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The effect of mineral nutrition on yield, nutritional value and consumer safety of radish microgreens under different photoperiods

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Abstract. The work was aimed to examine the effect of mineral nutrition on yield and content of phytochemicals with antioxidative properties in radish (*Raphanus sativus* var. *radicula*) microgreens grown under different photoperiods (16 h and 24 h). Microgreens were supplied with mineralized water or 50% Hoagland nutrient solution. The study has revealed that robust index (RI, dry mass per unit of stem volume) was increased by the presence of mineral nutrition as expected. However, the relationship between the content of phytochemicals that determine nutritional value of radish microgreens and amount of nutrients in the growing media depended on what phytochemical was under consideration. Thus, plants supplied with Hoagland solution had higher chlorophyll and carotenoid content, while plants supplied with water had higher concentration of such antioxidants as anthocyanins and proline and lower nitrate content. Continuous lighting synergistically increased plant response to nutrient deficiency in terms of elevated anthocyanin and proline content and decreased nitrate content, while enhanced RI, chlorophyll and anthocyanin content in plants supplied with nutrient solution. Thus, microgreen productivity and nitrate content as well as some phytochemicals adding nutritional value to radish have opposite dependence on the level of plant nutrient supply, which suggests growers to make a choice between higher yield or higher health benefits to human health and consumer safety of radish microgreens.

1 Introduction

In the world of nutrition and foods, microgreens are a top tend. Radish microgreens are quickly becoming one of the most popular microgreens among restaurant chefs and home growers. This is not only due to their crisp flavor and vibrant colors, but because radish microgreens are very nutritious. Compared to radish bulbs, radish microgreens are richer in antioxidants, phenolic compounds, ascorbic acid, carotenoids and anthocyanins [1].

Microgreens are usually grown in hydroponic cultivation systems with different nutrient solutions and culture vessels [2]. Information on the applied nutrient solution for microgreen cultivation is scarce, but many solutions currently used are based on a modified 25% [3-7] or

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50% Hoagland solution [8, 9]. Comparative study of the effects of fertilization on *Brassicaceae* microgreens revealed that the use of 50% Hoagland solution resulted in both a high yield and a desirable seedling height, while 12.5% Hoagland solution produced the least acceptable microgreens [10]. In the study of the effects of different Hoagland solution concentrations on the content of value adding compounds in microgreens it was shown that additional nutrient supplementation increased yield and chlorophyll content in rocket, Brussels sprouts, and cabbage microgreens [11]. The response of carotenoid content depended on cultivar and increased in Brussels sprouts and cabbage when nutrient content rose, but decreased in rocket.

Microgreens being suited for indoor production are widely grown in plant factories with artificial lighting. The same daily light integral can be achieved by using high-intensity light conditions for shorter photoperiods as with low-intensity lighting for a longer photoperiod. Few studies have been reported on the effects of photoperiod on microgreens growth and nutrition. Our recent research [12] have shown that growing *Brassicaceae* microgreens under continuous lighting (24-h photoperiod) provided by LEDs resulted in higher yield and robast index (RI) compared to shorter photoperiod, and led to increased content of antioxidants (carotenoids, anthocyanins, flavonoids, proline, antioxidant enzymes). Moreover, it was shown that continuous LED lighting CL significantly decrease nitrate content in arugula, broccoli, mizuna and radish microgreens [13]. Control of nitrate content in microgreens is required as one of the main criteria for “functional foods” is its biological safety. Low light and excessive mineral nutrition may result in higher rate of nutrient uptake than the rate of chemical reduction and consequent accumulation of nitrates [14, 15]. Although nitrates even in high concentrations are not toxic to plants, in the human body their excess is harmful [16].

The aim of the work was to examine the effect of mineral nutrition on the content of phytochemicals with antioxidative properties in radish (*Raphanus sativus* var. *radicula*) grown under different photoperiods (16 h and 24 h). Photoperiodic treatments were introduced in order to estimate how photoperiod may modify plant response to mineral nutrition level.

