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## Pre-harvest LED lighting strategies for reduced nitrate contents in leafy vegetables

Akvilė VIRŠILĖ<sup>1</sup>, Aušra BRAZAITYTĖ<sup>1</sup>, Julė JANKAUSKIENĖ<sup>1</sup>,  
Jurga MILIAUSKIENĖ<sup>1</sup>, Viktorija VAŠTAKAITĖ<sup>1</sup>, Ingrida ODMINYTĖ<sup>2</sup>,  
Algirdas NOVIČKOVAS<sup>3</sup>, Giedrė SAMUOLIENĖ<sup>1</sup>

<sup>1</sup>Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry  
Kauno 30, Babtai, Kaunas distr., Lithuania  
E-mail: a.virsile@lsdi.lt

<sup>2</sup>Vytautas Magnus University  
K. Donelaičio 58, Kaunas, Lithuania

<sup>3</sup>Institute of Applied Research, Vilnius University  
Saulėtekio 9-III, Vilnius, Lithuania

### Abstract

Short-term pre-harvest red light treatment was evaluated as a tool to reduce nitrate (NO<sub>3</sub>) contents in leafy vegetables, cultivated under low-light conditions in a greenhouse. Corn salad (*Valerianella locusta* L., 'Vit'), amaranth (*Amaranthus chlorostachys* Willd., 'Red Army') and tatsoi (*Brassica rapa* var. *rosularis* L., 'Rozetto F1') were cultivated under low-light conditions and 1–7 days before harvest were treated with 638 nm red light emitting diode light. The effects of light treatment on NO<sub>3</sub> and nitrite (NO<sub>2</sub>) contents, reducing enzyme activities and plant photosynthetic performance of different leafy vegetable species were explored seeking for comprehensive approach for the control of NO<sub>3</sub> metabolism. Nitrate, nitrite, total protein contents and reducing enzyme activity depend on plant species, lighting treatment duration and their interaction. A remarkable decrease in nitrates and an increase in NO<sub>3</sub> reductase activity were observed 3 days after red light treatment. It followed by a significant increase in NO<sub>2</sub> and protein contents in corn salad and amaranth. A medium correlation between photosynthetic rate and NO<sub>3</sub> contents was determined for tatsoi and corn salad. A negative statistically insignificant correlation between these indicators was established for amaranth. Short-term pre-harvest red light treatment can be applied as a technological tool to reduce NO<sub>3</sub> contents in green vegetables, cultivated under low-light conditions. The obtained results confirm the significant relationship between plant photosynthetic rate and nitrate metabolism, as well as indicate the sensitive, but differential physiological response of different vegetable species to the applied lighting.

Key words: light emitting diodes, nitrate reductase, nitrite reductase, nitrites, photosynthesis, proteins.

### Introduction

Nitrate (NO<sub>3</sub>) is classed by food safety authorities as a contaminant in fresh vegetables. NO<sub>3</sub> itself is fairly non-toxic for humans; however, its toxic metabolites, such as nitrite (NO<sub>2</sub>) and N-nitroso compounds, might have deleterious effects on health (Weightman, Hudson, 2013). Therefore, European Food Safety Authority (EFSA, 2008) suggested that acceptable daily consumption of NO<sub>3</sub> ions should not exceed 0.007 mg kg<sup>-1</sup> of body weight per day. The European Commission (Commission regulation (EU) No. 1258/2011) has set maximum limits for NO<sub>3</sub> concentrations in green vegetables, which tend to accumulate high levels of NO<sub>3</sub>. Brian and Ivy (2015) noticed that typical consumption patterns of fruits and

vegetables exceeded regulatory limits for dietary nitrate and this fact calls into question the rationale behind current nitrate and nitrite regulation. The nitrate problematics is important, as current diet is rich in a wide variety of leafy vegetables species; however, the modern horticultural technologies allow controlling the nutritional value of green vegetables. Therefore, the questions of nitrate contents in green vegetables revive in "plant factories", where plants are cultivated under fully controlled environmental conditions and in greenhouses in northern regions, where green vegetables are seasonally cultivated under low natural lighting conditions.

