Effect of end-of production continuous lighting on yield and nutritional value of Brassicaceae microgreens

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Abstract. The effect of continuous lighting applied in the end-of-production period on growth and nutritional quality of radish (Raphanus sativus var. radicula), broccoli (Brassica oleracea var. italic), mizuna (Brassica rapa. var. nipposinica) and arugula (Eruca sativa) was investigated in growth chambers under LED lighting. Microgreens were grown under 16 h photoperiod and 3 days before harvest half of plants were placed under continuous lighting conditions. Pre-harvest continuous lighting treatment increased yield, robustness index, and shorten time to harvest in radish, broccoli, mizuna and arugula microgreens. The end-of-production treatment has also led to higher content of compounds with antioxidative properties (flavonoids, proline) and increased the activity of antioxidant enzymes (CAT, APX, GPX) by inducing mild photooxidative stress. Increased antioxidative status added nutritional value to microgreens that can be used as functional foods providing health benefits. Pre-harvest treatment by continuous lighting is suggested as the practice than can allow producers to increase yield, aesthetic appeal, nutritional quality, and market value of Brassicacea microgreens.

1. Introduction

Microgreens (tender immature seedlings of edible vegetables and herbs) are gaining in popularity as a new culinary ingredient providing intense flavors, vivid colors, and crunchy texture when added to salads and other foods. Microgreens have fully developed cotyledons with or without the emergence of a rudimentary pair of first true leaves [1, 2]. Due to the high market value and increasing demand microgreen production is attractive to commercial producers [3]. Microgreens do not have a long shelf life and don't travel well so being close to market is a big advantage. Therefore, local production, especially for fresh-cut products, represents the most promising production strategy [4]. Many species of microgreens have health beneficial effects as they contain higher concentrations of phytochemicals than mature greens [1, 5]. Their health-promoting bioactive compounds include antioxidants, vitamins and minerals thus qualifying microgreens as functional food [1, 6].

Microgreens are produced in greenhouses and in plant factories with artificial lighting (PFAL), where natural sunlight is supplemented or replaced with electrical lighting. It was shown that light-emitting diode (LED) lighting has a great potential to stimulate plant growth and the synthesis of bioactive compounds beneficial to human health [7, 8]. Nowadays the

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timing and intensity of horticultural lighting can be controlled more precisely than ever before. However, plant species differ in their response to light environment [9]. Therefore, each species requires studies to be performed for the selection of optimal light setting to maximize efficiency, yield, and nutritional quality of plants. Our previous research [10] has shown that arugula, mizuna, broccoli and radish arugula can tolerate continuous lighting during production period (11 days). These microgreens grown under continuous lighting (24 h photoperiod) had higher yield and robust index and increased nutritional value compared to 16 h photoperiod, as they accumulated more antioxidative phytochemicals (carotenoids, anthocyanins, flavonoids, proline, antioxidant enzymes). Effects were more pronounced under LED lighting compared to fluorescent lamps. Some other research has also shown that LED continuous light of different spectral quality and intensity may be used to improve yield and nutritional quality of lettuce plants [11-14].

This study investigated the effect of pre-harvest LED continuous light treatment on growth, yield Our aim was to test if continuous lighting in the end-of-production (EOP) could be a practice that would allow growers to increase quality and market value of microgreens without negatively affecting growth and morphology.

2. Materials and methods

Radish (*Raphanus sativus* var. *radicula* Pers.), broccoli (*Brassica oleracea var. italica* Plenck*),* mizuna *(Brassica rapa.* var. *nipposinica* (L.H.Bailey) Hanelt) and arugula (*Eruca vesicaria* subsp. *sativa* (Mill.) Thell.) were grown under controlled environmental conditions in a growth chamber at the average air temperature of 23 ± 1 °C and relative air humidity of 60 ± 5%.

Coconut coir mats were used for microgreen culture. After sowing trays of microgreens were placed to germinate in darkness and were top-irrigated with water. Once cotyledons were fully expanded, a half-strength Hoagland nutrient solution (pH 6.2–6.4) was added to each tray daily until harvest.

Plants were grown under 16 h photoperiod with the photosynthetic photon flux density (PPFD) of 270 μmol m^{-2} s^{-1} provided by LEDs (LED GL V300, China) with light ratio (%) of red : green : blue – 50.3 : 21.1 : 17.6.

On day 8 after sowing (in the EOP period) half of plants were placed to the growth chamber with 24 h photoperiod (treatment 24 h EOP) and the same PPFD, where were kept until harvest on day 11.

