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Research Report

Supplemental Lighting with LEDs Improves the Biochemical Composition of Two *Valerianella locusta* (L.) Cultivars

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Abstract. In recent years, light-emitting diodes (LEDs) have been introduced as a new source of supplemental lighting in winter greenhouse crop production to improve yield and quality. The aim of this study was to determine the effect of light supplementation with LEDs on the yield and the concentration of several health-promoting compounds in two cultivars of lamb's lettuce (*Valerianella locusta*, L.), 'Noordhollandse' and 'Holländisher' in a greenhouse cultivation in winter. Four different LED spectra used to extend the photoperiod (16 h day/8 h night) were tested: 10R:0B (100% red), 9R:1B (90% red, 10% blue), 8R:2B (80% red, 20% blue), and 7R:3B (70% red, 30% blue). The control plants were illuminated by high-pressure sodium (HPS) lamps. The photosynthetic photon flux density at the plant canopy level was approximately 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in all treatments. The plants were harvested on the 45th day of light treatment (60 days after sowing). In both cultivars, the 10R:0B LED light treatment resulted in the highest yield, but low concentrations of ascorbic acid, xantophylls, and phenolic compounds. Supplemental lighting with HPS lamps resulted in high concentrations of nitrate and carotenoids, but low fresh mass, ascorbic acid, and total phenols content. The addition of blue light in the spectrum increased antioxidant properties; however, certain effects were cultivar-dependent. High yields with the most satisfactory composition of soluble sugars, ascorbic acid, carotenoids, and polyphenols, as well as high antioxidant properties and reduced nitrate levels, in the leaves of the tested cultivars were obtained with 8R:2B LED lighting. Thus, supplemental lighting with 80% red and 20% blue light may be advantageous in greenhouse-cultivated *V. locusta* 'Noordhollandse' and 'Holländisher' cultivars.

***Additional key words*:** antioxidants, ascorbic acid, carotenoids, greenhouse cultivation, HPLC, polyphenols

Introduction

Light provides the energy for carbon assimilation and the production of primary and secondary metabolites and also plays a signaling role in plant growth and development *via* photoreceptors (e.g. phytochromes, cryptochromes, phototropins, or UVR8) contributing to photomorphogenesis and the regulation of the synthesis of many compounds (Devlin et al., 2007; Ouzounis et al., 2015; Kong and Okajima, 2016). Thus, the insufficient quantity and low quality of light in autumn and winter may cause significant decreases in the yield and quality of greenhouse-grown crops. The use of artificial lighting in crop production helps to minimize the negative consequences of light deficiency.

In recent years, LED (light-emitting diode) light sources have been intensively studied for their potential to be used as supplemental lighting in greenhouses and their contribution to morphological, physiological, and biochemical properties of crop plants. Some experiments indicate the positive effects of LED light on fresh weight of plants (for example, lettuce or cabbage), and also on the improvement of nutritional quality (Li et al., 2012; Bian et al., 2015; Lee et al., 2016). The most frequent spectrums of light used in crop production are red and blue, which are correlated to the maxima of the absorption spectra of chlorophyll a (maximum absorption of 420 and 660 nm) and chlorophyll b (maximum absorption of 435 and 642 nm). For example, supplemental lighting with red LEDs in greenhouse cultivated lettuce resulted in an increase of sugars (Samuolienė et al., 2012), antioxidant capacity (Žukauskas et al., 2011; Samuolienė et

al., 2012), and phenolic compounds (Li and Kubota, 2009; Žukauskas et al., 2011; Samuolienė et al., 2012), and a decrease in the concentration of nitrates (Samuolienė et al., 2009a). Furthermore, some studies have shown the positive effects of supplemental blue LED light in lettuce cultivation, including enhancement of polyphenol concentrations (Jokhan et al., 2010), antioxidant potential (Stutte et al., 2009; Jokhan et al., 2010), and the levels of carotenoids (Li and Kubota, 2009). Increased levels of chlorophyll and ascorbic acid were also observed in non-heading Chinese cabbage grown under blue LED light (Li et al., 2012). The results of our previous research indicate that the extension of the photoperiod with LED light in greenhouse cultivation of lamb's lettuce (*Valerianella locusta*, L.) helped to obtain higher yields and quality. For example, the use of supplemental lighting with 90% red and 10% blue LEDs in winter cultivation of *V. locusta* contributed to higher yields and dry mass, as well as higher concentrations of soluble sugars and phenolic compounds than in plants grown under HPS lamps (Wojciechowska et al., 2015). We also found that the use of certain red/blue LED light combinations or white LED light may enhance the levels of folate in greenhouse-grown *V. locusta* (Długosz-Grochowska et al., 2016).

Though supplementation with LEDs can improve the quality of greenhouse-grown leafy vegetables, the light spectra required for maximal productivity and optimal nutritional quality in different species or varieties is still unknown (Olle and Viršile, 2013). The aim of this study was to determine the effect of light supplementation with LEDs on the yield and the concentration of several nutritional compounds in two cultivars of *V. locusta*. Our results will help to identify the optimal light spectrum for greenhouse cultivation of *V. locusta* cultivars 'Noordhollandse' and 'Holländisher' for maintaining high yield and a satisfactory biochemical composition.

