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Sole-Source LED Lighting and Fertility Impact Shoot and Root Tissue Mineral Elements in Chinese Kale (*Brassica oleracea* var. *alboglabra*)

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Abstract: The current study investigated the impacts of light quality and different levels of fertility on mineral nutrient concentrations in shoot and root tissues of Chinese kale (*Brassica oleracea* var. *alboglabra*). 'Green Lance' Chinese kale were grown under: 1) fluorescent/incandescent light; 2) 10% blue (447 ± 5 nm) / 90% red (627 ± 5 nm) LED light; 3) 20% blue / 80% red LED light; and 4) 40% blue / 60% red LED light as sole-source lighting at two different levels of fertility. All plants were harvested 30 d after seeding, and shoot and root tissues were analyzed for mineral nutrients. Lighting and fertility interacted to influence kale shoot and root mineral nutrient concentrations. Results indicate sole-source LED lighting used in production can impact mineral nutritional values of baby leafy greens now popular for the packaged market.

Keywords: blue light; calcium; iron; magnesium; potassium; red light

1. Introduction

The Inorganic elements participate in many different mechanisms in plant photosynthesis. Some elements participate in the structure of the photosynthetic apparatus, while others play vital roles in translocation of photosynthetic products and sink tissue formation (fruits, grains and storage organ).¹ Elements can be considered to have direct effects on photosynthesis when deficiencies of a particular element cause a rapid decline in photosynthetic activity. Direct effects of elemental deficiencies are usually considered reversible as reintroduction at a proper level results in resumption of photosynthetic activity. Indirect effects are not usually readily reversible. They occur over a longer period of time and involve elements not necessarily critical in the photosynthetic process, but instead are crucial in the production of metabolites or organs that are directly involved in photosynthesis. Chlorophyll loss and necrosis that accompany an elemental deficiency result in reduced leaf area and metabolic activity. Often, by the time symptoms are visible, chloroplast alteration is severe. The symptoms of many elemental deficiencies are simply the visual manifestations of decreased photosynthetic activity by a plant² which have impacts on light utilization.

Only a small percentage of solar spectral irradiance is captured by chlorophyll *a* and used in photosynthesis. Maximum light absorption by chlorophyll pigments and quantum yield of photosynthesis occur primarily in the blue and red regions of the visible light spectrum.³ Light-harvesting complexes composed of accessory pigments (chlorophyll *b*, lutein, and β -carotene)

improve light harvesting efficiency in the photosynthetically active radiation (PAR) spectrum and direct the flow of excitation energy to the reaction centers.⁴ However, absorption of excess light energy has the potential to damage photosynthetic machinery, and accessory pigments also play an important role in photoprotection.⁵ Damage to the photosynthetic apparatus by light intensity or quality (such as high ultraviolet light) will impact the product of metabolites and ATP used to drive elemental ion uptake and flux.

Light influences concentrations of plant elements by impacting the amount of carbohydrates produced and enzymatic activities within primary metabolic pathways.⁶ Absorption of PAR by photosystems I and II (PSI and PSII) result in H⁺ ions fluxes within the thylakoid which need to be counter balanced by fluxes of other cations. The generation of ATP in the light reactions of photosynthesis become a source of energy for active ion movements.⁷ Translocated carbohydrates are required for root respiration, which provides the energy needed for active uptake mechanisms.⁸ Recent research demonstrates that shoot tissue elemental concentrations can be impacted by both light quality and light intensity. Specialized photoreceptors in plants called phototropins change metabolic homeostasis and mobilize Ca²⁺ in response to blue light.⁹ Kopsell et al. demonstrated that blue/red LED lighting ratios in a sole-source light environment acted to increase sprouting broccoli (*Brassica oleracea* var *italica*) microgreen (21-d old) shoot tissue concentrations [mg/g dry mass (DM)] of calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), sulfur (S), boron (B), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn) as compared to broad spectrum incandescent/fluorescent lighting.¹⁰ Changing the light quality environment from blue/red light to only blue, and concomitantly reducing the light intensity from 350 µmols/m²/sec (blue/red LED) to 41 µmols/m²/sec (blue LED), for 5 days pre-harvest acted to significantly increase macro-element and micro-element concentrations in sprouting broccoli microgreens.¹¹ Increasing the photosynthetic photon flux density (PPFD) from 200 to 400 µmols/m²/sec resulted in increased concentrations (µg/plant) of B, copper (Cu), Fe, Mn, and Zn in a variety of tropical legume cover crops.¹²

