The benefits of red LEDs: improved nutritional quality due to accelerated senescence in lettuce

Giedrė Samuolienė¹, ², Akvilė Urbonavičiūtė¹, ², Aušra Brazaitytė¹, Julija Jankauskienė¹, Pavelas Duchovskis¹, ², Zenonas Bliznikas³, Artūras Žukauskas³

¹Lithuanian Institute of Horticulture, Kauno 30, LT-54333 Babtai, Kaunas distr., Lithuania, e-mail g.samuoliene@lsdi.lt
²Lithuanian University of Agriculture, LT-53361 Akademija, Kaunas distr.
³Institute of Materials Science and Applied Research, Vilnius University, Saulėtekio al. 9-III, LT-10222 Vilnius, Lithuania

The aim of this experiment was to enhance the action of photosynthetic system, and to improve the synthesis of primary and secondary metabolites by supplementation the traditional greenhouse lighting with red LEDs. This effect is very dependent on the duration of supplemental lighting and on other environmental conditions. Also we hypothesize, that the suspended supplemental lighting may affect the recovery of metabolic and photosynthetic systems of lettuce further grown under natural conditions in greenhouse. Lettuce (Lactuca sativa cv. ‘Grand rapids’) was grown in peat substrate within an industrial greenhouse at the Lithuanian Institute of Horticulture. The growth up to technical maturity of the plants (about 30 days) was performed under natural daylight. At the pre-harvest stage of 6 days, plants were additionally illuminated by red LEDs with the peak wavelength of 638 nm (200 μmol · m⁻² · s⁻¹ common environment and additional lighting) for 24 h. The temperature regime at night was 12/16 °C, and at day time 16/18 °C. We assume that the decline in nitrate contents, sugar and carotenoid accumulation, low hexoses/sucrose ratio, in supplemented red LEDs treatment induces senescence of lettuce leaves through sugar signalling pathways. In addition, environmental conditions may regulate senescence through pathways that are independent on photosynthesis and its primary metabolites. Signals, such as an increase in vitamin C concentration, can trigger senescence through photo-oxidative stress. This achieved effect by generating photo stress in lettuce leaves accelerated senescence and led to manipulate the synthesis of primary and secondary metabolites to economically advantage direction.

Key words: lettuce, nitrates, sugars, vitamin C.

Introduction. Quite a long while it is known that plant growth and development processes are regulated by light quality, quantity, and photoperiod. Together these three parameters strongly influence traits of horticultural interests, including plant stature, growth habits, the transition to flowering, and the end point of plant produc-
tivity (Casal and Yanovsky, 2005). Sustaining to other authors (Folta and Childers, 2008; Massa et al., 2008; Morrow, 2008; Wheeler, 2008; Demšar et al., 2004; Matsu-da et al., 2004) experiments with narrow bandwidth solid-state lighting, we can extrapolate the findings to the design of plant growth strategies that not only foster plant growth and development, but rather control plant growth and development. Solid-state lighting using light-emitting diodes (LEDs) represents a fundamentally different technology from the lamps currently used in horticulture. According to R. C. Morrow (Morrow, 2008), because of unique characteristics, LEDs can play a variety of roles in horticultural lighting. Below, we report on the application of a supplemental solid-state lighting in greenhouse. One of the most important indicators of nutritional quality of vegetables is low nitrate content. Although data on potential long-term health risk of nitrate are contradictory (Gangolli et al., 1994; Walker, 1990), national and international regulatory agencies are setting maximal allowed levels for nitrate in vegetables, which constitute the main dietary intake of nitrate (Santamaria, 2006). Generally, the largest amounts of nitrate are found in leaves and petioles and many leafy vegetables, such as lettuce, spinach, celery, rocket exhibit very high nitrate-accumulation capacity (in excess of > 2 500 mg kg^{-1}) (Santamaria, 2006). Commonly, the nitrate content in vegetables increases with increasing the geographical latitude of an agricultural region due to lower-irradiance artificial lighting and increased concentration of fertilizers in soil. In our experiments, we use supplemental narrow-bandwidth photosynthetic red light (the peak wavelength of 638 nm), which has the highest capacity in stimulating nitrate reductase (NR) activity (Lillo and Appenroth, 2001). As it is known, the differing capacities to accumulate nitrate can be controlled with differing location of the NR activity as well as to differing degree of nitrate absorption and transfer in the plant (Andrews, 1986).

As it is known primary metabolites of photosynthesis, such as sugars, serve as essential metabolic nutrients and structural components for most organisms (Dai et al., 1999). Sugars affect development and metabolic processes throughout the life cycle of the plant. Senescence is also thought to be regulated by sugars. It has been hypothesized that leaf senescence is characterized by a decline in chlorophyll content and in photosynthetic activity (Wingler et al., 2006; Wingler et al., 1998; van Doorn and Woltering, 2004). According to Himelblau and Amasino (2001), leaf senescence is not only a degenerative process, but it also plays a vital role in nutrient recycling, especially in the remobilization of nitrogen.

