

## **Effect of flashing amber light on the nutritional quality of green sprouts**

A. Urbonavičiūtė<sup>1</sup>, G. Samuolienė<sup>1</sup>, S. Sakalauskienė<sup>1</sup>, A. Brazaitytė<sup>1</sup>,  
J. Jankauskienė<sup>1</sup>, P. Duchovskis<sup>1</sup>, V. Ruzgas<sup>3</sup>,  
A. Stonkus<sup>2</sup>, P. Vitta<sup>2</sup>, A. Žukauskas<sup>2</sup> and G. Tamulaitis<sup>2</sup>

<sup>1</sup>Lithuanian Institute of Horticulture, 30 Kaunas str., LT–54333, Babtai, Kaunas distr., Lithuania. Tel. +370–37–555476, fax: +370–37–555176; e-mail: a.urbonaviciute@lsdi.lt

<sup>2</sup>Institute of Materials Science and Applied Research, Vilnius University, Saulėtekio al. 9–III, LT–10222 Vilnius, Lithuania

<sup>3</sup>Lithuanian University of Agriculture, Studentų g. 11, LT–53361 Akademija, Kaunas distr., Lithuania

**Abstract.** We report on the application of flashing amber (596 nm) light-emitting diodes (LEDs), supplemental to high pressure sodium lamps, for the cultivation of green sprouts, such as wheatgrass, barley grass, and leafy radish. The flashing light was found to significantly affect metabolism, thus conditioning the nutritional quality of the sprouts. In particular, it causes stressful conditions for the plants and within a short growth period can promote the synthesis of antioxidative compounds, such as vitamin C, phenolic compounds and carotenoids. However, the flashing amber light effect is dependent on the plant species.

**Key words:** Light-emitting diodes, wheatgrass, barley grass, leafy radish, antioxidant activity, vitamin C, carotenoids, saccharides

### **INTRODUCTION**

Light is one of the most important environmental factors which acts on plants as one source of energy. The complex, multiple photoreception system (chlorophylls, carotenoids, phytochrome, cryptochrome, phototropins, and other) (Chen et al., 2004; Spalding & Folta, 2005; Devlin et al., 2007) responds to light quantity and quality, duration, intermittence, and other parameters, thus determining plant morphogenetic changes, functioning of the photosynthetic apparatus, and the process of metabolic reactions. These photo-responses are of great importance for the development of advanced agrotechnologies. Solid-state lighting based on light-emitting diodes (LEDs) is one of the largest potential advancements in horticultural lighting in the last decades (Morrow, 2008). Efficiency, longevity, and versatile application possibilities are the features of LEDs that, when properly employed, are capable of providing performance well beyond any conventional lighting source (Bourget, 2008). LEDs enable not only tailoring a purposeful lighting spectra due to narrow-bandwidth emission, but also for adjusting such lighting parameters as the frequency and duration of light pulses due to fast switching. Flashing light offers technological and economical benefits as opposed to continuous illumination. For instance, flashing light has been proved to influence the

electron transport in Photosystem II (Szilard et al., 2005) and to have a pronounced effect on plant photosynthesis processes (Tennessen et al., 1995; Jao & Fang 2004). Moreover, by using electromagnetic pulses of certain duration and frequency, the resonant behaviour of biological reactions can be invoked and positive physiological effects can be attained (Vasilevski, 2003).

Wheatgrass, barley grass, and leafy radish, which are rich in various phytochemicals, are widely used as the 'green foods' in the USA, East Asian countries (Marsili et al., 2004; Paulickova et al., 2006) and Eastern Europe. The cultivating of such green foods with improved nutritional quality and functionality for healthy diets and therapeutics is one of the directions in the development of agricultural innovations (McGloughlin, 2008). In combination with other genetic and agrotechnical means, lighting might be an effective tool for photochemical-rich vegetable cultivation. To this end, spectral and temporal versatility of solid-state lighting can find novel applications. In this study, we supplemented high pressure sodium (HPS) lamp illumination with flashing amber (596 nm; almost same wavelength as that of HPS) light generated by LEDs for the cultivation of green sprouts with enhanced antioxidant potential.