Since long ago, nitrogen fertilization and light intensity have been identified as the major factors that

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influence  $\text{NO}_3$  content in vegetables (Santamaria, 2006). Light emitting diodes (LEDs) have provided new possibilities for horticulture and research. As a result, the effects of lighting spectra on various aspects of plant metabolism, as well as on  $\text{NO}_3$  contents were analysed (Olle, Viršile, 2013; Bian et al., 2015). Red light was identified as having the highest capacity for stimulating the activity of  $\text{NO}_3$  reductase, which means that red light can effectively reduce  $\text{NO}_3$  contents in plants (Lillo, 2008; Bian et al., 2015). Other authors have reported that blue light is also beneficial for lowering  $\text{NO}_3$  contents in lettuces, as the composition of red and blue light is more favourable for plant growth and photosynthesis (Ohashi-Kaneko et al., 2007; Qi et al., 2007; Lin et al., 2013). Bian et al. (2016) suggest that addition of green light to red and blue LEDs even enhanced the  $\text{NO}_3$  reduction effect.

Nitrate problematics also has the economic nuances, as investments in artificial lighting highly increase the costs of production. Therefore, various short-term plant treatment strategies have been developed, seeking to reduce  $\text{NO}_3$  contents in plant tissues. The removal, reduction or replacement of inorganic nitrogen by other ions in nutrient solution (Croitoru et al., 2015) were combined with artificial lighting (Liu, Yang, 2012), or  $\text{NO}_3$  nutrition was applied depending on the level of lighting (Demšar et al., 2004). Short-term pre-harvest LED lighting exposure was proved as an efficient tool to reduce  $\text{NO}_3$  contents in lettuces (Samuolienė et al., 2009; Zhou et al., 2012; Wanlai et al., 2013) and other green vegetables (Bliznikas et al., 2012). Continuous lighting in the pre-harvest stage was applied (Wanlai et al., 2013; Bian et al., 2016), seeking to increase photosynthetic efficiency and to reduce nitrate contents (Bian et al., 2016; Nicole et al., 2016). Photosynthesis and  $\text{NO}_3$  metabolism are highly interconnected, as carbohydrates provide energy and carbon skeleton for  $\text{NO}_3$  assimilation (Bian et al., 2015) as well as share the osmoregulative functions in the cell with  $\text{NO}_3$  ions (Umar, Iqbal, 2007; Bian et al., 2016). The decrease in  $\text{NO}_3$  contents is usually accompanied by increased carbohydrate and ascorbic acid level in vegetable tissues (Samuolienė et al., 2012; Zhou et al., 2012; Wanlai et al., 2013). Additional beneficial effects of pre-harvest LED light treatment have also been identified, as increase in leaf pigmentation due to higher levels of anthocyanins (Owen, Lopez, 2015; Nicole et al., 2016), flavonoids, tocopherols (Samuolienė et al., 2012), which also results in improved antioxidant properties of vegetables. Most of the research was performed with different lettuce species; however, the effect of light exposure on  $\text{NO}_3$  metabolism is also highly dependent on plant species (Bliznikas et al., 2012), light intensity (Wanlai et al., 2013), cultivation season (Samuolienė et al., 2012; Wojciechowska et al., 2016) which affects overall internal physiological activities.

Therefore, the objective of this study was to evaluate the effects of short-term pre-harvest red LED light treatment on the  $\text{NO}_3$  and  $\text{NO}_2$  contents, reducing enzyme activities and plant photosynthetic performance of different leafy vegetable species seeking for comprehensive approach for the control of  $\text{NO}_3$  metabolism.

## Materials and methods

**Plant cultivation.** Experiments were performed in the greenhouses of Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry from March to April of 2015. The seeds of corn salad (*Valerianella locusta* L., 'Vit'), red leaf amaranth (*Amaranthus chlorostachys* Willd., 'Red Army') and tatsoi (*Brassica rapa* var. *rosularis* L., 'Rozetto F1') were obtained from CN Seeds Ltd., United Kingdom. Leafy vegetables were cultivated in neutralized peat substrate (Profi mix (Durpeta, Lithuania: pH 6–6.5; N 115 mg L<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub> 55 mg L<sup>-1</sup>, K<sub>2</sub>O 160 mg L<sup>-1</sup> with microelements Fe, Mn, Cu, B, Mo and Zn) in plastic trays of 70 ml cell volume, three plants per cell. Equal soil humidity was maintained, once a week plants were fertilized with 0.2% ammonium nitrate ( $\text{NO}_3$ ) solution. During experiments,  $18 \pm 2/22 \pm 2^\circ\text{C}$  night/day temperature was maintained, relative air humidity was 55–70%. Average daily photosynthetic photon flux density (PPFD) of natural lighting in the greenhouse varied between 170–250  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

