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Growing of leaf lettuce (*Lactuca sativa* L.) under high-pressure sodium lamps with supplemental blue, cyan and green LEDs

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Abstract

The aim of this study was to evaluate the effect of combination of light-emitting diodes (LEDs) and high-pressure sodium (HPS) lighting on the growth of lettuce variety ‘Multigreen 3’ in a greenhouse. Supplemental blue light had an impact on the efficiency of photosynthetic performance in lettuce; however, different blue light wavelengths had uneven effect. The 470 nm light also had a positive impact on chlorophyll *a* to *b* ratio and higher specific leaf area (SLA) value and resulted in increased plant mass per leaf area. The overall growth performance, higher contents of soluble saccharides and higher growth rates, represented by net assimilation rate (NAR) value were determined in lettuce, cultivated under blue 455 nm light. The 505 nm light, being intermediate between blue and green wavelengths, had a significant positive effect on the content of photosynthetic pigments, higher NAR value thus also might be beneficial for supplemental baby leaf lettuce illumination. Green 535 nm light stimulated accumulation of soluble saccharides in lettuce and had controversial effect on photosynthetic pigment contents and assimilative indices, thus the evidence for the effect was inconclusive.

Key words: chlorophyll, greenhouse, growth analyses, LED, saccharides.

Introduction

Among various environmental factors, light is one of the most important variables affecting photosynthesis and its primary metabolites in plants (Kopsell, Kopsell, 2008; Perez-Balibrea et al., 2008). Through the photosensory system plants react to the physiologically active radiation within the range of 380–750 nm (Goins et al., 1997). Light quantity is often the limiting factor for plant growth and productivity in greenhouses. This is of relevant importance in the Northern countries, where natural irradiation level is low during autumn/winter season; therefore, the use of artificial lighting has been an important subject for investigation in the last century (Wheeler, 2008). Solid-state lighting, provided by light-emitting diodes (LEDs), has been potentially one of the biggest advancements in horticultural lighting over the last decades (Morrow, 2008). LED lighting systems have several unique advantages, including small size, relatively cool emitting surfaces, linearity between electrical input current and photon output and the ability to control spectral composition and flux in real time. These solid-state light sources are therefore ideal for use in plant lighting designs due to the possibility to select wavelengths corresponding to the plant photoreceptor absorption and to stimulate plant productivity or to influence plant morphology and metabolism (Bourget, 2008; Massa et al., 2008). The solid-state lighting technology, based on LEDs expanded the possibilities to analyze the effects of lighting parameters on physiological processes in plants (Samuolienė et al., 2011 a; b). The LED lighting may be customized for the specific horticultural objectives and optimized for maximum production without wasting energy on nonproductive wavelengths (Tamulaitis et al., 2004; 2005).

The grow lights available for greenhouse lighting are fluorescent, high-pressure sodium and metal halide lamps that differentially affect plant growth, development and photomorphogenesis (Goins et al., 1997). High-pressure sodium lamps mostly emit an orange-red light, but they lack blue and green spectral components (Tenessen et al., 1994). Meanwhile, blue light is important and involved in a wide range of plant processes such as phototropism, photomorphogenesis, stomatal opening, leaf photosynthetic functioning, chloroplast development, chlorophyll formation and chemical composition of plants (Whitelam, Halliday, 2007; Morrow, 2008). Overall, there is a trend to higher mass production and photosynthetic capacity in a blue light-containing irradiance (Tenessen et al., 1994). The quantum yield of green light for photosynthesis is quite low. However, green light is efficiently absorbed and used for photosynthesis in inner canopy levels (Folta, Maruhnich, 2007; Terashima et al., 2009) and thus may stimulate growth in different plants (Kim et al., 2006) and affect the morphogenetic processes through phytochrome and cryptochrome activity.

Lettuce is one of the major crops cultivated worldwide in greenhouses during the winter/spring season, when natural illumination level is low. Its photosynthetic and metabolic performance is highly dependent on various light parameters, thus, lettuce seems to be a model crop widely studied for light quality response (Brazaitytė et al., 2006; Wheeler, 2008). Therefore, the aim of this study was to evaluate the impact of high-pressure sodium lamps with supplemental blue, cyan and green LEDs on the growth of lettuce variety ‘Multigreen 3’ in a greenhouse.

Materials and methods

Chemicals. Calcium carbonate (CaCO_3) was obtained from “Lach-Ner” (Czech Republic). Sucrose, fructose, mannose and glucose were obtained from “Sigma-Aldrich” (Germany). Acetone was obtained from “Merck” (Germany). All standards and samples for high performance liquid chromatography (HPLC) were filtered through 0.25 μm nylon syringe filters (“Albet”, Germany).