Materials and Methods

Plant Materials and Growth Conditions

The experiment was conducted in the greenhouse of the Faculty of Biotechnology and Horticulture at the University of Agriculture in Kraków (50°03'N, 19°57'E), Poland. Seeds of lamb's lettuce (*Valerianella locusta*, L.) cultivars, 'Noordhollandse' and 'Holländisher', were sown on December 4, 2014 into cell trays filled with peat substrate (Klasmann KTS 2424, Geeste, Germany; chemical composition [mg·dm^{-3}]: N, 382.0; P, 452.0; K, 534.0; Mg, 156.0; Ca, 1272.0; Na, 32.0; S, 484.0; B, 0.5; Cu, 0.15; Fe, 3.5; Mn, 3.0; Zn, 0.7). Supplemental lighting was implemented just after germination (Dec. 15, 2014). Plants were harvested on January 29, 2015 after 45 days of supplemental lighting in a 16/8 hours (day/night) photoperiod. The average temperature during cultivation was 15.6°C and the humidity was approximately 58.7%.

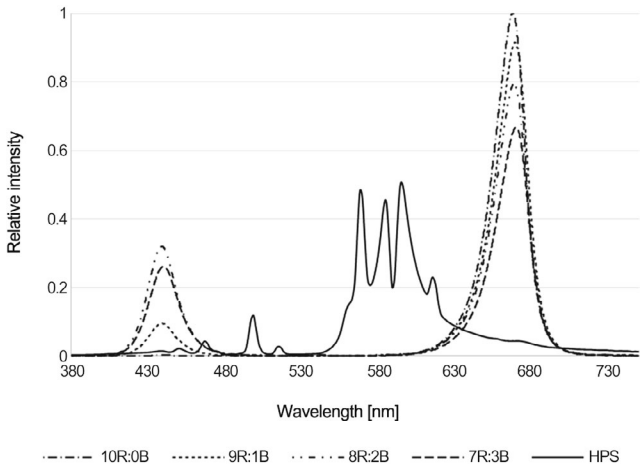

Fig. 1. Emission spectra of the four light-emitting diode treatments and the HPS lamp (control) used as supplemental lighting in greenhouse cultivation of *V. locusta* cultivars (10R:0B, 100% red; 9R:1B, 90% red and 10% blue; 8R:2B, 80% red and 20% blue; 7R:3B, 70% red and 30% blue; HPS, High Pressure Sodium lamp)

The supplemental radiation source was a SSL (solid state lighting) LED system (Grzesiak et al., 2014). Each LED lamp consisted of 48 100 W OSRAM OSLON diodes: red (peak at 660 nm) and blue (peak at 440 nm). The four LED treatments differed in the percentage of red and blue light: 100% red (10R:0B), 90% red and 10% blue (9R:1B), 80% red and 20% blue (8R:2B), or 70% red and 30% blue (7R:3B) (Fig. 1). Each treatment included four identical LED lamps (each lamp radiated 72 plants of each cultivar for a total of four replications). The control treatment used HPS lamp (SON AGRO 600W, Philips, Amsterdam, Netherlands) (Fig. 1). The photosynthetic photon flux density (PPFD) at the canopy level was approximately 200 μmol·m^{-2}·s^{-1} (measurements were taken with an LI-250A Light Meter with a LI-190 Quantum Sensor, LI-COR, Lincoln, Nebraska, USA).

Estimation of Yield and Dry Matter

To estimate yield, the fresh weight of 10 rosettes from each repetition was measured. The yield was expressed as grams of fresh weight per one rosette. Samples of 0.5 g of chopped plant material were dried at 105°C to obtain constant weight (about 4 hours) and then dry matter was measured. Dry matter measurements were expressed as percentage of fresh weight.

Biochemical Analyses

All chemicals, standards, and reagents were purchased from Sigma-Aldrich (Saint Louis, MO, USA), unless otherwise stated. Chemicals used for chromatographic analyses were

of HPLC gradient grade. Other reagents were of analytical grade.

Samples for biochemical analyses were collected at harvest (after 45 days of supplemental lighting).

***Content of soluble sugars*:** The concentration of soluble sugars in the tested materials was measured according to the anthrone colorimetric method described by Yemm and Willis (1954). In acidic conditions, the reaction of sugars with anthrone reagent results in a blue–green colour that can be measured at 625 nm. Samples of 10 g of chopped plant material were boiled (15 min) with 80 mL of 96% ethanol. Then, extracts were filtered (quality filter discs, 80 $g \cdot m^{-2}$, grade:289) and 2 mL aliquots were added to 10 mL of 0.02% anthrone reagent in sulfuric acid. Samples were heated in a boiling water bath (12 min) and, after cooling, the absorbance was measured at 625 nm on a spectrophotometer (HITACHI U2900, Tokyo, Japan). Results were expressed in mg of soluble sugars per 100 g fresh weight based on a glucose standard curve.

***Nitrate content*:** The potentiometric method was used to measure the content of nitrates. Samples of 5 g of chopped fresh material were shaken for 30 min in 100 mL of 0.02 M $Al_2(SO_4)_3 \cdot 18H_2O$. The concentration of nitrate ions was detected in filtered extracts by an ion-selective electrode (Orion Products) connected to an ORION 920A+ ionometer (Thermo-Electron Corp., Waltham, USA). Results were expressed as mg nitrate per kg of fresh weight.

