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Irradiance from Distinct Wavelength Light-emitting Diodes Affect Secondary Metabolites in Kale

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Abstract. **The use of light-emitting diodes (LEDs) for plant production is a new field of research that has great promise to optimize wavelength-specific lighting systems for precise management of plant physiological responses and important secondary metabolite production. In our experiment, hydroponically cultured kale plants (*Brassica oleracea* L. var. *acephala* D.C.) were grown under specific LED wavelength treatments of 730, 640, 525, 440, and 400 nm to determine changes in the accumulation of chlorophylls, carotenoids, and glucosinolates. Maximum accumulation, on a fresh mass basis, of chlorophyll *a* and *b* and lutein occurred at the wavelength of 640 nm, whereas β-carotene accumulation peaked under the 440-nm treatment. However, when lutein was measured on a dry mass basis, maximum accumulation was shifted to 440 nm. Sinigrin was the only glucosinolate to respond to wavelength treatments. Wavelength control using LED technology can affect the production of secondary metabolites such as carotenoids and glucosinolates with irradiance levels also a factor in kale. Management of irradiance and wavelength may hold promise to maximize nutritional potential of vegetable crops grown in controlled environments.**

Plant pigments have specific wavelength absorption patterns known as absorption spectra. Biosynthetic wavelengths for the production of plant pigments [chlorophylls (Chl) and carotenoids] are referred to as action spectra. Specifically, Chl *a* and *b* absorb wavelengths of light strongly in the red (maximum absorption at 663 and 642 nm, respectively) and blue region (maximum absorption at 430 and 453 nm, respectively) with less absorption occurring in the green wavelengths (Hopkins and Huner, 2004). The carotenoid pigments lutein (L) and β-carotene (BC) absorb strongly in the blue region with maximum absorption occurring at 448 and 454 nm (in acetone), respectively (Hopkins and Huner, 2004). Previously, maximum biosynthesis of plant pigments for the action spectrum for wheat (*Triticum aestivum* L.) occurred at 447 and 646 nm for Chls and BC, respectively (Ogawa et al., 1973). The action spectrum for white corn (*Zea mays* L.) revealed maximum absorption of Chls and BC to be 445 and 650 nm, respectively (Koski et al., 1951).

Environmental factors such as temperature and irradiance levels can have strong influences on the accumulation of plant pigments and glucosinolates (Antonious et al., 1996; Charron and Sams, 2004; Lefsrud et al., 2005, 2006). Previous research on the physiological impacts of wavelengths on plants has used filters, interference filters, and/or wavelength-specific bulbs. However, a weakness in these techniques has been a reduction in the accompanying irradiance levels with most techniques resulting in irradiance levels less than 13 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Ogawa et al., 1973; Virgin, 1993). Even under decreased irradiance levels, physiological changes have been reported for plants grown under varying wavelengths (Gauthier et al., 1997; Heraut-Bron et al., 2001; Quaderi and Reid, 2005; Walters and Horton, 1995).

With the development of light-emitting diodes (LEDs), specific wavelengths of light can now be applied at higher irradiance levels, thus producing more realistic results on the effects of wavelength on plant physiological responses. However, to date, there is limited information on plant responses to LED lighting. A few studies have demonstrated plant responses to red light using LED. Exposure to only red LED light resulted in both plant elongation and reduced biomass for lettuce (*Lactuca sativa* L.; Hoenecke et al., 1992) and pepper (*Capsicum annuum* L.; Brown et al., 1995).

Kale (*Brassica oleracea* L. var. *acephala* D.C.) is an excellent source of glucosinolates (GS) (Stoewsand, 1995) and carotenoids. Kale ranks as the highest source of L and BC of any vegetable (U.S. Dept. Agric., 2002). In plants, carotenoids are used as antenna pigments and quench the energetic triplet state of the Chl molecule to prevent damage to the photosynthetic system (Tracewell et al., 2001). In humans, dietary intake of foods rich in carotenoids is associated with reduced risk of lung cancer, cataracts, and age-related macular degeneration (Landrum and Bone, 2001; Le Marchand et al., 1993), whereas diets high in vegetables with GS reduce the risk of cancer (Stoewsand, 1995; van Poppel et al., 1999).

Light is critical for plant growth and development, and wavelengths can easily be controlled by growers in artificial growing environments. Therefore, the goal of this study was to investigate the influences of five different wavelengths of light using LED on plant biomass and accumulation patterns of Chls, carotenoids, and GS compounds in the leaf tissues of kale.

Material and Methods

'Winterbor' kale (Johnny's Selected Seed, Winslow, ME) was hydroponically grown in a controlled environmental (Model E15; Conviron, Winnipeg, Manitoba, Canada) using a half-strength Hoagland nutrient solution (described previously by Lefsrud et al., 2006). An air temperature of 20 ± 1°C and a cool-white fluorescent (17 W) and incandescent (40 W) pretreatment irradiance at 275 ± 10 $\mu mol \cdot m^{2} \cdot s^{-1}$ (at canopy height) under a 16-h photoperiod was used to culture kale plants for 7 d. A LED array capable of providing five wavelengths and independent control was acquired from a commercial source (ORBITEC, Madison, WI). The wavelengths provided by the LED were 730, 640, 525, 440, and 400 nm. The manufacturer reported irradiance intensity of 225, 47, and 154 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (LI-190; LI-COR Bioscience, Lincoln, NE) at 10 cm from the array and power use of 10.1, 9.8, and 9.9 W for the wavelengths of 640, 525, and 440 nm, respectively. Spectroradiometer measurements were taken by the manufacturer at 10 cm from the array with results of 15.2, 253.3, 6.5, 10.6, and 6.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PS-200; Apogee Instruments, Logan, UT) for 730, 640, 525, 440, and 400 nm, respectively. Photosynthetically active radiation (*PAR*) for each wavelength was measured at leaf canopy height (≈4 cm from the array) at the beginning of each treatment. Six plants were placed into 2-cm round holes at 10.6 × 9.5-cm spacing in each container lid. The experiment consisted of growing a single plant directly under the LED array (6 × 6 cm) with five guard plants surrounding the test plant. Irradiance was only supplied by the LED array with the chamber light sealed. The plants were grown for 7 d under the LED array with a 16-h photoperiod. Final harvest occurred at 10 h into the irradiance cycle.

