

## THE EFFECT OF UV-A SUPPLEMENTAL LIGHTING ON ANTIOXIDANT PROPERTIES OF *OCIMUM BASILICUM* L. MICROGREENS IN GREENHOUSE

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The effects of supplemental UV-A LED lighting on growth and antioxidant properties of two varieties of basil (*Ocimum basilicum* L.) microgreens were determined. Purple-leaf ‘Dark Opal’ and green-leaf ‘Sweet Genovese’ basil varieties were grown in greenhouse (14 days, 22/18 ± 2 °C day/night temperature, 40 ± 5 % relative air humidity) during winter season. The main lighting system (HPS lamps and natural daylight) was supplemented with ~13.0 μmol m<sup>-2</sup> s<sup>-1</sup> flux of UV-A 390 nm, and a total PPFD was ~125 μmol m<sup>-2</sup> s<sup>-1</sup> (16 h photoperiod) for 1 or 7 days before harvest, or entire growth period – 14 days. The results revealed that the influence of UV-A on growth and antioxidant properties depended on basil variety and duration of irradiation. Generally, UV-A irradiation for 7 days significantly ( $P \leq 0.05$ ) inhibited growth and hypocotyl elongation of green-leaf basil varieties, and for 14 days of both basil varieties. No significant differences on leaf chlorophyll index were determined. However, leaf flavonol index significantly increased in green-leaf basil varieties after 7 and 14 days UV-A irradiation. The total phenols and anthocyanin contents significantly decreased after 1 day UV-A irradiation in purple-leaf basil varieties, and the continuous decrease following UV-A irradiation for 7 or 14 days was determined. In addition, UV-A irradiation had negative effects on ABTS radical activity in purple-leaf basil varieties; however, the significantly higher ABTS radical scavenging activity after UV-A irradiation for 1 or 7 days in green-leaf basil varieties was determined. UV-A influenced higher ascorbic acid synthesis in purple-leaf basil varieties after 7 days irradiation, or after 14 days irradiation in both basil varieties. In summary, the supplemental UV-A LED lighting allows to protect basil microgreens from hypocotyl elongation, and enhances antioxidant properties in green-leaf basil varieties. Purple-leaf basil varieties showed to be more sensitive to UV-A irradiation, and less positive effects on antioxidant properties were determined.

*Keywords:* antioxidant, greenhouse, light emitting diode, microgreen, UV-A.

### INTRODUCTION

Light as a primary energy source for photosynthesis triggers photoprotective and development processes, also metabolic light acclimation responses of all plants (Lepetit and Dietzel, 2015). An appropriate lighting is a necessity of greenhouse industry, particularly in northern latitudes where the natural light is insufficient to produce high quality commercial crop of many horticultural products (Singh et al., 2015; Wojciechowska et al., 2015). The high intensity discharge (HID) lamps, such as high pressure sodium (HPS), are the most commonly used electric-powered light sources in greenhouse (Hernández and Kubota, 2015). HPS lamps are efficient in converting energy into photosynthetic light (Torres et al., 2010), and the spectra contains high amounts of yellow and orange with some red and low of blue lights (Ouzounis et al., 2015). Due to sensitivity range of plants to light extends from UV through visible spectrum to far red

radiation (Galvão and Fankhauser, 2015), the studies with different spectral compositions of supplemental light emitting diode (LED) lighting to HPS lamps were reported (Samuoliënė et al., 2011a; Bliznikas et al., 2012; Sirtautas et al., 2014a; Wojciechowska et al., 2015; Hernández and Kubota, 2015) and physiological and morphological plasticity of plants to light quality was confirmed. The advantages of LED in comparison with other light sources (fluorescent, HPS, metal halide lamps) include technical specifications such as longevity, safety, being solid state and emitting lower heat (Dąbrowski et al., 2015), and ability to be matched with plant photoreceptors (Olle and Viršilė, 2013). Using LED as a tool for investigations of photophysiological responses in plants, a digitally controlled and energy efficient lighting system for maximized productivity of horticultural plants can be created.