Our hypothesis is that accumulation of mineral elements in the shoot and root tissues of 30-day old (baby) leafy specialty vegetable crops will be higher under narrow-band LED light as compared to full spectrum fluorescent/incandescent light in controlled environments. Because of the increases in shoot tissue mineral elements of 21-day old sprouting broccoli microgreens grown under LED lighting in previous studies,^{10,11} the objective of this study was to measure the impact of different ratios of blue/red LED light on shoot and root tissue mineral elements in baby Chinese kale (*B. oleracea* var. *alboglabra*). A comparison among different blue/red LED ratio light treatments was also made with traditional fluorescent/incandescent in controlled environments. Plants were grown under ¼ or ½ strength Hoagland's nutrient solutions¹³ to establish any possible light by fertility experimental interactions.

2. Results

The acquisition of mineral nutrients into Chinese kale shoot tissue demonstrated a significant interaction when plants were grown under four light quality treatments within two fertility regimes. Micronutrients, Ca, K, Mg, P, and S (**Table 1**) were all affected by the interaction and in general had the highest concentrations in shoot tissue under the ½ strength fertility paired with the 20% blue/80% red LED light treatment. Interestingly, kale plants grown under the ¼ strength fertility paired with the 10% blue/90% red LED light treatments were significantly similar. For example, there was a less than 10% difference between ½ strength fertility paired with the 20% blue/80% red LED and ¼ strength fertility treatment combination for Ca, Mg, P, and S (**Table 1**). Conversely, the combination of fluorescent/incandescent light treatments with ¼ strength fertilizer were among the lowest mineral nutrients in kale shoot tissue. For example, K concentrations in shoot tissue under the 20% blue/80% red LED and ½ strength fertility treatments were 68.1% higher than in the fluorescent/incandescent light combined with the ¼ strength fertilizer treatment (**Table 1**). The K concentrations in shoot tissue under 20% blue/80% red LED and ½ strength fertility treatments were

also 50.4% higher compared to the fluorescent/incandescent light combined with the $\frac{1}{4}$ strength fertilizer treatment (**Table 1**). The micronutrients of Mn and Mo also had significant interactions between light and fertility treatments (**Table 1**). In general, there were significant increases in Mn and Mo when comparing LED lights and $\frac{1}{2}$ strength fertility treatments and LED lights and $\frac{1}{4}$ strength fertility and fluorescent/incandescent light combined with fertility. The lowest concentrations in kale shoot Mn and Mo occurred under the fluorescent/incandescent light combined with the $\frac{1}{4}$ strength fertilizer treatment (**Table 1**). There were 42.4 % and 57.8% decreases in Mo and Mn concentrations, respectively, when comparing the two treatment combinations (**Table 1**). However, there were not significant changes from the LED lights and $\frac{1}{2}$ strength fertility treatments and the $\frac{1}{4}$ strength fertility paired with the 10% blue/90% red LED light treatments (**Table 1**). Consequently, there were not significant changes in B and Cu in kale shoot tissue treated with differences in light and fertility treatments (**Table 1**).

There were limited interactions between light and fertility treatments when determining the mineral nutrient concentrations in the root tissue. For instance, there were only significant interactions for Mo and K that exhibited similar trends with differences between LED lights and $\frac{1}{2}$ strength fertility treatments and LED lights and $\frac{1}{4}$ strength fertility and fluorescent/incandescent light combined with fertility (**Table 2**). The combination of these treatments demonstrated a 42.7 and a 75.0% difference between concentration in the root tissue for Mo and K, respectively. On the other hand, Mg concentrations in kale root tissue demonstrate opposing results that indicated increases under LED lights combined with $\frac{1}{4}$ strength fertility and fluorescent/incandescent light combined with fertility (**Table 2**). For instance, concentrations of Mg were similar in the fluorescent/incandescent lights under either fertility treatment or LED lights combined with $\frac{1}{4}$ strength fertility. The least amount of Mg in kale root tissue occurred in the 40% blue/60% red LED light combined with $\frac{1}{2}$ strength fertility (**Table 2**).

