Sprouting Broccoli Accumulate Higher Concentrations of Nutritionally Important Metabolites under Narrow-band Light-emitting Diode Lighting

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ABSTRACT. Previous research in our group demonstrated that short-duration exposure to narrow-band blue wavelengths of light can improve the nutritional quality of sprouting broccoli (*Brassica oleracea* var. *italica*) microgreens. The objective of this study was to measure the impact of different percentages of blue light on the concentrations of nutritional quality parameters of sprouting broccoli microgreens and to compare incandescent/fluorescent light with light-emitting diodes (LEDs). Microgreen seeds were cultured hydroponically on growing pads under light treatments of: 1) fluorescent/incandescent light; 2) 5% blue (442 to 452 nm)/95% red (622 to 632 nm); 3) 5% blue/85% red/10% green (525 to 535 nm); 4) 20% blue/80% red; and 5) 20% blue/70% red/10% green in controlled environments. Microgreens were grown at an air temperature of 24 °C and a 16-hour photoperiod using a light intensity of 250 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for all light treatments. On emergence of the first true leaf, a nutrient solution of 42 $\text{mg}\cdot\text{L}^{-1}$ nitrogen (N) (20% Hoagland's #2 solution) was used to submerge the growing pads. Microgreens were harvested after 20 days under the light treatments and shoot tissues were processed and measured for nutritionally important shoot pigments, glucosinolates, and mineral nutrients. Microgreens under the fluorescent/incandescent light treatment had significantly lower shoot fresh mass than plants under the 5% blue/95% red, 5% blue/85% red/10% green, and the 20% blue/80% red LED light treatments. The highest concentrations of shoot tissue chlorophyll, β -carotene, lutein, total carotenoids, calcium (Ca), magnesium (Mg), phosphorus (P), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn), glucoiberin, glucoraphanin, 4-methoxyglucobrassicin, and neoglucobrassicin were found in microgreens grown under the 20% blue/80% red light treatment. In general, the fluorescent/incandescent light treatment resulted in significantly lower concentrations of most metabolites measured in the sprouting broccoli tissue. Results from the current study clearly support data from many previous reports that describe stimulation of primary and secondary metabolite biosynthesis by exposure to blue light wavelengths from LEDs.

Light is one of the most important environmental stimuli affecting plant growth and development (Christie, 2007; Kopsell and Sams, 2013). Plants have evolved specialized chlorophyll and carotenoid pigments to capture light energy to drive photosynthetic processes. Plants also have specialized photoreceptor pigments to respond to light quality and quantity through changes in developmental and physiological processes, commonly referred to as photomorphogenesis. Photosynthetically active radiation [PAR (400 to 700 nm)] are the wavelengths predominantly absorbed by leaf tissues. However, maximum light absorption by chlorophyll pigments and quantum yield of photosynthesis occur primarily in the blue and red regions of the visible light spectrum. Therefore, it is not

surprising that plants have evolved other specialized photoreceptors to regulate responses to these physiologically important wavelengths. Phytochromes are primarily red light photoreceptors and distinguish between red and far-red wavelengths to control physiological responses such as seed germination and flowering (Chaves et al., 2011; Vierstra and Zhang, 2011). Cryptochromes and phototropins are blue light receptors. Cryptochromes act as signaling molecules that regulate responses such as circadian rhythms and stem elongation, whereas phototropins control chloroplastic movements to maximize absorption of light (Briggs and Christie, 2002; Christie, 2007).

Plant responses to blue light stimuli include phototropisms, suppression of stem elongation, chloroplast movements, stomatal regulation, and genetic expression (Baum et al., 1999). Blue light exposure during plant growth is qualitatively required for normal photosynthesis and facilitates quantitative leaf responses similar to those normally associated with higher light intensities (Hogewoning et al., 2010). Blue light can also act as a powerful signal regulating stomatal operations with research showing blue light is up to 20 times more effective than red light at influencing stomatal opening (Sharkey and

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Raschke, 1981; Shimazaki et al., 2007). Blue light exposure can also cause significant changes in guard cell membrane transport activity through variations in Ca^{2+} , K^{+} , and H^{+} fluxes and corresponding impacts on pH conditions (Babourina et al., 2002).

Xanthophyll carotenoid pigments, specifically zeaxanthin (ZEA), can modulate blue light-dependent responses in plants (Tlačka et al., 1999). Moreover, ZEA is believed to be an important photoreceptor for blue light-activated plant responses (Briggs and Huala, 1999). Leafy specialty crops are excellent dietary sources of carotenoids, which function as free radical scavengers, enhance the immune response, suppress cancer development, and protect eye tissues (Yeum and Russell, 2002). Exposure to blue light (470 nm) also increased buckwheat (*Fagopyrum tataricum*) sprout carotenoid concentrations after 10 d of exposure (Tuan et al., 2013). The most studied bioactive components in the cruciferous vegetables are the glucosinolates (GS) and their hydrolyzed isothiocyanate products. Isothiocyanates are chemopreventive agents demonstrating anticarcinogenic activity in the human diet. Previously, research in our group showed that a 5-d exposure to only blue wavelengths (455 to 470 nm) significantly increased sprouting broccoli (*Brassica oleracea* var. *italica*) microgreen shoot tissue β -carotene, violaxanthin, total xanthophyll cycle pigments, glucoraphanin, epiprogoitrin, aliphatic glucosinolates, essential micronutrients of Cu, Fe, B, Mn, Mo, sodium (Na), Zn, and essential macronutrients of Ca, P, potassium (K), Mg, and S (Kopsell and Sams, 2013).