In addition to visible light spectra, ultraviolet (UV) radiation (~200–400 nm) is also involved in photophysiological effects of plants. The exposure of UV leads to generation of free radicals that damage DNA, proteins, lipids, chloroplasts and photosynthetic pigments (Hideg et al., 2013; Jenkins, 2014). Photosystem II (PS II) is known to be the most vulnerable constituent of thylakoid membrane being exposed to UV radiation (Yao et al., 2006). On the other hand, a lower level of UV enhances plant resistance to pathogens and herbivores (Tsormpatsidis et al., 2008; Ballaré, 2014).

It is well known, that UV-A (~315–400) radiation is the least hazardous part of UV, and cannot be held by ozone layer. UV-A radiation penetrates through plant leaf and is absorbed by chromophores associated with photosynthetic apparatus. The photoreceptors perceiving UV-A are common to blue lights (315–380 nm) (Lepetit and Dietzel, 2015). Phototropins (Phots) localize in plasma membrane and dissociate from it activated by UV-A light photons. Other photoreceptors, cryptochromes, perceive UV-A with optima 370 nm wavelength (Liu et al., 2010; Müller and Bouly, 2015). Zeitelupes, Flavin-binding Kelch and LOV Kelch proteins use Flavin mononucleotide as a chromophore (like phototropins do). Other proteins, phytochromes, use phytychromobilin which is synthesized in chloroplasts. UV-A acts in phototransformation between inactive Pr and active Pfr process as well as blue-far red lights (Casal et al., 2014; Huché-Thélier et al., 2015).

Many studies of investigating photophysiological processes in plants were conducted to improve nutritional quality of horticultural plants (Samuoliënė et al., 2011b; Son and Oh, 2013; Muneer et al., 2014; Sirtautas et al., 2014b; Carvalho and Folta, 2014; Chang and Chang, 2014; Demotes-Mainard et al., 2015; Duchovskis et al., 2015). The increased market of fresh vegetables ordered new investigations of growing technologies (Folta and Childers, 2008), and new types of vegetable species, such as microgreens, were suggested to be grown all year round (Brazaitytė et al., 2015; Pinto et al., 2015). Microgreens are type of specialty leafy greens harvested shortly after the first true leaves. Microgreens are qualified as 'functional food' due to good sources of phytochemical constituents such as flavonoids, phenols, anthocyanins, macro- and micronutrients (Xiao et al., 2012; Kopsell et al., 2012).

Little is known about the effects of UV-A on photophysiological responses in comparison with UV-B radiation of young age plants. In this study we hypothesized that UV-A radiation affects growth and development of microgreens, and the influence of UV-A depends on radiation duration and variety of plant. The aim of the study was to evaluate the effects of supplemental UV-A radiation on growth and antioxidant responses of two *Ocimum basilicum* L. varieties microgreens in greenhouse.

## MATERIALS AND METHODS

Experiments were performed at the Institute of Horticulture, Lithuanian Research Centre of Agriculture and Forestry. The purple-leaf 'Dark Opal' and green-leaf 'Sweet Genovese' forms of *Ocimum basilicum* L. were grown from seed to harvest time for 14 days in greenhouse during winter season. Day/night temperature of  $22/18 \pm 2$  °C was maintained and the relative air humidity was  $40 \pm 5$  %. 1 g of seeds (CN Seeds, Ltd., UK) were sowed in moist peat substrate (N 100–120, P<sub>2</sub>O<sub>5</sub> 30–80, K<sub>2</sub>O 120–200 mg L<sup>-1</sup>; microelements Fe, Mn, Cu, S, Mo, Zn; pH 5.5–6.5) (Profi 1, Durpeta, Lithuania) in the plastic pots (18 x 11 x 6 cm). Four pots for each basil variety were used for each light treatment. Plants were sprayed daily with a light mist of tap water. High pressure sodium (HPS) lamps (SON-T Agro, Philips, UK) irradiance was supplemented with  $\sim 13.0$   $\mu\text{mol m}^{-2} \text{s}^{-1}$  flux of UV-A 390 nm (NCSU034B, Nichia, Japan) light emitting diodes (LEDs) and the total photosynthetic photon flux density (PPFD) was  $\sim 125$   $\mu\text{mol m}^{-2} \text{s}^{-1}$  (photoperiod 16 h). Four lighting treatments were imposed: (1) HPS lamps at  $\sim 125$   $\mu\text{mol m}^{-2} \text{s}^{-1}$  (as a control); (2) HPS lamps supplemented UV-A LEDs for 1 day and (3) for 7 days (before harvest); (4) HPS lamps supplemented UV-A LEDs for 14 days (the entire growth period). PPFD was measured daily by photometer – radiometer RF 100 (Sonopan, Poland).