Light quality had a significant effect on kale shoot Fe and Zn concentrations (**Table 3**). In all instances, kale plants grown under LED light accumulated higher concentrations of Fe and Zn compared to the fluorescent/incandescent light treatments. The Fe concentrations were greatest in the 40% blue/60% red LED light treatments and increased by 34.9% over the fluorescent/incandescent light treatments (**Table 3**). The Zn concentrations were greatest under the 10% blue/90% red LED light ratio and increased by 42.1% compared to the fluorescent/incandescent light treatments (**Table 3**). Conversely, kale plant root concentrations of S, B, and Zn were significantly increased under the 10% blue/90% red LED light ratio treatment (**Table 4**). Kale plants demonstrated superior accumulation of S, B, and Zn in the root tissue under the 10% blue/90% red LED light ratio with increases of 34.0%, 39.3%, and 55.1%, respectively, compared to the fluorescent/incandescent light treatments (**Table 4**).

Fertility treatments of $\frac{1}{4}$ and $\frac{1}{2}$ strength fertilizer significantly impacted the concentrations of P, Mn, Fe, and Zn in kale root tissue (**Table 5**). In all instances, $\frac{1}{2}$ strength fertilizer increased the amount of these minerals in the root tissue compared to the $\frac{1}{4}$ strength fertilizer. Root tissue P, Mn, Fe, and Zn concentrations increased by 16.0%, 51.4%, 40.4%, and 20.5%, respectively (**Table 5**).

Table 1. The Effects of Four Light Quality and Two Fertility Treatments on Shoot Tissue Mineral Element Concentrations for 'Green Lance' Chinese Kale (*Brassica oleracea* var *alboglabra*) Grown in Controlled Environments^a.

	Ca	K	Mg	P	S	B	Cu	Fe	Mn	Mo	Zn
Light Quality ^b											
mg/g dry mass ^c											
1/4 Strength Fertility ^d											
Fluorescent/Incandescent	15.01 c	13.32 e	2.70 e	4.03 d	4.56 d	53.11 bc	2.56 a	33.03 c	57.74 b	1.29 d	17.70 b
10% Blue/90% Red LED	22.46 a	34.36 bc	4.04 ab	6.73 abc	9.48 a	51.89 bc	3.22 a	51.39 a	110.78 a	2.36 a	31.61 a
20% Blue/80% Red LED	18.79 b	23.28 d	3.37 cd	5.92 c	6.59 c	51.93 bc	2.93 a	42.51 abc	71.50 b	1.68 cd	28.41 a
40% Blue/60% Red LED	20.87 ab	30.12 c	3.95 ab	7.03 ab	7.92 b	56.22 ab	3.36 a	50.29 a	76.65 b	2.07 abc	26.95 a
1/2 Strength Fertility ^d											
Fluorescent/Incandescent	15.05 c	20.72 d	2.90 de	4.43 d	5.62 cd	51.27 bc	2.93 a	33.56 bc	74.61 b	1.56 cd	18.86 b
10% Blue/90% Red LED	18.84 b	36.80 ab	3.60 bc	6.43 bc	8.70 ab	50.31 c	3.34 a	46.50 ab	117.94 a	1.78 bcd	31.52 a
20% Blue/80% Red LED	22.78 a	41.74 a	4.45 a	7.43 a	9.12 ab	58.73 a	3.08 a	54.85 a	136.66 a	2.24 ab	30.20 a
40% Blue/60% Red LED	22.03 a	39.01 ab	4.19 a	6.89 ab	9.00 ab	53.54 abc	3.11 a	51.98 a	119.77	1.97 abc	32.17 a
SE _{α=0.05}	1.17	2.42	0.26	0.50	0.48	2.32	0.32	5.53	10.69	0.19	2.28
Source of variation ^e											
Light	***	***	***	***	***	ns	ns	**	***	**	***
Fertility	ns	***	ns	ns	**	ns	ns	***	ns	ns	ns
Light x Fertility	**	**	**	*	**	ns	ns	ns	*	*	ns

^aMean values represent 6 total plants per treatment for 2 replications of each of 3 experimental repeats. ^bAll light treatments at an intensity of 250±10 µmol/m²/sec; percentages indicate contributions to total light intensity (see text for light treatment details). ^cMeans followed by the same letter are not statistically different, $\alpha = 0.05$.

