horticulturae

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Effect of Temperature Variation and Blue and Red LEDs on the Elongation of Arugula and Mustard Microgreens

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Abstract: Recent studies using LED lighting at low to modest intensity have indicated that compared with red light, blue light can promote plant elongation in many crops as a shade avoidance response despite varying sensitivity with light environments, plant species, and growth stages. Currently, there is limited understanding of how temperature affects the blue light-mediated plant response. To clarify this point, two microgreen species (arugula and mustard) were grown indoors under two light quality × two temperature treatments: red LED light (peak at 670 nm) and blue LED light (peak at 450 nm) at 18 °C or 28 °C. A photosynthetic photon flux density of 110 μmol m^{-2} s^{-1} and a photoperiod of 12 h d^{-1} were used for all treatments. After 6 to 8 days of treatment, at both temperatures, blue vs. red light promoted plant elongation, as demonstrated by a greater plant elongation rate, final plant height, and hypocotyl length, in arugula but not in mustard. Blue vs. red light also promoted some shade-avoidance responses such as decreased cotyledon size in both species and increased petiole length and dry mass partitioning to hypocotyls in arugula only. The elongation promotion in arugula by blue light was greater at 18 °C than at 28 °C, showing interactions between light and temperature on most plant traits. For mustard, plant elongation was promoted at 28 °C compared to 18 °C independent of light treatment, showing no interactions between light and temperature on most plant traits. These results suggest that the blue light-mediated elongation as a shade-avoidance response is not reversed by high temperature, despite the varying sensitivity with temperatures and species.

Keywords: hypocotyl length; light wavelength; plant height; shade avoidance response; temperature interaction

Citation: Kong, Y.; Masabni, J.; Niu, G. Effect of Temperature Variation and Blue and Red LEDs on the Elongation of Arugula and Mustard Microgreens. *Horticulturae* **2023**, *9*, 608. https://doi.org/10.3390/horticulturae9050608

Academic Editor: Julė Jankauskienė

Received: 17 April 2023
Revised: 12 May 2023
Accepted: 16 May 2023
Published: 22 May 2023

1. Introduction

Compared with dark, both blue and red light can inhibit plant elongation by activating their main photoreceptor: phytochrome and cryptochrome, respectively [1]. In many previous studies using broad-band light sources (e.g., fluorescent lamps), blue light was also reported to have a stronger inhibitory effect on plant elongation than red light [2–7]. However, more and more recent studies using narrow-band LED lights indicate that compared with red light, blue light at low to modest photosynthetic photon flux density (PPFD; around 50–200 μmol m^{-2} s^{-1}) can promote plant elongation in many species such as arugula, cabbage, kale, petunia, calibrachoa, geranium, and marigold [8–17]. Possibly, a narrow-band light source such as the blue LED provided purer blue light than a broad-band light source such as the blue fluorescent lamp. The broad-band blue light source that normally contains low-level other wavelengths (e.g., a high ratio of red/far-red lights) can activate both phytochrome and cryptochrome, thus showing greater inhibitory effects on plant elongation relative to red light [3,15,17,18]. In contrast, the plant elongation promoted by blue light from LED lighting was found to be related to a low phytochrome activity,

which may reduce cryptochrome activity and increase phytotropin activity, due to a cross-talk between different light receptors [15,19–22]. The promoted plant elongation under blue vs. red light has been confirmed by a series of studies using LED lights at different photoperiods, light intensities, and peak wavelengths, although the response sensitivity varies with light environments, plant species, and growth stages [14,23–26]. However, whether the response sensitivity can be affected by temperature is still unknown and needs to be studied.

Recent studies on *Arabidopsis* indicate that phytochrome (especially phyB) is a major temperature sensor [27]. With the temperature increasing from 12 °C to 27 °C, the phytochrome activity was reduced and the plant stem was elongated [27]. Furthermore, phytochrome is the main receptor of red light, so the effect of red light on plant elongation can also be affected by temperature [27,28]. It has been found that the plant elongation response to red light was strictly temperature dependent; the red light promoted hypocotyl extension at 27 °C, whereas it repressed hypocotyl elongation at 17 °C or 22 °C [28]. On the contrary, blue light represses high temperature-mediated hypocotyl elongation [29]. In this case, at higher temperatures (e.g., ≥28 °C), plant elongation may not be promoted by blue light, but by red light for the plant species such as arugula, which was consistently taller under blue vs. red light from LED lighting at a PPFD of around 100 μmol m^{-2} s^{-1} in previous studies [17–19,23]. However, in those studies, plants were grown at a constant temperature of 23 °C. It is unclear whether monochromatic blue light can still promote stem elongation at a temperature higher than 23 °C in plant species such as arugula, compared with red light.

In previous studies, under LED lighting, plant elongation responses to blue light relative to red light also varied with different species. For example, differing from arugula, mustard seedlings were similarly tall under blue vs. red light at a PPFD of 50–100 μmol m^{-2} s^{-1} and an air temperature of 23 °C [17,19]. In fact, in those studies, mustard seedlings were elongated under both blue and red light. In other words, compared with blue light, red light showed a similar promotion effect on plant elongation in mustard, despite an inhibitory effect in other species such as arugula at 23 °C. A recent study on *Arabidopsis* indicated that with the intensity of red light increasing up to 100 μmol m^{-2} s^{-1}, hypocotyl elongation was inhibited at 17–22 °C, but was promoted at 27 °C [28]. Plant elongation response to the red light is very sensitive to temperature change and some species can distinguish differences of 1 °C [27]. Possibly, differing from arugula, mustard's elongation response to red light is more temperature sensitive. In this case, at a lower temperature (e.g., 18 °C), due to an increased inhibitory effect of red light, blue vs. red LEDs may promote stem elongation in mustard similarly to arugula.