After the lighting treatments, the edible biomass (cotyledons with stems) of both basil varieties (14 days old) was harvested. Ten randomly selected plants were used for hypocotyl length and plant height measurements. The leaf area was measured by an automatic meter (AT Delta-T Devices, UK). Fresh microgreens were dehydrated in drying oven (Venticell, MBT, Czech Republic) at 70 °C for 48 h to constant weight for measuring dry weight (DW) per plant. Non-destructive measurements of microgreen leaf chlorophyll and flavonol indexes were performed using Dualex meter (Force-A, France). The conjugated biological samples of fresh weight (FW) of randomly selected microgreens (0.5 g per sample) were used for phytochemical analysis. Three analytical replications were performed for each phytochemical measurement. Ascorbic acid was determined spectrophotometrically according to the method published by Janghel et al. (2007). In this assay ascorbic acid reduces methyl viologen to form blue coloured free radical ion. The absorbance of the radical ion was measured by UV/Vis spectrophotometer at 600 nm (M501, Spectronic Camspec Ltd., UK). The total phenol content in extracts was determined according to the Folin-Ciocalteu method as outlined Ragae et al. (2006). Frozen in liquid nitrogen microgreens FW samples were extracted with 80 % methanol (1:10), followed by centrifugation at 1680 RCF for 5 min. The supernatant was collected and quantification of total phenols was obtained through maximum absorbance at 765 nm using a spectrophotometer (M501, Spectronic Camspec Ltd., UK). The results were expressed as

gallic acid equivalents. The 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity was determined according to Kadri et al. (2013). The same to ones used in total phenol assay sample extracts were diluted with ABTS solution ( $A = 0.7 \pm 0.2$ ). The decrease of absorbance was measured after 15 min at 734 nm (M501, Spectronic Camspec Ltd., UK). The total anthocyanin content was estimated using pH-differential spectrophotometric method according to Stanciu et al. (2009). The absorption values of samples extracts were measured at 420, 520, 700 nm (Genesys 6, Thermospectronic, USA). Anthocyanin concentration was determined taking into account the molar extinction coefficient (25.740) and a molecular weight (485 g mol<sup>-1</sup>) for cyanidin 3-glucoside.

Data were averaged and statistically analysed by using one-way analysis of variance (ANOVA). The least significant difference (LSD) at the 0.95 level ( $P \leq 0.05$ ) was used to compare the means by Fisher's test. All data are expressed on a FW basis.

## RESULTS AND DISCUSSION

In this study UV-A LED irradiation as an additional light source to main lighting system in greenhouse was used; and the experiments of different duration of UV-A irradiation before harvest were performed. The results of the growth parameters of two basil varieties influenced by UV-A irradiation treatments are shown in Table 1. A 7 day UV-A irradiation of green-leaf, and 14 day (entire growth period) irradiation of both basil varieties led to significantly decreased plant height and hypocotyl length in comparison to control. In contrast, the irradiation of UV-A for 1 day significantly increased growth parameters of green-leaf basil. The UV-A inhibition effect on leaf expansion resulted in significantly decreased fresh and dry weights of basil after 14 days irradiation (data not shown).