Experiments to investigate the effect of light conditions on other biologically sensitive compounds, showed, that it is unlikely that the induction of senescence by light was caused by photo-oxidative stress (Ono et al., 2001). Otherwise, the vitamin C accumulation in metabolically active tissues, such as leaves, acts as the protective signalling molecule, coordinating the protective mechanism of an oxidative system (Pastori et al., 2003).

The aim of this experiment was to enhance the action of photosynthetic system, and to improve the synthesis of primary and secondary metabolites by supplementation the traditional greenhouse lighting with red LEDs. Also we hypothesize, that the suspended supplemental lighting may affect the recovery of metabolic and photosynthetic systems of lettuce further grown under natural conditions in greenhouse.
Object, methods and conditions. Plant Material and Growing Conditions. Lettuce (Lactuca sativa cv. ‘Grand rapids’) was grown in peat substrate within an industrial greenhouse (November, Lithuania, lat. 55° N). The growth up to technical maturity of the plants (about 30 days) was performed under natural daylight. Daylight was supplemented by high-pressure sodium lamps (Son-T Agro, Philips) at a PPF of about 130 µmol m$^{-2}$ s$^{-1}$ (from 5 to 10 am and from 17 to 24 pm). At the pre-harvest stage of 6 days, plants were additionally illuminated by red light-emitting diodes (LED) for 24 h. The solid-state illuminator contained high-power (3 W) red AlGaInP LEDs (LUXEON® III Star, model LXHL-LD3C, Philips Lumileds Lighting Company, San Jose, Cal.) with the peak wavelength of 638 nm (Žukauskas et al., 2008). The illumination system was coordinated to maintain at least 200 µmol m$^{-2}$·s$^{-1}$ common (environment and additional lighting) flux density at 30 cm distance from plants. The temperature regime at night was 12/16 °C, and at daytime 16/18 °C. Plants were fertilized with ammonium nitrate. Nutritional quality of the plants treated under the solid-state illuminator as well as of the reference (R) plants was assessed 6 days before harvesting and by measuring the concentrations of nitrate, carbohydrates, photosynthetic pigments and vitamin C (L-ascorbic acid). The recovery of treated compounds was observed after 5 days under natural greenhouse conditions.

Assessment procedures and sample preparation. Nitrate concentration was measured by potentiometric method (Geniatakis et al., 2003). Vitamin C was assessed by spectrophotometric method of Janghel et al. (2007). Carbohydrates (fructose (Fru), glucose (Glu), sucrose (Suc) and maltose (Mal)) were measured by high performance liquid chromatography (HPLC) method (Urbonavičiūtė et al., 2006). Photosynthetic pigments (chlorophyll $a$ and $b$ and carotenoids) were measured by spectrophotometric method of Wetshtein (Гавриленко, 2003).

Statistical Analysis. Data was processed using MS Excel software. Three analytical samples of nitrates, vitamin C and sugars and three biological samples of photosynthetic pigments were measured for each treatment. Standard deviations are indicated in figures by error bras.

Results. Estimating the obtained results it was noticed that after two-three days the decrease of nitrate concentration in 30 % was observed in lettuce leaves treated under red LEDs, comparing to reference plants (Fig. 1). Further treatment leads to nitrate content decrease both in reference and in treated lettuce. Though the reduction is weaker, the decrease of nitrates up to 15 % is observed. This effect also remains after 5 days of recovery period.

As shown in figure 2, the illumination lasting longer than three days influenced the accumulation of vitamin C more than up to 30 %. Also from the third day treatment the higher concentration of vitamin C was detected in treated plants comparing with reference once. From the fifth treatment day it remained stable (0,38 mg g$^{-1}$ of fresh weight) in treated plants. The drastic decrease (up to 30 %) was observed in reference plants after 5 days of recovery period (Fig. 2).
Significant higher common sugar content, especially of sucrose, was determined from the third treatment day in lettuce leaves supplementary treated with red light LEDs comparing with reference ones (Fig. 3). The hexoses and sucrose ratio in treated plants with red LEDs was varying from 1.1 to 2.9, whereas in reference plants it was much higher (fluctuated in the range of 5.0 to 10.0). Only after suspended lighting in treated plants this ratio increased. After 6 days of treatment the highest content of common sugars was estimated in treated plants, the increased sucrose amount lead to the lowest (1.1) ratio of hexoses and sucrose (Fig. 3).

The variation of pigment concentration was observed only within error limits. Notwithstanding more carotenoids were accumulated under red light treatment (Fig. 4.). The significant differences were obtained after evaluating the ratio of carotenoids and
the sum of chlorophylls \(a\) and \(b\). Already after two days treatment this ratio increased from 0.12 to 0.27–0.40 in lettuce leaves treated under LED. In reference plants it remained about 0.22 during all the experiment. The negative effect also remains after 5 days recovery period (Fig. 4.).