An emerging application of LED technology is for horticultural plant production in controlled environments (Martineau et al., 2012). Capacities such as spectral composition control, high light output, and little radiant heat emissions make LEDs a viable alternative to traditional controlled environment lighting such as gas-filled or filament bulbs (Morrow, 2008). LEDs now provide the ability to measure impacts of narrow-band wavelengths of light on nutritional values of a variety of specialty crops such as chili pepper [*Capsicum annuum* (Gangadhar et al., 2012)], cucumber [*Cucumis sativus* (Hogewoning et al., 2010)], baby kale [*B. oleracea* var. *acephala* (Lefsrud et al., 2008)], ginseng [*Panax ginseng* (Park et al., 2013)], brassica (*Brassica* sp.) microgreens (Kopsell and Sams, 2013; Samuolienė et al., 2013), lettuce [*Lactuca sativa* (Li and Kubota, 2009; Lin et al., 2013; Martineau et al., 2012; Massa et al., 2008; Samuolienė et al., 2012; Stutte et al., 2009)], and buckwheat sprouts (Tuan et al., 2013). Moreover, many studies demonstrate a clear relationship between blue light exposure and responses within primary and secondary metabolic pathways in specialty vegetable crops. Chlorophyll and carotenoid pigments absorb light most efficiently in the red and blue wavelengths. Researchers discovered in initial LED plant growth studies that crops such as wheat [*Triticum aestivum* (Goins et al., 1997; Tripathy and Brown, 1995)], radish (*Raphanus sativus*), lettuce, and spinach (*Spinacea oleracea*) (Yanagi et al., 1996; Yorio et al., 1998, 2001) produced the highest yields when red wavelengths were supplemented with blue (1% to 10%) LED wavelengths. Subsequent studies showed that biomass and leaf area could be further increased in lettuce with the addition of green wavelengths (up to 24% of total light intensity) (Kim et al., 2004). Our central hypothesis is that accumulation of primary and secondary metabolites in specialty vegetable crops will be higher under narrow-band LED light where plants only receive blue and red wavelengths as compared with full-spectrum

fluorescent/incandescent light in controlled environments. Because of the impacts short-duration blue light had on nutritional quality parameters of sprouting broccoli in a previous study (Kopsell and Sams, 2013) and earlier work showing the positive impacts of combining red/blue/green wavelengths, the objective of this study was to measure the impact of different ratios of blue/red/green narrow-band LED light on nutritionally important phytochemical compounds in sprouting broccoli microgreens. A comparison among LED light treatments is also made with traditional incandescent/fluorescent lighting in controlled environments.

Materials and Methods

SPROUTING BROCCOLI CULTURE AND HARVEST. Sprouting broccoli microgreens (Mountain Rose Herbs, Eugene, OR) were cultured on growing pads (Sure to Grow, Beachwood, OH) of polyethylene terephthalate fibers in controlled environment chambers (Model E15; Conviron, Winnipeg, Manitoba, Canada) according to Kopsell and Sams (2013). Briefly, a 7-g sample of sprouting broccoli seeds (≈ 2200 seeds) was sown evenly onto a growing pad ($25.4 \times 24.7 \times 0.89$ cm) set in a perforated tray ($26 \times 52 \times 6$ cm). Perforated trays were set into solid-bottom trays ($26 \times 52 \times 6$ cm) and filled with deionized water to create a hydroponic tray system for microgreen culture. Two tray systems were placed under each lighting treatment in controlled environment chambers (Model E15; Conviron) to germinate seeds at 24 ± 1 °C in darkness. Light treatments were initiated 24 h after seeding. Photosynthetically active radiation was measured with a spectroradiometer (Model SPEC-ultraviolet/PAR; Apogee Instruments, Logan, UT) at the center of each panel, and panel heights from the growing pads were adjusted to maintain a light intensity of 250 ± 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for all light treatments. The photoperiod was maintained at 16 h throughout the study and light treatments consisted of: 1) fluorescent/incandescent light; 2) 5% blue [447 ± 5 nm, full width half maximum (FWHM) = 20 nm]/95% red (627 ± 5 nm, FWHM = 20 nm); 3) 5% blue/85% red/10% green (530 ± 5 nm, FWHM = 30 nm); 4) 20% blue/80% red; and 5) 20% blue/70% red/10% green in controlled environments. The fluorescent/incandescent light treatment was measured to provide 5.6% blue (450 to 495 nm), 24.9% red (620 to 700 nm), and 22.5% green (495 to 570 nm). Eight replications per treatment were conducted starting 6 Aug. 2012 during two complete experimental runs of the study. On emergence of the first true leaf ≈ 8 d after sowing seeds, a nutrient solution of 42 $\text{mg}\cdot\text{L}^{-1}$ N [20% Hoagland's #2 solution (Hoagland and Arnon, 1950)] was used to submerge the growing pads. Each tray received 500 mL of the nutrient solution each day throughout the remainder of the study. Microgreen plants were harvested at the surface of the growing pads from all treatments 21 d after sowing seeds and stored at -80 °C.

SPROUTING BROCCOLI TISSUE PIGMENT EXTRACTION. Pigments were extracted from freeze-dried tissues (Model 6 L FreeZone; LabConCo, Kansas City, MO) and analyzed according to Kopsell et al. (2012). A 0.1-g tissue subsample was re-hydrated with 0.8 mL of ultrapure H_2O for 20 min. After incubation, 0.8 mL of the internal standard ethyl- β -8'-apo-carotenoate (Sigma-Aldrich, St. Louis, MO) was added to determine extraction efficiency. The addition of 2.5 mL of tetrahydrofuran (THF) was performed after sample hydration. The sample was then homogenized in a tissue grinding tube (Potter-Elvehjem;

Kimble Chase-Kontes Glass, Vineland, NJ) using ≈ 25 insertions with a pestle attached to a drill press set at 540 rpm. During homogenization, the tube was immersed in ice to dissipate heat. The tube was then placed into a clinical centrifuge for 3 min at 500 g_n . The supernatant was removed and the sample pellet was re-suspended in 2 mL THF and homogenized again with the same extraction technique. The procedure was repeated for a total of four extractions to obtain a colorless supernatant. The combined supernatants were reduced to 0.5 mL under a stream of nitrogen gas (N-EVAP 111; Organomation, Berlin, MA) and brought up to a final volume of 5 mL with acetone. A 2-mL aliquot was filtered through a 0.2- μ m polytetrafluoroethylene (PTFE) filter (Econofilter PTFE 25/20; Agilent Technologies, Santa Clara, CA) using a 5-mL syringe (Becton, Dickinson and Co., Franklin Lakes, NJ) before high-performance liquid chromatography (HPLC) analysis.

