

The Response of Baby Leaf Lettuce to Selenium Biofortification under Different Lighting Conditions[†]

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Abstract: Selenium (Se) is an essential microelement for human health having antioxidant and anticancerous properties. One of the ways to increase its concentration in plants is biofortification through various agronomic practices including artificial lighting. The aim of the study was to determine the responses of baby leaf lettuce to various Se content in hydroponic solution at different ratio of blue (B) and red (R) light of light-emitting diodes (LED) lighting. Lettuce (*Lactuca sativa*, 'Little Gem') were grown hydroponically under B:R light ratios - 10%B:90%R, 75%B:25%R. The photon flux density (PFD), photoperiod, temperature, and relative humidity in the growth chamber were maintained at 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 18 h, 21/17 \pm 2°C, and 60 \pm 5%, respectively. Two experiments with Se were performed using sodium selenate (Na_2SeO_4). Results of the first experiment (Se - 0, 1, 3 ppm) showed that the content of Se in lettuce was about 15 times higher at 3 ppm compared to 1 ppm. Similar trends were observed for both B and R ratios. However, even the lowest dose of Se in hydroponic solution inhibited lettuce growth and reduced photosynthesis and chlorophyll content. Therefore, a second experiment was performed with lower Se doses (0, 0.5, 1 ppm) at different growth stages (11th and 17th days after sowing (DAS)). It was found that when Se exposure was initiated at the 17thDAS, the lettuce accumulated a lower content of Se compared to the 11th DAS, but this did not have a negative effect on their growth. The B:R ratio of 10B:90R% resulted in a higher content of Se in the leaves. Overall, these results suggest that properly composed doses of Se, LED lighting and application time could be suitable way for cultivation of selenium-biofortified baby leaf lettuces without any adverse effects on growth.

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1. Introduction

Selenium (Se) is an essential microelement for humans health having antioxidant and anticancerous properties. Recommended Dietary Allowance (RDA) of Se is 55 - 70 $\mu\text{g Se day}^{-1}$ for adults. It is estimated that over 15 % of the world's population are Se deficient [1-5]. One of strategies for improving Se status is the biofortification of plants through various agronomic practices [1, 4, 5]. Hydroponic nutrient solution enriched with Se is useful for enhancement of Se. Studies with leafy vegetables showed that properly selected doses allow to increase Se content without negative effect on biomass and quality of plants [1, 4-8].

On other hand, leafy vegetables are widely cultivated in controlled environment agriculture (CEA), where artificial lighting is used [9]. The application of light-emitting diode (LED) lighting for regulation of light quality and quantity could be a tool for biofortification of various mineral nutrients including Se. Many studies are related to plant cul-

tivation under LEDs of blue (B) and red (R) light as they have the highest photon efficiency. Some of them have demonstrated the positive effect of short-term monochromatic blue light or its higher percentage in different light spectrum compositions on the mineral nutrient content in plants [10-16]. Others have reported that red LED or its higher percentage in blue–red lighting increased various mineral nutrients in marigold [15], lettuce [16], and basil [17]. However, there is a lack of information on how manipulation of the light spectrum affects changes in the Se content of various leafy vegetables. We found information from two studies concerning Se-enrichment in leafy vegetables under different lighting conditions. Bian and co-authors investigated the effects of monochromatic blue and red light, their ratio, and Se forms on Se uptake, nitrate content and photosynthesis in hydroponically grown lettuce [3]. He and co-authors [4] evaluated the combination of Se and different ratios of blue, red and green LEDs light on growth, biomass, phytochemicals and mineral nutrients in broccoli sprouts. The aim of this study was to determine the responses of baby leaf lettuce to various doses of Se in hydroponic solution at different ratio of blue and red light in light-emitting diodes lighting.