**Fig. 3.** The variation of carbohydrates concentration and ratio in lettuce leaves during 6 days treatment with supplemental red LEDs and after recovery period

**3 pav.** Sacharidų koncentracijos ir jų santykio kitimas salotų lapuose 6 dienas švitinant raudonaisiais LED ir salotoms atgaves pirmykštę būklę

**Fig. 4.** The variation of photosynthetic pigments concentration and ratio in lettuce leaves during 6 days treatment with supplemental red LEDs and after recovery period

**4 pav.** Fotosintezės pigmentų koncentracijos ir jų santykio kitimas salotų lapuose 6 dienais švitinant raudonaisiais LED ir salotoms atgaves pirmykštę būklę
**Discussion.** The observed drop in nitrate content (see Fig. 1) could be related to the increased activity of NR under conditions of enhanced photosynthesis intensity and due to maintaining phytochrome in the physiologically active Pfr isoform. Low radiant heat of solid-state sources of light allows using effectively much higher PPFs than those under conventional discharge or fluorescent lamps. The observed increase in concentration of carbohydrates in the treated plants from the third lighting day (see Fig. 3) is probably due to the increased photosynthesis intensity. Price et al. (2004) showed that glucose has a stronger effect on the regulation of genes associated with nitrogen metabolism than nitrogen supply. Besides, such an increase in carbohydrate concentration is favourable in terms of self-supporting NR activity through stimulation of gene expression (Cheng et al., 1992; Lillo and Appenroth, 2001). Moreover, carbohydrates are able to compete light in stimulating expression of genes involved in nitrogen assimilation (Thum et al., 2003). However, the supplemental lighting with red LEDs leads to increased carbohydrate content, especially of sucrose (see Fig. 3), and this can be a signal inducing the processes of senescence (Causin et al., 2006; Munde-Bosch, 2007). The sucrose metabolism is a physiological signal affecting further metabolic processes, (Koch, 2004) nutrient and gustatory properties. However, in this case low ratio of hexoses and sucrose (see Fig. 3) could accelerate the induction of senescence mechanism. Such carbohydrate distribution in lettuce leaves was conditioned not only by supplemented red light alone, but also by other environmental conditions, which affected decrease of photosynthesis system action and thereof processes of senescence were induced. Besides, according to Wingler and co-workers (Wingler et al., 2006), too early senescence would reduce plant's capacity to assimilate CO₂, whereas too late senescence would interfere with nutrient remobilisation, thereby compromising photosynthetic activity in the young leaves and reproductive success. Other authors propose that leaf senescence can be induced by low nutrient supply (Ono et al., 1996) or it can be maintained by carbon balance of a plant (Wingler et al., 2006). Otherwise, Wingler and co-workers assume that sucrose did not accumulate during developmental senescence (Wingler et al., 2006). The question remains – what causes strong accumulation of hexoses despite the decline in photosynthetic carbon assimilation in senescing leaves? A possible source of hexoses is the breakdown of starch. In addition, Jongebloed et al. (2004) have shown that phloem blockage by callose deposition could lead to an age-dependent sugar accumulation. Although it is maintained that red light makes positive effect on the formation and action of photosynthetic system (Spalding and Folta, 2005), according to our results, strong accumulation of carotenoids and thereof high ratio of carotenoids and sum of chlorophylls a and b and suspended growth (see Fig. 4) allow to presume that the excess of red light induced the senescence processes. According to Martin et al. (2002), the regulation of photosynthesis and plant development appears to depend on the carbon/nitrogen ratio instead of carbohydrate alone. These carbon/nitrogen interactions are likely to play an important role in the regulation of leaf senescence. Agreeably with other authors (Diaz et al., 2005), sugar and nitrogen contents show distinct changes during leaf senescence, with sugars accumulation (see Fig. 3) and
nitrate decline (see Fig. 1).

The tendency of increase in the vitamin C content in LED-treated lettuce leaves in contrary to reference ones was observed, and thus coheres with the faster senescence processes. Besides, vitamin C is not only an important nutrient but also plays a major role in the protection of plants against photo-oxidative stress, photoprotection, and phytohormones action (Conklin, 2001, Davey et al., 2000; Pastori et al., 2003). Interestingly, a noticeable increase in vitamin C content (see Fig., 2) is observed in lettuce leaves where LED treatment invoked an increase in the concentration of carbohydrates (see Fig. 3). However, Ono et al. (2001) suppose that the induction of senescence by red light could not be caused by photo-oxidative stress, since even higher light intensity in his experiment was low compared with natural conditions. Instead, illumination-depended changes in carbohydrate content (see Fig. 3) and in carotenoid/chlorophyll ratio (see Fig. 4) may have influenced senescence.

Conclusions. Nitrate decline, sugar and carotenoid accumulation, low hexoses/sucrose ratio, in supplemented red LEDs treatment induces lettuce leaves senescence through sugar signalling pathways. In addition, environmental conditions may regulate senescence through pathways that are independent from photosynthesis and its primary metabolites. An increase in vitamin C concentration can trigger senescence through photo-oxidative stress. This achieved effect by generating photo stress in lettuce leaves accelerated senescence and led to manipulate the synthesis of primary and secondary metabolites to economically advantage direction.

Acknowledgements. This study was supported by Lithuanian Science and Study foundation under the high technology project PHYTOLED (Nr. B24-2007).

References


Reikšminiai žodžiai: nitratai, sacharaidai, salotos, vitaminas C.