^d1/4 and 1/2 Strength Fertility describe concentrations based on Hoagland's #2 nutrient solution (see text for nutrient concentration details). ^eIndividual effects and interactions are given according to ANOVA tests, with significance as: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ns, not significant.

Table 2. The Effects of Four Light Quality and Two Fertility Treatments on Root Tissue Mineral Element Concentrations for 'Green Lance' Chinese Kale (*Brassica oleracea* var *alboglabra*) Grown in Controlled Environments^a.

Light Quality ^b	Ca	K	Mg	P	S	B	Cu	Fe	Mn	Mo	Zn
mg/g dry mass ^c											
1/4 Strength Fertility ^d											
Fluorescent/Incandescent	4.62 a	10.86 c	5.01 abc	3.85 b	5.30 b	41.67 b	15.77 a	101.23 bc	202.24 b	1.10 d	32.52 d
10% Blue/90% Red LED	4.75 a	30.31 ab	4.59 bc	5.78 a	11.02 a	70.61 a	18.50 a	93.24 c	487.78 ab	2.05 a	70.43 ab
20% Blue/80% Red LED	5.00 a	18.58 bc	5.37 ab	5.18 ab	6.14 b	39.06 b	17.49 a	75.94 c	274.91 b	1.36 cd	42.28 cd
40% Blue/60% Red LED	5.16 a	16.11 c	6.37 a	5.84 a	5.32 b	37.29 b	13.06 a	86.52 c	290.36 b	1.55 abcd	35.86 d
1/2 Strength Fertility ^d											
Fluorescent/Incandescent	4.94 a	13.73 c	5.41 ab	6.67 a	6.08 b	32.96 b	18.08 a	152.69 a	483.31 ab	1.39 cd	37.74 d
10% Blue/90% Red LED	3.79 a	37.80 a	4.44 d	6.01 a	9.24 ab	52.10 ab	14.71 a	150.84 a	704.97 a	1.49 bcd	77.23 a
20% Blue/80% Red LED	4.72 a	40.06 a	3.70 cd	6.53 a	8.18 ab	45.68 b	13.40 a	159.90 a	719.74 a	1.92 ab	50.12 bcd
40% Blue/60% Red LED	4.37 a	43.46 a	3.43 cd	6.37 a	8.58 ab	47.01 b	13.25 a	135.00 ab	677.21 a	1.77 abc	62.79 abc
SE _{α=0.05}	0.89	4.34	0.86	0.68	1.44	10.47	3.10	24.10	104.00	0.22	8.98
Source of variation ^e											
Light	ns	***	*	ns	*	*	ns	ns	ns	*	**
Fertility	ns	***	***	*	ns	ns	ns	***	***	ns	*
Light x Fertility	ns	*	*	ns	ns	ns	ns	ns	ns	*	ns

^aMean values represent 6 total plants per treatment for 2 replications of each of 3 experimental repeats. ^bAll light treatments at an intensity of 250 ± 10 $\mu\text{mol}/\text{m}^2/\text{sec}$; percentages indicate contributions to total light intensity (see text for light treatment details). ^cMeans followed by the same letter are not statistically different, $\alpha = 0.05$. ^d1/4 and 1/2 Strength Fertility describe concentrations based on Hoagland's #2 nutrient solution (see text for nutrient concentration details). ^eIndividual effects and interactions are given according to ANOVA tests, with significance as: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns, not significant.

Table 3. The Main Effects of Light Quality Treatment on Shoot Tissue Mineral Element Concentrations for 'Green Lance' Chinese Kale (*Brassica oleracea* var *alboglabra*) Grown in Controlled Environments^a.

	Fe	Zn
Light Quality ^b	µg/g dry mass ^c	
Fluorescent/Incandescent	33.29 b	18.28 b
10% Blue/90% Red LED	48.94 a	31.56 a
20% Blue/80% Red LED	48.68 a	29.31 a
40% Blue/60% Red LED	51.13 a	29.56 a
P-Value ^d	**	***
SE _{α=0.05}	4.51	1.76

^aMean values represent 6 total plants per treatment for 2 replications of each of 3 experimental repeats. ^bAll light treatments at an intensity of $250\pm10\text{ }\mu\text{mol/m}^2/\text{sec}$; percentages indicate contributions to total light intensity (see text for light treatment details). ^cMeans followed by the same letter are not statistically different, $\alpha = 0.05$.