Ten seedlings of each species were randomly selected and measured to determine hypocotyl length (HL), hypocotyl diameter (HD), first true leaf length (LL), fresh weight (FW) and dry weight (DW) for each light treatment. To determine FW seedlings were weighed as quickly as possible to limit the losses through evaporation and then samples were dried at 105°C in an oven until a constant DW was observed. The values of leaf mass per area (LMA) were calculated as a ratio of a dry mass of the lamina discs to their area. Four discs were cut from cotyledons with a 4-mm in diameter cork borer. The DW of the discs was determined after their drying to a constant weight at 105°C.

Robustness index (RI) = HD / HL × DW [4].

Five plants per treatment were randomly selected for the following measurements. Content of chlorophyll Chl*a* and *b* was measured in 96% ethanol extracts with a SF 2000 spectrophotometer (Spectrum, Russia) and calculated according to the known formulas [15].

The content of malondialdehyde (MDA), the final product of lipid peroxidation, was determined with a standard method based on the reaction of these substances with thiobarbituric acid that produces a trimethine complex with an absorption maximum at 532 nm [16].

For protein and antioxidant enzyme assays, leaf tissues (0.1 g) were ground in 4 ml of 50 mM potassium phosphate buffer (pH 7.8) using a chilled pestle and mortar. The homogenate was centrifuged at 14000 g for 20 min at 4°C and the supernatants thus collected was used for the assays of catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), and guaiacol peroxidase (GPX, EC 1.11.1.7) and protein determinations. The activity of CAT was measured by the method of [17], and was determined by monitoring the disappearance of H_2O_2 using the extinction coefficient 0.036 $mM^{-1}cm^{-1}$. CAT activity was expressed in μmol H_2O_2 per minute per mg of protein. The APX activity was assayed by following the reduction in a reaction mixture at 290 nm (extinction coefficient 2.8 $mM^{-1}cm^{-1}$) according to [18]. The GPX activity was determined by monitoring the increase in absorbance at 470 nm during the oxidation of guaiacol [19]. The APX and GPX activity values are expressed as unit U mg-*1* protein. The concentration of protein was determined by the method of Bradford using bovine serum albumin as a standard.

Hydrogen peroxide content was determined according to [20]. Leaf tissues (0,1 g) were homogenized in ice bath with 5 ml 0.1% (w/v) trichloroacetic acid. The homogenate was centrifuged at 12 000 *g* for 15 min at 4°C and 0.5 ml of supernatant was added to 0.5 ml potassium phosphate buffer (pH 7.0) and 1 ml 1M KI. The absorbance of supernatant was measured at 390 nm. The content of H_2O_2 was calculated by comparison with a standard calibration curve.

Free proline was extracted from 0,3 g of fresh leaf samples homogenized with 3% sulfosalicylic acid and estimated by using acid ninhydrin reagent according to [21].

The relative amounts of flavonoids were measured spectrophotometrically [22]. Fresh leaves tissues (0.1 g) were homogenized in 2 ml of 95% ethanol:1.5 N HCl (85:15, v:v). After overnight extraction at 4˚C in darkness, each sample was centrifuged at 10 000 g for 5 min. The supernatant was diluted 10 times and the absorbance was measured at 300 nm. Flavonoids content in the sample was expressed as absorbance at 530 nm g^{-1} fresh weight of tissue.

The experiment was carried out twice. The figures and tables show mean values and their standard errors. Difference between the mean values was considered significant at $p \leq 0.05$.

3. Results

Treatment of microgreens by continuous lighting for 3 days before harvest did not cause any visual leaf photodamage in all plant species. Radish, broccoli, mizuna and arugula 24 h EOP-treated plants were shorter and had higher FW and DW, therefore they outperformed plants grown under 16 h photoperiod during the whole production period in terms of RI and LMA (Figure 1).