Table 1. Growth parameters of basil microgreens under different lighting treatments in greenhouse

Lighting treatment	Hypocotyl length, cm	Plant height, cm	Leaf area, cm <sup>2</sup>
Purple-leaf 'Dark Opal'			
HPS (Control)	3.60 ± 0.35	4.62 ± 0.25	0.67 ± 0.05
HPS + 1 d. UV	3.46 ± 0.28	4.37 ± 0.30 <sup>b</sup>	0.66 ± 0.04
HPS + 7 d. UV	3.68 ± 0.31	4.65 ± 0.21	0.64 ± 0.01
HPS + 14 d. UV	2.61 ± 0.26 <sup>b</sup>	3.47 ± 0.26 <sup>b</sup>	0.53 ± 0.06 <sup>b</sup>
Green-leaf 'Sweet Genovese'			
HPS (Control)	2.64 ± 0.39	3.68 ± 0.45	1.15 ± 0.11
HPS + 1 d. UV	2.97 ± 0.42 <sup>a</sup>	4.05 ± 0.34 <sup>a</sup>	1.10 ± 0.04
HPS + 7 d. UV	2.19 ± 0.29 <sup>b</sup>	3.20 ± 0.29 <sup>b</sup>	1.02 ± 0.13
HPS + 14 d. UV	2.05 ± 0.35 <sup>b</sup>	3.02 ± 0.33 <sup>b</sup>	0.94 ± 0.18 <sup>b</sup>

<sup>a</sup> value is significantly ( $P \leq 0.05$ ) higher than control (HPS); <sup>b</sup> value is significantly lower than control

The decreased plant growth parameters observed in this study could be a consequence of cryptochromes activation by UV-A radiation (Lin et al. 1995). The results showed that substantial biological effects of UV-A irradiation on basil growth and development were variety-specific and duration-dependent. The decreased biomass accumulation and chlorophyll (Chl) a/b ratio of Brazilian joyweed plantlets by supplemental UV-A radiation to white light after eight weeks were determined (Silva et al., 2005). The reduction of total Chl contents and, conversely, increased Chl a/b ratio due to increased content of Chl b, after short time (40 and 80 min) directly irradiation in dark and re-irradiation at 4 days interval in two 25 days old cotton cultivars were determined (Ebrahim, 2005). Total Chl content decreased marginally in black gram plants exposed by short time (30 min) UV-A radiation for 30 days (Jayakumar et al., 2004). In contrary, in our study we used simultaneous UV-A irradiation for different duration time (1, 7 and 14 days), and no significant differences on Chl index were determined in both basil varieties (data not shown). In agreement with Hosseini Sarghein et al. (2008), simultaneous UV-A irradiation for 15 days slightly decreased Chl a and Chl b contents in pepper plants, but the reduction was insignificant. Yao et al. (2006) reported, that the effects on photosynthetic pigment was related to different UV bands and different species; and shorter UV-A wavelength imposed more negative impact than longer UV-A wavelength. Despite UV-A is an environmental stress factor experienced by plant leaves first, the sensitivity of foliar photosynthetic pigments is still under the studies.

On the other hand, UV absorbing compounds, such as flavonoids or anthocyanin, indicate the influence of UV-A in various plants. Moreover, these metabolites are natural antioxidants and play an important role in human health by scavenging oxygen free radicals (Steyn et al., 2002; Tsormpatsidis et al., 2008). UV-A radiation enhances the generation of active oxygen species (ROS) in plants, and results in oxidative damage to membrane lipids and proteins (Yao et al., 2006). The increased amounts of these metabolites reveal plant availability to develop adaptive mechanisms in case to reduce the damage of UV-A. In our experiments, the significantly higher leaf flavonol index of green-leaf basil after 7 and 14 days UV-A irradiation was determined (~29 % and 25 %, respectively) (Figure 1). No significant differences on leaf flavonol index of purple-leaf basil microgreens were determined; however, the higher indexes in comparison with green-leaf basil were measured.