Growth conditions and plant material. The green baby leaf lettuce (*Lactuca sativa* L.) ‘Multigreen 3’ was grown to harvest time (about 22 days) in November 2010 in an industrial greenhouse of Institute of Horticulture, Lithuanian Research Centre of Agricultural and Forestry Science when photosynthetic photon flux density (PPFD) of natural solar irradiation was $\sim 80\text{--}120 \mu\text{mol m}^{-2} \text{s}^{-1}$. High-pressure sodium (HPS) irradiance (“Son-T Agro”, “Philips”, USA) of $\sim 90 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD was supplemented with $\sim 15 \mu\text{mol m}^{-2} \text{s}^{-1}$ flux of blue 455 nm and 470 nm, cyan 505 nm and green 535 nm light-emitting diodes (LEDs). The surface area per light treatment was about 0.5 m^2 . Reference plants grew under natural illumination, supplemented with HPS lamps. The photoperiod of artificial irradiance was applied within 16 h. PPFD level was measured using a photometer-radiometer RF-100 (“Sonopan”, Poland). Lettuce was sown in 120 ml plastic vessels in peat substrate (pH 5–6), 3 seeds per vessel. The amount of nutrients (mg l^{-1}) in substrate was as follows: N 70, P 30, K 160, Ca 250, and Mg 50. The day/night temperature in greenhouse was $20/16 \pm 3^\circ\text{C}$ and relative air humidity was 50–60%.

Determination of soluble saccharides. Sucrose, fructose, mannose and glucose were analysed by HPLC method. The composite sample of lettuce leaves from five pots was used for analysis. About 1 g of fresh plant tissue was ground and diluted with 4 ml $+70^\circ\text{C}$ analytical grade water. The extraction was carried out for 24 h. The samples were filtered using cellulose acetate syringe filters (pore diameter 0.25 μm). Analyses were performed on a chromatograph “Shimadzu HPLC 10A” (“Shimadzu”, Japan) with a refractive index detector RID 10A (“Shimadzu”, Japan). Separation of soluble saccharides was performed on SC-1011 column ($300 \times 4.6 \text{ mm}$) (“Shodex”, Japan). Mobile phase – bi-distilled water; oven temperature was maintained at $+80^\circ\text{C}$. The sensitivity of the HPLC method was established using a method validation protocol (ICH, 2005).

Determination of photosynthetic pigments. Fresh leaf tissue was ground with 0.5 g CaCO_3 diluted 1:250 with pure acetone and filtered through a cellulose filter. Chlorophyll *a*, *b* and carotenoids were measured by spectrophotometric method (Gavrilenko, Zigalova, 2003). The absorption was measured at 644 nm, 662 nm and 440.5 nm, for chlorophyll *a*, *b* and carotenoids, respectively.

Growth analyses. Five randomly selected lettuce plants were measured for each analysis. The leaf area was measured by a leaf area meter “WinDias” (“Delta-T Devices”, UK). For the determination of dry mass, above-ground lettuce parts were dried in a drying oven (“Venticell-BMT”, Czech Republic) at $+105^\circ\text{C}$ for 24 h. The net assimilation rate (NAR) of a plant was defined as its growth rate per unit leaf area per day ($\text{g cm}^{-2} \text{d}^{-1}$). It can be calculated as: $\text{NAR} (\text{g cm}^{-2} \text{d}^{-1}) = \frac{1}{LA} \times \frac{dW}{dt}$, where LA is leaf area (cm^2) and $\frac{dW}{dt}$ – the change in plant dry mass per unit time. Specific leaf area (SLA) is the ratio of leaf area (cm^2) per plant to leaf dry weight.

Statistical analysis. Five biological and three analytical replications of soluble saccharides,

photosynthetic pigments and physiological indices were performed for each treatment. Data are presented as mean \pm standard error (SE). Data analysis was performed using STATISTICA 7.0 for Windows, one-way analysis of variance ANOVA, the Fisher’s LSD test from the control (HPS lighting) at the confidence level $p \leq 0.05$. Data was processed using software MS Excel (version 7.0).

