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THE EFFECTS OF LED LIGHTING ON NITRATES, NITRITES AND ORGANIC ACIDS IN TATSOI

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Abstract
Progressive type of controlled environment horticulture, such as plant factories, enables the precise control of cultivation environment parameters. The experiments were performed at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in the year of 2017. The aim of this study was to evaluate the effects of different irradiance levels produced by solid state light-emitting diodes (LEDs) on nitrates, nitrites and organic acids contents in tatsoi (*Brassica rapa* var. *rosularis*), cultivated in the controlled environment chambers. Plants were cultivated under combinations of red (640, 660 nm), blue (445 nm) and far red (731nm) LEDs at photosynthetic photon flux density (PPFD) level of 200 μmol m^{-2} s^{-1}. At the pre-harvest stage, PPFD was increased to 300 μmol m^{-2} s^{-1} by elevating the fluxes of different spectral components for 3 days. The contents of nitrates, nitrites and organic acids were evaluated. The results propose the metabolic interface between nitrates, nitrites and organic acids in green vegetables, sensitive for lighting conditions. The higher intensity of LED light at pre-harvest stage led to decreased contents of nitrates in tatsoi. The increased intensity of blue 455 nm light led to lower contents of nitrates and higher of organic acids in comparison to red 640 nm. In addition, the increased intensity of red 640 nm led to significantly higher ($p \leq 0.05$) contents of nitrites. The target management of LED light spectra and intensity at pre-harvest stage could be used to reduced nitrates and increased organic acids in tatsoi.
Key words: *Brassica rapa*, controlled environment, light emitting diodes.

Introduction

Light is one of the most important environmental factors necessary for the growth and development of plants. The technology of light-emitting diodes (LEDs) is one of the largest potential advantages in horticultural lighting in the last few decades (Morrow, 2008). Solid-state lighting systems have many unique advantages over existing horticultural lighting. LED lighting systems have the ability to control their spectral output, produce more light than incandescent lamps, they have high light output levels with low heat emission, when cooled properly, and the ability to maintain useful light output for years without replacement (Bourget, 2008; Morrow, 2008). LEDs emit narrow-band wavelengths from UV (~250 nm) to infrared (~1000 nm) (Bourget, 2008). This promising technology gives new possibilities to analyse the effects of lighting parameters on biochemical processes in green vegetables produced in growth chambers.

Light intensity and spectra affect plant growth and nutritional quality. Light intensity is known as the main factor affecting the accumulation of nitrates and nitrites in plants. Vegetables are the main source of nitrates and nitrites, accounting for between 72% and 94% of the total human nitrate intake (Hord, Tang, & Bryan, 2009; Reinik, Tamme, & Roasto, 2009; Yuming *et al*., 2017). Nitrate accumulation in plants depends on nitrate reductase (NR) activity, which can be caused by red light (Vaštakaitė & Viršilė, 2015). Nitrate accumulation in vegetables can be also affected by genetic and environmental factors such as plant variety, type and composition of soil, air temperature, harvesting time, storage (Boroujerdnia, Ansari, & Dehcordie, 2007; Konstantopouloua *et al*., 2010; Bahadoran *et al*., 2016). The consumption of high amounts of nitrates may increase the risk of developing stomach cancer or other diseases (Zhou, Liu, & Yang, 2012).

Vegetables cultivated under controlled environment conditions have a higher nitrate and nitrite content, but they also accumulate higher concentrations of useful substances for the human body, such as amino acids, vitamins, minerals, proteins, and organic acids (Wang *et al*., 2014; Yuming *et al*., 2017). Organic acids, depending on the concentration, provide the taste and smell of green vegetables (Flores *et al*., 2011; Wang *et al*., 2014).

Green vegetables are one of the main sources of vitamin C. Previous studies have shown that over 90% of vitamin C is consumed daily by vegetables and fruits (Hermsdorff *et al*., 2012). It has been noticed that ascorbic acid synthesis in plant tissues is caused by higher light intensity (Zhou, Liu, & Yang, 2012). Ascorbic acid molecules are found in various plant organs. Higher levels of ascorbic acid are found in photosynthetic plant organs (Gest, Gautier, & Stevens, 2013). Ascorbic acid performs many functions in the plant cell such as the synthesis of ethylene and gibberellins and is a co-factor for many enzymes (Arrigoni & De Tullio, 2000).

The aim of this study was to investigate how different light intensity and spectrum of light emitting diodes (LEDs) affect the level of nitrates, nitrites and organic acids in tatsoi (*Brassica rapa* var. *rosularis*).

