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Proceeding Paper

Zinc Biofortification of Hydroponic Mustard Microgreens Grown under Different Red and Blue LED Lighting Ratios †

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Abstract: Zn deficiency in soil and plants and its low nutritional status in the population encourage studies on the enrichment of agricultural products. Thus, the objective of this study was to evaluate the Zn doses (1, 5 and 10 mg L^{-1}) applied to the hydroponic solution and to increase the concentration of this element in the microgreen of mustard (*Brassica juncea* 'Red Lace'), depending on different blue–red (B:R) light ratios in light-emitting diode (LED) lighting. Mustards were grown hydroponically in controlled environment growth chambers under different B:R light ratios—10%B:90%R, 75%B:25%R. The results showed that the Zn content in mustard increased with increasing Zn dose in hydroponic solution. The B:R ratio at 75%:25% resulted in higher Zn content in mustard compared to 10%B:90%R. However, the higher percentage of blue light and the increasing concentration of Zn in the solution reduced the hypocotyl length and leaf area of mustard microgreens. The results suggest that the addition of Zn in a hydroponic solution and adapted LED lighting could be a suitable way for the cultivation of Zn-biofortified mustard microgreens.

Keywords: *Brassica juncea*; biofortification; growth; Zn; lighting

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1. Introduction

Zinc (Zn) is a microelement that plays a vital role in several organisms, being essential for human and plant growth. In plants, it is involved in the formation and activation of enzymes that impact growth, development, and production [1]. Another important issue is that Zn is a wide-distribution trace element in the human body, necessary for the activity of more than 200 enzymes involved in the maintenance of important metabolic pathways of the organism [2]. The recommended daily allowance (RDA) for Zn is estimated to range between 2 and 5 mg for infants and pre-school children and up to 8–11 mg per day for adult females and males, respectively [3,4]. Approximately one-third of the human population suffers from an inadequate intake of Zn and is significantly higher and widespread in developing countries primarily affecting young children and women [5,6]. Considering the impact of the lack of Zn in soil and plants and low nutritional status in the population, encourage studies on the enrichment of agricultural products with this element. As an alternative, agronomic biofortification, which consists of increasing the accumulation of target nutrients in edible plant tissues through fertilization or other eliciting factors has been increasingly proposed in recent years [7,8]. Microgreens become increasingly popular as a rich source of vitamins, bioactive compounds, and minerals [9–15] and are a promising crop category for Zn biofortification [12,13]. Data reported by other research [11,13] suggest that *Brassicaceae* species could be considered as a good source of Zn, and its actual concentration in young plant tissues is highly influenced by the availability of nutrients during the growth period [13,14].

Leafy greens including *Brassica* are consumed throughout the year and are widely grown in controlled environment agriculture (CEA) where artificial lighting is used [16]. Numerous studies have investigated and reviewed the effects of light-emitting diode (LED) lighting quality on plants [17,18]. Notably, blue and red LED light combinations are most commonly used, as these wavelengths are predominantly absorbed by photosynthetic pigments and sufficiently effective for plant growth and photosynthesis [18,19]. The application of LED lighting could be a promising tool for biofortifying various mineral nutrients including Zn, in plants. Previous several studies have shown a positive effect of blue and red light and their different percentage in diverse lighting spectrum on the mineral nutrients in various plants [16–22]. However, there is a lack of information on the effect of LED light spectrum on alteration in Zn content in leafy greens. Therefore, the objective of this study was to evaluate the Zn doses applied to the hydroponic solution and to increase the concentration of this element in the edible tissues of mustard microgreen depending on the different blue–red light ratios in light-emitting diode lighting.

2. Materials and Methods

The experiment was performed in closed, controlled environment, walk-in growth chambers (4 m × 6 m) in the phytotron complex at the Institute of Horticulture (IH), Research Centre for Agriculture and Forestry, Lithuania. The microclimate in the growth chamber was autonomously and independently controlled using the Phytotron Microclimate Control System developed in IH based on separate microcontrollers (AL-2-24MR-D, Mitsubishi Electric, Tokyo, Japan). The air temperature was measured with resistance temperature detectors (P-100; OMEGA Engineering Ltd., Norwalk, CT, USA), and data for these measurements were transmitted to the microcontrollers. The relative humidity and CO_2 concentration were measured by capacitive sensors (CO2RT(-D); Regin, Kållered, Sweden) and controlled by additional humidifiers. Data were collected every minute, processed, and stored on the operator panel (E1000, Mitsubishi Electric, Tokyo, Japan).