Our recent studies indicate that the promoted plant elongation under blue LED light at low to modest intensity is a shade-avoidance response [15,19]. In addition to an elongated stem, longer petiole, lighter color, smaller cotyledon, and greater dry mass partitioning to stem were also observed under blue vs. red LED at 23 °C and a photoperiod ≥16 h d^{-1} [14,17–19,23]. Besides blue LED, higher temperatures, especially at night, can also cause plant elongation similar to the shade-avoidance response through the inactivation of phytochromes [30]. It is well known that under natural conditions, the activity of phytochromes in plants decreases gradually at night. The rate of phytochrome inactivation is proportional to increasing temperature in the dark [27]. Under indoor conditions, when artificial light is turned off, it takes hours to inactivate phytochrome in the dark [31,32]. For indoor production, a 16-h photoperiod has been popularly adopted. However, it is unknown whether and how temperature variation affects blue LED-mediated plant elongation and other growth traits relevant to shade-avoidance responses for indoor-grown plants when plants are grown under a photoperiod shorter than 16 h (i.e., a darker period longer than 8 h)

Arugula and mustard are two popular microgreen species for indoor production. Taller microgreen plants can benefit machine harvesting [23]. LED lighting at low to modest PPFD (e.g., around 100 μmol m^{-2} s^{-1}) are commonly used for indoor microgreen production. Studying plant elongation responses to LED light under different environmental conditions

will provide useful information for microgreen growers. Despite many previous studies on plant elongation responses to blue vs. red LED light at a PPFD of around 100 μmol m^{-2} s^{-1} in arugula and mustard seedlings, the air temperature during plant growth was 23 ± 1 °C, and the photoperiod of LED lighting was 16–24 h d^{-1}. The objectives of this study were to examine whether blue vs. red light can still promote plant elongation in arugula at a temperature higher than 23 °C (e.g., 28 °C), whether blue vs. red light can promote plant elongation in mustard at a temperature lower than 23 °C (e.g., 18 °C), and whether and how temperature variation affects plant elongation and other traits relevant to the shade-avoidance response mediated by blue vs. red light for the two microgreen species grown indoor at a low to modest intensity (e.g., around 100 μmol m^{-2} s^{-1}) and a shorter photoperiod than 16 h d^{-1} (e.g., 12 h d^{-1}).

2. Materials and Methods

2.1. Plant Materials and Growing Conditions

Two microgreen species, arugula (*Eruca sativa*; Johnny's Selected Seeds, Winslow, ME, USA) and mustard (*Brassica juncea*; 'Red Mizuna' Kitazawa Seed Co., Ltd., Salt Lake City, UT, USA) were used for this study. The experiment was performed in walk-in growth chambers at the Texas A&M AgriLife Research and Extension Center in Dallas, TX, USA, and was replicated three times over time from April to June of 2022 (Table 1). Seeds were sown in 200-cube rockwools (Starter Plugs, Grodan Inc., Kingsville, ON, Canada), which were pre-soaked in tap water and placed in nursery trays (25 cm by 51 cm). Each tray had two species and each species occupied half rockwool cubes. The sown trays were moved to the growth chambers to begin light and temperature treatments until plant harvest when the cotyledons were fully expanded for all plants.

Table 1. Plant materials and key time points for this experiment.

Plant Species	Replication	Temperature Treatment	Seeding Date	Germination Time (d)	Harvest Time (d)
Arugula	1	HT	April 19	April 20 (1)	April 25 (6)
		LT	April 19	April 22 (3)	April 27 (8)
	2	HT	May 3	May 4 (1)	May 9 (6)
		LT	May 3	May 6 (3)	May 11 (8)
	3	HT	May 24	May 25 (1)	May 30 (6)
		LT	May 24	May 27 (3)	June 1 (8)
Mustard	1	HT	April 19	April 21 (2)	April 25 (6)
		LT	April 19	April 23 (4)	April 27 (8)
	2	HT	May 3	May 5 (2)	May 9 (6)
		LT	May 3	May 7 (4)	May 11 (8)
	3	HT	May 24	May 26 (2)	May 30 (6)
		LT	May 24	May 28 (4)	June 1 (8)

Note: Germination date means the time that seeds germinated in more than 95% of cells. For germination or harvest time, the data inside round brackets behind dates indicate the days after seeding. HT = high temperature treatment (28 °C); LT = low temperature treatment (18 °C).

The plants were sub-irrigated with nutrient solutions once every 2–5 d, with increasing frequency dependent upon the stage of plant growth. The nutrient solution (150.0 mg L^{-1} N; EC ≈ C 1.4 dS m^{-1}, pH = 6.3) was made by adding water-soluble fertilizer, Peters Professional® 20–10–20 (N—P_2O_5—K_2O; Jr. Peters, Allentown, PA, USA) to tap water (EC = 0.4 dS m^{-1}, pH = 7.8). The temperature and relative humidity (RH) inside the growth chamber are presented in Table 2.

Table 2. Environmental conditions under different treatments.

Temperature Treatment	Light Treatment	Shelf Environment		Room Environment		
		PPFD ($\mu mol\ m^{-2}s^{-1}$)	Air Temp. (°C)	Air Temp. (°C)	Max. RH (%)	Min. RH (%)
HT	Red light	109 ± 4	28.2 ± 0.1	27.9 ± 0.2	64.0 ± 4.6	55.5 ± 3.4
	Blue light	114 ± 5	28.3 ± 0.1			
LT	Red light	110 ± 3	17.4 ± 0.3	17.6 ± 0.2	87.3 ± 1.2	75.9 ± 0.9
	Blue light	111 ± 2	17.4 ± 0.3			

Note: Data in the table are means ± standard errors (n = 3). HT = high temperature; LT = low temperature; PPFD = photosynthetic photon flux density; temp.= temperature; max. = maximum; min. = minimum; RH = relative humidity.

2.2. Experimental Design and Treatments

The experiment was conducted with two factors (temperature and light) in a split-plot design for each species with three replicates over time (Table 1). The temperature and light treatments were allocated to the main plots (i.e., separated rooms in the walk-in chamber) and subplots (i.e., different shelves at the growth rack in each room), respectively. Two temperature treatments, low temperature (LT) and high temperature (HT) were set up by adjusting the room temperature with a target at 18 °C and 28 °C through air conditioners. The two light treatments included red light and blue light, which were emitted from the monochromatic red LED (450 nm) and blue LED (670 nm), respectively (Figure 1). The location of each light/temperature treatment was randomized among the rack shelves/walk-in rooms for the different replicates.