SPROUTING BROCCOLI TISSUE PIGMENT HPLC ANALYSIS. An HPLC unit with a photodiode array detector (1200 series; Agilent Technologies) was used for pigment separation. Chromatographic separations were achieved using an analytical scale (4.6 i.d. $\times$ 250 mm) 5- μ m, 200- $\text{\AA}$ polymeric RP-C₃₀ column (ProntoSIL; MAC-MOD Analytical, Chadds Ford, PA), which allowed for effective separation of chemically similar pigment compounds. The column was equipped with a 5- μ m guard cartridge (4.0 i.d. $\times$ 10 mm) and holder (ProntoSIL; MAC-MOD Analytical) and was maintained at 30 °C using a thermostatted column compartment. All separations were achieved isocratically using a binary mobile phase of 11% methyl *tert*-butyl ether, 88.99% MeOH, and 0.01% triethylamine (v/v/v). The flow rate was 1.0 mL·min⁻¹ with a run time of 58 min. Eluted compounds from a 10- μ L injection were detected at 453 (carotenoids and internal standard), 652 [chlorophyll *a* (Chl *a*)], and 665 [chlorophyll *b* (Chl *b*)] nm; and data were collected, recorded, and integrated using ChemStation software (Agilent Technologies). Peak assignment for individual pigments was performed by comparing retention times and line spectra obtained from photodiode array detection using external standards [antheraxanthin (ANT), β -carotene (BC), Chl *a*, Chl *b*, lutein (LUT), neoxanthin (NEO), violaxanthin (VIO), ZEA from ChromaDex, Irvine, CA]. Pigments were expressed on a fresh mass (FM) basis.

SPROUTING BROCCOLI 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). Digestion procedures followed those for organically based matrices (U.S. Environmental Protection Agency, 1996). Digestions were diluted with 2% HNO₃/0.5% HCl (v/v), and elemental measurements were made using an inductively coupled plasma mass spectrometry (ICP-MS) system (7500ce; Agilent Technologies). The ICP-MS system was equipped with an octapole collision/reaction cell, ICP-MS ChemStation software, a micromist nebulizer, a water-cooled quartz spray chamber, and an CETAC (ASX-510; CETAC, Omaha, NE) autosampler. The instrument was optimized daily in terms of sensitivity [lithium (Li), yttrium (Y), thallium (Tl)], level of oxide [cerium (Ce)], and doubly charged ion (Ce) using a tuning solution containing 10 μ g·L⁻¹ of Li, Y, Tl, Ce, and cobalt in a 2% HNO₃/0.5% HCl (v/v) matrix. Mineral elements were expressed on a dry mass (DM) basis.

SPROUTING BROCCOLI TISSUE GLUCOSINOLATE EXTRACTION. Glucosinolates were extracted from freeze-dried tissues and analyzed according to Kopsell and Sams (2013). For GS analysis, 0.2 g of freeze-dried tissue sample was combined with 1 mL benzyl GS solution (1 mM) as an internal standard, 2.0 mL MeOH, and 0.1 mL barium-lead acetate (0.6 M) in a 16 $\times$ 100-mm culture tube and shaken at 60 rpm for 1 h. Each tube was then centrifuged at 2000 g_n for 10 min. A 0.5-mL aliquot of supernatant was then added to a 1-mL column containing 0.3 mL DEAE Sephadex A-25 (Sigma-Aldrich). The sample was desulfated by the procedure of Raney and McGregor (1990).

SPROUTING BROCCOLI TISSUE GLUCOSINOLATE HPLC ANALYSIS. Extracted desulfoglucosinolates were separated using an HPLC unit with a photodiode array detector (1100 series; Agilent Technologies) using a reverse-phase 4.6 i.d. $\times$ 250 mm, 5- μ m Luna C₁₈ column (Phenomenex, Torrance, CA) at a wavelength of 230 nm. The column temperature was set at 40 °C with a flow rate of 1 mL·min⁻¹. The gradient elution parameters were 100% water for 1 min followed by a 15-min linear gradient to 75% water:25% acetonitrile. Solvent levels were then held constant for 5 min and returned to 100% water for the final 5 min. Desulfoglucosinolates were identified by comparison with retention times of authentic standards. Desulfated forms of sinigrin (2-propenyl GS), epiprogoitrin (2-hydroxy-3-butenyl GS), glucoiberin (3-methylsulfinylpropyl GS), glucoraphenin (4-methylsulphinyl-3-butenyl GS), glucobrassicin (3-indolylmethyl GS), 4-hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl GS), and neoglucobrassicin (1-methoxy-3-indolylmethyl GS) were provided by S. Palmieri (Istituto Sperimentale Industriali, Bologna, Italy). Response factors used were from the International Organization for Standardization Method 9167-1. Glucosinolates were expressed on a DM basis.

STATISTICAL ANALYSES. Data were analyzed using the PROC GLM procedure of SAS (Version 9.2; SAS Institute, Cary, NC). Differences among lighting treatments means were determined by least significant difference at $\alpha = 0.05$.

Results

IMPACT OF LIGHT TREATMENTS ON MICROGREEN BIOMASS. Light treatment significantly impacted sprouting broccoli microgreen shoot tissue FM ($P = 0.002$). The highest shoot tissue FM occurred under the light treatment of 5% blue/85% red/10% green and averaged 11.26 g FM per gram seed weight (Table 1). The lowest shoot tissue FM occurred under the fluorescent/incandescent light treatment and averaged 7.28 g FM per gram seed weight (Table 1). Plants under the fluorescent/incandescent light treatment had significantly lower FM than plants under the 5% blue/95% red, 5% blue/85% red/10% green, and the 20% blue/80% red LED light treatments (Table 1). Sprouting broccoli microgreen shoot tissue DM did not differ among any of the light treatment imposed in the current study (Table 1).

