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EFFECT OF SHORT-WAVELENGTH LIGHT ON LETTUCE GROWTH AND NUTROTIONAL QUALITY

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Abstract. Influence of short-wavelength light on growth, development and nutritional quality of lettuce was studied by growing lettuce *Lactuca sativa* L. cv. 'Grand rapids' in phytotron under LED-based illumination with bicomponent spectrum consisting of red component (640 nm) and short-wavelength component in cyan (500 nm), blue (460 nm), or near-UV (365 nm) regions. Biometric characteristics and concentrations of pigments and sugars were measured 39 days after germination. Drastic sensitivity of total carbohydrates concentration and relative ratio between amounts of different sugars on the spectral position of the short-wavelength component was observed. We also demonstrate that concentration of nitrites in plants grown under such bicomponent illumination is reduced in respect to the reference plants grown under illumination by conventional fluorescent lamps.

Key words: growth parameters, lettuce, light-emitting diodes, nutritional quality.

Introduction. Light is one of the most important factors playing the key role in plant growth and development. Light in different spectral regions selectively activates different photoreceptors that induce highly overlapping sets of genes indicating presence of shared signaling components (Lin, 2002). Spectral changes evoke different morphogenetic and photosynthetic responses, which can vary among different plant species (Shuerger et al., 1997). This photoresponse is important in agrotechnology, since feasibility of tailoring illumination spectra enables one to control plant growth, development, and nutritional quality. The recent progress in solid-state lighting based on light-emitting diodes (LEDs) facilitated and expanded the research in this field and created an outset for new progressive plant cultivation technologies. LEDs, in comparison with conventional high-pressure sodium (HPS) and fluorescent lamps, are environment friendly and long-lifetime light sources available in the spectral range covering entire visible and near-UV regions with a potential to supersede conventional lamps in efficiency (Tamulaitis et al., 2005).

It is well established that the red light is of major importance for plant growth (Kopcewicz, Lewak, 1998). Red light is important for the development of the photosynthetic apparatus in plants and affects plant morphogenesis due to light-induced transformations in phytochrome system (Furuya, 1993). Blue light, also essential for plants, affects the formation of chlorophyll, stomata opening, and photomorphogenesis (Dougher, Bugbee, 1998; Shuerger et al., 1997; Heo et al., 2002). As revealed in previous studies, a combination of red and blue light was favourable for growth of several crops (Kim et al., 2004; Yorio et al., 2001; Yanagi et al., 1996). Such two-component illumination promotes dry matter production in pepper, wheat, spinach, radish and lettuce (Matsuda et al., 2004). These results support the suggestion that cryptochromes work together with phytochromes to regulate photomorphogenic responses (Lin, 2002). The addition of green light (500 to 600 nm) was shown to have a significant positive effect on leave colour and biomass accumulation (Kim et al., 2004; Yorio et al., 2001; Yanagi et al., 1996). Nevertheless, agricultural applications of the LED-based lighting are still limited. One of the fundamental problems in application of this new technology is poor understanding of the performance of system controlling development of the growing plant, since the regulatory mechanisms and metabolic signalling under monochromatic illumination are still not explored in detail.

Lettuce is an important greenhouse vegetable, intensively grown during the seasons of low solar irradiation. Thus, elucidation of the illumination spectrum, which is optimal in view of lettuce growth rate, healthy development and nutritional quality, is of practical importance.

This study was aimed at evaluation of the effect of short-wavelength light on growth and development of lettuce. Using intentionally designed LED-based luminaries, we performed lettuce growth treatments under illumination with different two-component spectra. The basic red component (640 nm), which is of vital importance for photosynthesis, was supplemented by additional component in cyan (500 nm), blue (460 nm) or near-UV (365 nm) region. Biometric parameters and concentrations of chlorophylls, carotenoids, carbohydrates and nitrates in lettuce plants grown in these illumination conditions for 39 days were tested and compared.

Materials and methods. Lettuce *(Lactuca sativa* cv. 'Grand rapids') was grown in phytotron chambers in peat substrate. A photoperiod of 18 h was used and the temperature of 21/15°C (day/night) was maintained throughout the experiment. Reference plants were grown under illumination by fluorescent lamps (3 kJ, TMS022, *Philips*, USA). Three treatments under illumination with different spectral components were performed, as specified in Table 1. Spectra in all treatments contained a basal red component with peak emission at 640 nm delivered by AlGaInP LEDs (LUXEON® III Star, LXHL-LDE3C, *Philips Lumileds Lighting Company*). In Exp1, the red component was supplemented by near-UV LEDs (i-LED, NCCU033T, *Nichia Corporation*, Japan) with peak emission at 365 nm. In Exp2, blue LEDs (LUXEON® III Star, LXHL-LB3C, *Philips Lumileds Lighting Company*) with peak emission at 460 nm were used to supplement the red component. In Exp3, cyan LEDs (LUXEON®