^dIndividual effects and interactions are given according to ANOVA tests, with significance as: ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 4. The Main Effects of Light Quality Treatments on Root Tissue Mineral Element Concentrations for 'Green Lance' Chinese Kale (*Brassica oleracea* var *alboglabra*) Grown in Controlled Environments^a.

	S	B	Zn
Light Quality ^b	mg/g dry mass ^c	µg/g dry mass ^c	
Fluorescent/Incandescent	6.69 b	37.32 b	35.13 b
10% Blue/90% Red LED	10.13 a	61.36 a	73.83 a
20% Blue/80% Red LED	7.16 b	42.37 b	46.20 b
40% Blue/60% Red LED	6.95 b	42.15 b	49.33 b
P-Value ^d	*	*	**
SE _{α=0.05}	1.08	9.04	7.17

^aMean values represent 6 total plants per treatment for 2 replications of each of 3 experimental repeats. ^bAll light treatments at an intensity of $250\pm10\text{ }\mu\text{mol/m}^2/\text{sec}$; percentages indicate contributions to total light intensity (see text for light treatment details). ^cMeans followed by the same letter are not statistically different, $\alpha = 0.05$. ^dIndividual effects and interactions are given according to ANOVA tests, with significance as: * $P \leq 0.05$; ** $P \leq 0.01$.

Table 5. The Main Effects of Fertility Treatments on Root Tissue Mineral Element Concentrations for 'Green Lance' Chinese Kale (*Brassica oleracea* var *alboglabra*) Grown in Controlled Environments^a.

	P	Mn	Fe	Zn
Fertility	mg/g dry mass ^b		µg/g dry mass ^b	
1/4 Strength Fertility ^c	5.16 b	313.82 b	89.23 b	45.27 b
1/2 Strength Fertility ^c	6.14 a	646.31 a	149.61 a	56.97 a
P-Value ^d	*	***	***	*
SE _{α=0.05}	0.5	52	21.87	6.06

^aMean values represent 6 total plants per treatment for 2 replications of each of 3 experimental repeats. ^bMeans followed by the same letter are not statistically different, $\alpha = 0.05$. ^c1/4 and 1/2 Strength Fertility describe concentrations based on Hoagland's #2 nutrient solution (see text for nutrient concentration details). ^dIndividual effects and interactions are given according to ANOVA tests, with significance as: * $P \leq 0.05$; *** $P \leq 0.001$.

3. Discussion

Responses in kale biomass from the current study have been published previously.¹⁵ Kale shoot fresh mass (FM) was influenced by light treatment, fertility treatment, and their interaction. Kale shoot tissue FM under $\frac{1}{4}$ strength fertility was 17.30, 9.24, 11.03, and 9.11 g per plant for the light quality treatments of fluorescent/incandescent light, 10% blue / 90% red, 20% blue / 80% red, and 40% blue / 60% red, respectively. Kale shoot tissue FM under $\frac{1}{2}$ strength fertility was 25.74, 9.27, 13.92, and 11.63 g per plant for the light quality treatments of fluorescent/incandescent light, 10% blue / 90% red, 20% blue / 80% red, and 40% blue / 60% red, respectively.¹⁵

Previous LED research on leafy greens has focused on growth, morphological changes, yield, and phytonutrient concentrations. For example, Chen et al. indicated that there were significant differences in plant height, width, FM, DM, and leaf length and leaf width in lettuce grown under red and blue LED light at different daily light integrals.¹⁶ In another study, hypocotyl length, leaf area, FM, and DM were significantly affected by LED photosynthetic photon flux density (PPFD) in *Brassica* microgreens.¹⁷ Previous research has indicated that the addition of blue LED light increased production of phenolic acids in basil (*Ocimum basilicum*) and flavonoids in arugula (*Eruca vesicaria*).¹⁸ Kopsell et al. demonstrated that interactions of light quality, comparing fluorescent/incandescent and LED lights, and fertility significantly increased Chinese kale shoot biomass and pigment concentrations.¹⁵ Yan et al. demonstrated similar results comparing fluorescent/incandescent and LED lights with biomass accumulation but also discovered significant differences in vitamin C and soluble protein content in lettuce (*Lactuca sativa*).¹⁹

Limited research exists on how different LED light ratios affect the mineral nutrient concentrations and accumulation. Previous research on LEDs and mineral nutrients have focused on reduction of nitrate in the leaf and shoot tissues of hydroponically grown leafy greens, since the accumulation and concentrations are elevated in these growth systems. For example, a reduction of nitrate concentrations in lettuce leaf tissue was observed when plants were treated with red LED light.^{20,21} Previous research has also indicated that green LED light reduces nitrate concentrations in hydroponically grown lettuce.²² However, there is a lack of knowledge of how LED light ratios coupled with differing concentrations of a hydroponic nutrient solution affect mineral nutrient concentrations and accumulation in shoot and root tissues.