IMPACT OF LIGHT TREATMENTS ON MICROGREEN PIGMENTS. Light treatment significantly impacted sprouting broccoli microgreen shoot tissue Chl *a* ($P \leq 0.001$), Chl *b* ($P \leq 0.001$), and total Chl ($P \leq 0.001$). The highest concentrations of chlorophyll pigments were found under the light treatment of 20% blue/80% red (Table 2). Chlorophyll *a* did not differ between the fluorescent/incandescent light treatment and the 5% blue/95% red light treatment. Tissue Chl *a* concentrations under the 20% blue/80% red treatment were similar to concentrations under the 20% blue/70% red/10% green treatment

but were higher than all the other light treatments (Table 2). Tissue Chl *b* and total Chl concentrations were significantly higher for all LED light treatments when compared with the fluorescent/incandescent light treatment (Table 2).

Light treatment significantly impacted broccoli microgreen shoot tissue BC ($P = 0.003$), LUT ($P = 0.006$), NEO ($P \leq 0.001$), ANT ($P = 0.031$), VIO ($P \leq 0.001$), and total integrated carotenoids ($P = 0.025$). Sprouting broccoli microgreen shoot tissue ZEA was unaffected by light treatment (Table 3). The highest concentrations of BC and LUT were found under the 20% blue/80% red light treatment; the highest concentrations of ANT and total integrated carotenoid pigments were found under the 20% blue/70% red/10% green light treatment; the highest concentrations of NEO and VIO were found under the

fluorescent/incandescent light treatment (Table 3). Plants under the fluorescent/incandescent light treatment had significantly lower tissue BC and LUT when compared with all LED light treatments; however, tissue LUT did not differ among LED light treatments (Table 3). Tissue NEO concentrations were higher under the fluorescent/incandescent light treatment and the 5% blue/95% red light treatments when compared with the other LED light treatments (Table 3). Total integrated carotenoid pigments were highest for the 5% blue/95% red, 20% blue/80% red, and the 20% blue/70% red/10% green light treatments (Table 3).

IMPACT OF LIGHT TREATMENTS ON MICROGREEN ELEMENTAL NUTRIENTS. Light treatment significantly impacted broccoli microgreen shoot tissue Ca ($P \leq 0.001$), K ($P = 0.008$), Mg ($P = 0.001$), P ($P \leq 0.001$), and S ($P \leq 0.001$). The highest concentrations of shoot tissue Ca, Mg, P, and S were found under the 20% blue/80% red light treatment, whereas the highest concentrations of shoot tissue K were found under the 20% blue/70% red/10% green light treatment (Table 4). Tissue Ca, Mg, P, and S were significantly lower under the fluorescent/incandescent light treatment when compared with all other LED light treatments (Table 4). Tissue K was lower under fluorescent/incandescent light treatment when compared with the 20% blue/80% red and 20% blue/70% red/10% green light treatments (Table 4).

Light treatment significantly impacted broccoli microgreen shoot tissue B ($P = 0.004$), Fe ($P = 0.004$), Mn ($P = 0.009$), Mo ($P \leq 0.001$), and Zn ($P = 0.002$). Microgreen shoot tissue Cu was unaffected by light treatment. The highest concentrations of shoot tissue B, Cu, Fe, Mn, Mo, and Zn were found under the 20% blue/80% red light treatment (Table 5). Tissue B, Fe, Mn, Mo, and Zn were significantly lower under the fluorescent/incandescent light treatment when compared with all other LED light treatments (Table 5). Tissue Cu was lower under fluorescent/incandescent light treatment when compared with the 5% blue/85% red/10% green and 20% blue/80% red light treatments (Table 5).

IMPACT OF LIGHT TREATMENTS ON MICROGREEN GLUCOSINOLATES. Glucosinolates extracted from microgreen shoot tissues and identified as desulfoglucosinolates were glucoiberin (GI), progoitrin (PRO), epiprogoitrin (EPI), glucoraphanin (GR), sinigrin (SN), 4-hydroxyglucobrassicin (4OHGB), glucoerucin (GE), glucobrassicin (GB), 4-methoxyglucobrassicin (4MGB), and neoglucobrassicin (NGB) (Table 6). Light treatment significantly impacted broccoli microgreen shoot tissue GI ($P \leq 0.001$), GR ($P \leq 0.001$), GE ($P \leq 0.001$), GB ($P \leq 0.001$), 4MGB ($P \leq 0.001$), and NGB ($P = 0.001$). Light treatment did not affect concentrations of PRO, EPI, SN, or 4OHGB in the shoot tissues of the sprouting broccoli microgreens. The highest concentrations of shoot tissue GI, GR, 4MGB, and NGB were found under the 20% blue/80% red light treatment, whereas the highest concentrations of shoot tissue GE and GB were found under

Table 1. Mean values for shoot tissue fresh and dry mass of sprouting broccoli microgreens grown in controlled environments under broad-spectrum light and different specific narrow-band wavelengths from light-emitting diodes.

Light treatments ^z	Biomass ^y	
	Shoot tissue fresh mass	Shoot tissue dry mass
	(g)	
Fluorescent/incandescent	50.98 c*	7.17 a
5% blue/95% red	67.43 ab	7.85 a
5% blue/85% red/10% green	78.85 a	9.17 a
20% blue/80% red	71.83 a	7.99 a
20% blue/70% red/10% green	54.07 bc	7.14 a
LSD ($\alpha = 0.05$)	13.55	3.12

^zTreatments at a light intensity of $250 \pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Percentages indicate contributions of narrow-band wavelengths in total light intensity: blue [$447 \pm 5 \text{ nm}$, full width half maximum (FWHM) = 20 nm], red ($627 \pm 5 \text{ nm}$, FWHM = 20 nm), green ($530 \pm 5 \text{ nm}$, FWHM = 30 nm).