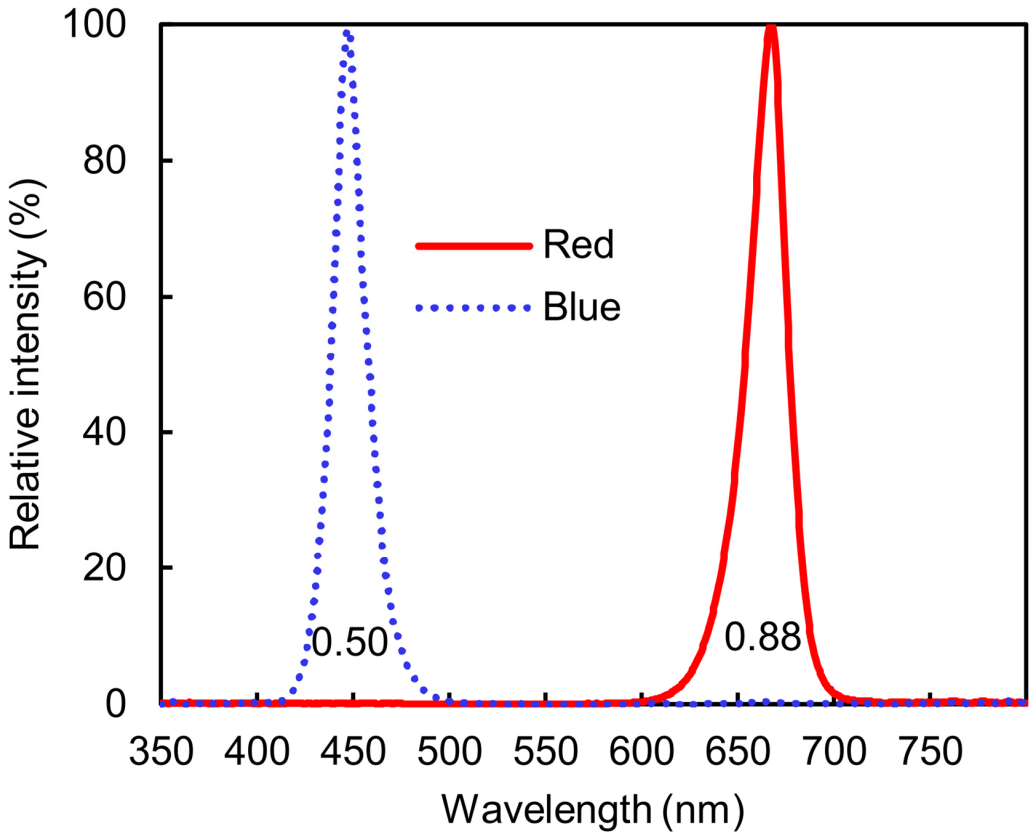

Figure 1. Light spectral distributions of red and blue LEDs in this experiment. The numbers inside the peak waves are values of phytochrome photostationary state (PPS), which are calculated based on light spectral distribution [15].

Both light treatments were set up at a PPFD of around 110 $\mu mol\ m^{-2}\ s^{-1}$ for 12 h d^{-1} (7:30–19:30). The light treatments were created by the tunable PHYTOFY® RL LED lighting

system (OSRAM GmbH, Munich, Germany) through PHYTOFY® RL control software (OSRAM GmbH).

2.3. Measurement of Biometric Parameters

Seed germination was checked daily. When seeds germinated over 50% of cells for each species under each light treatment, the number of cells with germinated seeds was counted and the percentage of germinated cells was calculated. For each replicate, both at the beginning of cotyledon unfolding and at the end of light treatments, plant heights were measured in 20 plants randomly selected from each light treatment for each species. The plant elongation rate (cm d^{-1}) was calculated following the method by Kong et al. [23] using Equation (1):

$$\text{Elongation rate} = \frac{\text{Final plant height} - \text{Initial plant height}}{\text{Days between the initial and final measurements}} \quad (1)$$

After 6–8 d light treatments, for each replicate, the sampled 20 plants from each species under each light treatment were used for the quantification of hypocotyl and cotyledon size and color. For the measurement of size and color, the detailed method by Kong et al. [23] was followed. Briefly, for the sampled plants, the aerial parts were cut at the root–shoot junction, and the cotyledons, with petioles, were separated from the hypocotyls. The hypocotyls, cotyledons, and a standard scale were scanned together using a scanner.

After scanning, the remaining plants from each species in each light treatment were used for quantification of fresh and dry mass accumulation and dry mass partitioning for each replicate. Considering a tiny weight for each plant, the remaining plants were separated into four groups (≈20 plants in each group) and all plants sampled from each group were used together as a subsample for fresh mass measurement. For each group, all harvested plants were counted and then cut at the root–shoot junction, and their total aerial fresh weight (FW) was determined, and individual aerial FW (mg $plant^{-1}$) was calculated. Then, the aerial plant parts were separated into cotyledons (with petioles) and hypocotyls (i.e., stems), and oven-dried separately at 65 °C to determine the dry weight (DW) of each component. Then, the individual aerial DW (mg $plant^{-1}$), and stem/leaf ratio were calculated.

After harvesting, based on the scanned images, hypocotyl length and diameter, petiole length, cotyledon area, maximum blade length and width, and hypocotyl and cotyledon color (i.e., RGB values) were determined using ImageJ 1.42 software (National Institute of Health, Bethesda, MD, USA). The hue angles of hypocotyls and cotyledons were calculated from the above RGB values using the formulas by Karcher and Richardson [33]. The hue angle ranges from 0 to 360°, and different angles indicate different colors: 0° = red, 60° = yellow, 120° = green, 180° = cyan, 240° = blue, and 300° = magenta [33].