Ascorbic acid content: The ascorbic acid content was determined according to the Polish Standard (1988) using Tillmans' method which uses 2,6-dichlorophenolindophenol sodium salt hydrate to oxidize *L*-ascorbic acid to *L*-dehydroascorbic acid in an acidic solution. After the reaction with ascorbic acid the blue reagent becomes colourless. Samples of 10 g of fresh weight were homogenized with 2% oxalic acid and titrated with 2,6-dichloroindophenolsodium salt hydrate (0.2 mg·mL, w/v). One mL of reagent corresponded to 88 μg of ascorbic acid and the results were expressed in mg of ascorbic acid per 100 g fresh weight.

***Antioxidant potential (FRAP assay)*:** The ferric reducing antioxidant power (FRAP) assay was performed according to the method by Benzie and Strain (1996). Ferric ions present in Fe^{3+}-TPTZ (2,4,6-tripyridyl-s-triazine) complexes are reduced by antioxidants to ferrous ions, obtaining blue Fe^{2+}-TPTZ complexes (with an absorption maximum at 595 nm). The FRAP working solution was prepared as follows: acetic buffer, pH 3.6, was mixed with TPTZ in ethanol (POCH, Gliwice, Poland) solution and 20 mM $FeCl_3$ solution in a ratio of 10:1:1. Aliquots of 20 μL of methanolic extracts were mixed with 3 mL of FRAP working solution, 0.3 mL of H_2O, and 80 μL of 80% methanol. Samples were incubated at 37°C (30 min) and the absorbance was measured at 595 nm. A trolox calibration curve was used. Results were calculated as mmol TE (trolox equivalent) per 1 g of fresh weight.

***Radical scavenging activity (DPPH assay)*:** Antioxidant activity was measured according to Pekkarinen et al. (1999) with 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals at 516 nm wavelength. Fresh weight samples (2.5 g) were homogenized with 10 mL of 80% methanol and then centrifuged at 4°C for 15 min at 2,700 × g. The radical scavenging activity of the extracts was measured at 516 nm after 30 min of incubation in the DPPH ethanolic solution. Results were expressed as the percentage of DPPH neutralization.

***Content of phenolic compounds*:** The total content of phenolics, phenylpropanoids, flavonols, and anthocyanins was measured in accordance with the Fukumoto and Mazza (2000) method. Samples of 2.5 g of fresh chopped plant material were homogenized with 10 mL of 80% methanol and centrifuged at 2,700 × g for 15 min at 4°C. Aliquots of 0.25 mL of supernatant were added to 0.25 mL of 0.1% HCl in ethanol and 4.5 mL of 2% HCl, mixed, and incubated for 15 min at room temperature. Then, absorbance was measured at 280, 320, 360, and 520 nm using a Hitachi U2900 UV-Vis Spectrophotometer (Tokyo, Japan). Calculations of total phenols, phenylpropanoids, flavonols, and anthocyanins were conducted according to calibration curves of chlorogenic acid, caffeic acid, quercetin, and cyanidine, respectively. Results were expressed as mg per 100 g of fresh weight.

***HPLC analysis of phenolic compounds*:** Chromatographic determination of several phenolic compounds was conducted using the LC-20AD Prominence HPLC System (Shimadzu, Kyoto, Japan) equipped with a DGU-20A5 degasser, a CTO-10AS VP column oven, and a SPD-M20A diode array detector. Separations were conducted with a Synergi 4u Fusion 250 mm (Phenomenex Inc., Torrance, CA, USA) column in low pressure gradient elution. The eluents used were: A, H_2O:MeOH:H_3PO_4 in a ratio of 1000:10:1 and B, MeOH:H_3PO_4 in a ratio of 1000:1. Extracts prepared for the measurement of phenolic content (see section "Content of phenolic compounds") were filtered through 0.22 μm syringe filters. Aliquots of 20 μL were injected into the column and eluted with a flow rate of 1 $mL\ min^{-1}$ in a gradient following the scheme: 0 min: 90% A 10% B, 20 min: 75% A 25% B, 30 min: 65% A 35% B, 40 min: 55% A 45% B, 50 min: 50% A 50% B, 60 min: 30% A 70% B, 62-85 min: 0% A 100% B. Peaks were identified by comparison of retention times and absorbance spectra with selected

standards (sinapic acid, hesperidin, *p*-coumaric acid, chlorogenic acid, diosmetin, and rutin) and quantified using their peak areas. Then, representative compounds which concentrations were significantly influenced by the light treatments were presented. The results were expressed in mg per 100 g of fresh weight.

***Content of chlorophylls and carotenoids*:** Freeze-drying was carried out in an Alpha 1-4 (Martin Christ Gefrier-trocknungsan-lagen GmbH, Germany) lyophilizer. Weighed plant samples were placed in the lyophilization chamber at 0.37 mbar for 48 h. The lyophilization was ceased after the temperature of the samples reached approximately 20°C. The dry mass was reduced to approximately 10.75 ± 1% of the original fresh weight.

The extracts were prepared using 0.5 g of freeze-dried material and 20 mL of n-hexane/ethanol (1:1, v/v) as a solvent in the presence of $MgCO_3$ to limit the degradation of chlorophyll over time. Lyophilized materials were ground in a mortar until the point of complete loss of colour and filtered through a sintered glass funnel into a volumetric flask to a total volume of 20 ml. Test tubes with the extracts were placed in darkness at -25°C.