## MATERIALS AND METHODS

A lighting unit containing light-emitting diodes (LEDs) and high pressure sodium (HPS) lamps was used for the growth experiments. HPS lamps (HS-T, 70W, Glamox, UK) were supplemented with amber LEDs (peak wavelength 596 nm; Luxeon LXHL-LL3C, Philips Lumileds Lighting Company). The LEDs were driven by a custom pulsed current source at a frequency of 2.9 Hz (250 ms 'on' and 100 ms 'off'). An 18 h photoperiod was maintained. The pulsed flux amounted to about 50% of the total photosynthetic flux density (about 35  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Reference plants were grown under solely HPS illumination with the photosynthetic flux density maintained at the same level. In the figures, labels 'HPS' and 'HPS+LED' refer to plants grown under solely HPS lamps and those supplemented with flashing amber LED light, respectively.

Experiments were performed in growth chambers, under controlled environmental conditions. The day/night temperature was maintained at 21/17°C. Green sprouts of wheatgrass (*Triticum aestivum* L. cv. 'Širvinta'), barley grass (*Hordeum vulgare* L. cv. 'Luoké'), and leafy radish (*Raphanus sativus* L. cv. 'Tamina') were sown in peat substrate. Lighting was applied starting from sowing and lasted for 7 days until harvesting, when the sprouts reached 7–9 cm in height. After harvesting, saccharides, vitamin C, phenolic compounds, and carotenoids content were assessed and the antioxidant activity of aboveground plant part extracts was evaluated.

Samples for the determination of saccharides content were prepared by hot-water extraction. Chromatographic analysis was carried out using a Shimadzu 10A high performance liquid chromatography (HPLC) system with a refraction index detector (Shimadzu, Japan) and an Adsorbosil NH<sub>2</sub>-column (150 mm x 4.6 mm; Alltech, USA) with mobile phase of 75% aqueous acetonitrile at a flow rate of 1 mL min<sup>-1</sup>.

Ascorbic acid content was evaluated using a spectrophotometric method of Janghel et al. (2007). The total content of phenolic compounds was determined in methanolic extracts of fresh leaves by a spectrophotometric Folin method (Ragae et

al., 2006). The antioxidant activity of methanolic extracts of the investigated leafy vegetables was evaluated spectrophotometrically as the ability to bind 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (Ragaei et al., 2006). A Genesys 6 spectrophotometer was used for the analysis (Thermospectronic, USA).

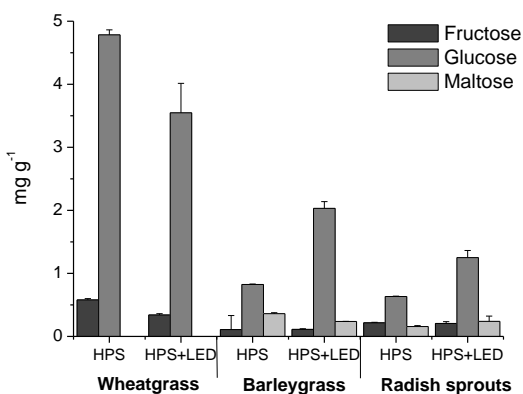
The carotenoids (violoxanthin, zeaxanthin, neoxanthin, lutein,  $\alpha$ -carotene (not detected) and  $\beta$ -carotene) content was determined using the HPLC method with gradient elution and diode array detection (Edelenbos et al., 2001). Extraction was performed in 80% iced acetone. Analysis was carried out using the Shimadzu 10A HPLC system and a Zorbax Extended-C18 column (150 mm x 4.6 mm; Agilent Technologies, Germany).

The error bars presented in figures and tables are the experimental uncertainties for the mean of five analytical measurements of a parameter. Data was processed using MS Excel software and Student's t-test for a confidence range of 95%.