2.4. Statistical Analysis

Analysis of variance was performed using the JMP 14 software (SAS, Cary, NC, USA) and data were presented as means ± SE (standard error). For each species, a two-way analysis of variance (ANOVA) was used to determine the effects of temperature, light, and temperature × light interaction on all plant traits evaluated in this study. When the temperature × light interaction was not significant, the temperature (or light) treatment effect was presented independently of the light (or temperature) treatment. When the temperature × light interaction was significant, the treatment combinations were separated using Tukey's HSD (honestly significant difference) test at $\alpha = 0.05$.

3. Results

For both species, the germination time was not different between red and blue light treatments but was delayed by two days in low vs. high temperature treatments (Table 1). Accordingly, plants in low temperature treatments were harvested two days later than those in high temperature treatments.

The plant elongation rate in the high vs. low temperature treatment was not different for arugula but was increased by 35% for mustard (Figure 2A,B). Compared with red light, blue light increased plant elongation rate by 37% at high temperatures and by 160% at low temperatures for arugula (Figure 2A). The varying promotion effect of blue light was due to an interaction between light and temperature. However, no interaction between light and temperature treatment was found for mustard, and blue vs. red light reduced plant elongation rate by 17%, regardless of the temperature (Figure 2B).

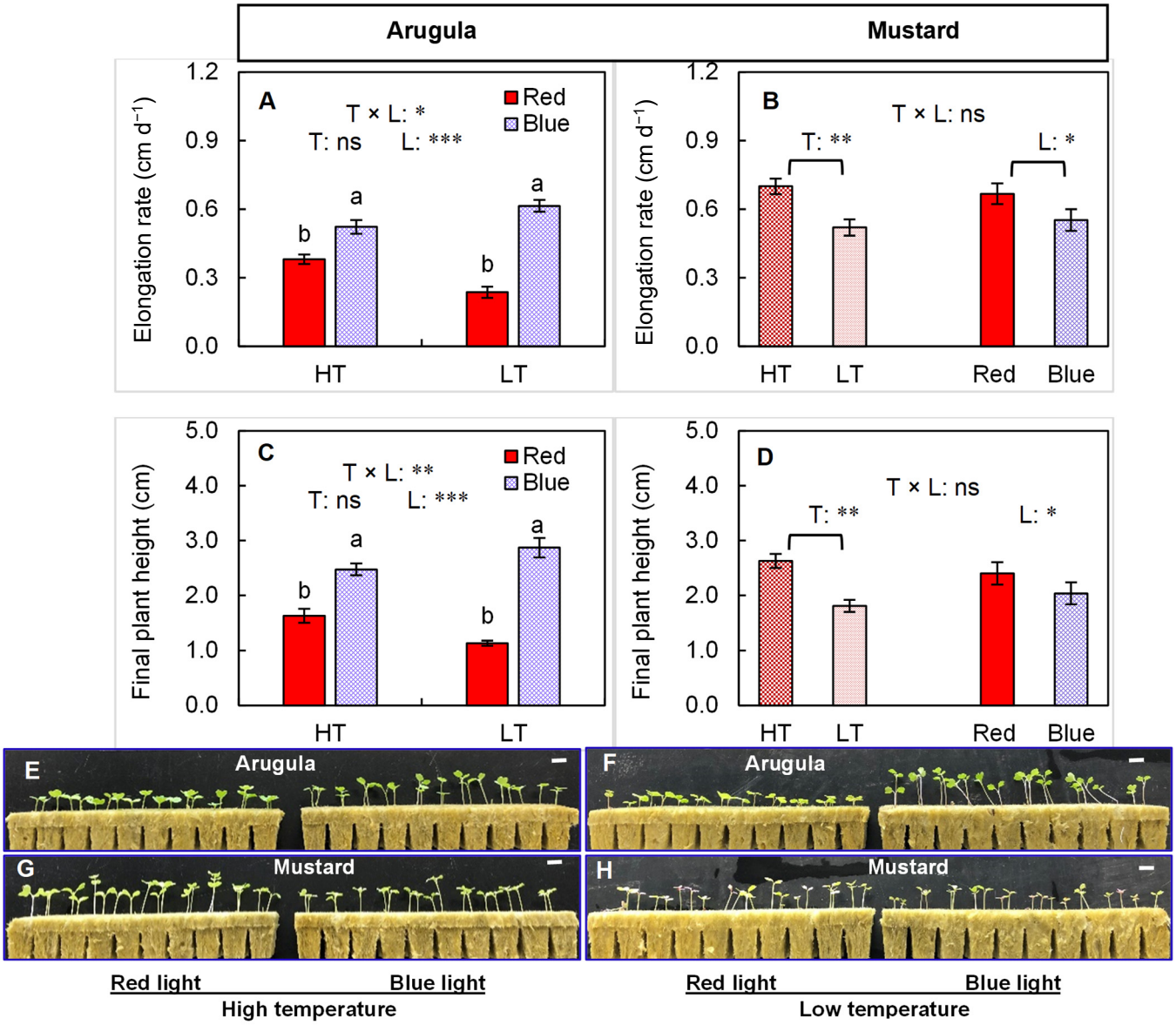

Figure 2. The elongation rate (**A**,**B**) and final height (**C**,**D**) under different light and temperature treatments, and the side-view pictures of plants (**E**,**H**) immediately before harvest. Data are means ± standard error. HT = high temperature; LT = low temperature. Treatment effects for each plant trait under different temperatures (T), lights (L), or the interactions of temperature and light (T × L) followed by ns, *, **, or *** denote that the treatment effects are not significant or significant at $\alpha = 0.05$, 0.01, or 0.001, respectively. When the treatment effects of T × L are significant, the four treatment combinations of T × L are separated using different lower-case letters at $\alpha = 0.05$ according to Tukey's HSD (honestly significant difference) test. The pictures were taken on two rows of plants growing in 20 rockwool cubes for each plant species and light treatment at high temperature on May 9 (**E**,**G**) and at low temperature on May 11 (**F**,**H**). The reference scale bars in the pictures are 1.6 cm.

