microgreens are unknown.

Traditional indoor production of pea microgreens in China indicates that despite an early-stage dark treatment, a three-day lighting treatment before harvesting can promote leaf expansion and greening (Zhang and Hu 2008). It appears that at least for some species (e.g., peas) dark treatment at the early growth stage may be an effective approach to grow

microgreens. This practice may not cause any negative effect on appearance quality traits such as leaf color. However, unlike peas, a large-seed species, many microgreen species have only medium- and small-size seeds (e.g., sunflower and arugula). A previous study indicates that in many cases, light sensitivity of de-etiolated seedlings increases with decrease of seed size (Kong and Zheng 2019b). In other words, smaller-seed species is more dependant on light for the development of de-etiolated seedlings (Kircher and Schopfer 2012). For medium- and small-seed species (e.g., sunflower and arugula), which have smaller seed size than peas, whether early-stage dark treatment can promote hypocotyl elongation of these microgreens without negatively affecting yield and quality is unknown.

Sunflower and arugula are popularly used for microgreen production. Dark treatment during the first 1–2 day after seeding is commonly adopted to promote seed germination in commercial production of these two species. However, there is no research on the effects of prolonged darkness at early growth stage (i.e., maintaining darkness after germination for some days) on the growth and morphology of sunflower and arugula microgreens. The objective of this study was to test whether early-stage dark treatment is an effective method for indoor microgreen production, by examining its effects on hypocotyl elongation, crop yield and a few related product quality metrics in sunflower and arugula.

Materials and Methods

Plant materials and growing conditions

The experiment was conducted in a walk-in growth chamber at the University of Guelph, ON, Canada from June to July of 2018. Two microgreen species, sunflower (*Helianthus*

annuus, ‘Black oil’) and arugula (*Eruca sativa*, ‘Rocket’), were used for the experiment; they had a 1000-seed weight of ≈ 62.0 and 1.9 g, respectively. Seeds were sown in 8.5 cm $\times$ 8.5 cm $\times$ 9.5 cm (height) pots containing Sunshine® Mix #5 substrate (Sun Gro Horticulture, MA, USA). Seeding rate was around 5.72 and 0.25 g/pot (i.e., 792 and 35 g m⁻²) for sunflower and arugula, respectively. Every 18 sown pots were placed inside one support tray, and then placed on benches in the chamber for the treatments. The plants were sub-irrigated with tap water for the first five days (i.e., dark treatment period) before nutrient solution was used. The irrigation frequency was once every 2–5 d, depending plant size. The nutrient solution was made from a 20–8–20 water soluble fertilizer (Master Plant-Prod Inc., Brampton, ON, Canada). The nutrient solution had a fertilizer:water mass ratio of 1:800. The temperature and relative humidity (RH) set points in the growth chamber were around 23 °C and 65%, respectively.

Experimental design and treatments

The experiment had a randomized complete block design (2 treatments $\times$ 6 blocks for each species). The six blocks were distributed to six separate compartments in the growth chamber. In each block, there were 36 pots, and each species had 18 pots. For each species, in each block, half-tray pots (9 pots, i.e., experimental unit) were under early-stage dark treatment, and another half tray of pots were under light treatment (i.e., control). For early-stage dark treatment, after seeding, the pots were covered with upside-down black trays for 5 days. Then the black covers were removed, and the plants continued growth under the same lighting as control until harvest.

For each block, the lighting was provided by the same LED fixtures as those described in

one of our recent studies (Kong and Zheng 2019b). Briefly, the lighting was a combination of red and blue LED, with red and blue light at around 85% and 15% of the total photosynthetic photo flux density (PPFD), respectively. The wavelength for red and blue light from the LED lighting ranged from 600–700 nm and 400–500 nm, and peaked at 660 nm and 455 nm, respectively. A PPFD of around $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the plant canopy level was achieved in each compartment by adjusting the lighting fixtures' light intensity outputs. The LED lighting was supplied 24 h d^{-1} (i.e., continuous lighting) to compensate the relatively low light intensity. In controlled environment using electric lighting as sole light source, 24-h lighting at a low to moderate light level (e.g., $100 \mu\text{mol m}^{-2} \text{s}^{-1}$) is a way of producing crops more economically (Sysoeva et al. 2010). The six compartments in the growth chamber were separated by curtains to prevent neighboring light effects.

