

# VII

## Publication [VII]

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## INFLUENCE OF BICOMPONENT COMPLEMENTARY ILLUMINATION ON DEVELOPMENT OF RADISH

**Akvilė URBONAVIČIŪTĖ<sup>1, 4</sup>, Paulo PINHO<sup>2</sup>, Giedrė SAMUOLIENĖ<sup>1, 4</sup>,  
Pavelas DUCHOVSKIS<sup>1, 4, 5</sup>, Pranciškus VITTA<sup>3</sup>, Andrius STONKUS<sup>3</sup>,  
Gintautas TAMULAITIS<sup>3</sup>, Artūras ŽUKAUSKAS<sup>3, 5</sup>, Liisa HALONEN<sup>2</sup>**

<sup>1</sup>*Lithuanian Institute of Horticulture, Babtai LT-54333, Kaunas distr., Lithuania .  
E-mail: a.urbonaviciute@lsdi.lt*

<sup>2</sup>*Helsinki University of Technology, Lighting Laboratory, 02150 Espoo, Finland*

<sup>3</sup>*Institute of Materials Science and Applied Research, Vilnius University, Vilnius,  
LT-10222, Lithuania*

<sup>4</sup>*Lithuanian University of Agriculture, LT-55307 Akademija, Kaunas distr.,  
Lithuania*

<sup>5</sup>*JSC "Hortiled"*

**Abstract.** Influence of bicomponent illumination supplemental to conventional high-pressure sodium lamps on growth and development of radish was studied. The supplemental illumination was delivered by purposefully designed luminaires containing two groups of light-emitting diodes emitting in red and at different spectral positions in short-wavelength region. Biometric parameters and concentrations of primary metabolites and photosynthetic pigments in leaves were measured. We observed that introduction of the short-wavelength components into the spectrum of illumination for radish cultivation is insufficient to compensate the stress caused by excessive illumination in photosynthetically active red region.

**Key words:** radish, HPS lamp, light-emitting diodes, phytohormones, carbohydrates, photosynthetic pigments.

**Introduction.** Light is one of the most important environmental factors, acting on plants not only as the sole source of energy, but also as a source of external information controlling their growth and development. Plants are empowered with an array of photoreceptors tracking many parameters of incoming light signals, including their presence or absence, spectrum, intensity, direction and duration (Huq, 2006). The optimal balance of these light parameters is essential for normal growth and development of plant. Therefore, an optimized lighting system is necessary for plant cultivation in greenhouses and phytotrons. Radish (*Raphanus sativus*) is one of the vegetables that are difficult to cultivate in artificial growth conditions, mainly because of disturbance of tuber formation processes. Temperature and light, including photoperiod, illumination level and spectrum are the main factors affecting assimilate distribution between leaves and storage organs (Craker et al, 1983; Franklin, Whitlam, 2003).

High-pressure sodium (HPS) lamps, which are commonly used in greenhouse plant cultivation, have the highest intensity in red/orange spectral region and are

efficient light sources for the time of plant flowering and fruiting. Nevertheless, the spectrum of HPS lamps is far from being optimal for storage organ formation. It is worth noting that there is still not known what are the requirements for the illumination spectrum necessary for normal radish development, since plants representing various life forms and life strategies require different lighting conditions (Tarakanov, 2006). The recent progress in solid-state lighting based on light-emitting diodes (LEDs) enables a more comprehensive study of plant photobiology in order to improve lighting systems used for plant cultivation. Such investigations of light effect on plant growth and development are important for agriculture.

**This study was aimed at** investigating possibilities of supplementing the HPS lamps with additional spectral components provided by LEDs. The study was carried out using purposefully designed luminaires consisting of HPS lamp and two groups of LEDs. LEDs with peak emission in red (640 nm), which is important for photosynthesis, were used in one group, while short-wavelength LEDs were used in another group. To reveal the influence of wavelength of the short-wavelength component, radish was grown simultaneously under three luminaires with different short-wavelength LEDs: UV (385 nm), blue (450 nm) and cyan (505 nm). Biometric parameters and concentrations of primary metabolites and photosynthetic pigments in leaves were measured.

**Materials and methods.** Radish (*Raphanus sativus* cv. 'Saxa') was grown in phytotron chambers in peat substrate. A photoperiod of 16 h was used and the temperature of 18/15 °C (day/night) was maintained throughout the experiment.