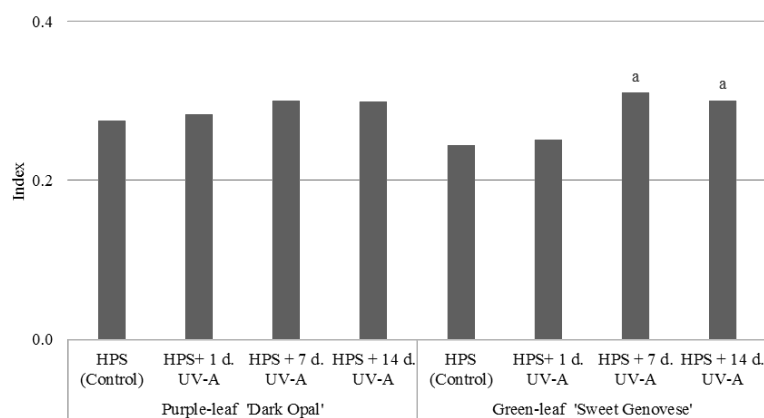


Figure 1. Leaf flavonol index of basil microgreens grown under different light treatments in greenhouse  
<sup>a</sup> value is significantly ( $P \leq 0.05$ ) higher than control (HPS)

In addition, the significantly higher concentration of total phenol (~14 %) after 7 days UV-A irradiation in green-leaf basil was determined (Figure 2). In contrast, UV-A led to significantly decreased amount of total phenol in purple-leaf basil regardless to duration (1, 7 and 14 days) of irradiation (~6 %, ~20 % and ~7%, respectively). The similar tendencies on total anthocyanin content were determined (Figure 2). No significant differences on total anthocyanin content in green-leaf basil were determined (Figure 2). However, the significantly decreased amounts of total anthocyanin in purple-leaf basil due to 1 and 7 days UV-A irradiation were determined (~12 % and ~10 %, respectively). Although green-leaf basil possess relatively high antioxidant properties, the presence of anthocyanin in purple varieties may enhance such properties (Flanigan and Niemeyer, 2014). According to our results, the amount of anthocyanin decreased with decreasing total phenols in purple-leaf basil. It is known, that the biosynthesis of phenolic compounds is linked to blue light receptors, cryptochromes, and phototropins, the same to UV-A light. In addition, phytochromes also participate in regulating anthocyanin accumulation (Iwai et al., 2010). The increased anthocyanin concentration following UV-A light treatment was determined in pepper plants, but the rise was not significant (Hosseini Sarghein et al., 2008). The accumulation of secondary metabolites may be dependent on UV-A wavelength and irradiation intensity. According to Brazaitytė et al. (2015), a significant increase of antioxidant properties in various microgreen species (also in green-leaf basil) was determined under 366 nm and 390 nm wavelengths and higher irradiation intensity (~12.4  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) under growth chamber conditions.

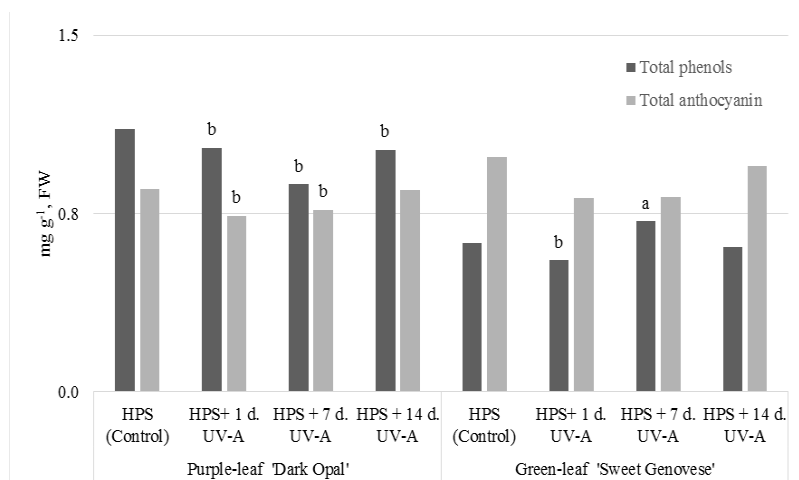


Figure 2. Total phenols and anthocyanin content in basil microgreens grown under different light treatments in greenhouse  
<sup>a</sup> value is significantly ( $P \leq 0.05$ ) higher than control (HPS); <sup>b</sup> value is significantly lower than control

Moreover, the significantly lower ABTS radical scavenging activity was also determined in purple-leaf basil after 1, 7 and 14 days irradiation of UV-A (~4 %, ~24 % and ~19 %, respectively) (Figure 3). On the contrary, the significantly higher ABTS radical scavenging activity in green-leaf basil after 1 and 7 day irradiation of UV-A was determined (~10 % and ~25 %, respectively).