Materials and Methods

The experiments were performed at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. Tatsoi were grown in a peat substrate (Profi 1, Durpeta, Lithuania) (pH 5–6) in plastic trays for 23 days. The average amounts of nutrients (mg L^{-1}) in the substrate were: N, 110; P_2O_5, 50; K_2O, 160; microelements Fe, Mn, Cu, B, Mo, and Zn. Electrical conductivity was 0.5 – 0.7 ms^{-1} cm^{-1}. Trays were arranged randomly and were systematically rotated every day to improve the uniformity of the light environment. Plants were watered with tap water when needed. Experiments were performed in controlled environment growth chambers. Day/night temperatures of 21/17 ± 2 °C were established with a 18-h photoperiod and a relative air humidity of 50 – 60%. As a light source, the different combinations of red (R; 640 nm, 660 nm), blue (B; 445 nm), far-red (FR; 731 nm) light emitting diodes were used. Three days before the harvest, the intensities of different spectrum components were increased seeking for improved parameters of nutrition quality (Table 1).

Nitrate and nitrite contents were evaluated according to the spectrophotometric method of Merino (2009). To prepare the dry material (DM) for determination of nitrates and nitrites, plant tissue samples were dried in the drying oven (Venticell, MBT, Czech Republic) at 70 °C for 48 h. Initial nitrite concentration and total nitrite after zinc reduction were determined by Griess reaction at 540 nm (M501, Camspec, UK). Nitrate and nitrite contents (mg kg^{-1}) were determined by the calibration method.

Determination of organic acid contents. Conjugated biological samples of fresh matter (FM) from randomly selected plants were used for biochemical analyses. Samples were taken from the central part of the tray, leaving plants at the edges of the tray as a guard. 0.3 g of FM was homogenized with liquid nitrogen, diluted with deionized water and placed in ultra-sonic bath for 30 min. The contents of organic acids in samples were determined according to the method of Wang *et al.* (2014) with modifications**.** The extracts were analysed using HPLC Shimadzu (10A) system with diode array detector. Column used Lichrosorb RP – 18, 5u, 4.6 mm×250 mm, 5 µm (Phenomenex, USA). Mobile phase – 0.01mol L^{-1} sulfuric acid in deionized water. The chromatogram was monitored by the photodiode array detector at 230 nm for ascorbic acid; and at 210 nm for citric acid, oxalic acid, malic acid and succinic acid. The concentrations of organic acids in FM samples were calculated according to the calibration curve of standards.

Photosynthetic photon flux density (PPFD) was measured daily by photometer-radiometer RF 100 (Sonopan, Poland).

All data are expressed on a FM basis. All measurements were evaluated for significance by an analysis of variance (ANOVA) followed by the least significant difference (LSD) test at the $p \leq 0.05$ level. Correlations were calculated by STATISTICA 7.

Results and Discussion

The results of nitrate, nitrite and organic acids in tatsoi influenced by different light treatments are shown in Table 2.

LED light treatments differentially affected the nitrate, nitrite and organic acid contents in tatsoi. The nitrate content was affected by an increased intensity of B 455 nm to PPFD level at 300 µmol m^{-2} s^{-1} treatment – it was ~4.2-fold lower in comparison to control (a PPFD level at 200 µmol m^{-2} s^{-1}). The increased intensity of R 640 nm LEDs resulted in a higher content of nitrates in comparison to B 455 nm, but slightly lower than in control. Moderate nitrate reduction was observed in the treatment, where all spectral components were proportionally increased to PPFD 300 µmol m^{-2} s^{-1}. However, other authors also confirm that a higher light intensity results in lower nitrate contents in various green vegetables. According to Vaštakaitė & Viršilė (2015), the higher LED light irradiation resulted in an increase of the nitrate content in tatsoi and mustard, red

Table 1

LED lighting parameters

Light treatment	PPFD, µmol m^{-2} s^{-1}									
	During the growing					3 days before the harvest				
	FR 731nm	B 445nm	R 640nm	R 660nm	P/ PPFD	FR 731nm	B 445nm	R 640nm	R 660nm	P/ PPFD
Control PAR 200 µmol m^{-2} s^{-1}	4	20	88	88	16/200	4	20	88	88	16/200
All up to PAR 300 µmol m^{-2} s^{-1}						6	30	132	132	16/300
450 nm to PAR 300 µmol m^{-2} s^{-1}						4	120	88	88	16/300
640 nm to PAR 300 µmol m^{-2} s^{-1}						4	20	188	88	16/300

FR – Far red, B – Blue, R – red, P – photoperiod, PPFD – photosynthetic photon flux density.