A Shimadzu LC-20AD chromatograph equipped with an LiChrosphere RP-18 (5 μm) column and a SPD-M20A-DAD photodiode-array detector was used to analyze the carotenoids. Detection was conducted in a full spectrum of wavelength from 350 nm to 800 nm. Separation of the compounds was performed in a gradient of three elution solutions: A, MeOH and H_2O in a ratio of 99:1; B, MeOH; C, acetonitrile and n-hexane in a ratio of 9:1. The flow rate of the elution solutions was 1.8 mL per min. Identification of carotenoids was conducted by comparing the retention times and absorption spectra of lutein and β-carotene standards (LGC Standards, Poland) with the chromatograms of the *V. locusta* extracts. Quantification of the identified compounds was performed by a comparison of the peak areas of the samples' chromatograms with the standards.

Identification of chlorophyll a and chlorophyll b was based on the absorption spectra available in the literature (Kopcewicz and Lewak, 2011). Changes in the content of the chlorophylls were expressed as a percentage of the control treatment.

Statistical Analysis

Yield estimation, dry weight, and the content of soluble sugars, nitrate, and ascorbic acid were conducted in quadruplicates within each light treatment. Analyses of FRAP, DPPH scavenging activity, and the contents of phenolic compounds and assimilation pigments were conducted in triplicates. Results were subjected to ANOVA analysis with Fisher's LSD post-hoc test for homogenous groups determination. The results were recognized as significant at $p < 0.05$.

Results

Yield, Dry Mass, and Content of Soluble Sugars

The yields of both *V. locusta* cultivars, 'Noordhollandse' and 'Holländisher' (Table 1), were significantly higher than the control when plants were cultivated under 10R:0B, 9R:1B, and 8R:2B LED light treatments. The 7R:3B LED light resulted in a similar fresh mass of rosettes as the control plants. The dry weight of the plants was similar under nearly all light treatments, except for that of 'Holländisher' which showed lower dry mass when cultivated under the 10R:0B

Table 1. Yield, dry weight, soluble sugars (reducing and non-reducing), nitrate, and ascorbic acid content in two *V. locusta* cultivars grown under LED (10R:0B, 100% red; 9R:1B, 90% red and 10% blue; 8R:2B, 80% red and 20% blue; 7R:3B, 70% red and 30% blue) or HPS lighting

Cultivar	Light treatment	Yield [g per rosette]	Dry weight [%]	Soluble sugars [mg·100g^{-1} f.w.]	Nitrate [mg NO_3^-·kg^{-1} f.w.]	Ascorbic acid [mg·100 g^{-1} f.w.]
Noordhollandse	10R:0B	3.7 a[x]	6.74	875.3 c	5591.2 c	29.48 c
	9R:1B	3.6 a	7.37	922.4 bc	6014.6 b	36.37 a
	8R:2B	3.5 a	6.81	1001.9 ab	5802.9 bc	38.28 a
	7R:3B	3.2 b	7.27	861.0 c	6413.6 a	33.15 b
	HPS	3.0 b	7.39	1061.3 a	6540.2 a	32.12 bc
Holländischer	10R:0B	3.7 ab	5.85 b	854.2 b	4978.1 c	29.04 d
	9R:1B	3.3 bc	7.18 a	949.1 b	5756.7 b	35.20 b
	8R:2B	3.9 a	7.39 a	1078.5 a	4413.6 d	38.43 a
	7R:3B	3.1 cd	6.85 a	843.8 b	4953.8 c	34.03 bc
	HPS	2.9 d	7.09 a	1146.5 a	6647.2 a	32.27 c

[x]Different letters indicate statistical significance due to LSD Fisher's test with $p < 0.05$. Statistical analysis conducted for each cultivar separately.

Table 2. Content of chlorophyll a and b, total xanthophylls, lutein, and β-carotene in *V. locusta* cultivars grown under different LED (10R:0B, 100% red; 9R:1B, 90% red and 10% blue; 8R:2B, 80% red and 20% blue; 7R:3B, 70% red and 30% blue) or HPS lights

Cultivar	Light treatment	Chlorophyll a [%]	Chlorophyll b [%]	Total Xantophylls [mg·100 g^{-1} f.w.]	Lutein [mg·100 g^{-1} f.w.]	β-carotene [mg·100 g^{-1} f.w.]
Noordhollandse	10R:0B	69.31 b[x]	71.88 ab	126.39 b	83.09 b	64.73 b
	9R:1B	76.81 b	57.63 b	115.38 b	89.40 b	66.82 b
	8R:2B	107.12 a	97.59 a	200.73 a	147.78 a	66.99 b
	7R:3B	97.18 a	91.91 a	184.44 a	133.65 a	92.91 a
	HPS	100.00 a	100.00 a	186.32 a	136.73 a	95.90 a
Holländischer	10R:0B	78.66 b	80.03 b	179.37 b	132.51 b	93.86
	9R:1B	81.82 b	77.51 b	183.79 b	137.40 b	94.89
	8R:2B	85.32 b	86.05 ab	195.15 ab	144.90 ab	102.62
	7R:3B	83.24 b	84.01 b	189.34 b	140.51 b	96.93
	HPS	100.00 a	100.00 a	225.82 a	166.44 a	103.87

[x]Different letters indicate statistical significance due to LSD Fisher's test with $p < 0.05$. Statistical analysis conducted for each cultivar separately.