^yMean values represent plants from 7 g of seed, eight replications per treatment; SE estimates: shoot tissue fresh mass = 4.50, shoot tissue dry mass = 1.04.

*Means followed by the same letter are not statistically different, $\alpha = 0.05$.

LSD = least significant difference.

Table 2. Mean values for chlorophyll pigment concentrations in the shoot tissue of sprouting broccoli microgreens grown in controlled environments under broad-spectrum light and different specific narrow-band wavelengths from light-emitting diodes.

Light treatments ^z	Shoot tissue chlorophyll ^y		
	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll
	(mg/100 g fresh mass)		
Fluorescent/incandescent	41.92 d*	18.86 c	60.78 d
5% blue/95% red	67.33 cd	30.87 b	98.20 c
5% blue/85% red/10% green	76.29 bc	32.98 b	109.27 bc
20% blue/80% red	107.91 a	42.17 a	150.09 a
20% blue/70% red/10% green	102.57 ab	38.18 ab	140.76 ab
LSD ($\alpha = 0.05$)	27.48	7.47	32.66

^zTreatments at a light intensity of $250 \pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Percentages indicate contributions of narrow-band wavelengths in total light intensity: blue [$447 \pm 5 \text{ nm}$, full width half maximum (FWHM) = 20 nm], red ($627 \pm 5 \text{ nm}$, FWHM = 20 nm), green ($530 \pm 5 \text{ nm}$, FWHM = 30 nm).

^yMean values represent plants from 7 g of seed, eight replications per treatment; SE estimates: chlorophyll *a* = 9.12, chlorophyll *b* = 2.48, total chlorophyll = 10.83.

*Means followed by the same letter are not statistically different, $\alpha = 0.05$.

LSD = least significant difference.

Table 3. Mean values for carotenoid pigment concentrations in the shoot tissue of sprouting broccoli microgreens grown in controlled environments under broad-spectrum light and different specific narrow-band wavelengths from light-emitting diodes.

Light treatments ^z	Shoot tissue carotenoids ^y						Total CAR
	BC	LUT	NEO	ZEA	ANT	VIO	
	(mg/100 g fresh mass)						
Fluorescent/incandescent	3.25 c ^x	6.36 b	3.64 a	0.16 a	0.82 bc	1.88 a	16.09 b
5% blue/95% red	4.87 ab	9.42 a	3.52 a	0.10 a	1.27 ab	1.27 b	20.44 a
5% blue/85% red/10% green	4.70 b	9.24 a	1.95 c	0.12 a	0.66 c	1.81 a	18.48 ab
20% blue/80% red	6.22 a	11.24 a	2.14 bc	0.15 a	1.21 ab	0.54 c	21.50 a
20% blue/70% red/10% green	5.78 ab	11.02 a	2.73 b	0.14 a	1.48 a	1.10 b	22.24 a
LSD ($\alpha = 0.05$)	1.37	2.48	0.67	0.08	0.54	0.37	3.83

^zTreatments at a light intensity of $250 \pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Percentages indicate contributions of narrow-band wavelengths in total light intensity: blue [$447 \pm 5 \text{ nm}$, full width half maximum (FWHM) = 20 nm], red ($627 \pm 5 \text{ nm}$, FWHM = 20 nm), green ($530 \pm 5 \text{ nm}$, FWHM = 30 nm).

^yMean values represent plants from 7 g of seed, eight replications per treatment; SE estimates: β -carotene (BC) = 0.45, lutein (LUT) = 0.82, neoxanthin (NEO) = 0.22, zeaxanthin (ZEA) = 0.03, antheraxanthin (ANT) = 0.18, violaxanthin (VIO) = 0.12, total integrated carotenoid pigments (Total CAR) = 1.27.

^xMeans followed by the same letter are not statistically different, $\alpha = 0.05$.

LSD = least significant difference.

Table 4. Mean values for macronutrient concentrations in the shoot tissue of sprouting broccoli microgreens grown in controlled environments under broad-spectrum light and different specific narrow-band wavelengths from light-emitting diodes.

Light treatments ^z	Shoot tissue macronutrients ^y				
	Calcium	Potassium	Magnesium	Phosphorus	Sulfur
	(mg·g ⁻¹ dry mass)				
Fluorescent/incandescent	11.43 c ^x	10.94 b	3.15 c	4.10 b	3.31 c
5% blue/95% red	15.75 b	16.47 ab	4.89 ab	6.91 a	5.25 b
5% blue/85% red/10% green	15.56 b	14.07 b	4.57 b	6.67 a	5.44 ab
20% blue/80% red	18.71 a	21.48 a	5.61 a	7.78 a	6.49 a
20% blue/70% red/10% green	17.82 ab	21.55 a	5.16 ab	6.98 a	6.27 ab
LSD ($\alpha = 0.05$)	2.73	6.14	0.98	1.37	1.17

^zTreatments at a light intensity of $250 \pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Percentages indicate contributions of narrow-band wavelengths in total light intensity: blue [$447 \pm 5 \text{ nm}$, full width half maximum (FWHM) = 20 nm], red ($627 \pm 5 \text{ nm}$, FWHM = 20 nm), green ($530 \pm 5 \text{ nm}$, FWHM = 30 nm).

^yMean values represent plants from 7 g of seed, eight replications per treatment; SE estimates: calcium = 0.91, potassium = 2.04, magnesium = 0.33, phosphorus = 0.45, sulfur = 0.39.

^xMeans followed by the same letter are not statistically different, $\alpha = 0.05$.

LSD = least significant difference.

Table 5. Mean values for micronutrient concentrations in the shoot tissue of sprouting broccoli microgreens grown in controlled environments under broad-spectrum light and different specific narrow-band wavelengths from light-emitting diodes.