**Red light emitting diode (LED) treatment.** Null (reference), 1, 3, 5 and 7 days before harvesting corn salad, tatsoi and amaranth were supplementary illuminated with an originally designed red light emitting diodes (LEDs) lighting unit (Bliznikas et al., 2009; Žukauskas et al., 2012). The unit was designed seeking to minimize radial heat emission at high light intensities, therefore it can be placed close to the plants (~30 cm above). The lighting unit consists of 638 nm LEDs LUXEON III Star, model LXHL-LD3C (Philips LLC, USA), that creates 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD and illuminates 3.5 m<sup>2</sup> area at 16 h photoperiod. Trays with plants were transferred under investigated lighting unit (4 trays per treatment, 72 plants per tray) in a randomized order. Each tray was treated as the replication of the treatment. Plants were harvested at baby leaf stage, when 5–6 leaves had formed: tatsoi was grown for 21 days, corn salad and amaranth for 25 days from sowing. After lighting exposure, photosynthetic and biochemical parameters were evaluated. The first fully developed leaves of randomly selected plants from each LED light treatment were used for biochemical analysis and measurements.

**Photosynthetic rate** ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was measured using the LI-6400XT (LI-COR, USA) photosynthesis system on the first fully matured leaf. The instrument was set for 400  $\mu\text{mol s}^{-1}$  airflow, 25°C cell temperature, 400  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ , 60% relative humidity in the cell and light intensity of 200  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ .

**Chlorophyll and flavonol indexes** were measured in the first fully matured leaf using Dualex meter (Dynamax, USA), 10 plants per treatment.

For **biochemical analysis**, the leaves from each light treatment were combined in single conjugated biological sample and three analytical replications were performed for each measurement. Total protein contents and  $\text{NO}_3$  reductase activity were determined in fresh plant matter. For  $\text{NO}_3$  analysis, plant material was dried at 70°C for 48 h. All data are expressed on a fresh weight (FW) basis.

**Nitrate ( $\text{NO}_3$ ) and nitrite ( $\text{NO}_2$ ) contents** were determined by spectrophotometric method. Samples were prepared by hot water (70°C, 1:100 w:w) extraction

from dry plant material in ultrasonic bath for 30 min. Initial  $\text{NO}_2$  concentration and total  $\text{NO}_2$  after zinc reduction were determined by diazotization-coupling Griess reaction (Merino, 2009) at 540 nm.  $\text{NO}_3$  and  $\text{NO}_2$  contents ( $\text{mg kg}^{-1}$ ) were determined according to the calibration curve and expressed on the FW basis.

*Total protein contents* were determined according to Bradford (1976) method. The fresh material was ground with liquid nitrogen and extracted with 50 mM phosphate buffer solution with 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM 2-merkaptoethanol, 100  $\mu\text{M}$  phenylmethylsulfonyl fluoride (PMSF). Homogenate was centrifuged for 10 min 4000 rpm  $\text{min}^{-1}$  and used for total protein content and enzyme activity determination. The sample was mixed with diluted Bradford reagent and absorbance at 595 nm was measured. Total protein contents ( $\text{mg g}^{-1}$  in FW) were determined according to the bovine serum albumin (BSA) calibration curve.

*Nitrate reductase (NR) activity* was determined by mixing 0.1 ml of protein extract with assay mix, containing 25 mM phosphate buffer (pH 7.3), 10 mM potassium nitrate ( $\text{KNO}_3$ ) and 0.5 mM EDTA, and adding 2.0 mM  $\beta$ -nicotinamide adenine dinucleotide ( $\beta$ -NADH), total volume of 2 ml. After incubation at 30°C for 5 min, reaction was stopped with 1 ml of 1% sulfanilamide in 3N HCl and 1 ml of 0.02% N-(1-naphthyl)-ethylenediamine dihydrochloride. After 10 min incubation in room temperature the absorption was measured at 540 nm and the contents of  $\text{NO}_2$  formed were determined according to a calibration curve of standard sodium nitrite solutions. Enzyme activity was evaluated as an amount of  $\text{NO}_2$  ions formed per hour ( $\mu\text{mol NO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ).