III Star, LXHL-LE3C, *Philips Lumileds Lighting Company*) with peak emission at 500 nm have been combined with the red light. The photosynthetic photon flux density was measured using a photometer-radiometer (RF-100, *Souopan*, Poland) and was maintained at 200 ± 5 mmol $m^{-2}s^{-1}$. The photon flux in each component was estimated by measuring spectra of each LED placed in the luminaries. Calculation of the contribution of the near-UV component was based on manufacturer's datasheet and operation conditions. Spectrometer (HR4000, *Ocean Optics Inc*., USA) was used to perform the spectral measurements.

T a b l e 1. **Photon flux densities of spectral components in different growth treatments**

1 l e n t e l ė. **Šviesos spektro komponenčių fotonų srauto tankis atliekant skirtingus auginimo bandymus**

Treatments Apšvietimo deriniai	Photon flux density of spectral components in % of a total flux density Komponentės fotonų srauto dalis, % bendro fotonų srauto tankio			
	640	500	460	365
R	-	-	-	-
Exp1	92	0	0	8
Exp2	86	0	14	0
Exp3	90	10	0	0

Biometric measurements were performed 39 days after germination (DAG). The total plant height, hypocotyls height, number of leaves, leaf area, and plant fresh weight were measured in five replications. The standard deviations are indicated in Table 2 and in Fig. 1, 2 by error bars. Net photosynthetic productivity was estimated from the plant dry mass and leaf area according to Kvģt (1971).

Concentrations of chlorophylls, carbohydrates and nitrates in leaves were also estimated. The chlorophyll *a*, *b* and carotenoid contents in green matter were determined in 100% acetone extracts by the spectrophotometrical Wettstein method (Гавриленко, 2003) using a spectrophotometer *Genesys 6* (*ThermoSpectronic*, USA). Three biological samples were measured. Standard deviations are indicated in Fig. 3 by error bars.

Samples for determination of carbohydrates were prepared by grinding 1 g of leaf fresh matter and extracting it with 4 mL hot bidistiled water. After 24 h, the extract was filtered through cellulose and membrane (pore diameter 0.2 μm) filters. Chromatographic analysis was carried out using a *Shimadzu* 10A HPLC system with refraction index detector (*Shimadzu*, Japan) and *Adsorbosil* NH_2-column (150 mm × 4.6 mm; *Alltech*, USA) with mobile phase of 75% aqueous acetonitrile at a flow rate of 1 mL/min. Error bars in Fig. 4 indicate the standard deviation of five analytical measurements.

Nitrates content in lettuce leaves was determined using the potentiometric method described by Geniatakis et al. (2003).

Results. The biometric parameters of lettuce plants grown under illumination by fluorescent lamps (R) and LED-based luminaries (Exp1 to Exp3) are summarized in Fig. 1 and Table 2. As evidenced in Fig. 1, the most compact plants were those grown under illumination with red and blue components (Exp2). Illumination in cyan region (Exp3) enhanced growth of hypocotyl by a factor of 2 in respect to other treatments. Lettuce plants grown under illumination in red supplemented by near-UV component were slight and spindled. Other biometric parameters (fresh weight, number of leaves and leaf area) varied within the experimental error (see Table 2).

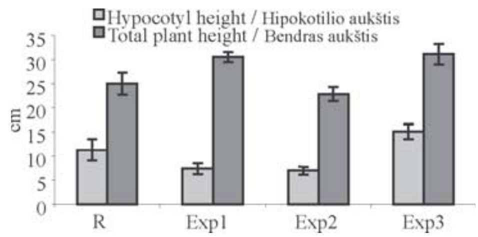

Fig. 1. Total plant height and hypocotyl height of lettuce grown under illumination by fluorescent lamp (R) and using different components of LED-based illuminator (Exp1 to Exp3, see Table 1 for details)

1 pav. Bendras salotų aukštis ir hipokotilio aukštis, kai augalai buvo auginami apšviečiant fluorescencinėmis lempomis (R) ir šviestuvu su skirtingomis šviestukų grupėmis (Exp1, 2, 3; žr. spektrines charakteristikas 1 lentelėje)

T a b l e 2. **Biometric parameters of lettuce plants**

2 l e n t e l ė. **Salotų biometriniai rodikliai**

Treatment Apšvietimo deriniai	Fresh weight Žalia masė, g	Leaf area Lapų plotas, cm^2	Number of leaves Lapų skaičius
R	15.7 ± 8.1	299 ± 135	6.6 ± 0.6
Exp1	16.7 ± 7.5	350 ± 182	7.4 ± 1.1
Exp2	15.9 ± 3.8	319 ± 84	6.4 ± 0.6
Exp3	14.3 ± 3.3	305 ± 86	6.4 ± 0.6