	Shoot tissue micronutrients ^y					
	Boron	Copper	Iron	Manganese	Molybdenum	Zinc
Light treatments ^z	(μg·g ⁻¹ dry mass)					
Fluorescent/incandescent	33.72 b ^x	2.56 b	43.56 b	20.71 b	0.63 b	22.94 c
5% blue/95% red	56.36 a	3.34 ab	72.48 a	39.88 a	0.97 a	33.23 ab
5% blue/85% red/10% green	53.79 a	3.60 a	70.54 a	39.98 a	0.94 a	30.57 b
20% blue/80% red	57.73 a	3.73 a	74.51 a	40.52 a	1.09 a	38.62 a
20% blue/70% red/10% green	52.11 a	3.06 ab	64.35 a	38.00 a	1.01 a	34.16 ab
LSD (α = 0.05)	11.96	1.02	15.57	11.43	0.16	6.55

^zTreatments at a light intensity of $250 \pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Percentages indicate contributions of narrow-band wavelengths in total light intensity: blue [$447 \pm 5 \text{ nm}$, full width half maximum (FWHM) = 20 nm], red ($627 \pm 5 \text{ nm}$, FWHM = 20 nm), green ($530 \pm 5 \text{ nm}$, FWHM = 30 nm).

^yMean values represent plants from 7 g of seed, eight replications per treatment; SE estimates: boron = 3.97, copper = 0.34, iron = 5.16, manganese = 3.79, molybdenum = 0.05, zinc = 2.17.

^xMeans followed by the same letter are not statistically different, $\alpha = 0.05$.

LSD = least significant difference.

the 5% blue/85% red/10% green light treatment (Table 6). Tissue GI, GR, GE, GB, and NGB concentrations were lower under the fluorescent/incandescent and 20% blue/70% red/10% green light treatments when compared with the other LED light treatments (Table 6). Tissue concentrations of 4MGB were lowest for plants in the fluorescent/incandescent light treatment compared with all other LED treatments (Table 6).

Light treatment significantly impacted the glucosinolate classes of aliphatic ($P \leq 0.001$), indoles ($P \leq 0.001$), and total GS ($P \leq 0.001$) in the sprouting broccoli microgreen shoot tissues. Light treatment did not affect concentrations of the total aromatic GS (gluconasturtiin) in the shoot tissues of the sprouting broccoli microgreens. The highest concentrations of shoot tissue aliphatic, indoles, and total GS were found under the 20% blue/80% red light treatment (Table 7). Tissue aliphatic, indole, and total GS concentrations were lower under fluorescent/incandescent and 20% blue/70% red/10% green light treatments when compared with the 5% blue/85% red/10% green and 20% blue/80% red light treatments (Table 7).

Discussion

Narrow-band wavelengths within the electromagnetic spectrum have strong influences on plant metabolic processes. Plants have evolved to respond to even subtle changes in radiational wavelengths. Ratios of red to far-red wavelengths impact seed germination and flowering (Chaves et al., 2011; Vierstra and Zhang, 2011), whereas differences in the intensities of blue wavelengths control phototropism responses, stem elongation, chloroplast movements, and stomatal operations (Baum et al., 1999; Briggs and Christie, 2002; Christie, 2007). LEDs now provide the ability to measure impacts of narrow-band wavelengths of light on plant physiology; moreover, successful plant production has been demonstrated using blue and red LED wavelengths.

A previous study by our group showed significant increases in tissue

Table 6. Mean values for individual glucosinolate concentrations in the shoot tissue of sprouting broccoli microgreens grown in controlled environments under broad-spectrum light and different specific narrow-band wavelengths from light-emitting diodes.

Light treatments ^z	Shoot tissue glucosinolates ^y									
	GI	PRO	EPI	GR	SN	4OHGB	GE	GB	4MGB	NGB
	(μg·g ⁻¹ dry mass)									
Fluorescent/incandescent	1.85 b*	0.16 b	0.42 a	4.53 b	0.15 b	4.81 a	0.25 d	6.74 c	1.96 c	1.61 b
5% blue/95% red	3.99 a	0.28 ab	0.58 a	10.34 a	0.20 b	4.78 a	1.51 bc	16.35 ab	4.86 b	3.14 a
5% blue/85% red/10% green	5.14 a	0.27 ab	0.73 a	12.75 a	0.45 a	6.36 a	2.73 a	21.21 a	5.59 b	3.43 a
20% blue/80% red	5.21 a	0.33 a	0.73 a	13.66 a	0.33 ab	5.71 a	2.22 ab	19.29 a	8.18 a	3.54 a
20% blue/70% red/10% green	2.35 b	0.21 ab	0.33 a	6.63 b	0.30 ab	5.20 a	0.55 cd	10.52 bc	4.17 b	1.92 b
LSD (α = 0.05)	1.52	0.15	0.44	3.64	0.24	1.60	1.08	6.58	2.14	1.07

^zTreatments at a light intensity of 250 ± 10 μmol·m⁻²·s⁻¹. Percentages indicate contributions of narrow-band wavelengths in total light intensity: blue [447 ± 5 nm, full width half maximum (FWHM) = 20 nm], red (627 ± 5 nm, FWHM = 20 nm), green (530 ± 5 nm, FWHM = 30 nm).

^yMean values represent plants from 7 g of seed, eight replications per treatment; SE estimates: glucoiberin (GI) = 0.54, progoitrin (PRO) = 0.53, epiprogoitrin (EPI) = 0.16, glucoraphanin (GR) = 1.29, sinigrin (SN) = 0.08, 4-hydroxyglucobrassicin (4OHGB) = 0.57, glucoerucin (GE) = 0.38, glucobrassicin (GB) = 2.35, 4-methoxyglucobrassicin (4MGB) = 0.76, neoglucobrassicin (NGB) = 0.38.

*Means followed by the same letter are not statistically different, α = 0.05.