Mustard (*Brassica juncea* L. 'Red Lace') microgreens were used in the experiment. About 2.5 g of seeds (CN Seeds, Cambridgeshire, UK) was sown on the surface of the hydroponic seed sprouting tray (23 cm × 30 cm) with perforated paper and deionized water (pH: 5.5–5.6) and covered with a plastic lid. After four days, trays were uncovered, and deionized water was exchanged with modified Hoagland nutrient solution containing the following average nutrient concentrations [mg L^{-1}]: N, 120; P, 20; K, 128; Ca, 72; Mg, 40; S, 53; Fe, 4; Mn, 0.08; Cu, 0.08; B, 0.16. Microgreens were grown in a hydroponic solution containing different concentrations of zinc (Zn): 1 mg L^{-1} (Zn1), 5 mg L^{-1} (Zn5), and 10 mg L^{-1} (Zn10). Zinc disodium ethylenediaminetetraacetate (Zn EDTA, $C_{10}H_{12}ZnN_2Na_2O_8 \cdot 2H_2O$)) was used in this experiment to maintain different Zn concentrations. In the hydroponic solution, pH was 5.5–6.5, and electrical conductivity (EC) was 1.3–1.7 mS cm^{-1} (GroLine HI9814, Hanna Instruments, Woonsocket, RI, USA). One hydroponic tray represented one replicate. Two trays were used under each radiation condition. The experiments were repeated twice. Seeds were germinated over 18 h with day/night temperatures (±SD) of 21/17 ± 2 °C and relative air humidity of 60 ± 5%.

Microgreens were cultivated under a controllable lighting fixture (HLRD, Hortiled, Kaunas, Lithuania) consisting of blue (B, peak = 447 nm) and red (R, peak = 660 nm) light-emitting diodes (LEDs). The total illuminated area for each treatment was 0.4 m^2. In experiments, R and B LEDs were used at different PFD ratios: 10%B:90%R, 75%B:25%R (treatments code B10R90, B75R25). All lighting treatments delivered the same total photon flux density (TPFD) of 220 μmol m^{-2} s^{-1}. The photon distribution of all lighting treatments was measured using a portable photometer-radiometer at the tray surface level (RF-100, Sonopan, Poland).

At 10 days after sowing, microgreen cotyledons were harvested with stems near the ground level. Samples were harvested from the center of the container, leaving plants in the 1.5 cm edge as a guard. The dry and fresh weight of microgreens was determined by

the gravimetric method using an electronic analytical balance (Mettler Toledo AG64, Columbus, OH, USA). The leaf area of microgreens was measured using the WinDIAS meter (Delta-T Devices Ltd., Cambridge, UK). Biometric measurements and fresh and dry weights were performed on thirty plants and nondestructive measurements on ten plants of three replications that were randomly selected from the edges and middle of each tray. Samples of microgreens used for mineral nutrients analysis as well as for dry weight were washed with deionized water and dried at 70 °C for 48 h in a drying oven (Venticell 222, MBT, Brno-Zábrdovice, Czech Republic). Conjugated experiment samples for mineral nutrients determination were stored in tightly closed 50 mL plastic bags until analysis. The analysis was performed in 3 biological with 3 analytical replications (total 9 analytical replications per treatment).

Nondestructive measurements of leaf chlorophyll (CHL) and flavonol (FLA) indexes were performed using the Dualex 4 Scientific® (FORCE-A, Orsay, France) meter.

Spectral reflectance was measured using a leaf spectrometer (CID Bio-Science, Camas, WA, USA) from 9:00 to 12:00 a.m. Reflection spectra obtained from the leaves were used to calculate various indexes according to formulas presented by produces. The anthocyanin reflectance index (ARI1), which shows changes in the anthocyanin content:

$$ARI1 = 1/R550 - 1/R700 \quad (1)$$

The carotenoid reflectance index (CRI2) shows changes in the carotenoid-to-chlorophyll ratio:

$$CRI2 = 1/R510 - 1/R700 \quad (2)$$

R700, R550, and R510 represent the leaf reflectance integrated over a 10 nm wavelength band centered on 700, 550, and 510 nm, respectively.