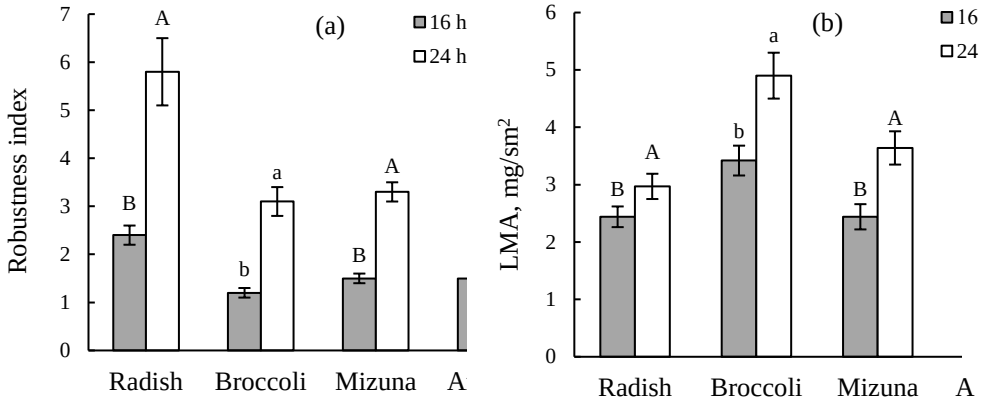

Fig. 1. The robustness index (a) and leaf mass per area (LMA) (b) of radish, broccoli, mizuna and arugula microgreens grown under 16 h photoperiod or under 16 h photoperiod with end-of-production treatment by continuous lighting (24 h EOP). The results are presented as the mean ± standard error. Different letters for each plant species indicate significant differences between the mean values at $p < 0.05$.

Total chlorophyll (Chl *a*+*b*) content of all four microgreens was not significantly affected by 24 h EOP (Table).

The first true leaf was bigger in all microgreen plants treated by 24 h EOP, and effect was the greatest (up to 2-fold) in radish and broccoli (Table).

In all 24 h EOP-treated microgreens flavonoids content was significantly increased (Figure 2). Broccoli and arugula 24 h EOP-treated plants had visible purple and blue coloration of abaxial leaf surfaces.

Results indicated that all microgreens treated by 24 h EOP had higher level of lipid peroxidation determined in terms of MDA content (Table). Broccoli and arugula had also higher H_2O_2 content compared to non-treated plants. In broccoli 86% increase in H_2O_2 content was observed, while arugula had 25% higher H_2O_2 content.

There were some differences in plant response to 24 h EOP between microgreen species in respect to antioxidant enzyme activities. The activity of CAT was increased in radish and arugula. APX activity was increased in all species except mizuna. GPX activity was increased by 24 h EOP treatment in all species to different extent, but the most dramatic increases were recorded in radish and arugula.

Higher proline content was observed in all 24 h EOP-treated plants, except for radish plants (Figure 2).

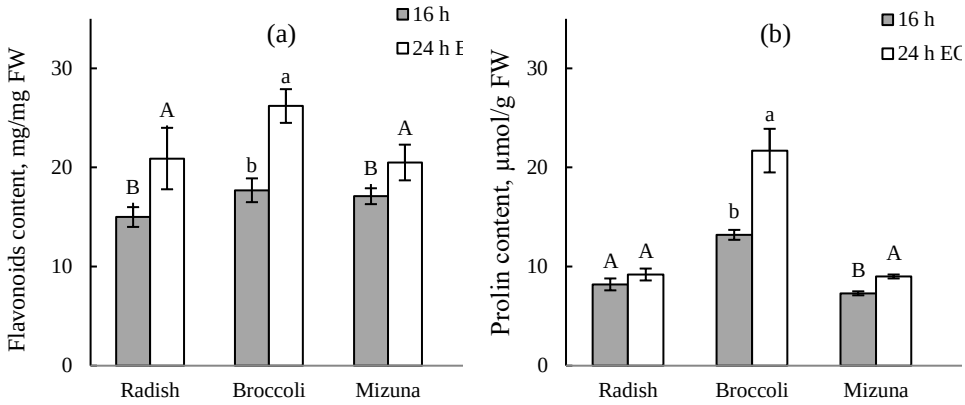

Fig. 2. Flavonoid (a) and proline (b) content of radish, broccoli, mizuna and arugula microgreens grown under 16 h photoperiod or under 16 h photoperiod with end-of-production treatment by continuous lighting (24 h EOP). The results are presented as the mean ± standard error. Different letters for each plant species indicate significant differences between the mean values at $p < 0.05$.

Table 1. The first true leaf length (LL), chlorophyll (Chl), malondialdehyde (MDA), H2O2 content and catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) enzyme activities of microgreens grown under 16 h photoperiod or under 16 h photoperiod with end-of-production treatment by continuous lighting (24 h EOP).