Growth, yield, and quality measurements

At 7 d and 12 d after seeding for sunflower and arugula, respectively, 18 pots (three pots each block) were randomly sampled from each treatment per species. Canopy height was measured from substrate level to canopy top at three locations for each pot. From each pot, six plants were randomly sampled to measure chlorophyll content index (CCI; SPAD 502; Spectrum Technologies, Inc., Aurora, IL, USA) of cotyledons for both species, and thickness of cotyledons for sunflower. Then, all the plants from the sampled pots were harvested at the substrate level, and the fresh weight (FW) of microgreens harvested from each pot were measured for calculating microgreen yield (kg m^{-2}) based on pot area. Six plants were randomly sampled from the harvested microgreens in each pot. They were separated into cotyledons (with petioles) and stems to measure FW and calculate individual plant FW. After

determining the FWs, the separated hypocotyls and cotyledons of the six sampled plants and the remaining harvested microgreens from each pot were put into three separate paper bags and oven-dried at 65 °C to constant weights to determine the dry weight (DW) of each component. Leafy index, leaf succulence, hypocotyl succulence, and seedling density were calculated using Eq. (1–4).

$$\text{Leafy index} = \frac{\text{FW of cotyledons (g)}}{\text{FW of hypocotyls (g)}} \quad (1)$$

$$\text{Leaf succulence (g H}_2\text{O g}^{-1}\text{DW)} = \frac{\text{cotyledon FW} - \text{cotyledon DW}}{\text{cotyledon DW}} \quad (2)$$

$$\text{Hypocotyl succulence (g H}_2\text{O g}^{-1}\text{DW)} = \frac{\text{hypocotyl FW} - \text{hypocotyl DW}}{\text{hypocotyl DW}} \quad (3)$$

$$\text{Seedling density (no/ pot)} = \frac{\text{DW of six sampled plants} + \text{DW of remianing plants}}{\text{DW of six sampled plants} / 6} \quad (4)$$

After harvesting, for each species, another six plants from each replicate per treatment were randomly selected from non-harvested pots for observation of stem and cotyledon morphology. For the morphology observations, the sampled plants were cut at the root-shoot junction, and the cotyledons, with petioles, were cut from the stems. The stems were then straightened and the cotyledons were fully unfolded using sticky, clear, plastic tape and laid on an A4 white sheet of paper with the upper cotyledon surface outward. The papers with stems, cotyledons, and a standard scale were then scanned using a CanoScan LiDE 25 scanner (Canon Canada Inc., Brampton, ON, Canada). The scanned images were processed using ImageJ 1.42 software (National Institute of Health, USA). Hypocotyl length, diameter, and hue angle, cotyledon length and width, and petiole length were determined from the scanned images. For hypocotyl hue angle measurements, the RGB values (i.e., the amount of red, green, and blue light emitted from each pixel in the image; measured on a scale of 0 to 255) were determined using ImageJ 1.42 software (National Institute of Health, USA), and

subsequently, hue angle were calculated from these RGB values using the formulas by Karcher and Richardson (2003). Hue angle is defined as an angle on a continuous, circular scale from 0 to 360° (0° = red, 60° = yellow, 120° = green, 180° = cyan, 240° = blue, 300° = magenta) (Karcher and Richardson 2003).

For each species another six to ten plants from each replicate per treatment were randomly selected from non-harvested pots to determine soluble solids content (SSC, °Brix). The sampled plants were ground in a mortar, and the filtered juice was used for the measurement using a digital pocket refractometer (PAL-1, Spectrum Technologies Inc., Aurora, IL, USA).

Statistical analysis

Data were subjected to one-way analysis of variance using the Data Processing System Software (DPS, version 7.05; Refine Information Tech. Co., Hangzhou, China) and were presented as means $\pm$ SE (standard error).

Results

Plant height

Comparing to light treatment, dark treatment increased canopy height by 17% for sunflower, but had no effect on the height of arugula (Fig.1 A). Hypocotyl length increased for both species under dark vs. light treatment, by 26% and 28% for sunflower and arugula, respectively (Fig.1 B). The plants under dark vs. light treatment appeared lodging-susceptible, especially for arugula, which might partly explain the different responses in canopy height and hypocotyl length for arugula.