2. Materials and Methods

Experiments were performed in closed controlled environment walk-in growth chambers (4 × 6 m) in the phytotron complex at the Institute of Horticulture (IH), Research Centre for Agriculture and Forestry, Lithuania. Lettuce (*Lactuca sativa*, ‘Little Gem’) (CN Seeds, United Kingdom) grown as baby leaf were used in the experiments. One lettuce seed were sown in rockwool cubes (2×2×3.5 cm) soaked with deionized water. Seeds were germinated and seedlings were grown under high pressure sodium lamps (HPS – SonT Agro 400w, Philips) at 18-hour photoperiod with day/night temperatures (\pm SD) of 21/17 \pm 2°C and relative air humidity of 60 \pm 5%. Nutrient solution was added at cotyledon stage. Similar size lettuce seedlings were transplanted into 9-L hydroponic containers at 11th day after sowing (DAS). The experiments were repeated twice. Modified Hoagland nutrient solution with the average concentration of nutrients [mg L⁻¹] N, 120; P, 20; K, 128; Ca, 88; Mg, 40; S, 53; Fe, 1.6; Mn, 0.08; Cu, 0.08; B, 0.16; Zn, 0.8; Mo, 0.2 were used from seedling stage till the end of experiments. The pH was 5.5–6.5, and the electrical conductivity (EC) was 1.3–1.7 mS cm⁻¹ (GroLine HI9814, Hanna Instruments, USA).

Transplanted lettuces were cultivated under a controllable lighting fixture (HLRD, Hortiled, Lithuania), consisting of blue (B - 447 nm) and red (R - 660 nm) light-emitting diodes (LED). In experiments, red and blue 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 μ mol m⁻² s⁻¹. The photon distributions of all lighting treatments were measured using a portable photometer-radiometer at the tray surface level (RF-100, Sonopan, Poland).

Two experiments with selenium (Se) were performed using sodium selenate (Na₂SeO₄). The first experiment (EXP1): Se of 0, 1, 3 ppm were applied at 11th DAS and lasted till 21st DAS. The second experiment (EXP2): Se of 0, 0.5, 1 ppm were applied at 11th DAS and 17th and lasted till 23rd DAS.

Lettuces were harvested at the ground level. Six plants were randomly selected from each container and used for biometric measurements and the determination of fresh and dry weights. Samples of lettuces used for elemental composition 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, Czech Republic). Samples for elemental composition were stored in tightly closed 50 mL plastic bags until analysis. The dry and fresh weight of lettuces was determined by the gravimetric method using an electronic analytical balance (Mettler Toledo AG64, Columbus, OH, USA) and the leaf area was measured using the WinDIAS meter (Delta-T Devices Ltd., UK).

Nondestructive measurements of leaf chlorophyll (CHL) and flavonol (FLA) indexes were performed using the Dualex 4 Scientific® (FORCE-A, Orsay, France) meter. Photosynthetic rate (Pr, μ mol CO₂ m⁻² s⁻¹) was measured using a portable photosynthesis system (LI-COR 6400XT, United States).

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. [18] and Barbosa et al. [19]. The Se bioconcentration factor (BCF) and transfer factors (TF) of the roots and shoots was calculated according to Bian et al. [3].

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

Literature data showed that the supplement of Se can significantly increase its content in leafy vegetables, such as lettuce, spinach, basil without a negative effect on their yield. However, higher doses of Se result in toxicity in plants, and there is a narrow boundary between beneficial and toxic Se concentrations [1, 3, 5, 6, 8]. Our EXP1 results revealed that even a 1 ppm Se dose in nutrient solution negatively affected the growth parameters of baby leaf lettuce under both lighting conditions (Table 1). 3 ppm Se dose more than double decreased leaf area and shoot fresh weight in comparison to plants without Se application. Meanwhile, lower Se doses and different its application time did not affect the number of lettuce leaves (EXP2) (Table 2). There was observed trend that after the application of Se at 17DAS, above mentioned indices and root length were slightly higher in comparison with 11DAS. The light most affected the leaf area and shoot fresh weight. Higher percentage of blue light (B75R25) in LED lighting resulted in decrease of such indexes. According to literature such a blue light effect was found for other plants as well [10, 17].