Parameter	Radish		Broccoli		Mizuna		Arugula	
	16 h	24 h EOP	16 h	24 h EOP	16 h	24 h EOP	16 h	24 h EOP
LL, mm	7.9± 2.0	14.3± 1.5*	3.6± 0.6	6.9± 0.5*	21.1± 2.1	29.9± 1.3*	4.5± 0.5	5.1± 0.5*
Chl *a*+*b*, mg/mg DW	4.5± 0.4	3.9± 0.2	5.1± 0.1	4.9± 0.4	4.6± 0.2	4.5± 0.5	4.4± 0.6	4.7± 0.2
MDA, µmol/g FW	13.9± 0.6	17.6± 1.4*	18.2± 0.8	50.0± 8.4*	11.6± 0.5	13.7± 0.9*	41.7± 3.6	59.0± 7.3*
H_2O_2, µmol/g FW	0.47± 0.04	0.43± 0.02	0.51± 0.05	0.95± 0.06*	0.40± 0.01	0.40± 0.03	0.71± 0.05	0.89± 0.08*
CAT, µmol/(mg protein min)	27± 5	42± 6*	72± 5	81± 9	31± 5	40± 7	48± 5	73± 7*
APX, µmol/(mg protein min)	171± 25	256± 20*	230± 28	316± 56*	239± 19	214± 16	225± 18	316± 43*
GPX, µmol/(mg protein min)	70± 18	102± 8*	220± 11	263± 15*	184± 18	232± 25*	29± 6	75± 13*

*$p < 0.05$ 24 h EOP-treatments vs. 16 h-treatments

4. Discussion

Pre-harvest continuous lighting treatment increased aboveground fresh and dry weight and LMA of all studied species compared to plants grown under 16 h photoperiod during the whole production period. The matter of fact that 24 h EOP-treated plants received higher daily light integral (DLI) as the light intensity was similar in both light settings. Higher DLI

did not cause any leaf photodamages, but provided additional light for photosynthetic activity and biomass accumulation. Thus, all four plant species had higher yield. There are no data on the sensitivity of broccoli, radish and mizuna to continuous lighting, but it was reported that continuous lighting had no detrimental effects on the yield and quality of rocket leaves while improved efficiency of rocket indoor cultivation [23]. RI relates individual components of hypocotyl (length and diameter) to DW and serves as a proxy for plant robustness. The 24 h EOP treatment increased RI in all four microgreen species due to decreased plant height and increased DW. Beside robustness higher RI also ensures aesthetic appeal of microgreens.

In many research it was noted that continuous lighting increases developmental rate of plants [24]. We observed significant acceleration of the first true leaf emergence in all 24 h EOP-treated plants, especially in radish and mizuna. Thus, it is suggested that 24 h EOP treatment may reduce the time to harvest in radish, broccoli, mizuna and arugula.

In this study Chl content of all studied species was not significantly affected by the 24 h EOP treatment. The slight decrease of Chl content in radish, broccoli and mizuna was not visually detected, i.e. 24 h EOP treatment did not decrease overall visual quality of microgreens. The tendency of Chl content decrease in response to continuous lighting is rather expected as it reduces the light absorption efficiency by photosynthetic apparatus and may serve as one of mechanisms to protect plants from excess light.

Plants treated by 24 h EOP had higher H_2O_2 and MDA content than plants grown under 16 h photoperiod, which means that they experienced mild oxidative stress. Plants usually accumulate antioxidant bioactive compounds in response to stress. EOP-treated plants of all species had increased proline, flavonoid contents and elevated activities of antioxidant enzymes (CAT, APX, GPX). It is consistent with previous findings that higher concentrations of H_2O_2 and higher activity of CAT and APX were recorded for tomato plants treated by 24 h photoperiod compared to plants grown under 16 h photoperiod with the same DLI [25]. Thus, continuous lighting applied in the EOP added nutritional value to microgreens of all four species by increasing their antioxidant properties. Antioxidants by scavenging free radicals serve as essential compounds for human health, protecting the human organism against reactive oxygen species [26].

5. Conclusions

This study investigated the responses of four microgreen species to continuous lighting applied in the end-of-production period provided by LEDs with light ratio (%) of red : green : blue – 50.3 : 21.1 : 17.6 and PPFD of 270 μmol m^{-2} s^{-1}. Pre-harvest continuous lighting treatment increased yield, robustness index, and shorten time to harvest in radish, broccoli, mizuna and arugula microgreens. The end-of-production treatment has also led to higher content of compounds with antioxidative properties (flavonoids, proline) and increased the activity of antioxidant enzymes (CAT, APX, GPX) by inducing mild photooxidative stress. Increased antioxidative status added nutritional value to microgreens that can be used as functional foods providing health benefits. Pre-harvest treatment by continuous lighting is suggested as the practice than can allow producers to increase yield, aesthetic appeal, nutritional quality, and market value of *Brassicacea* microgreens.

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