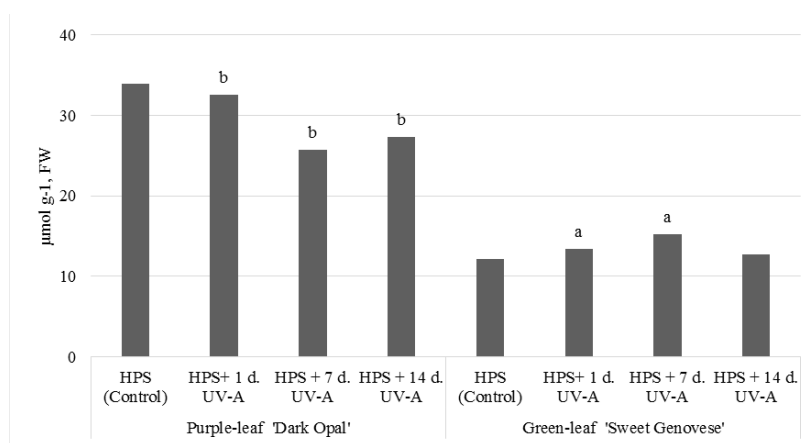


Figure 3. ABTS radical scavenging activity in basil microgreens grown under different lighting treatments in greenhouse  
<sup>a</sup> value is significantly ( $P \leq 0.05$ ) higher than control (HPS); <sup>b</sup> value is significantly lower than control

The irradiation of UV-A had impact to ascorbic acid synthesis in basil microgreens (Figure 4). In both varieties, the significantly higher concentrations of ascorbic acid (~18 % and ~8 %, respectively) after 14 days UV-A irradiation were determined; either in purple-leaf basil after 7 day irradiation (~10 %). Ascorbic acid has a crucial role in antioxidant defense preventing the accumulation of ROS as well as in stress protection, and the capacity of plant leaves to synthesize, regenerate and accumulate ascorbic acid depends on light conditions (Bartoli et al., 2006).

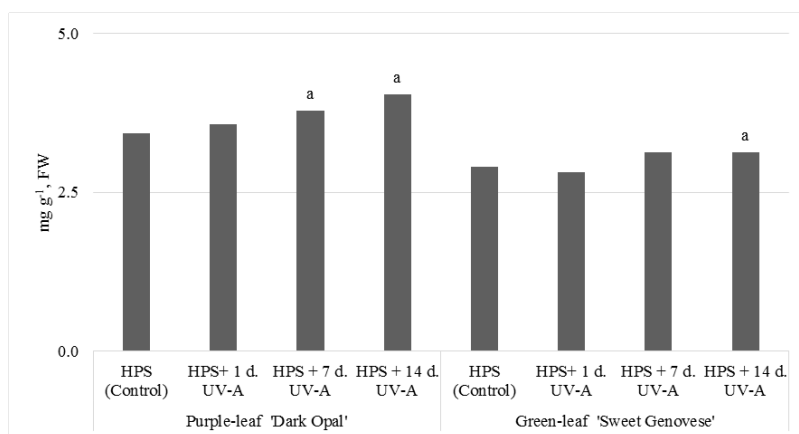


Figure 4. Ascorbic acid concentrations in basil microgreens grown under different lighting treatments in greenhouse  
<sup>a</sup> value is significantly ( $P \leq 0.05$ ) higher than control (HPS)

## CONCLUSIONS

The main lighting system with the UV-A LEDs allows to protect basil microgreens from undesirable hypocotyl elongation in greenhouse. UV-A LEDs light improved antioxidant properties (total phenol and anthocyanin contents, ascorbic acid concentration and ABTS radical scavenging activity) in young basils. However, the effect of UV-A irradiation on phytochemical substances strongly depended on basil variety. Supplemental UV-A in the greenhouse is beneficial improving antioxidant properties of green-leaf basils, but does not have positive impact on red-leaf basils.

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