*Nitrite ( $\text{NO}_2$ ) reductase (NiR) activity* was determined (Takahashi et al., 2001) by mixing protein extract with 50 mM phosphate buffer (pH 7.5), 1 mM sodium nitrite ( $\text{NaNO}_2$ ) and 1 mM methyl viologen. Reaction was initiated by adding 57.4 mM sodium dithionite ( $\text{Na}_2\text{S}_2\text{O}_4$ ) in 290 mM sodium bicarbonate ( $\text{NaHCO}_3$ ) solution. After 5 min incubation at 30°C temperature, 20  $\mu\text{l}$  of reaction mixture was transferred to new vial, containing 480  $\mu\text{l}$  of water and vortexed. Immediately, 500  $\mu\text{l}$  1% sulfanilamide in 3N HCl and 500  $\mu\text{l}$  0.02% N-(1-naphthyl)-ethylenediamine dihydrochloride were added. After 10 min absorption was measured at 540 nm. Enzyme activity was expressed as the amount of  $\text{NO}_2$  ions formed per hour ( $\mu\text{mol NO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ).

*Statistical analysis.* Values are presented as mean  $\pm$  standard deviation in fresh weight (FW). For data evaluation the *Student's t*-test and determination coefficients (simple linear regression) at  $p < 0.05$  were used (Raudonius, 2017). Softwares *MS Excel*, version 7.0, and *Statistica*, version 7.0 were used for data processing.

## Results and discussion

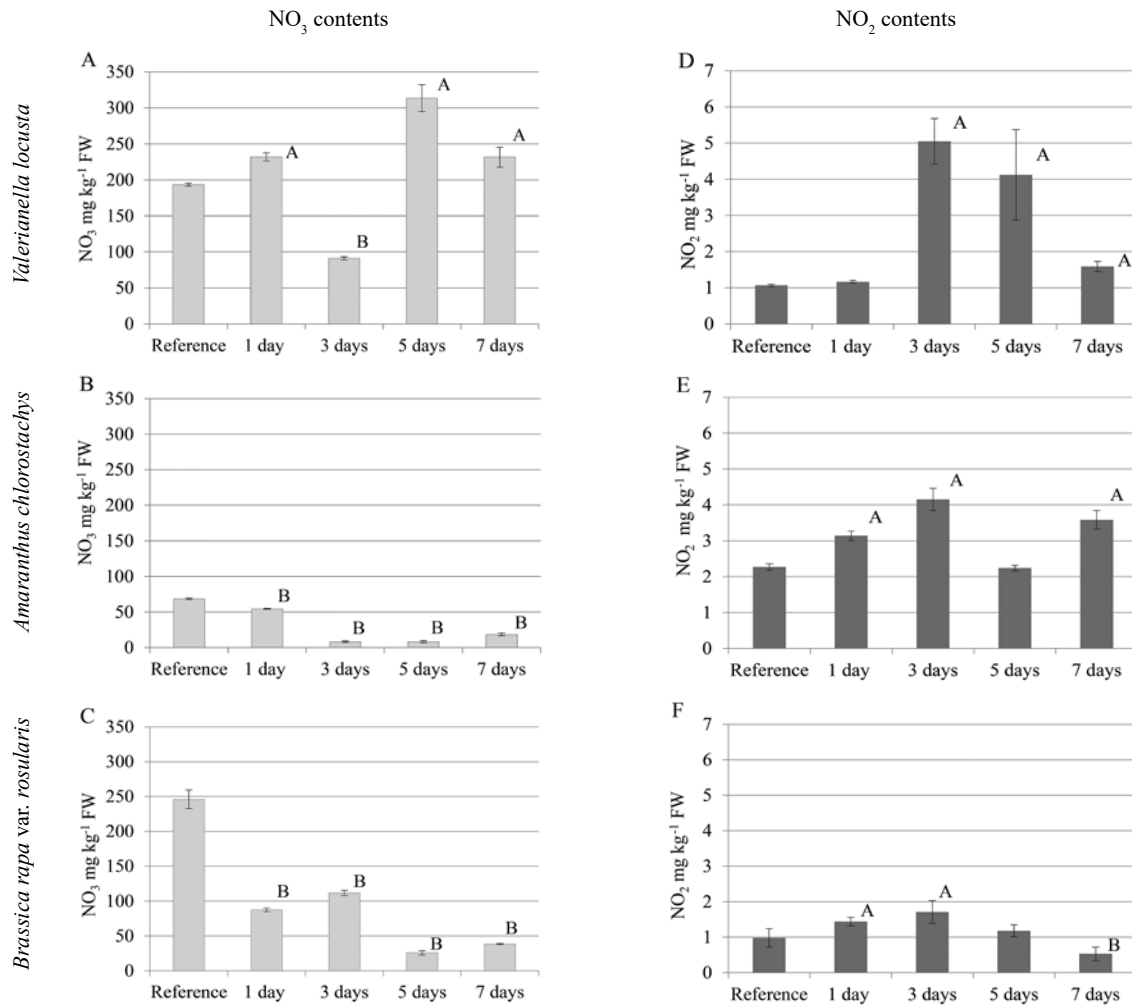
Artificial lighting with 638 nm red LEDs, applied for 1–7 days before harvesting can help reduce energy costs for plant cultivation in commercial greenhouses, compared to conventional lighting during the whole cultivation cycle. The effect of applied lighting was dependant on plant species and duration of lighting exposure (Fig.). A 3-day pre-harvest lighting reduced

$\text{NO}_3$  contents in corn salad, amaranth and tatsoi by 2.1, 8.1 and 2.2 times, respectively, as compared to the reference non-illuminated plants.