Crop yield

Comparing to light treatment, dark treatment increased microgreen yield by 13% for sunflower, and reduced by 24% for arugula (Fig.2 A). Similarly, individual FW increased by 21% for sunflower, and reduced by 36% for arugula under dark vs. light treatment (Fig.2 B). There was no difference in microgreen density (≈ 80.0 and 80.3 seedlings/pot or ≈ 11114 and 11073 seedlings m^{-2} for sunflower and arugula, respectively) between the two treatments for both species (data not shown). Apparently, for both species, the variation in microgreen yield under dark vs. light treatment was resulted from the change in individual plant FW.

Product quality

Dark vs. light treatment resulted in a smaller cotyledon for arugula, with a 41% shorter petiole length and 25% shorter blade length and width, but did not change cotyledon size for sunflower (Fig. 3A-B and D). Leafy index of microgreens was lower under dark vs. light treatment for both species (Fig. 3C). There were no differences in hypocotyl diameter and CCI between dark and light treatments for both species (Fig. 3E and G). However, dark vs. light treatment reduced hypocotyl hue angle from around 65° to 38° for arugula; the stems appeared light red under the dark treatment and light green under the light treatment (Fig. 3F, and Fig.4).

Dark vs. light treatment increased hypocotyl succulence by 23% for arugula and cotyledon succulence by 14% for sunflower (Fig. 5). However, there were no differences in hypocotyl succulence for sunflower and cotyledon succulence for arugula under dark vs. light treatment. Also, dark vs. light treatment did not change SSC for both species.

Discussion

Early-stage dark promotes hypocotyl elongation of microgreens

A previous study indicated that among shoot growth traits, shoot length has the highest plasticity in response to RB-LED light vs. dark (Kong and Zheng 2019b). After germination, seedlings growing in darkness develop long hypocotyls, and light inhibition of hypocotyl elongation is one of the most common phenomena for de-etiolated seedlings (Maloof et al. 2001). In darkness, the limited resources in the seed are mainly allocated to hypocotyl growth (Kircher and Schopfer 2012). In the present study, when black covers were removed from dark-treated microgreens, the plants were much taller than those under light treatment. Since the increased hypocotyl elongation was not reversible, subsequent lighting did not eliminate the difference in hypocotyl length of plants under early-stage dark vs. light treatment. This hypocotyl elongation promoted by early-stage dark treatment would potentially benefit machine harvest.

It is worthwhile to note that for arugula, early-stage dark treatment did not increase its final canopy height despite increased hypocotyl length. There are at least two possible reasons. One is that the arugula plants under early-stage dark treatment were so lodging-susceptible that most hypocotyls did not grow straight, which reduced the contribution of hypocotyl elongation to canopy height. Another is that dark treatment reduced cotyledon size (including petiole length, maximum blade length and width) of arugula microgreens, which could partly offset the contribution of promoted hypocotyl elongation to plant height.

The effect of early-stage dark treatment on yield differs among plant species

In the present study, early-stage dark relative to light treatment increased yield for

sunflower, but reduced yield for arugula microgreens. The different yield responses between the two microgreen species might be related to their different leafy index. The leafy index for arugula was above 1.0 (i.e., 1.4–2.0), but below 1.0 (i.e., 0.8–0.98) for sunflower. This suggests that hypocotyls are heavier than cotyledons for sunflower microgreens, but hypocotyls are much lighter than cotyledons for arugula microgreens. Apparently, the biomass of hypocotyls and cotyledons played an important role in the yield of sunflower and arugula, respectively. So it is possible that early-stage dark treatment increased sunflower yield by promoting hypocotyl elongation, but reduced arugula yield by inhibiting cotyledon expansion.

The increased yield of sunflower microgreens under early-stage dark treatment indicates that deprivation of light even after germination for a short period might be an economically efficient way to improve this species' productivity due to the reduction in lighting cost. However, precautions should be taken in adopting the early-stage dark treatment for promoting hypocotyl elongation of arugula microgreens, since it resulted in yield decrease. In the present study, arugula had much smaller seed size than sunflower. A previous study on 18 genotypes of vegetable seedlings indicated that the sensitivity of plant response to LED light vs. dark decreases non-linearly with increasing seed size (Kong and Zheng 2019b). Possibly, smaller-seed genotypes depend more on energy from light to drive the development of de-etiolated seedlings due to less seed storage than larger-seed genotypes (Kircher and Schopfer 2012).