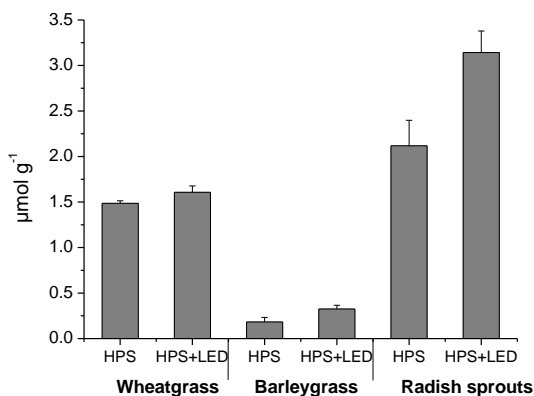
## RESULTS AND DISCUSSION

No significant variation in biometric growth parameters of the sprouts due to flashing lighting was found with a short growth period. At the same time, the content of nutrient compounds altered in many cases. The results of the biochemical assessment are presented in Figs 1 to 5. Fig. 1 shows that the accumulation of saccharides, which are metabolic products of photosynthesis, was altered, depending on species. In wheatgrass, grown under supplemental flashing amber light, fructose and glucose content dropped by 1.6 and 1.3 times, respectively; whereas in barley grass and radish sprouts the content of glucose was higher by about 2.0–2.5 times in respect to reference plants. In barley grass grown under flashing light, a slight decrease in maltose content was also observed.

Although light in the yellow region was claimed to suppress chlorophyll or chloroplast formation (Dougher & Bugbee, 2001), our experiments show no universal inhibitory effect on saccharides content in the sprouts.



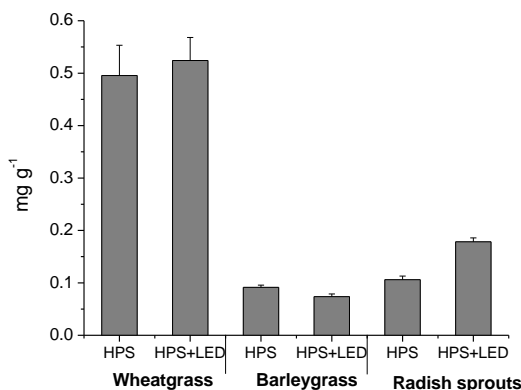
**Fig. 1.** Saccharides content in green sprouts grown under solely HPS lamps and with supplementation by flashing amber LED light.



**Fig. 2.** Antioxidant activity of the extracts, as the ability to bind DPPH free radicals, in green sprouts grown under solely HPS lamps and with supplementation by flashing amber LED light.

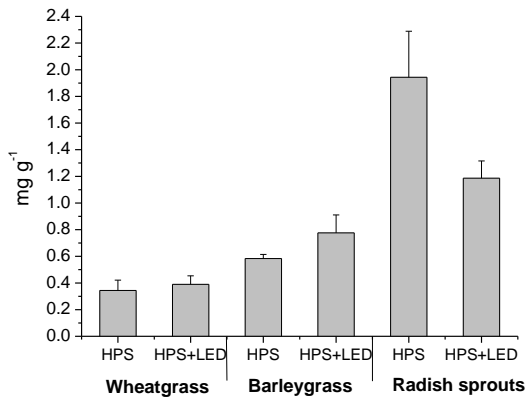
Moreover, the increase in monosaccharides content in barley grass and radish sprouts indicates effective utilization of amber light in assimilation processes. High content of monosaccharides, particularly of glucose, not only displays a higher activity of photosynthesis (Smeekens, 2000), but also has nutritional importance, since in vegetable food, they are more valuable sugars than disaccharides.