Table 3. Antioxidant potential, antiradical activity, and the content of total phenols and three phenolic fractions in *V. locusta* cultivars grown in a greenhouse under different LED (10R:0B, 100% red; 9R:1B, 90% red and 10% blue; 8R:2B, 80% red and 20% blue; 7R:3B, 70% red and 30% blue) or HPS lights

Cultivar	Light treatment	FRAP [mmol TE·g^{-1} f.w.]	DPPH scavenging activity [%]	Total phenols [mg·100 g^{-1} f.w.]	Phenylpropanoids [mg·100 g^{-1} f.w.]	Flavonols [mg·100 g^{-1} f.w.]	Antocyanins [mg·100 g^{-1} f.w.]
Noordhollandse	10R:0B	6486.8 bc[x]	10.01 a	84.55 c	23.18 b	2.34	1.86
	9R:1B	6442.3 bc	6.56 b	93.33 abc	25.10 b	2.34	1.86
	8R:2B	9258.8 a	10.01 a	96.36 ab	28.77 a	2.26	1.80
	7R:3B	8236.0 ab	9.04 ab	99.60 a	29.58 a	2.24	1.78
	HPS	5552.9 c	9.60 a	86.06 bc	24.22 b	1.95	1.55
Holländischer	10R:0B	6220.0 ab	4.04 b	80.71 c	21.32 c	2.13 b	1.70 b
	9R:1B	7628.2 a	8.51 a	98.99 a	28.44 a	2.18 b	2.32 a
	8R:2B	7568.9 a	7.67 a	85.86 bc	26.16 ab	2.65 a	1.74 b
	7R:3B	6709.1 ab	8.27 a	89.70 b	27.96 a	2.91 a	2.11 ab
	HPS	5982.8 b	4.27 b	90.10 b	22.79 bc	2.86 a	2.27 a

[x]Different letters indicate statistical significance due to LSD Fisher's test with $p < 0.05$. Statistical analysis conducted for each cultivar separately.

LED light.

Both cultivars accumulated the highest levels of soluble sugars (Table 1) under the control (HPS) treatment, but the difference between values obtained in the control plants and those grown under 8R:2B LED lighting were insignificant. Plants cultivated under the other LED treatments accumulated significantly lower amounts of soluble sugars compared to the control.

Nitrate and Ascorbic Acid Contents

In both cultivars, nitrate concentrations were lower when cultivated under the LED treatments than for the HPS control, except for 'Noordhollandse' where the 7R:3B LED treatment resulted in similar nitrate levels to those detected in the control plants (Table1).

The concentrations of ascorbic acid in the leaves of both cultivars were the highest when plants were illuminated by 8R:2B LED light, and additionally in the case of 'Noordhollandse' when the 9R:1B LED treatment was used (Table 1). Furthermore, a higher ascorbic acid level was detected in 'Holländisher' cultivated with the 9R:1B treatment than in the control plants. The lowest ascorbic acid content was obtained in plants of both cultivars illuminated by 10R:0B LED light.

Antioxidant and Antiradical Properties

The FRAP values were significantly higher for the 'Noordhollandse' (Table 3) cultivar grown under the 8R:2B and 7R:3B LED treatments than for the control plants. The 'Holländisher' cultivar had the highest FRAP values for the plants illuminated by the 9R:1B and 8R:2B LED lights,

Table 4. Content of six phenolic compounds in V. locusta leaves grown in a greenhouse supplemented with different LED (10R:0B, 100% red; 9R:1B, 90% red and 10% blue; 8R:2B, 80% red and 20% blue; 7R:3B, 70% red and 30% blue) or HPS lighting

Cultivar	Light treatment	Sinapic acid [mg·100 g^{-1} f.w.]	Hesperidin [mg·100 g^{-1} f.w.]	p-coumaric acid [mg·100 g^{-1} f.w.]	Chlorogenic acid [mg·100 g^{-1} f.w.]	Diosmetin [mg·100 g^{-1} f.w.]	Rutin [mg·100 g^{-1} f.w.]
Noordhollandse	10R:0B	0.23	0.42 b	0.55 bc	15.31 b	2.61 c	0.48 b
	9R:1B	0.24	0.48 ab	0.54 c	17.14 b	4.16 ab	0.93 a
	8R:2B	0.30	0.51 ab	0.68 a	26.29 a	3.86 b	0.66 b
	7R:3B	0.30	0.41 b	0.59 bc	23.80 a	4.35 a	0.58 b
	HPS	0.29	0.57 a	0.61 b	14.60 b	2.97 c	0.55 b
Holländischer	10R:0B	0.26 b[x]	0.33 c	0.56 b	11.52 b	2.47 d	0.42 b
	9R:1B	0.33 a	0.52 a	0.57 b	21.98 a	4.04 a	0.62 a
	8R:2B	0.33 a	0.36 bc	0.64 a	21.66 a	3.19 bc	0.53 a
	7R:3B	0.35 a	0.40 bc	0.66 a	22.55 a	3.63 ab	0.54 a
	HPS	0.35 a	0.48 ab	0.54 b	10.39 b	2.87 cd	0.39 b

[x]Different letters indicate statistical significance due to LSD Fisher's test with $p < 0.05$. Statistical analysis conducted for each cultivar separately.

which were significantly higher than the control.