2 Materials and methods

Radish (*Raphanus sativus* var. *radicula* Pers.) plants were grown in growth chambers at the average temperature of 22±1°C and relative air humidity of 60±5%. Coconut coir mats were used for microgreen culture. Seeded trays were placed for germination in darkness for 3 days and irrigated with demineralized water. Then plants were illuminated by LEDs (LED GL V300, China) with light ratio (%) of red:green:blue – 50:21:18 with light intensity of 270 μmol m-2 s^{-1}. Plants were grown under photoperiods 16/8 h (day/night) or 24/0 h. Once cotyledons were expanded, half of plants were supplied with deionized water and another half of plants with 50% Hoagland nutrient solution. Thus, there were altogether four treatments: (1) 16-h + water, (2) 16-h + nutrient solution, (3) 24-h + water, (4) 24-h + nutrient solution.

On day 12, ten plants from each treatment were randomly selected for the measurements of hypocotyl length and diameter, shoot fresh weight (FW) and dry weight (DW). The robustness index (RI) was calculated as

$$\text{RI} = \text{Hypocotyl diameter} / \text{Hypocotyl length} \times \text{DW [17]} \qquad (1)$$

The content of chlorophyll *a+b* and carotenoids was measured in ethanol extracts with a spectrophotometer SF2000 (Spectrum, Russia) according to [18]. Anthocyanins were extracted from leaves according to [19]. The absorbance of supernatant was measured at 533 (absorption maximum for anthocyanins) and 657 nm (absorption maximum for chlorophyll degradation products). The results were calculated according to [20]. Free proline content was estimated spectrophotometrically by using acid ninhydrin reagent according to [21]. Malondialdehyde (MDA) content was determined at an absorption maximum at 532 nm according to [22]. The content of hydrogen peroxide H_2O_2 was determined according to [23] at an absorption maximum at 390 nm.

The content of nitrates was measured by potentiometric method with pH-meter Anion-4100 (Infraspak-Analit, Russia). Dry shoot tissue (0.3 g) was dissolved in 15 ml of a 1% potassium aluminium sulphate solution and stirred for 5 min. Then the readings of the electromotive force of the solution and pNO_3^- were taken. The measurements were carried out using ion-selective nitrate and silver chloride electrodes connected to calibrated potentiometer. The results are expressed in mg kg^{-1} FW.

The experiment was conducted two times. The tables show mean values and their standard errors. Difference between the mean values was considered significant at $p \leq 0.5$.

3 Results

Plants supplied with nutrient solution had 44% and 24% longer hypocotyl than plants irrigated by water under 16-h and 24-h photoperiods, correspondingly (Fig. 1, Table 1). Aboveground FW of plants treated with nutrient solution was more than 70% higher than that of plants supplied with water under both photoperiods. In respect of shoot DW plants supplied with nutrient solution surpassed watered plants by 64 and 78%, correspondingly under 16-h and 24-h photoperiods. They also had more than 30% higher RI regardless of photoperiod. Absolute RI values for plants supplied with water or nutrient solution were much higher for those grown under 24-h photoperiod compared to 16-h photoperiod (Table 1).

Table 1. Hypocotyl length, fresh (FW) and dry (DW) shoot weight, and robustness index (RI) of radish microgreens.

	16-h photoperiod		**24-h photoperiod**	
	Water	**Nutrient solution**	**Water**	**Nutrient solution**
Hypocotyl length, mm	27.2 ± 1.31^{c}	48.4 ± 1.2^{a}	28.1 ± 1.42^{c}	37.2 ± 1.25^{b}
FW, mg	64.3 ± 3.0^{c}	230.0 ± 2.0^{b}	67.6 ± 4.0^{c}	300.0 ± 10.0^{a}
DW, mg	7.6 ± 0.4^{c}	21.0 ± 2.6^{b}	8.1 ± 0.7^{c}	38.0 ± 1.0^{a}
RI	0.25 ± 0.03^{d}	0.40 ± 0.03^{c}	0.54 ± 0.05^{b}	0.80 ± 0.03^{a}

Here and later different letters indicate significant differences between the mean values at $p \leq 0.05$

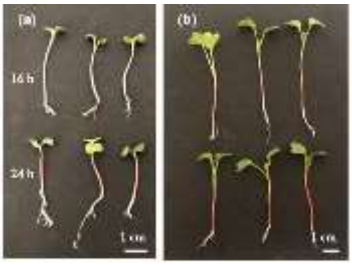

Fig. 1. Radish seedlings grown as microgreens under 16-h (upper row) or 24-h (lower row) photoperiod irrigated by (a) demineralised water or (b) 50% nutrient solution.