Se doze of 3 ppm (EXP1) significantly decreased photosynthetic rate (Pr) and chlorophyll index (CHL) in comparison to lettuces grown without Se, except CHL under B75R25 (Table 1). Application of Se 0.5 ppm at 17DAS (EXP2) had positive effect on these indexes in comparison with 11DAS (Table 2). Moreover, a trend was found that a higher percentage of blue light (B75R25) in LED lighting resulted in positive effect on them. These results are consistent with other studies showing that a lower concentration of Se is beneficial for photosynthesis process, while excessive doses could cause damaging effect on it [1, 3, 5, 8].

Table 1. Effect of different blue-red light ratio in LED lighting and selenium doses on baby leaf lettuce.

Variables	Treatment						Source of Variance		
	B10R90			B75R25			L	Se	L×Se
	Se0	Se1	Se3	Se0	Se1	Se3			
LN	7.00±0.00 ^a	6.67±0.29 ^{ab}	6.00±0.00 ^b	7.3±0.58 ^a	6.00±0.00 ^b	5.00±0.00 ^c	*	*	*
LA	157.8±15.5 ^a	124.2±9.1 ^b	70.5±7.7 ^{cd}	120.6±14.6 ^b	98.2±4.8 ^{bc}	43.5±5.4 ^d	*	*	
SFW	5.04±0.09 ^a	3.70±0.23 ^b	2.08±0.28 ^c	3.7±0.46 ^b	2.67±0.27 ^c	1.18±0.15 ^d	*	*	
RL	24.78±1.10 ^{ab}	22.57±2.68 ^{abc}	17.17±2.36 ^{bc}	26.15±5.85 ^a	25.52±3.19 ^{ab}	14.65±1.34 ^c		*	
Pr	17.76±1.21 ^a	14.05±0.7 ^b	7.46±0.33 ^c	16.77±0.62 ^a	16.76±0.67 ^a	7.68±0.55 ^c		*	*
CHL	25.26±0.88 ^a	23.02±1.05 ^{ab}	19.07±0.31 ^b	25.77±0.85 ^a	25.85±1.25 ^a	24.81±3.52 ^a	*	*	
FLA	0.26±0.02 ^d	0.55±0.06 ^{ab}	0.34±0.05 ^{cd}	0.66±0.02 ^a	0.48±0.05 ^{abc}	0.41±0.15 ^{bcd}	*	*	*
P	6.37±0.70 ^d	8.95±1.69 ^{bc}	7.61±0.31 ^{cd}	14.47±0.61 ^a	14.36±0.45 ^a	10.13±0.75 ^b	*	*	*
K	12.75±0.36 ^d	15.67±0.27 ^c	21.38±0.32 ^b	22.09±0.21 ^b	21.96±0.26 ^b	27.05±1.07 ^a	*	*	*
Ca	2.53±0.87 ^c	5.96±2.11 ^b	8.95±0.16 ^a	10.14±0.21 ^a	10.34±0.13 ^a	9.67±0.64 ^a	*	*	*
Mg	2.31±0.41 ^d	3.23±0.32 ^c	4.48±0.14 ^{ab}	4.23±0.03 ^b	4.29±0.08 ^b	4.96±0.25 ^a	*	*	*
S	0.76±0.04 ^{ab}	0.72±0.09 ^b	0.88±0.06 ^a	0.48±0.01 ^c	0.48±0.02 ^c	0.68±0.04 ^b	*	*	
Mn	0.021±0.004 ^c	0.031±0.007 ^{bc}	0.035±0.004 ^b	0.049±0.002 ^a	0.050±0.004 ^a	0.048±0.002 ^a	*	*	*
Fe	0.046±0.007 ^c	0.081±0.033 ^{bc}	0.098±0.011 ^{abc}	0.123±0.010 ^{ab}	0.140±0.026 ^{ab}	0.160±0.034 ^a	*	*	
Zn	0.037±0.002 ^c	0.064±0.018 ^b	0.066±0.002 ^b	0.100±0.005 ^a	0.101±0.002 ^a	0.081±0.004 ^{ab}	*	*	*