LSD = least significant difference.

Table 7. Mean values for glucosinolate class concentrations in the shoot tissue of sprouting broccoli microgreens grown in controlled environments under broad-spectrum light and different specific narrow-band wavelengths from light-emitting diodes.

Light treatments ^z	Shoot tissue glucosinolates ^y			
	Aliphatic	Aromatic	Indoles	Total GS
	(μg·g ⁻¹ dry mass)			
Fluorescent/incandescent	7.36 c*	0.01 a	15.11 c	22.47 c
5% blue/95% red	16.89 ab	0.08 a	29.13 ab	46.09 ab
5% blue/85% red/10% green	22.07 a	0.01 a	36.58 a	58.65 a
20% blue/80% red	22.47 a	0.06 a	36.72 a	59.24 a
20% blue/70% red/10% green	10.37 bc	0.02 a	21.80 bc	32.19 bc
LSD (α = 0.05)	6.67	0.08	9.46	15.81

^zTreatments at a light intensity of 250 ± 10 μmol·m⁻²·s⁻¹. Percentages indicate contributions of narrow-band wavelengths in total light intensity: blue [447 ± 5 nm, full width half maximum (FWHM) = 20 nm], red (627 ± 5 nm, FWHM = 20 nm), green (530 ± 5 nm, FWHM = 30 nm).

^yMean values represent plants from 7 g of seed, eight replications per treatment; SE estimates: aliphatic = 2.37, aromatic = 0.03, indoles = 3.37, total integrated glucosinolates (Total GS) = 5.63.

*Means followed by the same letter are not statistically different, α = 0.05.

LSD = least significant difference.

pigments, GS compounds, and mineral elements in sprouting broccoli microgreens exposure to short-duration blue light after culture under blue and red LED light (Kopsell and Sams, 2013). The present study examined the impacts of different percentages of blue to red light, with and without green light, on primary and secondary metabolites in sprouting broccoli microgreens. The study also compared plant responses under fluorescent/incandescent light. Sprouting broccoli microgreen shoot tissue FM under the LED light treatments was similar to Kopsell and Sams (2013). Shoot FM was significantly greater under LED light treatments than under the fluorescent/incandescent light treatment, although no changes in shoot tissue DM were found among the light treatments. Shoot tissue FM for lettuce (*L. sativa* var. *capitata*) grown under similar light intensities as the current study were higher under blue, red, and white LED lights as compared with fluorescent light (Lin et al., 2013). Chili pepper 'Cheongyang' plants grown under lower light intensity than the current study, but of equal physiological age, had significantly higher leaf tissue FM under red and blue LED lighting than under fluorescent lighting (Gangadhar et al., 2012). Taken together, these results demonstrate an advantageous

impact of narrow-band LED wavelengths on shoot tissue FM during early seedling growth. There were no impacts of light treatment on shoot tissue DM accumulation in the current study, which is similar to results from Lin et al. (2013). Results are also similar to Samuolienė et al. (2013) who reported no impacts on DM for brassica microgreens [kohlrabi (*B. oleracea* var. *gongylodes* 'Delicacy Purple'), mustard (*B. juncea* 'Red Lion'), red pak choi (*B. rapa* var. *chinensis* 'Rubi F₁'), and tatsoi (*B. rapa* var. *rosularis*)] grown under 7.5% blue/92% red/0.5% far-red LED light at irradiance intensities of 330 to 545 μmol·m⁻²·s⁻¹. Exposing seedlings to only red and blue wavelengths would maximize photosynthetic processes and eliminate the

need for photo-protection and energy dissipation from exposure to other wavelengths found in spectra emitted from fluorescent/incandescent bulbs. Reducing possible light stress in the growing environment by using narrow-band wavelengths may allow for greater water uptake and increases in FM tissue accumulation in the microgreen seedling.

Chlorophyll *a* and *b* pigments in leaf tissue maximize light absorption in red (663 and 642 nm, respectively) and blue (430 and 453 nm, respectively) wavelengths (Lefsrud et al., 2008). Wavelengths of maximum light absorption by chlorophyll correlate to maximum photosynthetic efficiency. Lefsrud et al. (2008) demonstrated Chl *a* and Chl *b* concentrations were highest in kale seedlings exposed to the narrow-band LED wavelengths of 640 and 440 nm, establishing a positive correlation between wavelength and chlorophyll accumulation in brassica seedlings. Results from the current study demonstrate dramatic increases in chlorophyll pigment concentrations under LED light when compared with fluorescent/incandescent bulbs. These results contrast other studies in which chlorophyll pigment concentrations in lettuce and chili pepper seedlings did not differ among fluorescent and red and blue LED light

treatments (Gangadhar et al., 2012; Lin et al., 2013). Data in the current study show a cumulative effect of increasing percentages of blue wavelengths in the lighting background. Concentrations of Chl *a*, Chl *b*, and total Chl were significantly higher under the 20% blue/80% red light treatment when compared with other lighting treatments. Chlorophyll pigments in the sprouting broccoli microgreens in increasing concentrations for the light treatments were: fluorescent/incandescent less than 5% blue/95% red = 5% blue/85% red/10% green less than 20% blue/70% red/10% green = 20% red/80% blue. Both Chl *a* and Chl *b* absorb higher amounts of light in higher energy blue wavelengths. The data may show that plants produce higher concentrations of chlorophyll pigments in response to higher levels of blue wavelengths in the light environment.