At harvest, plant height was not affected by temperature treatment for arugula but was increased by 45% in high vs. low temperature treatment for mustard (Figure 2C,D). Plants grown under blue vs. red light were longer by 52% at high temperatures and by 124% at low temperatures for arugula. The varying promotion effect of blue light was due to a light × temperature interaction effect. In contrast, for mustard, plants grown under blue light were 15% shorter than those under red light, and the light treatment effect was not affected by temperature treatment. The trends of plant elongation growth in light and temperature treatment were also supported by the side-view pictures taken at plant harvesting time (Figure 2E,H).

For arugula, all traits relevant to plant size were not affected by temperature treatment but by light treatment, and there were interactive effects between light and temperature treatment on all plant size traits except blade width (Figure 3A,C,E,G,I). Specifically, compared with red light, blue light increased hypocotyl length by 42% and 102%, and petiole length by 55% and 104% for plants grown at high and low temperatures, respectively, showing a greater promotion effect at lower temperatures. Differing from hypocotyl and petiole length, blue vs. red light reduced cotyledon area by 31% and 19% at high and low temperatures, respectively, and reduced blade length by 16% at high temperatures but not at low temperatures. Regardless of temperature, blue vs. red light reduced blade width by 16% for arugula plants.

Differing from arugula, for mustard, all traits relevant to plant size were affected by either or both temperature and light treatments, and there were no interactive effects between light and temperature on all the plant traits except cotyledon area (Figure 3B,D,F, H,J). Specifically, hypocotyl increased by 41% and petiole lengths increased by 54% in high vs. low temperature treatment and did not change in blue vs. red light treatment. Blade length was not affected by temperature treatment. Temperature interacted with light affecting the cotyledon area. Blue vs. red light reduced the cotyledon area by 29% at high temperatures and by 27% at low temperatures. Blue vs. red light reduced blade length by 13%, regardless of temperature treatment. Blade width was increased by 23% in high vs. low temperature treatment and reduced by 16% in blue vs. red light treatment.

For cotyledon color, hue angle was not affected by temperature for arugula, but was greater (80° vs. 60°) in high vs. low temperature treatment regardless of light treatment for mustard (Figure 4A,B), indicating decreased leaf redness of mustard with increased temperature. Compared with red light, blue light increased hue angle from 75° to 80° (i.e., reduced leaf yellowness) at low temperatures rather than high temperatures for arugula due to an interaction between light and temperature. However, blue vs. red light did not affect leaf color regardless of temperature treatment for mustard.

For hypocotyl color, the hue angle in high vs. low temperature treatment was greater (59° vs. 34°; reduced stem redness) regardless of light treatment for arugula but was greater (66° vs. 47°; reduced stem redness) under blue light rather than red light treatment for mustard (Figure 4C,D). Compared with red light, blue light reduced the hue angle from 63° to 57° (i.e., increased stem redness) regardless of temperature treatment for arugula, and from 60° to 47° (i.e., increased stem redness) at low temperature rather than high temperature treatment for mustard.

For fresh mass, aerial fresh weight was not affected by temperature treatments for arugula but was increased by 65% at high vs. low temperatures regardless of light treatment for mustard (Figure 5A,B). Compared with red light, blue light reduced aerial fresh weight by 37% at high temperatures and by 20% at low temperatures for arugula, due to an interaction between temperature and light treatment. However, for mustard, blue vs. red light reduced aerial fresh weight (by 34%) regardless of temperature treatment.