The radical scavenging activity, measured as an ability to scavenge DPPH free radicals, was different for both cultivars. 'Noordhollandse' had similar values irrespective of the treatment, except for plants grown under the 9R:1B LED treatment in which the DPPH scavenging activity was the weakest and significantly lower than the control plants. 'Holländisher' had the highest radical scavenging activity in the plants illuminated by LED lamps with blue diodes (9R:1B, 8R:2B, and 7R:3B), whereas the other treatment (10R:0B) and the control resulted in a lower antiradical activity.

Phenolic Compounds

The highest concentrations of total phenols (Table 3) in 'Noordhollandse' leaves were detected in plants grown under the 7R:3B LED light, but this value was not significantly different in comparison with the 8R:2B and 9R:1B LED treatments. In 'Holländischer', the 9R:1B LED treatment resulted in the highest total accumulation of phenols and a high level of phenylpropanoids. An increased content of phenylpropanoids was observed in the leaves of both cultivars illuminated with 8R:2B and 7R:3B LED light. Supplemental lighting with LEDs did not significantly affect the levels of flavonols and anthocyanins in 'Noordhollandse'. In 'Holländischer', the use of blue LED light (8R:2B and 7R:3B) increased the content of flavonols in comparison to other LED light treatments, but high flavonols were also found in the control plants grown under the HPS lamps.

The content of chlorogenic acid showed the greatest difference among the six phenolic compounds. In comparison to the control (HPS), around 50% more chlorogenic acid was identified in the 8R:2B LED light treated plants in both cultivars. Moreover, in both cultivars, no significant differences in *p*-coumaric acid, chlorogenic acid, and rutin contents were observed between the HPS control and the 10R:0B treatment. Plants grown under the 8R:2B LED light had the lowest concentration of *p*-coumaric acid. The only phenolic compound that was increased by HPS light (and also 9R:1B LED light) was hesperidin.

Assimilation Pigments

The contents of both chlorophyll a and b, as well as total xanthophylls with a representative of lutein, in 'Noordhollandse' were the highest when plants were treated with 8R:2B, 7R:3B, or HPS lights (Table 2). High concentrations of β-carotene were detected in plants cultivated under 7R:3B and HPS lights. In 'Holländisher', the levels of chlorophyll a and b, total xanthophylls, and lutein were the highest when cultivated under HPS lamps. However, the contents of chlorophyll b, total xanthophylls, and lutein were also high in the plants grown under 8R:2B LED light and were not significantly different from the HPS control. The supplemental lighting used in this study did not significantly influence the concentrations of β-carotene in the leaves of this cultivar.

Discussion

Extending the photoperiod with supplemental lighting in autumn-winter greenhouse crop production is necessary for obtaining high quality and yield. The comparison of the effect of four different LED light spectra with the HPS light on the fresh weight of the two cultivars indicated that the use of the HPS lamps commonly used in greenhouses is the least effective in promoting vegetative growth of *V. locusta*. A greater percentage of red light in the spectrum (10R:0B, 9R:1B, and 8R:2B) contributed to higher yields. Higher fresh

weight of the plants corresponded with a higher content of soluble sugars only in the plants treated with 8R:2B LED light. The higher R:B ratio resulted in lower soluble sugars and higher yields. However, very similar dry weights were observed in all plants grown under all treatments; thus, the lower content of soluble sugars might be due to a dilution effect (higher water contents in the tissues that had lower soluble sugars).

Nitrate ions are potentially hazardous to humans when present in excess in consumed food (Mensinga et al., 2003). The enzymes responsible for nitrate utilization in plant tissues are nitrate and nitrite reductases. Nitrate reductase is the key enzyme in this process and both protein synthesis and enzyme activity may be influenced by light (Lillo, 2004; Nemie-Feyissa et al., 2013); therefore, studies on the ability of different light treatments to reduce the concentration of nitrates have been conducted. Nitrate levels in plants have been shown to be effectively reduced by red light (Lillo and Appenroth, 2001), whereas blue light was less effective (Urbonavičiūtė et al., 2009). However, a significant decrease of nitrate concentration under a mixture of red and blue LED lights has been reported (Chen et al., 2014). Our previous research on the effect of supplemental lighting on the concentrations of nitrate ions indicated that the use of LED light (90% red and 10% blue) may significantly decrease the nitrate content in comparison to HPS light (Wojciechowska et al., 2016). The results of the current study confirmed the positive effect of the 9R:1B LED light on reducing nitrate concentrations in both *V. locusta* cultivars compared to HPS light. An even lower nitrate concentration was achieved with the 8R:2B and 10R:0B LED lights. The negative health effects of nitrate in the human body may be reduced by the presence of ascorbic acid (Pokluda, 2006). In our research, low values of nitrate ions coincided with high ascorbic acid concentrations in the plants grown under the 8R:2B LED light in both cultivars.