The contents of mineral nutrients in lettuces were determined using a modified microwave-assisted digestion technique combined with ICP–OES methods as described by Araújo et al. [23] and Barbosa et al. [24]. The content of Zn in mustard was recounted as mg g^{-1} dry weight. The Zn bioconcentration factor (BCF) and transfer factors (TF) of the roots and shoots were calculated according to Bian et al. [25].

Statistical analysis was performed using Microsoft Excel 2016 and Addinsoft XLSTAT 2019.1 XLSTAT statistical and data analysis (Long Island, NY, USA). Two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test ($p < 0.05$) for multiple comparisons was used to evaluate differences between means of measurements.

3. Results and Discussion

According to the literature data, plants accumulate a higher amount of Zn with increasing Zn concentration in the growth medium [6]. The data from our study confirm this statement and show that the Zn content in mustard microgreens increased with an increasing dose of Zn in the hydroponic solution (Table 1). Moreover, the higher proportion of B light in LED lighting enhanced this effect.

Previous research indicates a different response of Zn accumulation in plants depending on applied illumination; a higher proportion of R light promoted Zn accumulation in Chinese kale [26], more Zn was found in einkorn seedlings at a higher proportion of B light [27], while in *Brassica* seedlings the B:R light ratio did not effect Zn content [28]. The bioconcentration factor (BCF) indicates the capability of plants to accumulate mineral nutrients in roots and the translocation factor (TF)—in the aboveground tissue. In mustard microgreens, BCF_{Zn} and TF_{Zn} tend to decrease with increasing Zn concentration in solution, and the values determined were highly dependent on the applied illumination (Table 1). BCF_{Zn} and TF_{Zn} were higher under B75R25 LED lighting, except for Zn5 and Zn1 treatments, respectively. In general, several studies have shown the positive effect of B light on the accumulation of mineral nutrients in various microgreens [21,29].

Table 1. Effect of different blue-red light ratios in LED lighting and zinc doses on zinc content (Zn), bioconcentration (BCF_{Zn}), and translocation (TF_{Zn}) factors in mustard microgreens.

Variables	Treatment						Source of Variance		
	B10R90			B75R25			L	Zn	L×Zn
	Zn1	Zn5	Zn10	Zn1	Zn5	Zn10			
Zn, mg g^{-1} dry weight	0.118 ^{f}	0.312 ^{d}	0.514 ^{b}	0.160 ^{e}	0.418 ^{c}	0.621 ^{a}	*	*	*
BCF_{Zn}	46.5 ^{d}	58.1 ^{b}	38.8 ^{f}	90.5 ^{a}	53.2 ^{c}	44.4 ^{e}	*	*	*
TF_{Zn}	2.53 ^{a}	1.07 ^{e}	1.33 ^{d}	1.76 ^{b}	1.57 ^{c}	1.40 ^{d}	*	*	*

B10R90, B75R25—a percentage of blue (B) and red (R) light. Zn1, Zn5, Zn10—zinc doses 1, 5, 10 mg L^{-1} respectively. L—blue and red light. All values in the table are expressed as mean ± standard error (n = 9). Means with different letters are significantly different at the $p < 0.05$ level by Tukey's honestly significant difference test. * significant at $p < 0.05$.

Numerous studies have shown that R, B, or R:B LED light can affect the accumulation of bioactive compounds and pigmentation of microgreens. In this study, applied lighting along with the increasing concentration of Zn in the solution had the opposite effect on the chlorophyll index (CHL) (Table 2).

Table 2. Effect of different blue-red light ratios in LED lighting and zinc doses on the chlorophyll (CHL), flavonols (FLA), anthocyanin reflectance (ARI1), and carotenoid-to-chlorophyll ratio (CRI2) indexes of mustard microgreens.