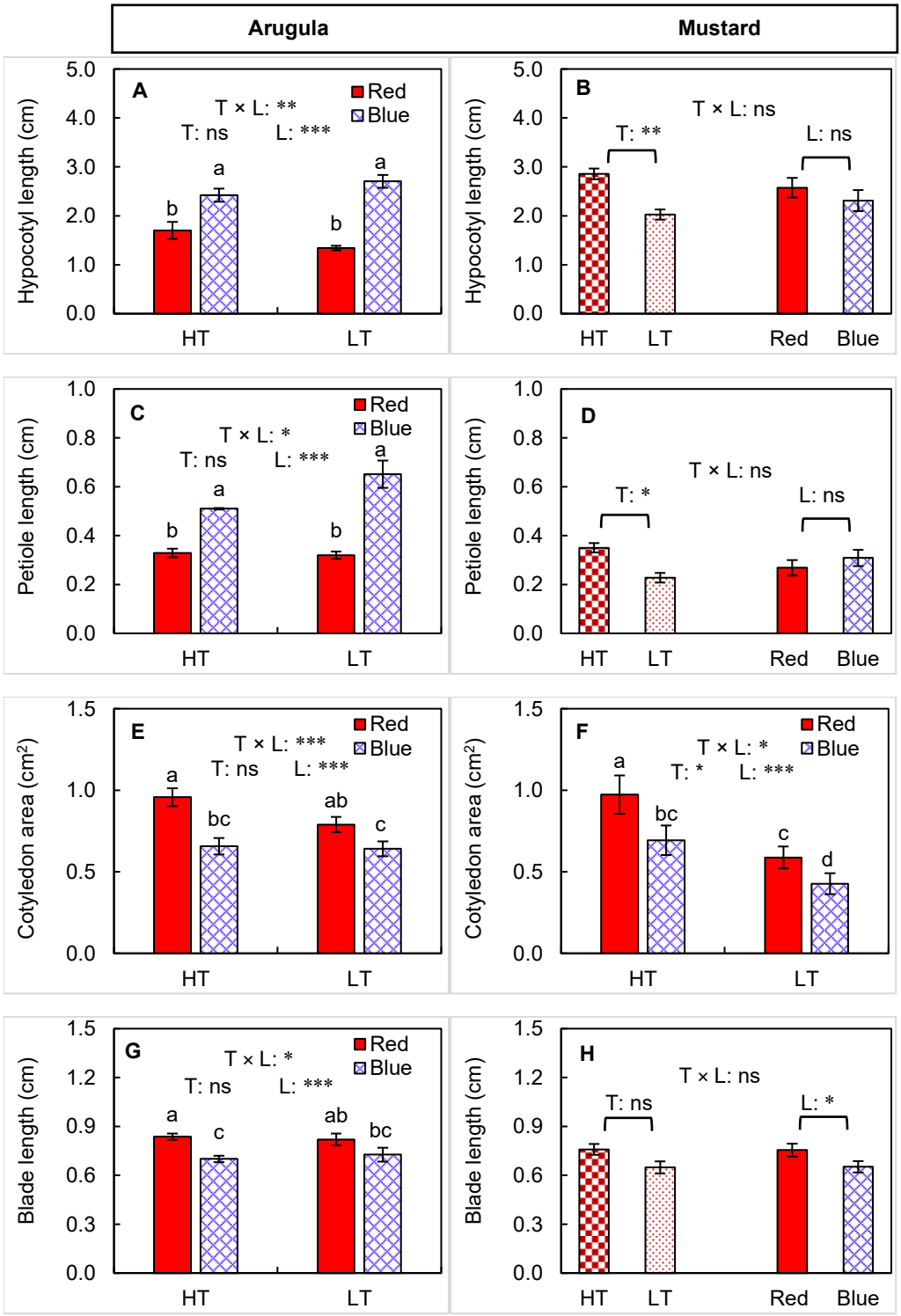

Figure 3. Hypocotyl length (**A**,**B**), petiole length (**C**,**D**), cotyledon area (E,**F**), and blade length (**G**,**H**) under different light and temperature treatments. Data are means ± standard error. HT = high temperature; LT = low temperature. Treatment effects for each plant trait under different temperatures (T), lights (L), or the interactions of temperature and light (T × L) followed by ns, *, **, or *** denote that the treatment effects are not significant or significant at α = 0.05, 0.01, or 0.001, respectively. When the treatment effects of T × L are significant, the four treatment combinations of T × L are separated using different lower-case letters at α = 0.05 according to Tukey's HSD (honestly significant difference) test.

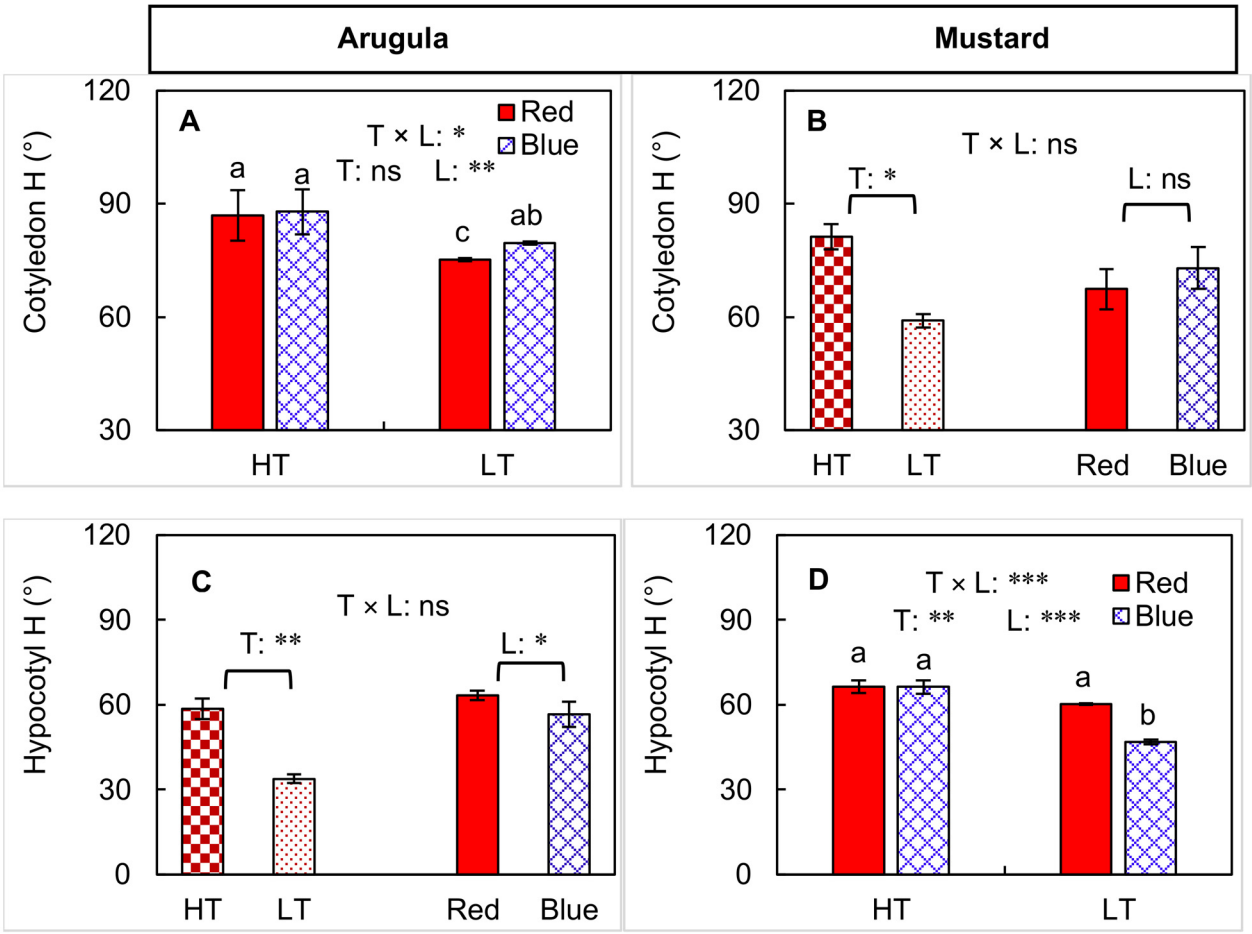

Figure 4. Color of cotyledon (**A**,**B**) and hypocotyl (**C**,**D**) under different light and temperature treatments. Data are means ± standard error. Plant color was indicated by hue angle (H). HT = high temperature; LT = low temperature. Treatment effects for each plant trait under different temperatures (T), lights (L), or the interactions of temperature and light (T × L) followed by ns, *, **, or *** denote that the treatment effects are not significant or significant at α = 0.05, 0.01, or 0.001, respectively. When the treatment effects of T × L are significant, the four treatment combinations of T × L are separated using different lower-case letters at α = 0.05 according to Tukey's HSD (honestly significant difference) test.