Application of nutrient solution increased total chlorophyll content by 14% under 16-h photoperiod and by 24% under 24-h photoperiod (Table 2). Similarly, carotenoid content was increased by nutrient supply by 25 and 28%, correspondingly in plants grown under 16 h and 24 h photoperiod. 24 h photoperiod decreased chlorophyll and carotenoid content in plants irrigated by water, and did not affect it significantly in plants supplied with nutrient solution. In watered plants chlorophyll content was decreased by continuous lighting, but carotenoid content increased. Chlorophyll *a/b* ratio was higher in plants supplied with nutrient solution.

In the case of demineralized water anthocyanin content was higher by more than 70% regardless of photoperiod, while generally anthocyanin content was higher under 24-h photoperiod (Table 2). Proline content was also higher by 66 and 53% in water supplied plants under 16-h and 24-h photoperiods.

Table 2. Content of chlorophylls, carotenoids, anthocyanins, proline, malondialdehyde, hydrogen peroxide, and nitrates in radish microgreens.

	16-h photoperiod		**24-h photoperiod**	
	Water	**Nutrient solution**	**Water**	**Nutrient solution**
Chlorophyll *a+b* content, mg g^{-1}DW	$5.6±0.3^{b}$	$6.5±0.2^{a}$	$4.7±0.5^{c}$	$6.2±0.3^{a}$
Chlorophyll *a/b* ratio	$2.18±0.12^{bc}$	$2.39±0.15^{b}$	$2.10±0.09^{c}$	$2.73±0.08^{a}$
Carotenoid content, mg g^{-1}DW	$0.60±0.05^{c}$	$0.80±0.10^{ab}$	$0.72±0.09^{b}$	$1.00±0.10^{a}$
Anthocyanin content, (A_{530}-$0.25A_{657}$) g^{-1}FW	$0.9±0.2^{b}$	$0.2±0.1^{d}$	$1.6±0.1^{a}$	$0.4±0.1^{c}$
Proline content, µmol g^{-1}	$28.4±0.9^{b}$	$9.7±0.8^{d}$	$35.4±3.4^{a}$	$16.7±2.3^{c}$
MDA, µmol/g FW	$35.5±1.2^{a}$	$18.4±1.3^{b}$	$35.8±3.7^{a}$	$18.0±0.5^{b}$
H_2O_2, µmol/g FW	$0.71±0.02^{b}$	$0.47±0.05^{c}$	$0.85±0.04^{a}$	$0.55±0.04^{c}$
Nitrate, mg kg^{-1}FW	$1058±60^{c}$	$1885±55^{a}$	$836±50^{d}$	$1690±41^{b}$

MDA content was twice as much in watered plants compared to those supplied with nutrient solution regardless of photoperiod (Table 2). Similarly, the content of H_2O_2 was 35% higher.

In contrast, plants cultivated with 50% Hoagland nutrient solution had double amount of nitrates compared to water-supplied plants regardless of photoperiod (Table 2). A 24-h photoperiod decreased the nitrate content in both plants treated by water and nutrient solution.

4 Discussion

The higher FW, DW and RI (dry mass per unit of stem volume) of plants supplied with nutrient solution compared to plants supplied with demineralized water were rather expected as mineral nutrition is an important factor that influences plant growth and productivity. There are many observations of the decrease of microgreens fresh biomass due to nutrient deficiency [11, 24, 25]. Interestingly that water-supplied plants grown under 24-h photoperiod had higher RI than plants supplied with nutrient solution grown under 16-h photoperiod. Thus, higher DLI provided by continuous lighting more than compensated nutrient deficiency in respect of resulted plant biomass. Higher DLI provides additional light for photosynthesis and therefore usually plant biomass increases with an increase of DLI to a saturation point [26]. Thus, if plants tolerate continuous lighting and do not develop leaf injuries, they benefit from constant supply of energy for carbon assimilation and accumulate larger biomass. In our study radish did not suffer from continuous lighting and therefore the highest RI was in plants grown under continuous light and supplied with nutrient solution.