Variables	Treatment						Source of Variance		
	B10R90			B75R25			L	Zn	L×Zn
	Zn1	Zn5	Zn10	Zn1	Zn5	Zn10			
CHL	20.33 ^{bc}	20.13 ^{bc}	17.97 ^{c}	20.09 ^{bc}	21.67 ^{ab}	23.47 ^{a}	*	*	
FLA	0.85 ^{a}	0.81 ^{a}	0.80 ^{a}	0.71 ^{a}	0.73 ^{a}	0.78 ^{a}		*	
ARI1	0.074 ^{a}	0.083 ^{a}	0.050 ^{b}	0.050 ^{b}	0.046 ^{b}	0.048 ^{b}	*	*	*
CRI2	0.074 ^{a}	0.080 ^{a}	0.057 ^{b}	0.059 ^{b}	0.054 ^{b}	0.056 ^{b}	*	*	*

B10R90, B75R25—a percentage of blue (B) and red (R) light. Zn1, Zn5, Zn10—zinc doses 1, 5, 10 mg L^{-1} respectively. L—blue and red light. All values in the table are expressed as mean ± standard error (n = 3). Means with different letters are significantly different at the $p < 0.05$ level by Tukey's honestly significant difference test. * significant at $p < 0.05$.

An increased B light percentage in LED lighting resulted in decreased values of flavonol index (FLA), anthocyanin reflectance index (ARI1), and carotenoid reflectance index (CRI2) in mustard microgreens. Other research results indicate the contrasting genotypic responses of microgreens to B and R light regarding photosynthesis and accumulation of bioactive compounds; an increased B light proportion increased FLA and decreased CRI2 in kale microgreens, and decreased the values of ARI1, CRI2 in mustard microgreens [30].

The results from assaying growth parameters show that having a higher percentage of B light (B75R25) in the illumination spectrum and increasing concentration of Zn in the solution resulted in reduced length of hypocotyl and leaf area of mustard microgreens (Table 3). There was an observed trend that increasing Zn concentration in solution increased the growth response of mustard microgreens as they were grown under B10R90 light. As is known the length of the hypocotyl is the main quality attribute in microgreens cultivation [12] however the result of this study shows that a higher proportion of B light in B:R lighting and increasing Zn concentration in solution had a stronger inhibitory effect on hypocotyl elongation. A stronger inhibitory effect of B light on hypocotyl elongation has been found in other studies [30]. Also, other authors have supposed that increasing the proportion of B light reduces the fresh mass of various plants [22,31]. In this study, a higher proportion of B light in B:R lighting tended to reduce mustard leaf area and shoot fresh weight.

Table 3. Effect of different blue-red light ratios in LED lighting and zinc doses on the growth parameters of mustard microgreens.

Variables	Treatment						Source of Variance		
	B10R90			B75R25			L	Zn	L×Zn
	Zn1	Zn5	Zn10	Zn1	Zn5	Zn10			
HL	2.18 ^{bc}	2.63 ^{ab}	3.16 ^{a}	2.28 ^{bc}	2.09 ^{bc}	1.78 ^{c}	*	*	
LA	2.25 ^{abc}	2.40 ^{ab}	2.52 ^{a}	2.13 ^{bcd}	1.91 ^{cd}	1.89 ^{d}	*	*	
SFW	60.45 ^{ab}	63.75 ^{a}	61.43 ^{ab}	52.04 ^{ab}	48.45 ^{b}	53.34 ^{ab}		*	
SDW	3.84 ^{a}	4.01 ^{a}	3.96 ^{a}	3.15 ^{a}	2.91 ^{a}	3.53 ^{a}		*	

B10R90, B75R25—a percentage of blue (B) and red (R) light. Zn1, Zn5, Zn10—zinc doses 1, 5, 10 mg L^{-1} respectively. L—blue and red light. HL—hypocotyl length, cm; LA—leaf area, cm^2; SFW—shoot fresh weight, mg; SDW—shoot dry weight, mg. All values in the table are expressed as mean ± standard error (n = 3). Means with different letters are significantly different at the $p < 0.05$ level by Tukey's honestly significant difference test. * significant at $p < 0.05$.

4. Conclusions

In this study, the amount of Zn in mustard microgreens increased with the increasing concentration of Zn in the hydroponic solution. The higher proportion of blue light (B75R25) in the applied LED lighting enhanced this effect. However, this lighting regime has reduced the growth parameters and bioactive compounds of mustard microgreens. The results suggest that Zn biofortification could be a technique in which the inherent Zn status of the edible parts of microgreens could be improved through a proper dose application in the hydroponic solution and adapted LED lighting.

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