For dry mass, the aerial dry weight responded to all treatments similarly to fresh weights for arugula (Figure 5C); it was not affected by temperature treatments and was reduced by 43% at high temperature and by 33% at low temperature under blue vs. red light due to an interaction between temperature treatment and light treatment. Unlike arugula, for mustard, the aerial dry weight showed different responses from fresh weight (Figure 5D). Its aerial dry weight was increased by 73% at high vs. low temperature under red light rather than blue light and was reduced by 37% under blue vs. red light at high temperature rather than low temperature due to an interaction between temperature treatment and light treatment.

For dry mass partitioning, the stem/leaf dry weight ratio was not affected by temperature treatment for either arugula or mustard (Figure 5E,F). Compared with red light, blue light increased the stem/leaf dry weight ratio by 47% at high temperatures and by 86% at low temperatures for arugula due to an interaction between temperature treatment and light treatment. However, blue vs. red light did not affect the stem/leaf dry weight ratio regardless of temperature for mustard.

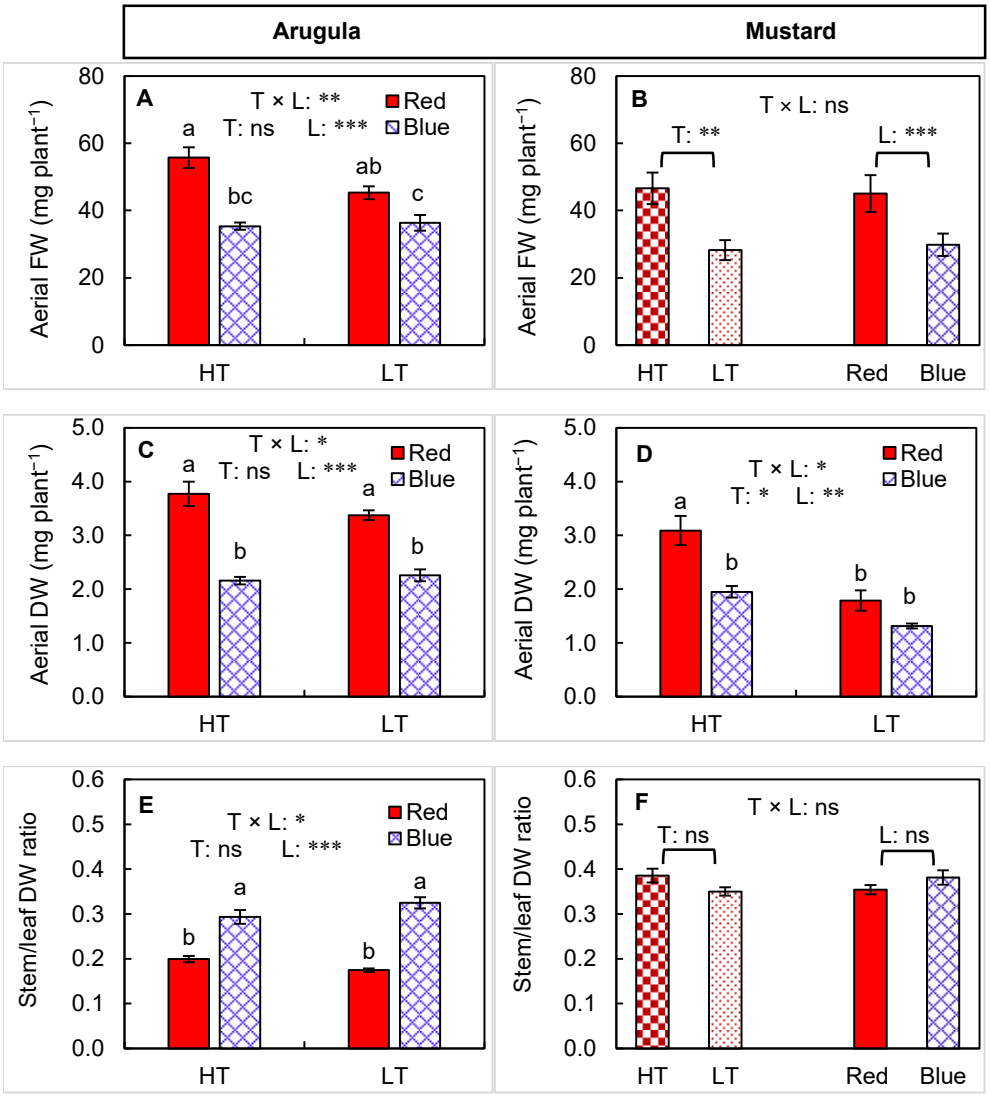

Figure 5. Aerial fresh weight (FW; (**A**,**B**)), aerial dry weight (DW; (**C**,**D**)), and ratio of stem/leaf DW (**E**,**F**) under different light and temperature treatments. Data are means ± standard error. HT = high temperature; LT = low temperature. Treatment effects for each plant trait under different temperatures (T), lights (L), or the interactions of temperature and light (T × L) followed by ns, *, **, or *** denote that the treatment effects are not significant or significant at α = 0.05, 0.01, or 0.001, respectively. When the treatment effects of T × L are significant, the four treatment combinations of T × L are separated using different lower-case letters at α = 0.05 according to Tukey's HSD (honestly significant difference) test.