Reference plants (R) were grown under illumination by high-pressure sodium (HPS) lamps *Son-T agro* (PHILIPS, Massachusetts, USA). In three test growth runs, a part of the HPS photon flux (70%) was replaced by illumination delivered by LEDs (Table 1). Each of three luminaires contained a basal red component with peak emission at 640 nm delivered by AlGaInP LEDs (LUXEON® III Star, LXHL-LDE3C, Philips Lumileds Lighting Company). The red component contained approximately 90% of the total LED emission flux in all treatments, while the rest 10% were delivered in cyan, UV, or blue regions. In treatment L1, cyan LEDs (LUXEON® III Star, LXHL-LE3C, Philips Lumileds Lighting Company) with peak emission at 505 nm have been used. In treatment L2, the illumination spectrum was supplemented by near-UV LEDs (i-LED, NCCU033T, Nichia Corporation, Japan) with peak emission at 385 nm. In treatment L3, blue LEDs (LUXEON® III Star, LXHL-LB3C, Philips Lumileds Lighting Company) with peak emission at 460nm were used.

Table 1. **Photon flux densities of illumination components**  
1 lentelė. **Apšvietimo spektrinių komponentų fotonų srauto tankis**

Experiment Bandymas	LED treatment Šviestukų deriniai	Photon flux density Fotonų srauto tankis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		
		LED	Son-T agro	total bendras
R	-	-	225	225
L1	Red (640 nm) + Cyan (505 nm)	162	65	227
L2	Red (640 nm) + UV (385 nm)	158	65	223
L3	Red (640 nm) + Blue (450 nm)	160	62	222

Biometric measurements and analysis of plant primary metabolites were performed 25 days after germination (DAG). The total plant height, hypocotyl height, leaf area, and plant fresh weight were measured in five replicates. The standard deviations of these measurements are indicated in Table 2.

Concentrations of chlorophylls, carotenoids and carbohydrates 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. 1 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 bidistilled water. After 24 h, the extract was filtered through cellulose and membrane (pore diameter 0.2  $\mu\text{m}$ ) filters. Chromatographic analysis was carried out using a *Shimadzu* 10A HPLC system with refraction index detector (*Shimadzu*, Japan) and *Adsorbosil*  $\text{NH}_2$ -column (150 mm  $\times$  4.6 mm; *Alltech*, USA) with mobile phase of 75% aqueous acetonitrile at a flow rate of 1 mL/min. Error bars in Fig. 2 indicate the standard deviation of five analytical measurements.

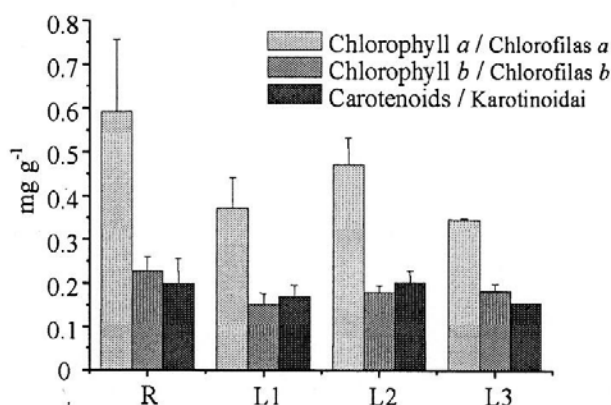
Samples for phytohormone determination were prepared by grinding 1 to 2 g of fresh tissue per sample into powder under liquid nitrogen treatment. The extracts were centrifuged and pre-purified using solid-phase extraction with  $\text{NH}_2$ - cartridge columns. The prepared samples were stored in vials at 4 °C. Analysis of gibberellic acid ( $\text{GA}_3$ ), indolyl-3-acetic acid (IAA), abscisic acid (ABA) and zeatin was performed using HP 1050 Series liquid chromatography system with variable wavelength UV-VIS detector (*Agilent Technologies*, Germany). *Intersil* ODS-2 column (150  $\times$  4.6 mm<sup>2</sup>) (*Alltech*, USA) was used for phytohormones separation. Mobile phase consisted of 45% methanol containing 1% acetic acid. Flow rate was maintained at 1 mL/min. The wavelengths of 254 nm for  $\text{GA}_3$  and ABA detection, 270 nm for zeatin and 280 nm for IAA detection were set. Error bars in Fig. 3 correspond to the standard deviations of analytical measurements of the phytohormones identified.

**Results.** Light effect on growth parameters of radish is presented in Table 2. The reference plants (R) showed better growth patterns: they were significantly higher, formed the largest leaf area and the thickest hypocotyl. Radish in treatments L2 and L3 treatments were dwarf and slight, and accumulated very little of biomass. Meanwhile, radish in treatment L1 accumulated almost the same fresh weight as in reference plants, though its leaf area was by a factor of 3.5 and the total height by a factor of two smaller than that in reference treatment. All the radish plants, including reference ones, did not accumulate the biomass in hypocotyls. Therefore, no storage root was formed.