The highest rate of accumulation of assimilation pigments in the leaves of the 'Noordhollandse' cultivar were obtained when plants were grown under 8R:2B, 7R:3B, and HPS lights. In 'Holländisher', the highest concentrations of chlorophylls, xanthophylls, and lutein occurred when the plants were illuminated by HPS light. Interestingly, illumination with the 8R:2B LED light resulted in lower, but non-significant, concentrations of chlorophyll b, xanthophylls, and lutein. The synthesis of carotenoids in photosynthetic organs is regulated by light in multiple ways at transcriptional and post-transcriptional levels (Pizarro and Stange, 2009). Lutein and β-carotene play a crucial role in the leaves as components of light-harvesting complexes and also may act as photoprotectants (Young, 1991). Interestingly, in our experiment, the levels of β-carotene were less influenced by the light source than lutein and xanthophylls. Both β-carotene and lutein are synthesized in green tissues from lycopene via different metabolic pathways: one leads to lutein formation, and the other leads to the synthesis of β-carotene and further compounds such as zeaxanthin, violaxanthin, and finally abscisic acid (DellaPenna, 2004). Therefore, the different reactions of lycopene may be regulated by different wavelengths of light. Another explanation may be the transitory nature of β-carotene, which is an important intermediate in the formation of xanthophylls: zeaxanthin and violaxanthin. Lower levels of β-carotene may result from rapid transformation of this compound to xanthophylls, especially because zeaxanthin may also act as a photoreceptor. Differences in lutein and β-carotene concentrations affected by the quality of light were also observed in kale, where red LED light stimulated the synthesis of lutein, and blue LED light stimulated the formation of β-carotene (Lefsrud et al., 2008).

Dietary polyphenols may promote better health due to their high antioxidant and radical scavenging activity. These compounds are involved in plant defensive mechanisms. The synthesis of phenolic compounds in plants is regulated by light at certain stages. At the first stage of the polyphenol synthesis pathway, light regulates the activity of phenylalanine ammonia-lyase (PAL; EC 4.3.1.24), which catalyzes the deamination of p-phenylalanine to trans-cinnamic acid. The expression and activity of chalcone synthase (CHS; EC 2.3.1.74), which is responsible for condensation of the CoA-ester of cinnamic acid (or its derivatives) with malonyl-CoA forming naringenin chalcone as a major product, is also regulated by light (Dao et al., 2011). In our experiment, the two *V. locusta* cultivars showed different patterns of accumulation of polyphenols in the leaves in response to the different light treatments. However, both cultivars grown under the 10R:0B LED light synthesized the lowest levels of total phenols, phenylpropanoids, and flavonols, which suggests a prominant role of blue light in the regulation of polyphenol synthesis in plants. Similarly, low concentrations of these compounds were detected in plants grown under HPS light, which also lacks radiation in blue wavelengths (Fig. 1). These results are in accordance with other studies which have shown positive effects of blue light on the synthesis of total phenolic compounds in young barley leaves (Samuolienė et al., 2009b), leafy radish (Urbonavičiūtė et al., 2009), and lettuce (Son and Oh, 2013). Interestingly, the synthesis of flavonols and anthocyanins in 'Holländisher' seemed to be more sensitive to the light treatments than in 'Noordhollandse'. Interestingly, there were noticeable differences in the effects of the light treatments on the accumulation of the six phenolic compounds measured in our study. Further research will aid our understanding of the light-mediated regulation of the different branches of polyphenol synthesis in plants. The dominant phenolic compound found in *V. locusta* leaves was chlorogenic acid. In our study,

blue wavelengths showed the best results for enhancing the content of chlorogenic acid, but this result is inconsistent with other research. For example, blue light did not increase chlorogenic acid concentrations in lettuce and basil (Taulavuori et al., 2016) or Chinese cabbage (Kim et al., 2015). Because we observed a significant increase in chlorogenic acid with blue light (8R:2B and 7R:3B) in both cultivars , it is possible that this response may be species-specific. In both cultivars, the addition of blue light stimulated the synthesis of diosmetin, which is a free flavonoid. Further studies on the influence of blue light on the concentrations of other compounds, such as rutin, in *V. locusta* leaves are needed.

In summary, supplemental illumination of lamb's lettuce with the 8R:2B LED light resulted in high yields with high antioxidant and antiradical activities, a low nitrate concentration, and the most satisfactory composition of soluble sugars, ascorbic acid, carotenoids, and polyphenols in both 'Noordhollandse' and 'Holländischer' cultivars.

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Literature Cited

Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70-76

Bian ZH, Yang QC, Liu WK (2015) Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: A review. J Sci Food Agric 95:869-877

Chen X, Guo W, Xue X, Wang L, Qiao X (2014) Growth and quality responses of "Green Oak Leaf" lettuce as affected by monochromic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED). Sci Hortic 172:168-175

Dao TTH, Linthorst HJM, Verpoorte R (2011) Chalcone synthase and its functions in plant resistance. Phytochem Rev 10: 397-412

DellaPenna D (2004) Carotenoid Synthesis and Function in Plants: Insights from Mutant Studies in Arabidopsis thaliana. *In* HA Frank, AJ Young, G Britton, RJ Cogdell, eds, The Photochemistry of Carotenoids. Dordrecht: Kluwer Academic Publishers, pp 20-37

Devlin PF, Christie JM, Terry MJ (2007) Many hands make light work. J Exp Bot 58:3071-3077

Długosz-Grochowska O, Kołton A., Wojciechowska R (2016) Modifying folate and polyphenol concentrations in Lamb's lettuce by the use of LED supplemental lighting during cultivation in greenhouses. J Funct Foods 26:228-237

Fukumoto L, Mazza G (2000) Assessing antioxidant and prooxidant activities of phenolic compounds. J Agric Food Chem 48:3597-3604

Grzesiak W, Żupnik M, Wojciechowska R (2014) Practical implementation of the programmable plant irradiation system, with multiple research stations, based on SSL LED technology. Prace Instytutu Elektrotechniki 267:97-106

Jokhan M, Shoji K, Goto F, Hashida S-N, Yoshihara T (2010) Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. HortScience 45:1809-1814