Strong negative correlation was determined between lighting duration and  $\text{NO}_3$  contents in amaranth and tatsoi ( $R = -0.7962$  and  $R = -0.8021$  respectively,  $p \leq 0.05$ ). Corn salad adapted to high PPFD red light and illuminating more than 3 days, no further  $\text{NO}_3$  reduction was observed (Fig. A). The decrease in  $\text{NO}_3$  contents after 3 days of lighting was followed by the increase in  $\text{NO}_2$  contents.  $\text{NO}_2$  contents (Fig. D–F) after 3 days of red light treatment were by 4.8, 1.8 and 1.7 times higher in corn salad, amaranth and tatsoi, respectively. However, no direct correlation between  $\text{NO}_3$  and  $\text{NO}_2$  contents was determined when illuminating plants with red light for 1–7 days. Burns et al. (2011), analysing the genotype and environment effects on  $\text{NO}_3$  assimilation processes, concluded that variability in  $\text{NO}_3$  accumulation under different environmental conditions arises more from differences in uptake of nitrate than from differences in efficiency of its chemical reduction. However, the changes in  $\text{NO}_3$  and  $\text{NO}_2$  contents and enzymatic activity during 1–7 days of light exposure indicate an important direct and indirect role of light on  $\text{NO}_3$  metabolism. The remarkable increase in  $\text{NO}_2$  contents suggests the need for more detailed analysis of this contaminant in green leafy vegetables. After analysis of numerous lettuce and spinach samples Iammarino et al. (2014) have also raised the idea about determining maximum admissible level for nitrites in leafy vegetables, as high  $\text{NO}_2$  contents do not necessarily correlate with high levels of  $\text{NO}_3$ .

The reduction of  $\text{NO}_3$  ions occurs in two step processes.  $\text{NO}_3$  is first reduced by cytosolic nitrate reductase (NR) to  $\text{NO}_2$ , which is then imported into the chloroplast and reduced further by nitrite reductase (NiR) into ammonium (Krapp, 2015). NR is considered as the key enzyme in this process (Wojciechowska et al., 2016). The obtained results show that NR and NiR activities are regulated independently.  $\text{NO}_3$ ,  $\text{NO}_2$ , total protein contents and reducing enzyme activity depend on plant species, lighting treatment duration and their interaction (Tables 1 and 2). Short-term red LED light treatment enhanced NR activity. The highest enzyme activity was determined after 5–7 days of supplemental red LED light exposure in amaranth and tatsoi. NR activity in amaranth increased 1.79 times after 5 days of lighting, in tatsoi – 1.70 times after 7 days of illumination. NiR activity was also determined the highest after 5 days in amaranth and after 7 days in tatsoi; it was increased 1.67 and 2.06 times, respectively, as compared to non-illuminated plants. In corn salad, NR activity changed insignificantly; NiR activity was determined lower, as compared to non-illuminated plants.

The analysis of total protein contents showed that lighting duration had statistically significant effect. The most pronounced effect was determined in corn salad after 5 days and in amaranth after 7 days' illumination with red LED light. Protein contents increased 1.54 and 1.34 times, as compared to the reference. The increase in protein contents did not correlate with  $\text{NO}_3$  contents



Note. A – value significantly higher, B – value significantly lower than reference (non-illuminated plants) according to Student's *t*-test,  $p \leq 0.05$ ; each data point represents mean  $\pm$  standard deviation,  $n = 3$ .