Results and discussion

Our investigations revealed that blue, cyan and green LEDs, supplemental for the HPS irradiance in the greenhouse had significant effect on the contents of soluble saccharides (Table 1), photosynthetic pigments (Table 2) and assimilative indices (Table 3) in green baby leaf lettuce ‘Multigreen 3’. The amount of soluble saccharides was found to be remarkably higher in lettuce, irradiated with the supplemental blue 455 nm light, as compared to other irradiance treatments. The amount of sucrose was determined 6 times higher, glucose 2.9 times, mannose 3.5 times and fructose 1.5 times higher in the lettuce, grown under supplemental 455 nm LEDs as compared to these, grown under sole HPS artificial irradiance. Supplemental 470 nm blue light, though being close to 455 nm blue light, had a less pronounced effect: the content of sucrose was found to be 1.8 times higher, glucose 2.3 times, mannose 3.3 times higher, as compared to soluble saccharide contents in lettuce without supplemental LED illumination. The 505 nm supplemental cyan LED light had the weakest stimulating effect on the accumulation of almost all soluble saccharides, when supplemental green 535 nm LED light had no significant effect on the contents of sucrose and fructose, but resulted in 3 times higher glucose and 5 times higher mannose concentration (Table 1). Saccharides are the main carbon and energy source in plants (Salerno, Curatti, 2003; Weber et al., 2005) and act as physiological signal affecting further metabolic processes, simultaneously affecting nutrient and gustatory properties of vegetable food (Koch, 2004).

Higher value of the chlorophyll *a* to *b* ratio usually represents better plant photosynthetic performance. However, the higher level of soluble saccharides in the leaves of baby leaf lettuce (Table 1), supplementary irradiated with blue 455 nm LED light match the reduced chlorophyll *a* to *b* ratio (Table 2). The decrease in exports of leaf sucrose and subsequent increase in leaf sucrose and sucrose cycling are responsible for photosynthesis down-regulation in plants (Moore et al., 1999).

In lettuce, supplementary irradiated with blue 470 nm light, the chlorophyll *a* to *b* ratio was found to be significantly higher. However, such an increase is rather due to the reduced chlorophyll *b* concentration in leaves than the increased chlorophyll *a* contents. Supplemental 505 nm light had the most pronounced effect on the photosynthetic pigment contents in lettuce: the concentrations of chlorophyll *a*, chlorophyll *b* and carotenoids was determined to be ~ 1.2 times higher as compared to lettuce, grown without supplemental LED irradiance. The addition of green light may improve plant growth and photosynthesis by perceived specific photoreceptors and induces the pattern of investment of captured resources and the ability of the plant to capture further resources (Kim et al., 2006). However, supplemental green 535 nm light had no significant effect on chlorophylls. Only on the contents of carotenoid pigments significant effects were found.

Supplemental lighting also had effects on biometric lettuce parameters, determining differences in assimilative indices. SLA represents the mean leaf area

Table 1. The content of soluble saccharides amount (mg g⁻¹ fresh mass) in baby leaf lettuce ‘Multigreen 3’ under high-pressure sodium (HPS) and supplemental light-emitting diode (LED) light

	Soluble saccharides			
	sucrose	glucose	mannose	fructose
HPS	0.69 ± 0.03	2.49 ± 0.04	1.20 ± 0.03	0.23 ± 0.04
HPS + 455 nm	4.18 ± 0.04*	7.32 ± 0.02*	4.33 ± 0.04*	0.36 ± 0.03*
HPS + 470 nm	1.22 ± 0.05*	5.70 ± 0.03*	3.96 ± 0.03*	0.28 ± 0.01*
HPS + 505 nm	1.08 ± 0.04*	3.47 ± 0.04*	2.93 ± 0.05*	0.28 ± 0.03*
HPS + 535 nm	0.77 ± 0.03	7.50 ± 0.04*	5.96 ± 0.05*	0.29 ± 0.04*
LSD ₀₅	0.18	0.21	0.12	0.02

Note. * – values (mean ± SE) in each column are significantly different ($p \leq 0.05$) from HPS by Fisher’s LSD test.

Table 2. The content of photosynthetic pigments (mg g⁻¹ fresh mass) in baby leaf lettuce ‘Multigreen 3’ under high-pressure sodium (HPS) and supplemental light-emitting diode (LED) light

	Photosynthetic pigments			
	chlorophyll <i>a</i> to <i>b</i> ratio	content of chlorophyll <i>a</i>	content of chlorophyll <i>b</i>	content of carotenoids
HPS	2.63 ± 0.01	0.71 ± 0.01	0.27 ± 0.01	0.24 ± 0.02
HPS + 455 nm	2.31 ± 0.03	0.67 ± 0.04	0.29 ± 0.03	0.24 ± 0.03
HPS + 470 nm	2.91 ± 0.02*	0.70 ± 0.02	0.24 ± 0.02	0.24 ± 0.01
HPS + 505 nm	2.75 ± 0.03	0.88 ± 0.04*	0.32 ± 0.01*	0.29 ± 0.03*
HPS + 535 nm	2.50 ± 0.03	0.60 ± 0.03	0.24 ± 0.03	0.20 ± 0.03*
LSD ₀₅	0.25	0.11	0.03	0.03

Explanation under Table 1

per unit of leaf weight. Poorter (2002) observed that an increase in SLA implies that leaves invest less mass per unit area. Evaluating the supplemental lighting effect on baby leaf lettuce assimilation indices, the significant blue 470 nm light effect on SLA was observed (Table 3). Higher SLA value represents the thicker leaf or higher leaf mass investment per unit of leaf area (Poorter, 2002). Also higher SLA value in lettuce plants is associated with the altered ability to capture more light per unit mass and is more common in the shade-grown plants (Shipley, 2002).