4. Discussion

4.1. Blue vs. Red LED Can Still Promote Plant Elongation in Arugula at a Higher Temperature

Similar to previous studies on arugula at 23 °C [17,19], in the present study at 28 °C, blue light can still promote plant elongation in this species, demonstrated by increased final plant height, elongation rate, and hypocotyl length, compared with red light. It appeared that phytochrome in arugula was still deactivated under blue light but activated under red light as confirmed by previous studies [19]. Recent studies indicate that even under red light *Arabidopsis* plants decreased phytochrome activity and increased stem elongation with temperature increasing from 12 °C to 27 °C [27,28]. Possibly, differing from *Arabidopsis*, arugula plants under red light did not decrease phytochrome activity to a great degree at a higher temperature. This was partly supported by the result from the current study that temperature treatment had no effect on plant elongation for arugula. Unlike *Arabidopsis*,

arugula appears to be a temperature-insensitive species, at least during the de-etiolation stage in terms of plant elongation response.

The promoted elongation by blue vs. red light was considered one of the shade-avoidance responses [15,19]. This conclusion was also supported by the results on arugula in the present study. In addition to promoting elongation growth, blue vs. red light also increased petiole length and dry mass partitioning to stem, but reduced cotyledon size showing typical shade-avoidance responses [34]. The reduced cotyledon size under blue vs. red light may negatively affect the appearance quality of microgreens to some degree because microgreens with large leaf sizes are normally more attractive to most consumers.

4.2. At a Lower Temperature Blue vs. Red LED Still Cannot Promote Plant Elongation in Mustard

Previous studies indicated that, unlike arugula, mustard plant elongation was not promoted under blue vs. red light, showing a similar plant height at 23 °C [17,19]. At 18 °C in the present study, blue vs. red LED still did not promote plant elongation for mustard. Possibly, at low temperatures, mustard plants under red LED did not increase phytochrome activity to a greater degree than those under blue LED. Similar to *Arabidopsis* [27,28], mustard is a temperature-sensitive species in terms of plant elongation response that was affected by temperature treatment in the present study. Unlike green-leafed arugula, red-leafed mustard plants contain many red pigments, which might increase the reflection and then reduce the transmission of red light to its main photoreceptors, phytochromes [23]. Furthermore, differing from the previous study, blue vs. red light even slightly reduced the plant height and elongation rate for mustard in the present study, despite similar hypocotyl length and petiole length. This might result from decreased cotyledon size under blue vs. red light, since the former two traits (i.e., plant height and elongation rate) were measured from rockwool surface to plant top.

For mustard, although blue light did not promote plant elongation relative to red light, it reduced cotyledon size showing one shade-avoidance response similar to arugula [17,19]. In addition to promoted hypocotyl elongation, the decreased cotyledon size is also an obvious shade-avoidance response for plants at the de-etiolation stage [35]. It appears that different plant species may have different shade-avoidance strategies under certain light conditions since elongation growth is just one of the most striking shade-avoidance responses [36].

4.3. Interactions between Light and Temperature Vary with Plant Species and Plant Traits

There were interactive effects between temperature and light treatment on all plant elongation traits for arugula rather than mustard in the present study. This indicated that plant elongation mediated by blue vs. red light was affected by temperature variation for arugula, but not for mustard. In arugula, temperature increase did not reverse blue light-promoted plant elongation relative to the red light but did reduce the promotion magnitude. In the present study, although plant elongation of arugula was not affected by the temperature at a significant level, it tended to increase under red light and decrease under blue light at a higher temperature. Possibly, for arugula, blue light tends to inhibit plant elongation at higher temperatures, but red light does so at lower temperatures, showing a similarity to *Arabidopsis* [27–29]. Differing from arugula, mustard plant elongation was mediated by light quality and temperature independently. For mustard, temperature variation had a stronger effect on plant elongation than light quality. It appears that different plant species have developed different sensing systems to mediate plant elongation to adapt to varying environmental conditions [37]. For plant elongation mediation during indoor microgreen production, caution needs to be taken since arugula is a light-sensitive species and mustard is temperature-sensitive species.

For most plant traits relevant to the shade-avoidance response, temperature and light treatment interacted on arugula, but not on mustard. However, there were also a few exceptions for both species. In arugula, no interactions between temperature and light treatment were observed on blade width and hypocotyl color. In mustard, interactions

between temperature and light treatment were observed on the cotyledon area, hypocotyl color, and dry mass. For the two common exceptions in both species, leaf size and stem color appeared to be more variable than other plant traits in response to temperature change and light quality, possibly because they are more accurately mediated by the two factors [38,39]. For the only exception in mustard, dry mass was reduced by blue vs. red light at higher temperatures, but not at lower temperatures, showing an interaction between light and temperature. Possibly, plants consumed greater dry mass for plant elongation growth under blue light than red light especially at higher temperatures, since the two light treatments had a similar PPFD, and photosynthesis contributed little to dry mass accumulation during the de-etiolation stage. However, the dry mass was not different between blue and red light in our previous studies [17,19]. This may be due to a shorter photoperiod (12 h vs. 16 or 24 h) used in the present study, although the underlying mechanism is unclear.

5. Conclusions

In summary, for microgreens grown indoors at a PPFD of 110 μmol $m^{-2}s^{-1}$ and a photoperiod of 12 h d^{-1}, blue vs. red LED light can promote plant elongation at either higher temperature (28 °C) or lower temperature (18 °C) for arugula, but not for mustard. In addition, blue vs. red light can also promote shade-avoidance responses such as decreased cotyledon size for both species. For arugula, the elongation promotion effects by blue light are more obvious at lower than higher temperatures. For mustard, plant elongation is promoted by increased temperature regardless of light treatment. There are interactions between light and temperature on most plant traits for arugula, but not for mustard. Therefore, the blue light-promoted elongation growth associated with shade-avoidance responses cannot be reversed by temperature variation, but the response sensitivity varies with temperature and species. When using LED light to accurately mediate plant elongation for indoor microgreen production, the effects of other factors such as temperature and species also need to be taken into consideration.

Author Contributions: Y.K. designed and carried out the experiments; G.N. and J.M. provided resources; Y.K. drafted the manuscripts with input from G.N. All authors have read and agreed to the published version of the manuscript.

Funding: This project was supported primarily by internal funding and was partially funded by USDA Hatch project TEX07726.

Acknowledgments: We appreciate student worker Kelven Hua's help with data collection during this study.

Conflicts of Interest: The authors declare no conflict of interest.

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