Early-stage dark effects on microgreens quality vary with attributes and species

For appearance quality, one obvious effect of early-stage dark treatment was reduced

cotyledon size of arugula microgreens, despite promoted hypocotyl elongation. Similarly, in *Arabidopsis*, a dark- vs. light-grown seedling can increase cell elongation rate in hypocotyl, while reduce cell expansion and cell division in cotyledon (Von Arnim and Deng 1996). In a given species, specific effects of dark vs. light can differ drastically between organs or cell types, and even between neighboring cells (Von Arnim and Deng 1996). However, the cotyledon size reduction was not found in sunflower microgreens under early-stage dark treatment. Possibly, cotyledon expansion of sunflower depended more on seed storage due to its larger seed size compared with arugula. Another obvious effect of early-stage dark treatment on appearance quality was reduced greenness and increased redness in the hypocotyls of arugula microgreens despite no effect on cotyledon greenness. This suggests that several-day lighting before harvesting could fully promote chlorophyll biosynthesis in cotyledons of microgreens that experienced early-stage dark treatment. In this case, the plants under early-stage dark treatment finally reached similar leaf greenness as those under early-stage light treatment. However, the greenness recovery did not occur in hypocotyls of arugula. Similarly, different color response between cotyledon and hypocotyl was also found in pea shoots (Virgin 1993). Differing from pea shoots, the hypocotyls of arugula microgreens experienced early-stage dark treatment appeared reddish rather than pure white. This might be a result of the increased anthocyanin biosynthesis under before-harvest lighting, or the white background due to reduced chlorophyll biosynthesis under early-stage dark treatment. The exact explanation still needs further study. Nevertheless, this light redness of hypocotyl may increase the appearance attraction of microgreens.

Microgreen succulence was increased by early-stage dark treatment for both species. This

improved succulent texture by early-stage darkness could potentially improve the palatability of microgreens. In contrast, increased light levels have been reported to reduce tissue succulence in pea shoots (Kong et al. 2018), pea pods (Kong and Zheng 2019a), broccoli and tomato seedlings (Dorais and Gosselin 2002) and turf (Hurdzan 1969). The increase in succulence from early-stage dark treatment was found only in cotyledons for sunflower and hypocotyls for arugula microgreens, suggesting different organ sensitivity among species. As mentioned before, the contribution to microgreen yield is small from the biomass of cotyledons for sunflower and that of hypocotyls for arugula. Consequently, the increased succulence in the organs by early-stage dark treatment should have little effects on the yields of the two microgreen species. It is worthwhile to note that early-stage dark treatment did not reduce SSC, a common indicator of sugar level, for both species. Possibly, early-stage light availability mainly affected photomorphogenesis, but had little effects on photosynthesis, and thereby photo-assimilate (e.g., sugar). The similar SSC indicates that dark treatment might affect the sweetness of the two microgreens to a small degree. Nevertheless, some biochemical measurements on sugar, anthocyanin, and chlorophyll contents are necessary for further evaluation of the effects of early-stage dark treatment on microgreen phytochemical related qualities.

Conclusion

In summary, for indoor microgreen production using sole-source LED lighting, early-stage dark treatment promoted hypocotyl elongation in both sunflower and arugula, but had different effects on yield and quality between the two species. For sunflower, early-stage

dark treatment increased microgreen yield and cotyledon succulence. However, for arugula, early-stage dark treatment reduced microgreen yield and cotyledon size, despite increased redness and succulence of hypocotyls. Therefore, early-stage dark treatment is an effective way to promote hypocotyl elongation without compromising microgreen yield and at least appearance quality for larger-seed species (e.g., sunflower); however, precautions should be taken for smaller-seed species (e.g., arugula) due to its potential negative effects on yield and quality.