Se	-	0.029±0.001 ^b	0.527±0.008 ^a	-	0.038±0.001 ^b	0.542±0.041 ^a	*	
BCF _{Se}	-	136.3±41.5 ^{ab}	65.2±13.7 ^{bc}	-	185.2±54.2 ^a	122.6±7.4 ^{ab}	*	*
TF _{Se}	-	0.22±0.07 ^c	2.78±0.60 ^a	-	0.22±0.07 ^c	1.48±0.20 ^b	*	* *

B10R90, B75R25 – a percentage of blue (B) and red (R) light. Se0, Se1, Se3 – selenium doses 0, 1, 3 ppm respectively. L – blue and red light. LN - leaf number; LA - leaf area, cm²; SFW - shoot fresh weight, g; RL - root length, cm; Pr - photosynthetic rate, μmol CO₂ m² s⁻¹; CHL - chlorophyll index, FLA - flavonols index; Mineral nutrients P, K, Ca, Mg, S, Mn, Fe, Zn, Se expressed as LL (dry weight); BCF_{Se} - bioconcentration factor of Se; TF_{Se} – translocation factor of Se. All values in the table are expressed as mean ± standard error (n = 6). 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.

Table 2. Effect of different blue-red light ratio in LED lighting, selenium doses and their application time on baby leaf lettuce.