The photosynthetic pigment contents showed similar patterns (Fig. 1). However, the content of chlorophyll *a* was significantly higher in the leaves of reference plants, illuminated with solely HPS lamps. Moreover, the chlorophyll *a/b* ratio in plants grown with supplemental LED illumination was lower than that in reference plants. This is an indication of decreased activity of photosynthetic system. The lowest chlorophyll *a/b* ratio (20% lower than that in reference plants) was observed in radish grown in treatment with short-wavelength component in blue region (treatment L3).

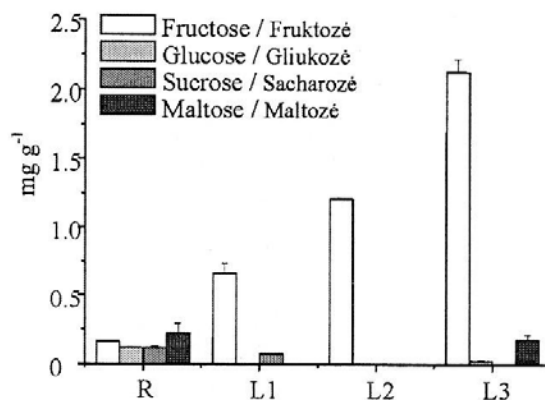
Table 2. **Biometric parameters of radish plants**  
2 lentelė. **Ridikėlių biometriniai rodikliai**

Treatment Bandymas	Fresh weight Žalia masė (g)	Leaf area Lapų plotas (cm <sup>2</sup> )	Total plant height Bendras augalo aukštis (cm)	Hypocotyl height Hipokotilio aukštis (cm)	Hypocotyl diameter Hipokotilio skersmuo (cm)
R	0.9 ± 0.3	72.0 ± 20.1	18.8 ± 2.1	15.4 ± 2.3	0.35 ± 0.19
L1	1.0 ± 0.4	21.5 ± 12.3	10.8 ± 1.0	7.7 ± 1.8	0.24 ± 0.12
L2	0.3 ± 0.1	7.4 ± 1.4	9.3 ± 1.3	4.9 ± 0.8	0.13 ± 0.03
L3	0.3 ± 0.0	7.4 ± 1.9	7.6 ± 0.6	5.0 ± 0.8	0.31 ± 0.20



**Fig. 1.** Content of photosynthetic pigments in radish leaves  
**1 pav.** Fotosintetinių pigmentų kiekis ridikėlių lapuose

The most striking illumination effect was observed on carbohydrate contents. Contents of fructose, glucose, sucrose and maltose are presented in Fig. 2.



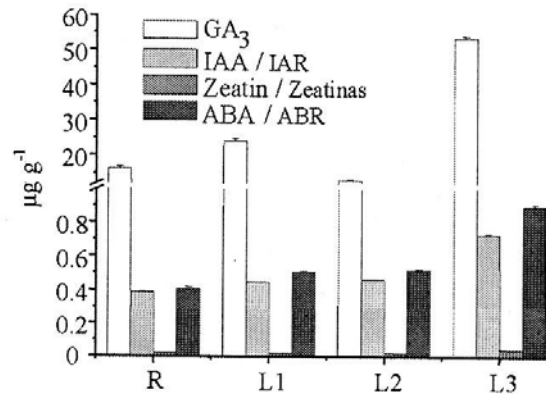
**Fig. 2.** Content of carbohydrates in radish leaves 25 days after germination  
**2 pav.** Angliavandenių kiekis ridikėlių lapuose praėjus 25 dienoms po sudygimo

Reference plant leaves contained similar amount of all these sugars. This balance reflects normal radish growth. In growth runs under illumination with supplemental red and short-wavelength components, the sugars content was dominated by fructose, which is known to be a stress sugar. The content of fructose was 4, 7.5 and 13 times higher in treatments L1, L2 and L3, respectively. Only a small amount of sucrose



was observed in the treatments with supplemental red and UV LEDs (L1) and some sucrose and maltose in radish leaves illuminated with supplemental red and cyan LEDs (L3). Such distribution of carbohydrates in leaves is a consequence of disorganized metabolism.

The conclusion on disorganized vital processes in radish grown under supplemental bicomponent illumination is in consistence with the results on determination of phytohormone concentrations. The concentration of ABA and zeatin in treatments L1 and L2 were slightly higher than that in reference growth, however, concentrations of these phytohormones in treatment L3 was higher by a factor of two.