Amount of chlorophyll in leaf tissue is influenced by nutrient availability and therefore higher chlorophyll content in plants supplied with nutrient solution is also expected. However, it should be noted that significant differences in our experiments were observed between very contrasting treatments such as demineralized water and 50% nutrient solution. In the experiments with 25, 50 and 100% Hoagland solution increased nutrient supply did not result in higher contents of chlorophyll in radish cress [27]. Thus, effect of fertigation on chlorophyll content is not simple.

Continuous lighting affects the chlorophyll content the opposite way. In order to reduce the light absorption and protect photosynthetic apparatus from excessive light chlorophyll content decreases. In our experiment the decrease was recorded only in plants irrigated by water, but not nutrient solution and the decrease was not observed visually. It is worth mentioning as the perception of green pigmentation of leaves by consumers is important.

Carotenoid content was higher in plants supplied with nutrient solution. Similar results were obtained for cress plants when highest carotenoid content was observed in plants grown in the nutrient solution with the highest mineral content [27]. Higher concentration of carotenoids under continuous lighting is explained by their protective role against excessive light as they serve as scavengers of free radicals [28].

Nutrient deficiency is an abiotic stress and therfore results in adaptive plant response, which is able to modify the biochemical composition of microgreens in respect of secondary metabolites [29]. Frequently is results in elevated concentrations of value adding compounds [30]. This was the case in our experiments where higher concentrations of MDA and H_2O_2 in plants supplied with water indicate that they had higher level of lipid peroxidation. At the same time contents of anthcyanins and proline were higher in plants supplied with water. Similar results on higher anthocyanin content were reported for cress, rocket, cabbage, and lettuce under nutrient deficiency [11, 27, 31].

Continuous lighting imposes photo-oxidative pressure on plants and induces mild oxidative stress in plants. In response to stress plants accumulate antioxidant bioactive compounds. We recorded higher concentrations of proline and anthocyanin under 24-h photoperiod. Earlier we also reported higher flavonoid contents and elevated activities of antioxidant enzymes (catalase, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase) in *Brassicacea* microgreens grown under continuous lighting [12].

Mineral deficiency has resulted in lower nitrate content in radish microgreens supplied with water as nitrate accumulation depends on nitrogen concentration in the nutrient solution. It should be noted that nitrate content in plants supplied with 50% nutrient solution was still below the limits imposed by corresponding regulations. Nitrate accumulation was also decreased by continuous lighting. Several mechanisms involving are suggested to explain this effect of [15, 32]. Thus, the lowest nitrate content was recorded in watered plants grown under 24-h photoperiod. Hight nitrate content is potentially harmful to human health, therefore lower nitrate content adds value to microgreens in the marketplace ensuring the safety of the product.

5 Conclusions

In the present study radish microgreens were grown under 16-h and 24-h photoperiods and supplied with mineralized water or 50% Hoagland nutrient solution. Thus, plant response to different levels of mineral nutrition modified by photoperiod was investigated. The study has revealed that RI was increased by the supply of plants with mineral nutrition, as expected. However, the relationship between the content of phytochemicals that determines nutritional value of radish microgreens and amount of nutrients in the growing media depended on what phytochemical is under consideration. Thus, plants supplied with Hoagland solution had higher chlorophyll and carotenoid content, while plants supplied with water had higher concentration of such antioxidants as anthocyanins and proline and lower nitrate content. Continuous lighting synergistically increased plant response to nutrient deficiency in terms of elevated anthocyanin and proline content and decreased nitrate content, while enhanced RI, chlorophyll and anthocyanin content in plants supplied with nutrient solution. Thus, microgreen productivity and nitrate content as well as some phytochemicals adding nutritional value have opposite dependence on the level of plant nutrient supply, which suggests growers to make choice between higher yield and photosynthetic pigment content or higher health benefits to human health and consumer safety of radish microgreens.

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