Variables	Treatment										Source of Variance						
	B10R90					B75R25					L	Se	DAS	L×Se	L×DAS	Se×DAS	L×Se×DAS
	Se0	Se0.5		Se1		Se0	Se0.5		Se1								
		11 DAS	17 DAS	11 DAS	17 DAS		11 DAS	17 DAS	11 DAS	17 DAS							
LN	6.83±0.29 ^a	6.00±0.00 ^a	6.83±0.29 ^a	6.67±0.58 ^a	6.17±0.29 ^a	6.33±0.58 ^a	6.50±0.50 ^a	6.50±0.50 ^a	6.00±0.00 ^a	6.17±0.29 ^a							
LA	133.7±11.6 ^a	128.1±7.1 ^a	158.3±8.2 ^a	137.0±8.6 ^a	140.2±6.8 ^a	88.4±18.4 ^b	70.0±7.2 ^b	79.3±7.5 ^b	59.2±8.4 ^b	83.1±9.5 ^b	*	*	*				
SFW	4.25±0.31 ^b	3.78±0.28 ^b	5.23±0.37 ^a	3.95±0.37 ^b	4.20±0.44 ^b	2.53±0.18 ^c	2.28±0.26 ^{cd}	2.61±0.31 ^c	1.62±0.31 ^d	2.37±0.27 ^{cd}	*	*	*		*	*	
RL	25.62±4.00 ^{ab}	23.63±2.34 ^{ab}	29.25±0.80 ^a	22.28±0.73 ^{ab}	27.53±0.60 ^{ab}	23.20±2.31 ^{ab}	21.82±4.17 ^{ab}	26.73±1.81 ^{ab}	20.08±2.80 ^b	26.80±1.95 ^{ab}	*		*		*	*	
Pr	14.20±0.77 ^d	16.01±1.11 ^{bcd}	18.79±0.96 ^{ab}	15.04±1.72 ^{cd}	14.11±1.50 ^d	17.58±1.12 ^{abc}	14.92±0.23 ^{cd}	20.06±0.46 ^a	16.24±0.48 ^{bcd}	16.07±0.75 ^{bcd}	*	*	*	*	*	*	
CHL	24.02±0.18 ^{de}	24.40±0.94 ^{cde}	26.08±0.61 ^{bc}	23.09±1.36 ^e	23.09±1.36 ^e	25.57±0.14 ^{bcd}	27.38±0.37 ^{ab}	28.40±0.51 ^a	23.75±0.83 ^{de}	26.11±0.96 ^{bc}	*	*	*		*	*	
FLA	0.33±0.05 ^{bc}	0.35±0.06 ^{bc}	0.27±0.01 ^c	0.40±0.03 ^{bc}	0.36±0.04 ^{bc}	0.43±0.07 ^b	0.62±0.07 ^a	0.67±0.03 ^a	0.43±0.04 ^b	0.64±0.08 ^a	*	*		*	*		*
P	12.12±0.17 ^{cd}	12.55±0.27 ^c	10.31±0.23 ^e	10.99±0.48 ^{cde}	10.97±0.61 ^{de}	14.23±0.26 ^b	16.03±0.99 ^a	15.77±0.63 ^{ab}	11.29±0.14 ^{cde}	16.06±1.00 ^a	*	*	*	*	*	*	*
K	21.91±0.57 ^{ab}	22.04±0.63 ^{ab}	20.91±0.14 ^b	21.43±0.70 ^{ab}	21.46±0.96 ^{ab}	23.21±0.39 ^a	22.72±0.43 ^{ab}	22.84±1.54 ^{ab}	23.10±0.80 ^a	23.30±0.57 ^a	*						
Ca	8.99±0.86 ^{bcd}	9.37±0.08 ^{bc}	7.89±0.05 ^d	8.29±0.37 ^{cd}	7.92±0.37 ^d	9.95±0.50 ^{ab}	11.16±0.27 ^a	11.17±0.19 ^a	8.61±0.43 ^{bcd}	11.31±0.48 ^a	*	*		*	*	*	*
Mg	3.68±0.30 ^{bc}	3.78±0.11 ^{abc}	3.55±0.07 ^{bc}	3.40±0.01 ^c	3.32±0.11 ^c	4.12±0.27 ^{ab}	4.19±0.13 ^{ab}	4.41±0.32 ^a	4.14±0.19 ^{ab}	4.41±0.25 ^a	*		*				
S	0.44±0.02 ^{cd}	0.46±0.01 ^{bcd}	0.40±0.01 ^d	0.44±0.01 ^{bcd}	0.48±0.01 ^{bcd}	0.67±0.05 ^a	0.52±0.03 ^{bc}	0.52±0.05 ^{bc}	0.52±0.05 ^{bc}	0.54±0.01 ^b	*	*		*			
Mn	0.050±0.008 ^{abc}	0.043±0.001 ^{abc}	0.041±0.001 ^{bc}	0.042±0.003 ^{bc}	0.040±0.002 ^c	0.053±0.004 ^{abc}	0.062±0.003 ^a	0.059±0.011 ^{abc}	0.049±0.003 ^{abc}	0.059±0.015 ^{ab}	*	*		*			
Fe	0.14±0.05 ^a	0.15±0.01 ^a	0.12±0.02 ^a	0.15±0.05 ^a	0.10±0.01 ^a	0.15±0.02 ^a	0.20±0.03 ^a	0.18±0.07 ^a	0.17±0.02 ^a	0.15±0.02 ^a	*						
Zn	0.082±0.004 ^{bcd}	0.073±0.006 ^d	0.076±0.008 ^d	0.078±0.005 ^{cd}	0.071±0.004 ^d	0.094±0.004 ^{abc}	0.104±0.012 ^a	0.098±0.005 ^{ab}	0.074±0.003 ^d	0.095±0.006 ^{ab}	*	*		*			*
Se	-	0.004±0.000 ^{fg}	0.014±0.001 ^d	0.052±0.003 ^a	0.032±0.001 ^b	-	0.004±0.000 ^f	0.009±0.002 ^e	0.019±0.002 ^c	0.020±0.001 ^c	*	*		*	*	*	*
BCF _{Se}	-	244.2±68.8 ^{bcd}	91.2±19.3 ^{ef}	147.2±26.7 ^{de}	142.6±45.3 ^{de}	-	364.7±55.9 ^{ab}	394.4±37.9 ^a	285.0±76.6 ^{abc}	195.3±44.5 ^{cde}	*	*	*	*			*
TF _{Se}	-	0.031±0.011 ^c	0.313±0.039 ^{ab}	0.361±0.083 ^a	0.241±0.082 ^b	-	0.022±0.004 ^c	0.044±0.012 ^c	0.070±0.018 ^c	0.109±0.031 ^c	*	*	*	*			*