The antioxidant properties of the sprouts also exhibited diverse dependence on flashing light (Fig. 2). No significant effect on wheatgrass or barley grass extract ability to bind free 2,2-diphenyl-2-picrylhydrazyl (DPPH) radicals was observed. In radish sprouts, which have the highest antioxidant potential, the radical binding activity was increased by about 1.5 times due to the effect of flashing LED illumination. This is in line with the enhanced synthesis of phenolic compounds (see Fig. 3). Such behaviour can be attributed to light-induced stress that is known to stimulate the antioxidant activity, mostly due to natural mechanisms of defence against the photo-oxidative damage (Wu et al., 2007).

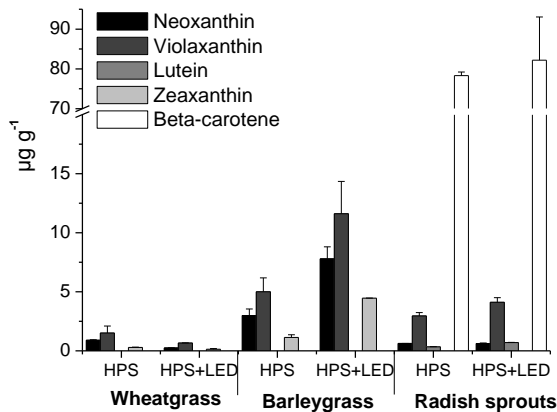


**Fig. 3.** Content of phenolic compounds in the fresh matter of green sprouts grown under solely HPS lamps and with supplementation with flashing amber LED light.

Phenolic compound content in the sprouts was found to be moderately affected by flashing light (Fig. 3). In wheatgrass and barley grass the changes were within the experimental errors, meanwhile in radish sprouts an increase of about 30% was observed under supplementary flashing lighting. Probably, the photosynthetic apparatus of younger leaves can respond to light conditions by rapid adaptation responses, including formation of new pigments and synthesis of phenolic compounds (Lichtenthaler, 2007). Vitamin C content was also affected by lighting conditions, depending on species (Fig. 4). Again, almost no difference was found in wheatgrass and barley grass, whereas flashing light invoked a significant drop in vitamin C content in radish leaves (by about 1.6 times). Vitamin C acts in plants as a key component in excess photonic energy dissipation mechanisms such as the xanthophylls cycle (Yabuta et al., 2007). The increase in the vitamin C contents coincides with the increased content of the violoxanthin and neoxanthin pool (Fig. 5) in HPS+LED grown barley grass, which is common under stressful light conditions (Lichtenthaler, 2007).



**Fig. 4.** Vitamin C concentration in green sprouts grown under solely HPS lamps and with supplementation with flashing amber LED light.



**Fig. 5.** Carotenoid contents in green sprouts grown under solely HPS lamps and with supplementation with flashing amber LED light.

The flashing amber LED light had no significant effect on carotenoid synthesis in wheatgrass and radish sprouts (Fig. 5). Contrarily, in barley grass, a statistically significant increase in concentration of xanthophylls cycle pigments, such as neoxanthin (by approximately 2.5 times), violaxanthin (by 2.3 times), and zeaxanthin (by 3.9 times) was found. This trend corresponds to the statements that the increase in the violaxanthin cycle pigments indicate the plant needs to utilize the low light efficiently (Demmig-Adams & Adams, 2002).

## CONCLUSIONS

The above results show that the biochemical content of the investigated sprouts responds to photostress invoked by flashing light in different ways, depending on species. In particular, accumulation of antioxidants and other metabolites can undergo either positive (by up to 2–4 times) or negative (by up to 1.6 times) variation; whereas in some cases no effect was observed. Nevertheless, our pilot results imply that selective phytochemical enrichment of plants might be possible within a short growth period and without a noticeable change in productivity. However, on an industrial scale, further optimization of the flashing photostress conditions (e.g., wavelength, photon flux and temporal parameters) is needed for the production of green sprouts with improved nutritional quality.

ACKNOWLEDGEMENT. The work was supported by the EU-Asia Link Programme under the ENLIGHTEN project and by the Lithuanian Science and Studies Foundation under the PHYTOLED project.