Table 3. The assimilative indices in baby leaf lettuce ‘Multigreen 3’ under high-pressure sodium (HPS) and supplemental light-emitting diode (LED) light

	Assimilative indices	
	specific leaf area (SLA) cm ² g ⁻¹	net assimilation rate (NAR) g cm ⁻² d ⁻¹
HPS	796 ± 74	0.073 ± 0.002
HPS + 455 nm	801 ± 88	0.084 ± 0.005*
HPS + 470 nm	981 ± 105*	0.078 ± 0.002
HPS + 505 nm	813 ± 103	0.084 ± 0.008*
HPS + 535 nm	736 ± 65	0.086 ± 0.003*
LSD ₀₅	138	0.006

Explanation under Table 1

The observed positive effect of artificial cyan 505 nm and green 535 nm light on the NAR, describing the growth rate per unit leaf area per day, indicates that the activity of photosynthetic system was accelerated by effective penetration of light of these wavelengths through the plant tissues. Plants absorb from 43% to 87% of the green light (Nishio, 2000) and use this energy for photosynthesis efficiently in lower canopy levels and deeper within leaf mesophyll (Folta, Maruhnich, 2007). Kim et al. (2006) reported the stimulating green light effect on lettuce photosynthesis; however, this trend was observed only at low green light levels: green light flux >50% of total PPFD had the inhibiting effect. Different supplemental blue light wavelengths had different effects on growth rate: 455 nm supplemental blue light resulted in increased NAR value, when 470 nm light had no significant effect.

Conclusion

The combination of light-emitting diodes (LEDs) and high-pressure sodium (HPS) irradiance was efficient for the cultivation of ‘Multigreen 3’ variety of lettuce (*Lactuca sativa* L.) in a greenhouse. Supplemental blue light improved photosynthetic performance in lettuce; however, different blue light wavelengths had uneven effect. The 470 nm light resulted in higher chlorophyll *a* to *b* ratio and higher specific leaf area (SLA) value, and determined higher plant mass per leaf area. The overall growth performance, high contents of saccharides and higher growth rates, represented by net assimilation rate (NAR) value were determined in lettuce, cultivated under blue 455 nm light. The 505 nm light, being intermediate between blue and green wavelengths, had the most pronounced effect on photosynthetic pigment contents, higher NAR value and thus also might be beneficial for supplemental baby leaf lettuce illumination. Green 535 nm light stimulated soluble saccharide accumulation in lettuce, had inconsistent effect on photosynthetic pigment contents and assimilative indices, thus the evidence of the effect was inconclusive.

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Salotų auginimas po aukšto slėgio natrio lempomis, šviesos spektrą papildžius mėlynais, ciano ir žaliais kietakūniais šviestukais

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Santrauka

Tyrimo tikslas – įvertinti aukšto slėgio natrio lempų ir papildomų mėlynų, ciano ir žalių kietakūnių šviestukų apšvietimo įtaką veislės ‘Multigreen 3’ sėjamosios salotos (*Lactuca sativa* L.) augimui šiltnamyje. Papildoma mėlyna šviesa turėjo įtakos fotosintezės efektyvumui salotose, bet skirtingos mėlynos šviesos bangos turėjo nevienodą poveikį. 470 nm šviesa taip pat turėjo teigiamą įtaką chlorofilo *a* ir *b* santykiui bei specifiniam lapų plotui, lėmė didesnį augalo biomasės ir lapų ploto santykį. Augimo efektyvumas, didesnis tirpių angliavandenių kiekis ir augimo greitis, kuris nusakomas santykiniu augimo greičiu, nustatyti po mėlynais 455 nm LED. 505 nm šviesa, kuri yra tarp mėlynos ir žalios šviesos, turėjo žymią teigiamą įtaką fotosintetinių pigmentų kiekiui. Padidėjęs santykinis augimo greitis taip pat buvo sąlygotas salotų apšvietimo. Žalia 535 nm šviesa stimuliavo angliavandenių kaupimąsi salotose ir turėjo nevienareikšmę įtaką fotosintetiniams pigmentams bei asimiliaciniam indeksams. Tyrimo rezultatai kol kas nepatvirtino žalios šviesos įtakos.

Reikšminiai žodžiai: angliavandeniai, augimo analizės, chlorofilai, kietakūniai šviestukai, šiltnamis.