B10R90, B75R25 – a percentage of blue (B) and red (R) light. Se0, Se1, Se3 – selenium doses 0, 1, 3 ppm respectively. DAS – days after sowing. L – blue and red light. LN - leaf number; LA - leaf area, cm²; SFW - shoot fresh weight, g; RL - root length, cm; Pr - photosynthetic rate, μmol CO₂ m² s⁻¹; CHL - chlorophyll index, FLA - flavonols index; Mineral nutrients P, K, Ca, Mg, S, Mn, Fe, Zn, Se expressed as mg g⁻¹ DW (dry weight); BCF_{Se} - bioconcentration factor of Se; TF_{Se} – translocation factor of Se. All values in the table are expressed as mean ± standard error (n = 6). 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 .

Some literature data indicated that Se doses had no impact on phenolic compound content, but positively affected anthocyanin content in basil [1]. Meanwhile, others reported increase of phenolic compounds in lettuce and spinach [6, 8] and flavonoids in lettuce [6]. Our results revealed that effect of Se doses on flavonols index (FLA) depended on B:R light ratio. The B10R90 resulted in the highest FLA when 1 ppm Se dose were applied and B75R25 without Se at EXP1 (Table 1). Generally, higher percentage of blue light caused higher FLA value in both experiments (Table 1 and 2). He and co-authors reported that Se supplementation under higher blue and red light ratio significantly increased total phenolic and flavonoids content in broccoli sprouts [4]. Thought DAS had no significant effect on FLA, but 1 ppm Se dose applied at 17DAS resulted in significantly higher FLA in comparison to 11DAS under B75R25 (Table 2).

Literature data showed positive effect of blue light on mineral nutrients content [10–16]. Some studies revealed effect of different Se doses on mineral nutrients content, but it depended on the species and doses [4, 7, 8]. Our results showed a trend that Se doses positively affected mineral nutrients content under higher percentage of blue light (B75R25) (Table 1 and 2). Meanwhile, DAS had no significant effect on mineral nutrients content. Light had no impact on Se content in lettuce leaves when effects of 1 and 3 ppm were compared (EXP1) (Table 1). The higher Se content were determined at B10R90 and Se dose of 1 ppm as compared to 0.5 ppm (EXP2) (Table 2). Other studies showed positive effect of blue light on Se content in leafy vegetables [3, 4]. 0.5 ppm Se applied at 17DAS resulted in higher Se content compared to 11DAS. The bioconcentration factor (BCF) indicates the capability of plants to accumulate mineral nutrients in roots and translocation factor (TF) indicates the capability accumulate it in the aboveground tissue [4]. BCF_{Se} were higher under B75R25 in both experiments. However, translocation factors (TF) were the highest under B10R90 (Table 1 and 2). Bian and co-authors [3] demonstrated the potential role of wider light spectra in Se uptake in plants and suggested for further investigations.

4. Conclusions

The content of Se in lettuce was the highest at 3 ppm under both blue and red light ratios. However, such a dose of Se inhibited the growth of lettuce and reduced the rate of photosynthesis and chlorophyll content. When 1 ppm Se was applied at 17th DAS under B:R ratios 10B:90R% , lettuce accumulated lower Se content compared to the 11th DAS, but this did not have a negative effect on their growth. Overall, these results suggest that properly composed doses of Se, LED lighting and application time could be suitable way for cultivation of selenium-biofortified baby leaf lettuces without any adverse effects on growth.

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