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Table 1. Mean pigment (mg/100 g^{-1} fresh mass) and glucosinolate (mg/100 g^{-1} dry mass) concentrations[z] in the leaf tissues of 'Winterbor' kale grown under specific wavelength using LEDs.

Wavelength (nm)	Pigment (mg/100 g^{-1} FM)				Glucosinolate (mg/100 g^{-1} DM)
	Lutein	β-carotene	Chl *a*[y]	Chl *b*[y]	Sinigrin
730	6.9 ± 1.0	2.7 ± 0.4	31.1 ± 10.1	16.0 ± 6.0	21.7 ± 21.7
640	11.2 ± 0.4	3.7 ± 0.4	85.7 ± 7.2	66.2 ± 2.8	32.0 ± 16.6
525	7.8 ± 0.8	3.3 ± 0.4	51.2 ± 10.5	31.8 ± 6.5	0.8 ± 0.8
440	9.8 ± 0.7	4.0 ± 0.4	63.8 ± 4.3	33.9 ± 5.1	ND[y]
400	8.1 ± 1.1	3.4 ± 0.3	57.9 ± 3.7	28.8 ± 3.9	ND[y]

[z]Mean composition of sampled leaf tissue of three replications and one plant ± standard error.
[y]Chl *a* = chlorophyll *a*; Chl *b* = chlorophyll *b*; ND = not detected.
LEDs = light-emitting diodes; FM = fresh mass; DM = dry mass.

The experiment was replicated three separate times for all five wavelengths. At harvest, shoot tissues from the single plant directly underneath the LED array were harvested according to Lefsrud et al. (2006). Leaves between 1 and 2 weeks old were collected based on pigment accumulation rate reported by Lefsrud et al. (2007). Carotenoids and Chl levels were determined according to Kopsell et al. (2004) and glucosinolates according to Charron and Sams (2004) using high-performance liquid chromatograph analysis. Main effects were analyzed by one-way analysis of variance.

Results and Discussion

Irradiance levels were measured for all treatment wavelengths using a light sensor (QSO-ELEC; Apogee Instruments). *PAR* levels at 4 cm from the LED array for the 730-, 640-, 525-, 440-, and 400-nm treatments were 1.4, 226.5, 5.7, 10.5, and 2.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. The LED array produced a small amount of background light (0.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR* irradiance) when the system was electrified but turned off during the skotoperiod. The LED manufacturer reported using a LI-COR light senor, which was different than the one we used to calculate experimental irradiance from the LED array; therefore, the difference between the manufacture's reported irradiance values and our measured values could be the result of the difference in light sensors used.

The effects of wavelength treatments on the concentrations of L, BC, and Chl pigments were measured for the kale shoot tissues (Table 1). Lutein concentrations responded to changes in wavelength ($P = 0.031$), but BC was not significant. Maximum L accumulation on a fresh weight (FM) basis (11.2 mg/100 g^{-1} FM) occurred under the highest *PAR* at 640 nm, whereas the lowest L concentrations (6.9 mg/100 g^{-1} FM) occurred under the lowest *PAR* at 730 nm wavelength. The concentrations of Chl *a* ($P = 0.007$), Chl *b* ($P \leq 0.001$), and total Chl ($P = 0.002$) pigments were influenced by changes in wavelength *PAR*. Maximum Chl accumulation occurred under the highest *PAR* at 640 nm. Glucosinolates were measured in the kale shoot tissues (data not shown). Sinigrin ($P = 0.050$), an aliphatic GS, responded to wavelength treatment (Table 1). The enzyme myrosinase catalyzes the hydrolysis of sinigrin to produce allyl isothiocyanate, which reduces the incident of certain cancers (Stoewsand, 1995). Wavelength did not affect the accumulation of any of the other GS compounds (data not shown).

In our study, peak accumulation of the Chls, L, and sinigrin occurred under the 640-nm wavelength, which corresponded to the maximum measured irradiance. A second peak was also measured at 440 nm, corresponding to the second highest irradiance, for Chls and L. Although not significant, the peak for BC was reversed with the major peak at 440 nm and another at 640 nm. Our research produced two peaks of maximum carotenoid pigment concentrations at 440 and 640 nm, which closely conforms to the action spectrum reported for wheat (Ogawa et al., 1973). However, the Chls only had one peak of maximum concentration occurring at 640 nm, which closely conforms to the maximum irradiance level from the LEDs.

Irradiance can be a major factor in pigment accumulation within kale leaves with previous research by Lefsrud et al. (2006) demonstrating that irradiance levels affected concentrations of L, BC, and Chl when measured on a FM basis. Based on Lefsrud et al. (2006) irradiance research, the maximum levels of accumulation should have occurred at our maximum irradiance at 640 nm and decreased linearly in relation to irradiance, but this was not observed in this experiment. The low irradiance levels at 400- and 525-nm wavelengths, pigment accumulations were not statistically different from the 440 nm, although the irradiance was statistically different. Further research is required to determine if the results measured in this study were a result of wavelength or simply irradiance levels. In either case, this study demonstrates LED arrays may facilitate investigation on the impact of specific wavelengths on secondary metabolite production in vegetable crops.

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