**Fig. 3.** Phytohormone concentration in radish leaves, grown under different illumination conditions

**3 pav.** Fitohormonų koncentracija ridikėlių, augusių skirtingo apšvietimo sąlygomis, lapuose

Compared to the reference plants, the contents of gibberelic acid (GA<sub>3</sub>), representing total amount of unidentified gibberelins isomers, was lower in treatment L1, but 3 times higher in treatment L3. Concentrations of the stress hormone ABA were higher in all treatments with supplemental illumination, especially in treatment L3.

**Discussion.** Light is a powerful tool, regulating plant physiological processes. Our results confirm that light might be a limiting factor for metabolism and assimilate partitioning in radish. It is previously observed that excessive red light disturbs radish tuber formation. The excessive intensity of red light stimulates biomass accumulation in aboveground parts of plant, instead of its storage in root formation (Drozdova et al., 2001; Bukhov et al., 1996). Our experimental results indicate that substitution of large part of illumination by HPS lamps with bicomponent illumination in red and short-wavelength regions negatively affected not only tuber formation, but also leaf area formation and biomass accumulation. Such lighting conditions with high flux of red light obviously created stressful conditions for radish growth and development and triggered stress-avoidance responses (Tarakanov, 2006). High contents of fructose in radish leaves indicate disturbance in sucrose metabolism, which is typical for the reaction to the exposure of other abiotic stress factors (Rolland et al., 2006). These results are in consistence with phytohormone contents. The significant increase in concentrations of ABA, the stress hormone (Roitsch, Ehness, 2000; Rook et al., 2006),

and of zeatin, which is responsible for alteration in source/sink relationships (Roitsch, Ehness, 2000) and lethal cell death processes (del Pozo et al., 2005), indicate inappropriate lighting conditions. The misbalance in action of photosynthetic system and primary metabolism resulted in blocked assimilate export to sink organs. As carbohydrate partitioning between source and sink organs and tissues is essential for growth and development in higher plants (Roitsch, Ehness, 2000; Drozdova et al., 2004), no tuber formation was observed in our cultivated radish.

Cryptochromes, principally thought of as red/far-red reversible pigments, are extremely sensitive to the entire illumination spectrum and even small variations in the spectrum can initiate responses in cryptochrome system (Folta, Maruhnich, 2007). Thus, variation of illumination intensity in short-wavelength region in treatments L1 to L3 should make a significant influence on the cryptochrome system. A positive effect on radish growth has been previously observed (Drozdova et al., 2001; Bukhov et al., 1996). However, this positive effect is obviously not sufficient to counterbalance the negative influence due to excessive intensity in red region. Illumination in cyan region resulted in slightly improved biometric parameters in comparison with those observed in plants grown under illumination with short-wavelength components in blue and UV regions. There are some previous reports on positive effect of green light for plant cultivation (Kim et al., 2004), thus, it is possible, that cyan light, being closer to the green region, has the same biological effect.

**Conclusion.** Introduction of short-wavelength components into the spectrum of illumination for radish cultivation is insufficient to compensate the stress caused by excessive illumination in photosynthetically active red region. Spectral position of the short-wavelength component in the region from 385 to 505 nm has no crucial influence on radish development, though illumination in cyan region (505 nm) is more favorable for biomass accumulation than illumination at shorter wavelengths. Bicomponent illumination is inappropriate for radish cultivation. Illumination with more sophisticated illumination spectrum should be selected for healthy growth and rhizocarp formation of radish in artificial conditions.

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## **DVIKOMPONENČIO PAPILDOMO APŠVIETIMO ĮTAKA RIDIKĖLIŲ VYSTYMUISI**

**A. Urbonavičiūtė, P. Pinho, G. Samuolienė, P. Duchovskis, P. Vitta,  
A. Stonkus, G. Tamulaitis, A. Žukauskas, L. Halonen**

### *Santrauka*

Ištirta dvikomponenčio apšvietimo, papildančio įprastines aukšto slėgio natrio lempas, įtaka ridikėlių augimui ir vystymuisi. Tuo tikslu sukurtame apšvietimo modulyje įrengtos dvi šviestukų (šviesos diodų) grupės, skleidžiančios raudoną ir skirtingo bangos ilgio trumpabangę šviesą. Atlikti biometriniai augalų matavimai, nustatytas fotosintetinių pigmentų ir pirminių metabolitų kiekis lapuose. Nustatyta, kad trumpabangės šviesos komponentų srautas buvo nepakankamas, kad kompensuotų stresą, kurį ridikėliams sukėlė fotosintetiškai aktyvios raudonos šviesos srauto perteklius.

**Reikšminiai žodžiai:** ridikėliai, aukšto slėgio natrio lempos, šviesą emituojantys diodai, fitohormonai, cukrūs, fotosintetiniai pigmentai.

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