Even though there is limited research information on different nutrient solution concentrations and LED lights, other studies have demonstrated how differing LED light ratios affect the uptake of mineral nutrients in leaf tissues. For example, Gerovac et al. indicated that LED light quality; ratio of red, green, far-red, blue; and LED light intensity had significant effects on macronutrient concentrations in *Brassica* microgreens.²³ Previous research has also indicated sprouting broccoli shoot tissue macronutrients were significantly affected when grown under red and blue LED or five day preharvest blue LED light treatments.¹¹ Metallo et al. found similar results with LED and white light and duration treatments for K concentrations in kale plants.²⁴ Data indicated that 95% red/5% blue at the 37-day treatments increased K concentrations to 4.87% compared to 3.61% in the white light treatment. In the current study, the interaction of light and fertility had a profound effect on the uptake of macronutrients in kale shoot tissue. Another study demonstrated that LED light treatment affect mineral nutrients such as Ca, K, Mg, P, and S in microgreen production.¹⁰ Similar results were discovered in the current study that indicated increases in mineral nutrients under LED lights compared to fluorescent/incandescent. Thus, LED light quality and an adequate fertility program can lead to a significant impact on increasing macronutrient uptake and concentrations in plant tissues, increasing the quality and nutritional content in edible kale tissue. In the current study, the results indicate that the biomass dilution effect is not a factor when increasing the nutrient solution concentrations under LED light quality conditions verses fluorescent/incandescent light, with adequate light intensities. Under this study and previous studies, the light intensities for growing

leafy greens such as kale, have been approximately 250 to 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$, indicating that within this light intensity range, plants given the correct LED light quality and increased fertility can have elevated concentrations of mineral nutrients.

There were less effects of the interaction of light and fertility on micronutrient concentrations in kale shoot tissue. Previous research indicated that the interaction of light quality and intensity decreased concentrations of B, Fe, and Zn in kohlrabi (*B. oleracea* var *gongylodes*), mizuna (*B. rapa* var *japonica*), and mustard [*B. juncea* (L.) Czern. 'Garnet Giant'].²³ These results indicate that decreases in micronutrient concentrations may have been caused by increases in biomass under increased light intensity and pinpointed light quality giving a biomass dilution effect under these conditions.

LED light research on leafy greens has indicated that light ratios can be manipulated to impact mineral nutrient uptake and stimulate secondary metabolic pathways associated with nutritional quality factors. Several previous studies within our collaborative research efforts demonstrate the ability to increase secondary metabolic pathways and mineral nutrient uptake associated with nutritional quality factors.^{10,11,15,24} However, the current research study is the first to examine how novel LED light ratios and differing fertilizer regimes affect mineral nutrients uptake into plant root and shoot tissues. By manipulating light ratios and mineral nutrient concentrations, it is proven that plants can be manipulated with novel LED light ratios coupled with lower mineral nutrients for a more sustainable approach to plant growth.

should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

4. Materials and Methods

Chinese Kale Culture and Harvest.