**Figure.** The effect of 1–7 days' pre-harvest red light emitting diode (LED) treatment on nitrate (NO<sub>3</sub>) and nitrite (NO<sub>2</sub>) contents in leafy vegetables

**Table 1.** Nitrate reductase (NR), nitrite reductase (NiR) activities and total protein contents in leafy vegetables after 1–7 days' pre-harvest red LED lighting treatment

	Duration of red light exposure	<i>Valerianella locusta</i>	<i>Amaranthus chlorostachys</i>	<i>Brassica rapa var. rosularis</i>
NR $\mu\text{mol NO}_2 \text{ g}^{-1} \text{ h}^{-1}$	reference	5.23 $\pm$ 0.46	8.88 $\pm$ 0.34	5.87 $\pm$ 0.15
	1 day	5.49 $\pm$ 0.10	10.28 $\pm$ 0.56 A	8.97 $\pm$ 1.41 A
	3 days	3.98 $\pm$ 0.09 B	9.61 $\pm$ 0.87	7.33 $\pm$ 0.78 A
	5 days	6.79 $\pm$ 0.24 A	15.79 $\pm$ 0.62 A	6.38 $\pm$ 0.15 A
	7 days	4.37 $\pm$ 0.50 B	12.31 $\pm$ 0.26 A	10.00 $\pm$ 1.53 A
NiR $\mu\text{mol NO}_2 \text{ g}^{-1} \text{ h}^{-1}$	reference	23.26 $\pm$ 1.23	27.12 $\pm$ 1.44	8.21 $\pm$ 0.44
	1 day	18.14 $\pm$ 1.02 B	15.18 $\pm$ 0.85 B	3.76 $\pm$ 0.12 B
	3 days	20.53 $\pm$ 1.11 B	15.79 $\pm$ 0.93 B	3.63 $\pm$ 0.20 B
	5 days	11.78 $\pm$ 0.68 B	45.27 $\pm$ 2.63 A	14.67 $\pm$ 0.85 A
	7 days	20.51 $\pm$ 1.23 B	35.28 $\pm$ 2.22 A	16.95 $\pm$ 1.02 A
Total proteins $\text{mg kg}^{-1}$	reference	1.45 $\pm$ 0.13	5.14 $\pm$ 0.13	6.14 $\pm$ 0.02
	1 day	2.30 $\pm$ 0.09 A	6.74 $\pm$ 0.35 A	3.76 $\pm$ 0.12 B
	3 days	2.09 $\pm$ 0.15 A	6.04 $\pm$ 0.33 A	6.14 $\pm$ 0.05
	5 days	2.24 $\pm$ 0.15 A	6.66 $\pm$ 0.22 A	4.93 $\pm$ 0.16 B
	7 days	1.70 $\pm$ 0.03 A	6.91 $\pm$ 0.20 A	3.79 $\pm$ 0.13 B

Note. A – value significantly higher, B – value significantly lower than reference (non-illuminated plants) according to Student's *t*-test,  $p \leq 0.05$ ; each data point represents mean  $\pm$  standard deviation,  $n = 3$ .

**Table 2.** Determination coefficient *R* between nitrate reductase (NR), nitrite reductase (NiR), nitrate, nitrite and protein contents in leafy vegetables

<i>Valerianella locusta</i>			
	total proteins	nitrites	nitrates
NR	0.3229	0.0023	0.8268
NiR	-0.7019*	-0.4095	-0.6968*
Nitrates	0.1710	-0.2448	–
Nitrites	0.3870	–	–
<i>Amaranthus chlorostachys</i>			
	total proteins	nitrites	nitrates
NR	0.5674*	-0.3250	-0.6075*
NiR	0.2379	-0.5471*	-0.3740
Nitrates	-0.5057	-0.4201	–
Nitrites	0.2399	–	–
<i>Brassica rapa var. rosularis</i>			
	total proteins	nitrites	nitrates
NR	-0.7221*	-0.2995	-0.4750
NiR	-0.3951	-0.7529*	-0.4886
Nitrates	0.6598	0.0676	–
Nitrites	0.3473	–	–

\* –  $p \leq 0.05$ 

in plant tissues. However, strong negative correlation was determined between total protein content and NiR in corn salad and NR in tatsoi (Table 2). In amaranth, positive correlation between NR activity and total protein contents was determined. Liu et al. (2016) analysed primary metabolism of  $\text{NO}_3^-$  in lettuce, cultivated under different light sources, and proposed that wide spectrum lamps, such as fluorescent, determined the lowest  $\text{NO}_3^-$  contents in lettuce tissues, but stimulated uptake of  $\text{NO}_3^-$  from soil and protein synthesis, when red and blue LED light inhibited protein synthesis. These trends, as well as obtained results confirm the differential response of plant species and varieties to light exposures.  $\text{NO}_3^-$  reduction upon light treatments was more efficient in species naturally containing higher concentrations of antioxidant phenols, anthocyanins (Samuoliene et al., 2011; Bliznikas et al., 2012), as pre-harvest changes in lighting conditions require plant adaptation and might evoke photostress conditions.