The photosynthetic apparatus is highly dynamic with the ability to respond rapidly to changes in light stimuli (Szabó et al., 2005). Carotenoid pigments are integrated into light-harvesting complexes of chloroplasts and participate in light harvesting and photo-protection (Croce et al., 1999; Demmig-Adams and Adams, 1996; Frank and Cogdell, 1996). Leaf tissues of most plant species accumulate high concentrations of BC, LUT, and NEO along with minor concentrations of ZEA, ANT, and VIO carotenoids (Sandmann, 2001). The dominate carotenoids found in sprouting broccoli microgreens are BC and LUT (Kopsell and Sams, 2013). In the current study, BC and LUT responded similarly to the lighting treatments. The fluorescent/incandescent lighting treatment resulted in significantly lower BC and LUT when compared with all other LED treatments. However, there were no differences in BC or LUT among the LED lighting treatments, which is consistent with the finding of Tuan et al. (2013) where BC and LUT concentrations in buckwheat sprouts did not differ among red and blue LED light treatments. Gangadhar et al. (2012) also showed that total carotenoid pigments in chili pepper seedlings were higher under LED lighting treatments than for fluorescent light. However, Lin et al. (2013) reported no differences in total carotenoid concentrations in lettuce among LED and fluorescent light treatments. In the current study, the minor carotenoid pigments of ZEA, ANT, and VIO did not differ under the light treatments or were slightly higher under the fluorescent/incandescent lighting treatment. The xanthophyll cycle controls the energy dissipation of excess light excitation through reversible de-epoxidation and epoxidation of ZEA, ANT, and VIO (Latowski et al., 2004). After exposure to high light conditions, VIO is converted to ZEA to dissipate high thermal energy through the intermediate ANT through de-epoxidation reactions (Havaux et al., 2007; Kopsell et al., 2012). In the current study, there was no impact of light treatment on the pool of total xanthophyll cycle pigments (data not shown) or on ZEA concentrations, indicating that none of the light treatment imposed stress on the microgreens. Total integrated carotenoid pigments in the sprouting broccoli microgreens in increasing concentrations for the light treatments were: fluorescent/incandescent less than 5% blue/95% red = 5% blue/85% red/10% green = 20% blue/70% red/10% green = 20% red/80% blue. Park et al. (2013) showed blue light from LEDs to positively influence sucrose metabolism in ginseng roots, which would indicate higher photosynthetic efficiency and biosynthesis of carotenoid compounds. The data demonstrate the potential to produce carotenoid-dense microgreens using LEDs of optimal blue and red light.

The perception of blue light wavelengths by plants results in fundamental photomorphogenic changes. Plant responses to blue light are preceded or coincide with significant changes in electrochemical potentials of cells and tissues resulting from rapid changes in membrane potentials and stimulation of ion transport mechanisms (Babourina et al., 2002; Spalding, 2000). Furthermore, blue light acts as a powerful light signal controlling stomatal operations (Sharkey and Raschke, 1981). On photosynthetic saturation of guard cells, additions of blue light, but not red light, proved effective at inducing membrane transport and proton extrusion through increased H⁺-ATPase, which stimulated K⁺ uptake channels (Assmann et al., 1985; Dietrich et al., 2002). Blue light irradiance also induced H⁺ and Ca²⁺ transporters in *Arabidopsis thaliana* (Babourina et al., 2002). In the current study, the lowest concentrations of shoot tissue Ca, K, Mg, P, S, B, Fe, Mn, Mo, and Zn were found under the fluorescent/incandescent light treatment, which were significantly lower than the higher blue light LED treatments. Kopsell and Sams (2013) also demonstrated increases in macronutrient and micronutrient concentration in the sprouting broccoli microgreens under illumination of only blue wavelengths. Results for macronutrient and micronutrient concentration in the sprouting broccoli microgreens in the current study provide further support on the impacts of blue light wavelengths on proton pumping, membrane permeability, and ion channel activities. It also demonstrates the potential to produce nutrient-dense microgreens under higher percentages of blue wavelengths in the light environment.

The major aliphatic glucosinolate found in broccoli is GR. The isothiocyanate derived from GR is sulforaphane, which induces up-regulation of phase II detoxification enzymes and is the central cancer-preventive agent in broccoli (Fahey et al., 1997). The bioavailability of sulforaphane from broccoli sprouts is superior to that of prepared broccoli supplements and validates the valuable nutritional impacts of whole foods (Clarke et al., 2011; Fahey et al., 1997). The light environment can have impacts of glucosinolate concentrations within brassica species (Charron and Sams, 2004; Kopsell and Sams, 2013; Lefsrud et al., 2008), and data in the current study further illustrate the potential to manipulate GS concentrations through light treatments. The LED light treatments of 5% blue/95% red, 5% blue/85% red/10% green, and 20% blue/80% red had significantly higher shoot tissue individual GS than the broccoli microgreens grown under the fluorescent/incandescent light treatment, in most cases dramatically higher (Table 6). The classes of GS compounds followed similar trends with light treatments of 5% blue/95% red, 5% blue/85% red/10% green, and 20% blue/80% red having significantly higher aliphatic, indole, and total GS than the fluorescent/incandescent light treatment (Table 7). Glucosinolate classes are divided based on amino acids side chain derivatives. Aliphatic GSs (GI, PRO, EPI, GR, SN) are derived from Alanine, Leucine, Isoleucine, Valine, and Methionine; aromatic GSs are derived from Phenylalanine or Tyrosine; and indole GSs (GB, NGB, 4OHGB) are derived from Tryptophan (Sønderby et al., 2010). In a recent study by Park et al. (2013), the impacts of LED lighting on metabolic profiles in ginseng roots revealed that blue light treatments were associated with higher metabolism of many amino acids. It is possible that the LED light treatments in the current study influenced GS side chain elongation and modifications through impacts on amino acid metabolism. This study further demonstrates the potential to

produce glucosinolate-dense brassica microgreens under higher percentages of blue wavelengths in the light environment.

Results from the current study clearly support data from many previous reports that describe stimulation of primary and secondary metabolite biosynthesis after exposure to blue light wavelengths from LEDs. Perception of energy-rich blue light by specialized photoreceptors will trigger a cascade of metabolic responses in plants. Stimulation of primary and secondary metabolic pathways associated with nutritional quality factors using blue LED wavelengths appears promising. Management of the light environment may be a viable means to improve the nutritional contributions of specialty vegetable crops.

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