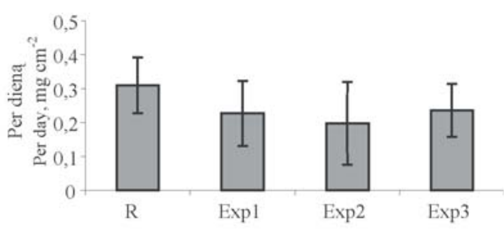

Fig. 2. Photosynthetic productivity in lettuce plants

2 pav. Salotų fotosintezės produktyvumas

The results of photosynthetic productivity calculation did not show significant differences between treatments after 39 DAG, though this parameter was possibly slightly higher in plants grown under illumination by fluorescent lamps (see Fig. 2).

Contents of chlorophylls *a*, *b* and carotenoids are presented in Fig. 3. Statistically significant difference was observed only for chlorophyll *a*. Its content in plants grown under illumination by fluorescent lamps was higher than in all other experiments.

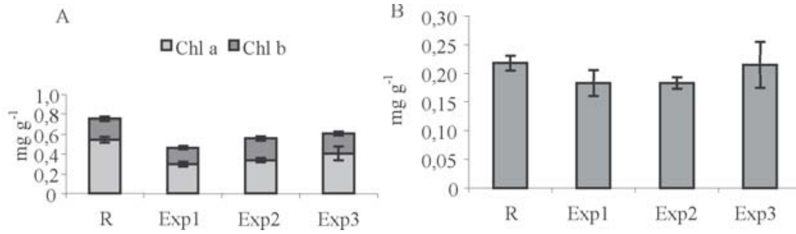

Fig. 3. Concentration of photosynthetic pigments in lettuce leaves: chlorophyll *a* and *b* (A) and carotenoids (B)

3 pav. Fotosintezės pigmentų koncentracija salotų lapuose: chlorofilai *a* ir *b* (A) ir karotinoidai (B)

The most striking sensitivity on illumination spectrum was observed for carbohydrates content (Fig. 4). In respect to the reference growth run under fluorescent lamp illumination, the total concentration of carbohydrates was considerably lower in treatments Exp1 (red and near-UV components) and Exp3 (red and cyan components) and significantly higher in Exp2 (red and blue components). Note that substitution of the blue component by the cyan component in the illumination spectrum results in decrease of a total carbohydrate content by a factor of 3.5. It is also worth noting that the plants grown under red illumination supplemented by the cyan component contained essentially only one type of carbohydrates: fructose. The results indicate that the lighting in reference (R) and Exp2 treatments was suitable for lettuce cultivation. Therefore, intensive monosugar synthesis was observed in the lettuce plants grown in these treatments. In addition, substantial amounts of disugars (maltose in reference treatment and sucrose in Exp2) were found in these plants.

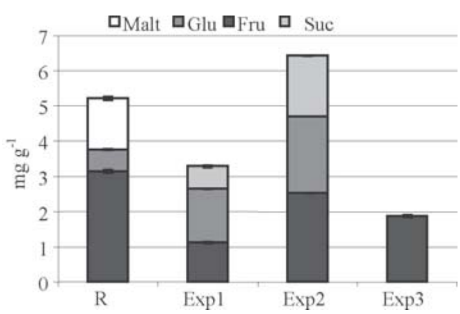

Fig. 4. Carbohydrate contents in lettuce leaves 39 days after germination

4 pav. Angliavandenių kiekis salotų lapuose praėjus 39 dienoms nuo sudygimo

Another interesting feature of plant sensitivity to illumination spectrum is presented in Fig. 5, where concentration of nitrates in lettuce leaves in four treatments is compared. Content of nitrites in different treatments using LED-based illumination is similar within the experimental error, however, it is lower, especially in treatments Exp2 and Exp3, than that in plants grown under fluorescent lamp illumination.