Table 2

The contents of nitrates, nitrites and organic acids tatsoi cultivated under different LED light treatments

Light treatment	Nitrate content mg^{-2} kg^{-1}	Nitrite content mg^{-2} kg^{-1}	Ascorbic acid content mg^{-2} g^{-1}	Citric acid content mg^{-2} g^{-1}	Oksalic acid content mg^{-2} g^{-1}	Malic acid content mg^{-2} g^{-1}	Sucinic acid content mg^{-2} g^{-1}
Control PAR 200 μmol $m^{-2}s^{-1}$	205.63	0.94	0.19	0.78	1.83	2.06	0.08
All up to PAR 300 μmol $m^{-2}s^{-1}$	**83.04***	0.73	0.19	**2.27****	**0.86***	**1.3***	**0.21****
450 nm to PAR 300 μmol $m^{-2}s^{-1}$	**48.39***	0.91	**0.11***	**2.56****	**1.09***	**1.06***	0.11
640 nm to AR 300 μmol $m^{-2}s^{-1}$	**193.47***	**7.52****	**0.13***	**2.38****	**0.96***	**1.33***	0.14
$LSD_{0.05}$ =	7.91	0.48	0.06	0.08	0.19	0.11	0.05

*the value is significantly ($p \leq 0.05$) lower than control; ** the value is significantly ($p \leq 0.01$) higher than control.

pak choi microgreens, while Samuolienė *et al.* (2013) stated that the lowest investigated (100 μmol m^{-2} s^{-1}) LED light intensity resulted in the highest nitrate contents in *Brassica* microgreens.

Nitrite content in tatsoi was determined ~ 8-fold higher, when R 640 nm LED light intensity was increased to PPFD 300 μmol m^{-2} s^{-1}, compared to the control plants. No significant differences were determined when B 455 nm or all spectral components were increased to PPFD level at 300 μmol m^{-2} s^{-1} (Table 2). Wanlai, Liu &, Qichang (2013) also reported decreased nitrate contents in lettuce, a few days before the harvest was exposed to high intensity of red and blue LED light.

The data of organic acid analysis in tatsoi leaves showed that ascorbic acids and other organic acid contents in tatsoi can be changed by tailoring LED lighting parameters (Table 2). Ascorbic acid content was significantly affected by increased B 450 nm and R 640 nm spectral components (to PPFD 300 μmol m^{-2} s^{-1}): it was determined ~1.7, ~1.4-fold lower than control, respectively. The increased light intensity due to proportionally elevated intensity of all spectral components had no effect comparing to control. Several studies have observed the increase in ascorbic acid contents in tatsoi microgreens grown under a higher intensity of LED light (Vaštakaitė & Viršilė, 2015; Brazaitytė *et al.*, 2016). In our study, the accumulated ascorbic acid content was not only affected by the light intensity, but also by the wavelengths of LED light. The variation of ascorbic acid contents in plant tissues is affected by the complex relationships among effects of light stress and plant genetics, according to Solfanelli *et al.* (2006).

The LED light treatments affected the contents of citric acid in tatsoi. The significantly higher contents of citric acid in tatsoi grown under the treatments of all spectra components to PPFD level at 300 μmol m^{-2} s^{-1}, B 455 nm and R 640 nm on PPFD level at 300 μmol m^{-2} s^{-1} were determined in comparison to control (~2.9, ~3.2, ~3 -fold, respectively). On the contrary, the higher intensity of LEDs led to lower contents of oxalic acid and malic acid contents in tatsoi. The contents of oxalic acid were found to be ~2.1, ~1.6, ~1.9-fold and malic acid ~1.5, ~1.9, and ~1.5-fold lower in comparison to control. Succinic acid content was significantly affected by lighting treatment, where the intensity of all lighting components was increased to PPFD 300 μmol m^{-2} s^{-1} proportionally. The significantly higher (~2.6-fold) content of succinic acid in tatsoi grown under increased intensity of all LED components to 300 μmol m^{-2} s^{-1} were determined in comparison to plants grown under PPFD level at 200 μmol m^{-2} s^{-1}.

Table 3

The correlation among nitrates, nitrites and organic acid

Variable	Nitrate	Nitrite	Oxalic acid	Malic acid	Ascorbic acid	Succinic acid
Nitrite	0.52	1				
Oxalic acid	0.54	-0.32	1			
Malic acid	0.76	-0.15	**0.87**	1		
Ascorbic acid	**0.23**	-0.39	0.32	**0.59**	1	
Succinic acid	-0.37	0	**-0.68**	-0.38	0.37	1
Citric acid	**-0.65**	0.30	**-0.92**	**-0.98**	-0.56	0.47

Bolded correlations are significant at $p \leq 0.05$.

The correlation analysis revealed interdependencies between nitrate, nitrite and organic acid contents (Table 3). Strong, statistically significant negative correlation was determined between nitrate and citric acid contents, and weak positive, but statistically significant – between nitrate and ascorbic acid contents. Strong correlation was found between the contents of oxalic acid and malic, succinic and citric acid.

Conclusions

Our study revealed that increased LED light intensity at pre-harvest (3 days before harvest) resulted in decreased nitrate contents in tatsoi. The increased intensity of blue 455 nm LED light had a more pronounced effect compared to elevated R 640 nm light. However, this negatively affected ascorbic acid content in plant leaves. Oxalic and malic acid contents were the most sensitive to the differences in the light spectrum. Further analysis of nitrate and organic acid metabolism under different LED light spectrum and intensity should be explored seeking to develop strategies for reduced nitrate and increased ascorbic acid contents in tatsoi.

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