The photosynthetic parameters (Table 3) are also affected by pre-harvest red LED light treatment.

Medium correlation between photosynthetic rate and  $\text{NO}_3^-$  contents was determined in tatsoi and corn salad. A negative statistically insignificant correlation

**Table 3.** Photosynthetic rate, chlorophyll and flavonol indexes in leafy vegetables after 1–7 days' pre-harvest treatment with red 638 nm light

	Duration of red light exposure	<i>Valerianella locusta</i>	<i>Amaranthus chlorostachys</i>	<i>Brassica rapa var. rosularis</i>
Photosynthetic rate $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$	Reference	11.80 ± 1.63	2.54 ± 1.15	4.65 ± 0.50
	1 day	12.56 ± 1.46	7.21 ± 3.50	4.13 ± 0.64
	3 days	11.40 ± 1.14	9.44 ± 3.67 A	2.86 ± 0.28 B
	5 days	13.71 ± 0.54	7.44 ± 3.79	3.22 ± 0.98
	7 days	13.91 ± 1.41	5.61 ± 0.96 A	2.74 ± 0.20 B
Chlorophyll index	Reference	36.72 ± 0.48	21.90 ± 0.38	36.94 ± 2.24
	1 day	26.97 ± 0.59	36.18 ± 1.06 A	35.04 ± 2.64
	3 days	29.46 ± 0.91 B	25.78 ± 1.81 A	36.12 ± 2.54
	5 days	29.63 ± 1.83 B	24.75 ± 0.82 A	35.64 ± 2.43
	7 days	33.53 ± 3.95 B	20.38 ± 0.91 B	27.80 ± 3.55 B
Flavonol index	Reference	0.34 ± 0.03	0.18 ± 0.01	0.61 ± 0.03
	1 day	0.33 ± 0.03	0.19 ± 0.03	0.71 ± 0.09
	3 days	0.39 ± 0.01	0.26 ± 0.05 A	0.84 ± 0.06 A
	5 days	0.39 ± 0.06	0.23 ± 0.02 A	0.94 ± 0.04 A
	7 days	0.48 ± 0.03 A	0.28 ± 0.03 A	0.99 ± 0.03 A

Note. A – value significantly higher, B – value significantly lower than reference (non-illuminated plants) according to Student's *t*-test,  $p \leq 0.05$ ; each data point represents mean ± standard deviation,  $n = 3$ .

between these indicators was established for amaranth (Table 4). In corn salad, no statistically significant differences in photosynthetic rate were observed. In amaranth, photosynthesis was remarkably higher after 1–3 days of red LED treatment (photosynthetic rate was 3.7 times higher, as compared to non-illuminated plants). Further lighting (5–7 days) resulted in slight decrease in photosynthetic rate, but it was still higher, as compared to the reference. In tatsoi, photosynthetic rate was negatively affected by pre-harvest light treatment. No direct correlation between photosynthetic rate and chlorophyll

**Table 4.** Determination coefficient between nitrates and photosynthetic rate in leafy vegetables

	<i>Valerianella locusta</i>	<i>Amaranthus chlorostachys</i>	<i>Brassica rapa var. rosularis</i>
	nitrates		
Photosynthesis intensity	0.5696*	-0.5117	0.6324*

\* –  $p \leq 0.05$

content was observed, as the changes in chlorophyll content are the result of longer-term exposure, when photosynthetic rate changes immediately under different lighting conditions. In corn salad chlorophyll index decreased with the duration of pre-harvest light exposure. In amaranth, chlorophyll index increased during the first days of treatment, but significantly decreased after 7 days' exposure. In tatsoi, remarkable decrease in chlorophyll index was determined after 7 days' exposure.

The increase in flavonol index in leaves reflects plant adaptation to unfavourable conditions. High PPFD flux of red light can act as a photostressor for plants and evoke the response of antioxidant system (Petruša et al., 2013). Strong positive correlation ( $R = 0.73\text{--}0.93$ ) was determined between flavonol index and lighting duration in all green vegetables, which shows active adaptation processes.

## Conclusions

1. Short-term pre-harvest red light treatment can be applied as a technological tool to reduce nitrate ( $\text{NO}_3$ ) contents in leafy vegetables, cultivated under low natural light conditions.