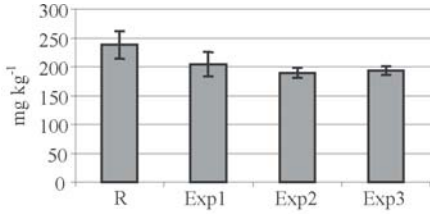

Fig. 5. **Content of nitrates in lettuce leaves**
5 pav. **Nitratų kiekis salotų lapuose**

Discussion. The results of our study confirm that plant growth can be modulated by employing tailored spectra provided by different LEDs. Even illumination with spectrum, consisting of two components, if properly selected, can be beneficial in respect to illumination using fluorescence lamps. Optimal growth was observed in treatment Exp2, where plants were illuminated by red and blue spectral components. Plants were compact, without any spindling. These results are in correspondence with presumption that red and blue light co-act inhibiting cell elongation in stem and leaves (Spalding, Folta, 2005), and the optimal illumination spectrum should contain 90% of red and 10% of blue light energy (Kopcewicz, Lewak, 1998). Blue light promotes stomatal opening more efficiently than the light in other spectral regions and this stimulation of stomatal opening may contribute to the enhanced photosynthesis rates and dry matter production (Goins et al., 1997). It was observed that supplementing red light with blue light promotes dry matter production (Matsuda et al., 2004). However, the biomass production in our experiments was not significantly affected by additional short-wavelength lighting. No significant differences in leaf area or leaf number were observed.

In spite of relatively insignificant variations of growth characteristics, the additional short-wavelength illumination has a drastic influence on carbohydrates amount and composition. The highest concentration of carbohydrates was observed in lettuce grown under red light supplemented by blue spectral component. Increased content of hexoses and sucrose indicate enhanced vital processes in these plants, since sucrose metabolism lies at the very heart of the sensitive self-regulatory system of plant development (Koch, 2004).

High total concentration of carbohydrates is one of desirable parameters in view of food quality. However, the quality also depends on the percentage of monosugars. Note that cultivation of lettuce with additional component in cyan spectral region results in suppression of production of disugars. Fructose distinctively dominated all other carbohydrates in this growth treatment, though the total concentration of carbohydrates was considerably lower than that in other treatments. Possibility of intentional monitoring of the trade-off between quantity and quality of sugars by selecting the illumination spectrum is worth of further detailed study. This feature might be of practical importance for lettuce commercially grown under artificial lighting.

Another positive effect of selective illumination is reduction of nitrates content in respect to plants grown under fluorescent lamp illumination. This effect of the order of 15–20% was observed for all combinations of red and short-wavelength components under study. This observation is in line with suggestion that the red illumination component, which is effectively absorbed by phytochrome, plays the key role in the stimulation of nitrate reductase (Lillo, 2004) and with previous observation that supplementation of the red component with blue light promotes the uptake or assimilation of nitrogen in rice plants (Ohashi et al., 2006). Reduction of nitrates content is definitely of importance for improvements of nutritional quality of lettuce. Possibly, the effect can be pushed above 20% by further optimisation of the illumination spectrum.

In conclusion, exploitation of intentionally designed LED-based luminary for cultivation of lettuce plant under different combinations of red and short-wavelength components in the illumination spectrum enabled us to elucidate the influence of light in short-wavelength visible and near-UV regions on growth and development of lettuce. Combination of red and blue components was found to be favourable for lettuce growth, especially in respect to its nutritional quality. Development of plants cultivated under this illumination was similar to development of reference plants grown under conventional illumination by fluorescent lamps. The most striking sensitivity to illumination spectrum was observed for production of carbohydrates. Moreover, in respect to the reference plants, content of nitrates in lettuce grown under the bicomponent illumination in red and short-wavelength region was by 15–20% lower, with no significant difference among treatments, where the short-wavelength component was cyan, blue or UV.

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TRUMPABANGĖS ŠVIESOS POVEIKIS SALOTŲ AUGIMUI IR MAISTINEI KOKYBEI

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Santrauka

Fitotrono komplekse tirtas trumpabangės šviesos poveikis salotų (*Lactuca sativa* L.) veislės ‘Grand rapids’ augimui, vystymuisi ir maistinei kokybei. Apšvietimui naudota bikomponenčio spektro šviesa, emituojama šviestukų (šviesos diodų) moduliuose, kuriuose raudona šviesa (640 nm) papildyta trumpabangėmis komponentėmis ciano (žaliai mėlynojoje, 500 nm), mėlynojoje (460 nm) ir artimojoje ultravioletinėje (365 nm) spektro dalyse. Biometriniai matavimai, fotosintezės pigmentų, cukrų ir nitratų analizės atliktos praėjus 39 dienoms po sudygimo. Nustatyti esminiai bendro angliavandenių kiekio ir atskirų angliavandenių santykio skirtumai salotose, išaugintose apšviečiant augalus šviesa, kurios spektre yra skirtingo bangos ilgio trumpabangės komponentės. Salotose, augintose panaudojant apšvietimą su bikomponenčiu spektru, nitratų koncentracija buvo reikšmingai mažesnė negu kontroliniuose augaluose, augintuose po įprastinėmis fluorescencinėmis lempomis.

Reikminiai žodžiai: biometriniai rodikliai, maistinė kokybė, salotos, šviestukai.