Kim YJ, Kim YB, Xiaouhua L, Choi SR, Park S, Park JS, Lim YP, Park SU (2015) Accumulation of phenylpropanoids by white, blue, and red light irradiation and their organ-specific distribution in Chinese cabbage (*Brassica rapa* ssp. *Pekinensis*). J Agric Food Chem 63:6772-6778

Kong SG, Okajima K (2016) Diverse photoreceptors and light responses in plants. J Plant Res 129:111-114

Kopcewicz J, Lewak S (2011) Plant Physiology. Wydawnictwo Naukowe PWN, Warszawa (in Polish)

Lee MJ, Son KH, Oh MM (2016) Increase in biomass and bioactive compounds in lettuce under various ratios of red to far-red LED light supplemented with blue LED light. Hortic Environ Biotechnol 57:139-147

Lefsrud M, Kopsell DA, Sams CE (2008) Irradiance from distinct wavelength light-emitting diodes affect secondary metabolites in Kale. HortScience 43:2243-2244

Li H, Tang C, Xu Z, Liu X, Han X (2012) Effects of different light sources on the growth of non-heading chinese cabbage (*Brassica campestris* L.). J Agric Sci 4:262-273

Li Q, Kubota C (2009) Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ Exp Bot 67:59-64

Lillo C (2004) Light regulation of nitrate uptake, assimilation and metabolism. *In* S Amâncio, I Stulen, eds, Nitrogen acquisition and assimilation in higher plants, pp 149-184

Lillo C, Appenroth KJ (2001) Light regulation of nitrate reductase in higher plants: which photoreceptors are involved? Plant Biol 3:455-465

Mensinga TC, Speijers GJA, Meulenbelt J (2003) Health implications of exposure to environmental nitrogenous compounds. Toxicol Rev 22:41-51

Nemie-Feyissa D, Królicka A, Førland N, Hansen M, Heidari B, Lillo C (2013) Post-translational control of nitrate reductase activity responding to light and photosynthesis evolved already in the early vascular plants. J Plant Physiol 170:662-667

Olle M, Viršile A (2013) The effects of light-emitting diode lighting on greenhouse plant growth and quality. Agric Food Sci 22:223-234

Ouzounis T, Rosenquist E, Ottosen CO (2015) Spectral effects of artificial light on plant physiology and secondary metabolism: a review. HortScience 50:1128-1135

Pekkarinen SS, Stoeckmann H, Schwarz K, Heininen IM, Hopia AI (1999) Antioxidant activity and partioning of phenolic acids in bulk and emulsified methyl linoleate. J Agric Food Chem 47:3036-3043

Pizarro L, Stange C (2009) Light-dependent regulation of carotenoid biosynthesis in plants. Cienc Investig Agrar 36:143-162

Pokluda R (2006) As assessement of the nutritional value of vegetables using and ascorbate-nitrate index. Veg Crop Res Bull 64:29-37

Polish Standard PN-A-04019:1998 (1988) Polish Committee for Standardization. Food products – Determination of vitamin C

Samuolienė G, Sirtautas R, Brazaitytė A, Viršilė A, Duchovskis P (2012) Supplementary red-LED lighting and the changes in phytochemical content of two baby leaf lettuce varieties during three seasons. J Food Agric Environ 10:701-706

Samuolienė G, Urbonavičiūtė A, Brazaitytė A (2009a) The benefits of red LEDs : improved nutritional quality due to accelerated senescence in lettuce. Sci Work Lith Inst Hortic Lith Univ Agric 28:111-120

Samuolienė G, Urbonavičiūtė A, Duchovskis P, Bliznikas Z, Vitta P, Zukauskas A (2009b) Decrease in nitrate concentration in leafy vegetables under a solid-state illuminator. HortScience, 44: 1857-1860

Son KH, Oh MM (2013) Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. HortScience 48:988-995

Stutte GW, Edney S, Skerritt T (2009) Photoregulation of bioprotectant content of red leaf lettuce with light-emitting diodes. HortScience 44:79-82

Taulavuori K, Hyöky V, Oksanen J, Taulavuori E, Julkunen-Tiitto R (2016) Species-specific differences in synthesis of flavonoids and phenolic acids under increasing periods of enhanced blue light. Environ Exp Bot 121:145-150

Urbonavičiūtė A, Samuolienė G, Brazaitytė A, Žukauskas A (2009) The effect of light quality on nutritional aspects of leafy radish. Sci Work Lith Inst Hortic Lith Univ Agric 28:147-156

Wojciechowska R, Długosz-Grochowska O, Kołton A, Żupnik M (2015) Effects of LED supplemental lighting on yield and some quality parameters of lamb's lettuce grown in two winter cycles. Sci Hortic 187:80-86

Wojciechowska R, Kołton A, Długosz-Grochowska O, Knop E (2016) Nitrate content in *Valerianella locusta* L. plants is affected by supplemental LED lighting. Sci Hortic 211:179-186

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 58:508-514

Young AJ (1991) The photoprotective role of carotenoids in higher plants. Phys Plant 83:702-708

Žukauskas A, Bliznikas Z, Breivė K, Novičkovas A, Samuolienė G, Urbonavičiūtė A, Brazaitytė A, Jankauskienė J, Duchovskis P (2011) Effect of supplementary pre-harvest LED lighting on the antioxidant properties of lettuce cultivars. Acta Hortic 907:87-90