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References

- Alrifai, O., Hao, X., Marcone, M. F. and Tsao, R. 2019. Current review of the modulatory effects of LED lights on photosynthesis of secondary metabolites and future perspectives of microgreen vegetables. *J. Agric. Food Chem.* **67**: 6075-6090.
- Dorais, M. and Gosselin, A. 2002. Physiological response of greenhouse vegetable crops to supplemental lighting. *Acta Hort.* **580**: 59-67.
- Fankhauser, C. and Chory, J. 1997. Light control of plant development. *Annu. Rev. Cell Dev.*

- Biol. **13**: 203-229.
- Gao, M., He, R., Shi, R., Zhang, Y., Song, S., Su, W. and Liu, H. 2021. Differential Effects of Low Light Intensity on Broccoli Microgreens Growth and Phytochemicals. *Agronomy*. **11**: 537.
- Hurdzan, M. J. 1969. Effect of light intensity on seedling growth in red fescue (*festuca rubra* L.), The University of Vermont. Master's dissertation, Burlington, USA.
- Jones-Baumgardt, C., Llewellyn, D., Ying, Q. and Zheng, Y. 2019. Intensity of sole-source light-emitting diodes affects growth, yield, and quality of Brassicaceae microgreens. *HortScience*. **54**: 1168-1174.
- Kami, C., Lorrain, S., Hornitschek, P. and Fankhauser, C. 2016. Light-regulated plant growth and development, p. 29-66. *Current Topics in Developmental Biology*. Academic Press, New York, USA.
- Karcher, D. E. and Richardson, M. D. 2003. Quantifying turfgrass color using digital image analysis. *Crop Sci.* **43**: 943-951.
- Kircher, S. and Schopfer, P. 2012. Photosynthetic sucrose acts as cotyledon-derived long-distance signal to control root growth during early seedling development in *Arabidopsis*. *Proc. Natl. Acad. Sci.* **109**: 11217-11221.
- Kong, Y., Kamath, D. and Zheng, Y. 2019. Blue versus red light can promote elongation growth independent of photoperiod: A study in four Brassica microgreens species. *HortScience*. **54**: 1955-1961.
- Kong, Y., Llewellyn, D. and Zheng, Y. 2018. Response of growth, yield and quality of pea shoots to supplemental LED lighting during winter greenhouse production. *Can. J.*

Plant Sci. **98**: 732-740.

Kong, Y. and Zheng, Y. 2019a. Response of growth, yield and quality of edible-podded snow peas to supplemental light-emitting diode lighting during winter greenhouse production. Can. J. Plant Sci. **99**: 676-687.

Kong, Y. and Zheng, Y. 2019b. Variation of phenotypic responses to lighting using combination of red and blue light-emitting diodes versus darkness in seedlings of 18 vegetable genotypes. Can. J. Plant Sci. **99**: 159-172.

Kozai, T., Niu, G. and Takagaki, M. 2015. Plant factory: an indoor vertical farming system for efficient quality food production. Academic press, Amsterdam.

Kyriacou, M. C., Roupael, Y., Di Gioia, F., Kyrtziz, A., Serio, F., Renna, M., De Pascale, S. and Santamaria, P. 2016. Micro-scale vegetable production and the rise of microgreens. Trends Food Sci. Technol. **57**: 103-115.

Maloof, J. N., Borevitz, J. O., Dabi, T., Lutes, J., Nehring, R. B., Redfern, J. L., Trainer, G. T., Wilson, J. M., Asami, T. and Berry, C. C. 2001. Natural variation in light sensitivity of *Arabidopsis*. Nat. Genet. **29**: 441-446.

Rajan, P., Lada, R. R. and MacDonald, M. T. 2019. Advancement in Indoor Vertical Farming for Microgreen Production. Am. J. Plant Sci. **10**: 1397.

Seluzicki, A., Burko, Y. and Chory, J. 2017. Dancing in the dark: Darkness as a signal in plants. Plant Cell Environ. **40**: 2487-2501.

Sysoeva, M. I., Markovskaya, E. F. and Shibaeva, T. G. 2010. Plants under continuous light. Plant Stress. **4**: 5-17.