'Green Lance' Chinese kale (Johnny's Selected Seeds, Winslow, ME, USA) were seeded into growing cubes (Oasis® Hortcubes®, Smithers-Oasis North America, Kent, OH, USA) and grown in controlled environment chambers (Model E15; Conviron, Winnipeg, Manitoba, Canada) at the University of Tennessee, Knoxville, TN. Seeds were cultured at an air temperature of 23 °C with a 16-h photoperiod using a light intensity of 250 $\mu\text{mol}/\text{m}^2/\text{sec}$ from fluorescent and incandescent bulbs. Five days after germination, seedlings were fertilized with a complete nutrient solution [elemental concentrations were (mg/L): N (52.5), P (7.7), K (58.7), Ca (40.1), Mg (12.3), S (16), Fe (0.25), B (0.12), Mo (0.003), Cu (0.005), Mn (0.12), and Zn (0.012)]. After 15 days, seedlings were transferred to 10 L plastic containers (Rubbermaid Inc., Wooster, OH, USA). Six plants were placed into 2 cm round holes set at 10.6 cm x 9.5 cm spacing on each container lid to constitute an experimental unit. The plants were grown in 9 L of a modified nutrient solution.¹³ The $\frac{1}{4}$ Hoagland's nutrient fertility treatment (solution #2) elemental concentrations were (mg/L): N (52.5), P (7.7), K (58.7), Ca (40.1), Mg (12.3), S (16), Fe (0.25), B (0.12), Mo (0.003), Cu (0.005), Mn (0.12), and Zn (0.012). The $\frac{1}{2}$ Hoagland's nutrient fertility treatment elemental concentrations were (mg/L): N (105.0), P (15.5), K (117.3), Ca (80.2), Mg (24.6), S (32.0), Fe (0.5), B (0.25), Mo (0.005), Cu (0.01), Mn (0.25), and Zn (0.025). The nutrient solutions were aerated with standard aquarium air pumps connected to air stones via plastic tubing.

Kale plants were grown under four different light treatments which consisted of: 1) fluorescent/incandescent light; 2) 10% blue (447 ± 5 nm, full width half maximum (FWHM) = 20 nm) / 90% red (627 ± 5 nm, FWHM = 20 nm); 3) 20% blue / 80% red; 4) 40% blue / 60% red. A spectroradiometer (model SPEC-UV/PAR; Apogee Instruments, Logan, UT) was used to adjust and maintain a light intensity of $250 \pm 10 \mu\text{mol}/\text{m}^2/\text{sec}$ at the center of each LED panel and the fluorescent/incandescent light treatment at canopy height. The fluorescent/incandescent light treatment was composed of cool-white fluorescent bulbs (160 W) and incandescent bulbs (60 W) and measured 15.3% blue (400-500 nm) and 26.4% red (600-700 nm) of total irradiance. The total irradiance of $250 \mu\text{mol}/\text{m}^2/\text{sec}$ resulted in a total energy output of 52.3, 49.4, 51.3 and 55.1 W/m² for the light treatment of fluorescent/incandescent, 10% blue / 90% red LEDs, 20% blue / 80% red LEDs,

and 40% blue / 60% red LEDs, respectively. Treatments provided a red/blue light ratio of 1.7 for fluorescent/incandescent, 9 for 10% blue / 90% red, 4 for 20% blue / 80% red, and 1.5 for 40% blue / 60% red light treatments. Kale plants were harvested from each container at 30 days after seeding from all treatments. Plants were weighed for biomass accumulation and stored at -80 °C prior to tissue pigment analyses.

Chinese Kale Tissue Mineral Element Analysis.

A 0.5 g subsample of ground freeze-dried tissue was combined with 10 mL HNO₃ (70%) and sealed in a closed vessel microwave digestion system (ETHOS series; Milestone, Shelton, CT, USA). Digestion procedures followed those for organically based matrices.¹⁴ Digestions were diluted with 2% HNO₃ / 0.5% HCl (v/v), and elemental measurements were made using an Agilent 7500ce ICP-MS system (Agilent Technologies, Santa Clara, CA, USA). The ICP-MS system was equipped with an octapole collision/reaction cell, Agilent 7500 ICP-MS ChemStation software, a micromist nebulizer, a water-cooled quartz spray chamber, and a CETAC (ASX-510; CETAC, Omaha, NE, USA) autosampler. The instrument was optimized daily in terms of sensitivity (Li, Y, Tl), level of oxide (Ce), and doubly charged ion (Ce) using a tuning solution containing 10 µg/L of Li, Y, Tl, Ce, and Co in a 2% HNO₃/0.5% HCl (v/v) matrix. Mineral elements were expressed on a DM basis and calculated as concentration (mg/g; µg/g).

Statistical Analyses.

The experimental design was a randomized complete block in a two (fertility treatment) x four (light treatment) factorial arrangement. The study was repeated three times. Data were analyzed using PROC GLM procedure of SAS (version 9.4; SAS Institute, Cary, NC, USA). Differences among light and fertility treatments means were determined by least significant difference (LSD_{α=0.05}). Treatment by experimental run interactions were not detected, therefore data from each experimental repeat was combined and analyzed together.

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