## REFERENCES

- Bourget, M.C. 2008. An Introduction to light emitting diodes. *Hortscience* **43**, 1944–1946.
- Chen, M., Chory, J. & Fankhauser, C. 2004. Light signal transduction in higher plants. *Annu. Rev. Genet.* **38**, 87–117.
- Demmig-Adams, B. & Adams, W.W. 2002. Antioxidants in photosynthesis and human nutrition. *Science* **298**, 2149–2153.
- Devlin, P.F., Christie, J.M. & Terry, M.J. 2007. Many hands make light work. *Journal of Experimental Botany* **58**, 3071–3077.
- Dougher, T.A.O. & Bugbee, B. 2001. Evidence for yellow light suppression of lettuce growth. *Photochemistry and Photobiology* **73**, 208–212.
- Edelenbos, M., Christensen, L.P. & Grevsen, K. 2001. HPLC determination of chlorophyll and carotenoid pigments in processes and green pea cultivars (*Pisum sativum* L.). *J.Agric. Food Chem.* **49**, 4768–4774.
- Janghel, E.K., Gupta, V.K., Rai, M.K. & Rai, J.K. 2007. Micro determination of ascorbic acid using methyl viologen. *Talanta* **2**, 1013–1016.
- Jao, R.C. & Fang, W. 2004. Effects of Frequency and duty ratio on the growth of Potatoe plantlets in vitro using light emitting diodes. *Hortscience* **39**, 375–379.
- Lichtenthaler, H. K. 2007. biosynthesis, accumulation and emission of carotenoids,  $\alpha$ -tocopherol, psiloxanthin, and isoprene in leaves under high photosynthetic irradiance. *Photosynth. Res.* **92**, 163–179.

- Marsili, V. Calzuola, I. & Gianfranceschi, G.L. 2004. Nutritional Relevance of Wheat sprouts containing high levels of organic phosphates and antioxidant compounds. *Journal of Cilinical Gastroenterology* **38**, S123–S126.
- McGloughlin, M. 2008. Nutritionally improved Agricultural Crops. *Plant Physiology* **147**, 939–953.
- Morrow, R.C. 2008. LED Lighting in horticulture. *Hortscience* **47**, 1947–1950.
- Paulickova, I., Eherenbergerova, J., Fieldlerova, V., Gabrovska, D., Havlova, P., Holasova, M., Kopacek, J., Ouhrabkova, J., Pinkrova, J., Rysova, J., Vaclova, K. & Winterova, R. 2006. Evaluation of Barely grass as a potential source of some nutritional substances. *Czech J. Food Sci.* **25**, 65–72.
- Ragae, S., Abdel-Aal, E.M., Noaman, M. 2006. Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry* **95**, 32–38.
- Smeekens, S. 2000. Sugar-induced signal transduction in plants. *Annu. Rev. Plant Physiol. Plant. Mol. Biol.* **51**, 49–81.
- Spalding, E.P. & Folta, K.M. 2005. Illuminating topics in plant photobiology. *Plant Cell Environment.* **28**, 39–53.
- Szilard, A., Sass, L., Hideg, E. & Vass, I. 2005. Photoinactivation of Photosystem I Iby flashing light. *Photosynthesis Research* **84**, 15–20.
- Tennessen, D.J., Bula, R.J. & Sharkley, T.D. 1995. Efficiency of photosynthesis in continuous and pulsed light emitting diode irradiation. *Photosynthesis research* **44**, 261–269.
- Vasilevski, G. 2003. Perspectives of the application of biophysical methods in sustainable agriculture. *Bulg.J.Plant Physiol.* **Special issue**, 179–186.
- Wu M.C., Hou, C.Y., Jiang, C.M., Wang, Y.T., Wang, C.Y., Chen, H.H. & Chang, H.M. 2007. A novel approach of LED light radiation improves the antioxidant activity of pea seedlings. *Food Chemistry* **101**, 1753–1758.