2. The obtained results show a close interrelation between  $\text{NO}_3$  metabolism and photosynthesis parameters under light treatments, but no single trend was determined in all vegetable species. The higher photosynthetic photon flux density (PPFD) lighting, applied on mature plants before harvesting, evoked the adaptation processes and the response of antioxidant system and might act as a photostressor for more sensitive vegetable species.

3. The effect of light on  $\text{NO}_3$  reduction is closely linked to overall plant light sensitivity and internal physiological activities. In corn salad (*Valerianella locusta* L.), the applied lighting had no remarkable effect on photosynthetic rate and enzyme activity, thus the  $\text{NO}_3$  reduction was observed only after 3 days of lighting and further increased, as plants adapted to the applied lighting. In amaranth (*Amaranthus chlorostachys* Willd.) and tatsoi (*Brassica rapa* var. *rosularis* L.), nitrate reductase activity was promoted under red LED lighting and remarkable  $\text{NO}_3$  reduction was observed.

4. The correlation between the photosynthetic rate and  $\text{NO}_3$  contents in all plants confirm the significant interrelation between photosynthesis and  $\text{NO}_3$  metabolism; however, the differential response of the vegetables species to the applied lighting was also indicated.

5. Further comprehensive research is necessary to evaluate species and variety specific effects of the red light treatment on nitrate content reduction in different green vegetables.

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## LED apšvietimo taikymas prieš derliaus nuėmimą, siekiant sumažinti nitratų kiekį žalumyninėse daržovėse

A. Viršilė<sup>1</sup>, A. Brazaitytė<sup>1</sup>, J. Jankauskienė<sup>1</sup>, J. Miliauskienė<sup>1</sup>, V. Vaštakaitė<sup>1</sup>, I. Odminytė<sup>2</sup>, A. Novičkovas<sup>3</sup>, G. Samuolienė<sup>1</sup>

<sup>1</sup>Lietuvos agrarinių ir miškų mokslų centro Sodininkystės ir daržininkystės institutas

<sup>2</sup>Vytauto Didžiojo universitetas

<sup>3</sup>Vilniaus universiteto Taikomųjų mokslų institutas

### Santrauka

Salotinė sultenė (*Valerianella locusta* L. 'Vit'), trispalvis burnotis (*Amaranthus chlorostachys* Willd. 'Red Army') ir skrotelinis kopūstas (*Brassica rapa* var. *rosularis* L. 'Rozetto F1'), išauginti silpno natūralaus apšvietimo sąlygomis šiltnamyje, 1–7 dienas prieš derliaus nuėmimą buvo papildomai apšviesti 638 nm bangos ilgio raudoną šviesą skleidžiančių diodų (LED) šviesa. Siekiant išsamiai įvertinti nitratų metabolizmo valdymo galimybes žalumyninėse daržovėse, analizuotas taikyto apšvietimo poveikis nitratų ( $\text{NO}_3$ ), nitritų ( $\text{NO}_2$ ), redukuojančių fermentų aktyvumui ir augalo fotosintetiniam aktyvumui įvairių veislių daržovėse.  $\text{NO}_3$ ,  $\text{NO}_2$ , suminis baltymų kiekis ir redukuojančių fermentų aktyvumas priklausė nuo augalo rūšies, apšvietimo trukmės ir jų sąveikos. Reikšmingas  $\text{NO}_3$  kiekio sumažėjimas ir reduktazės aktyvumo padidėjimas nustatytas po trijų dienų trukmės šviesos poveikio. Po apšvietimo buvo nustatytas reikšmingas  $\text{NO}_2$  ir suminio baltymų kiekio padidėjimas salotinėse sultenėse ir trispalviuose burnočiuose. Skroteliniuose kopūstuose ir salotinėse sultenėse nustatyta vidutinė koreliacija tarp fotosintezės intensyvumo ir  $\text{NO}_3$  kiekio. Burnočiuose tarp šių rodiklių nustatyta neigiamas neesminė koreliacija. Trumpalaikis apšvietimas raudona LED šviesa prieš derliaus nuėmimą gali būti taikomas kaip efektyvus būdas sumažinti nitratų kiekį žalumyninėse daržovėse, išaugintose silpno natūralaus apšvietimo sąlygomis. Tyrimo rezultatai patvirtina reikšmingą ryšį tarp augalo fotosintezės bei nitratų apykaitos ir kartu jautrią bei nevienodą skirtingų augalų rūšių fiziologinę reakciją į taikomą apšvietimą.

Reikšminiai žodžiai: baltymai, fotosintezė, nitratai, nitratų reduktazė, nitritų reduktazė, šviesą skleidžiantys diodai.