Treadwell, D., Hochmuth, R., Landrum, L. and Laughlin, W. 2015. Microgreens: A new

- specialty crop. University of Florida IFAS Extension HS1164.
<https://edis.ifas.ufl.edu/pdffiles/HS/HS116400.pdf>.
- Turner, E. R., Luo, Y. and Buchanan, R. L. 2020. Microgreen nutrition, food safety, and shelf life: A review. *J. Food Sci.* **85**: 870-882.
- Virgin, H. I. 1993. Effectiveness of light of different wavelengths to induce chlorophyll biosynthesis in rapidly and slowly greening tissues. *Physiol. Plant.* **89**: 761-766.
- Von Arnim, A. and Deng, X.-W. 1996. Light control of seedling development. *Annu. Rev. Plant Biol.* **47**: 215-243.
- Ying, Q., Kong, Y., Jones-Baumgardt, C. and Zheng, Y. 2020a. Responses of yield and appearance quality of four Brassicaceae microgreens to varied blue light proportion in red and blue light-emitting diodes lighting. *Sci. Hort.* **259**: 108857.
- Ying, Q., Kong, Y. and Zheng, Y. 2020b. Applying blue light alone, or in combination with far-red light, during nighttime increases elongation without compromising yield and quality of indoor-grown microgreens. *HortScience*. **1**: 1-6.
- Ying, Q., Kong, Y. and Zheng, Y. 2020c. Overnight supplemental blue, rather than far-red, light improves microgreen yield and appearance quality without compromising nutritional quality during winter greenhouse production. *HortScience*. **55**: 1468-1474.
- Zhang, G. and Hu, Y. 2008. *Production Technology of Sprouts and Microgreens*. Jin Dun Publishing House, Beijing, China.
- Zhang, X., Bian, Z., Yuan, X., Chen, X. and Lu, C. 2020. A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. *Trends Food Sci. Technol.* **99**: 203-216.

Figure captions:

Fig. 1 Plant height (A) and hypocotyl length (B) of sunflower and arugula microgreens under early-stage dark treatment vs. no dark treatment. Data are means $\pm$ SE (n = 6). For each species, bars marked with ns or *, **, *** denote that treatment effects are not significant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Fig. 2 Yield (A) and individual plant fresh weight (FW; B) of sunflower and arugula microgreens under early-stage dark treatment vs. no dark treatment. Data are means $\pm$ SE (n = 6). For each species, bars marked with ns or *, **, *** denote that treatment effects are not significant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Fig. 3 Some appearance quality traits of sunflower and arugula microgreens under early-stage dark treatment vs. no dark treatment. Data are means $\pm$ SE (n = 6). Leafy index = ratio of cotyledon/ hypocotyl fresh weight; CCI: chlorophyll content index. For each species, bars marked with ns or *, **, *** denote that treatment effects are not significant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Fig. 4 Residual stems after harvesting of arugula microgreens under early-stage dark treatment vs. no dark treatment. The pictures were taken immediately after harvesting (at 12 d after seeding).

Fig. 5 Hypocotyl succulence, leaf succulence, and soluble solids content (SSC) of sunflower and arugula microgreens under early-stage dark treatment vs. no dark treatment. Data are means $\pm$ SE (n = 6). For each species, bars marked with ns or *, **, *** denote that treatment effects are not significant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Fig. 1

Fig. 1 Plant height (A) and hypocotyl length (B) of sunflower and arugula microgreens under early-stage dark treatment vs. no dark treatment. Data are means $\pm$ SE ($n = 6$). For each species, bars marked with ns or *, **, *** denote that treatment effects are not significant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Fig. 2

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Fig.3

Fig. 3 Some appearance quality traits of sunflower and arugula microgreens under early-stage dark treatment vs. no dark treatment. Data are means $\pm$ SE (n = 6). Leafy index = ratio of cotyledon/ hypocotyl fresh weight; CCI: chlorophyll content index. For each species, bars marked with ns or *, **, *** denote that treatment effects are not significant or significant at $P \leq 0.05$, 0.01 , 0.001 , respectively.

Fig. 4

Fig. 4 Residual stems after harvesting of arugula microgreens under early-stage dark treatment vs. no dark treatment. The pictures were taken immediately after harvesting (at 12 d after seeding).

215x279mm (600 x 600 DPI)

Fig. 5

Fig. 5 Hypocotyl succulence, leaf succulence, and soluble solids content (SSC) of sunflower and arugula microgreens under early-stage dark treatment vs. no dark treatment. Data are means $\pm$ SE (n = 6). For each species, bars marked with ns or *, **, *** denote that treatment effects are not significant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.