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Selenium distribution and nitrate metabolism in hydroponic lettuce (*Lactuca sativa* L.): Effects of selenium forms and light spectra

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A deficiency in selenium (Se) in the human diet is a worldwide problem. The intake of Se-rich vegetables can be a safe way to combat Se deficiency for humans. However, most leafy vegetables can accumulate a high content of nitrates, which poses a potential threat to human health. Light is an important environmental factor that regulates the uptake and distribution of mineral elements and nitrogen metabolism in plants. However, the effects of Se forms and light conditions, especially light spectra, on the uptake and translocation of Se and on nitrate reduction are poorly understood. In this study, lettuce (*Lactuca sativa* L.) was treated with exogenous Se applied as selenate (10 mmol L⁻¹) and selenite (0.5 mmol L⁻¹) and grown under five different light spectra: fluorescent light (FL), monochromatic red LED light (R), monochromatic blue LED light (B), and mixed red and blue LED light with a red to blue light ratio at 4 (R/B=4), 8 (R/B=8), and 12 (R/B=12), respectively. The effects of light spectra and Se forms on plant growth, photosynthetic performance, Se accumulation and nitrate reduction were investigated. The results showed that the light spectra and Se forms had significant interactions for plant growth, foliar Se accumulation and nitrate reduction. The Se concentration and nitrate content in the leaves were negatively correlated with the percentage of red light from the light sources. Compared to Se applied as selenite, exogenous Se applied as selenate was more effective in reducing nitrate *via* promoting nitrate reductase and glutamate synthase activities. The lowest nitrate content and highest plant biomass were observed under R/B=8 for both the selenate and selenite treatments. The significant effect of the light spectra on the root concentration factor and translocation factor of Se resulted in marked variations in the Se concentrations in the roots and leaves. Compared with FL, red and blue LED light led to significant decreases in the foliar Se concentration. The results from this study suggest that the light spectra can contribute to Se distribution and accumulation to produce vegetables with better food quality.

Keywords: selenium, light spectra, nitrates, nitrogen metabolism enzymes, LEDs, *Lactuca sativa* L.

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1. Introduction

Selenium (Se) is an essential micro-nutrient for maintaining human health (Birringer *et al.* 2002). When enters in metabolism, Se can enhance the anti-carcinogenic capacity of the human body (Clark *et al.* 1996; Diwadkar-Navsariwala *et al.* 2006). Vegetables and cereals are important sources

of Se for humans. However, the low bioavailability of Se in the soil in some areas restricts Se accumulation in vegetables and cereals (Hawkesford and Zhao 2007), which leads to inadequate Se intake levels to possibly prevent cancer (Clark *et al.* 1996). Se deficiency in the diet is a worldwide problem, especially in China, the UK, Eastern Europe and Australia (Pedrero *et al.* 2006). Therefore, there is an increasing demand for Se-enriched food (da Silva *et al.* 2017). Vegetables play important roles in the human diet. The consumption of Se-rich vegetables could be a safe and effective way to solve the problem of Se deficiency. It is known that plants can uptake Se in the form of selenate, selenite and organic species (Schiavon and Pilon-Smits 2017). The exogenous application of Se has been particularly effective in increasing the Se concentration in plants, but this effect varies among species (McKenzie *et al.* 2015; White 2016). An excessive intake of Se can also lead to chronic toxicity for humans, with the recommended daily maximum intake of dietary Se not exceeding 400 mg per day (Combs *et al.* 2001). The target to regulate the Se concentration in vegetables is still therefore unknown. Consequently, there is an urgent need to develop adaptive agricultural strategies to regulate Se uptake and distribution in vegetables.

Nitrate is one of the main forms of nitrogen used for plant growth and development and is widely used in vegetable production, especially in leafy vegetables grown in hydroponic systems (Bian *et al.* 2015). Lettuce (*Lactuca sativa* L.) is the main crop grown in greenhouses and is consumed worldwide due to its flavour and high levels of phytochemicals. However, lettuce is a hyperaccumulator of nitrate and easily accumulates a large amount of nitrate in its leaves (van Eysinga and van der Meijs 1985). The daily consumption of vegetables with high amounts of nitrate is associated with a higher risk for cancers and methemoglobinemia (Eichholzer and Gutzwiller 1998; Inoue-Choi *et al.* 2015). In our previous study, we reported that exogenous Se application had a positive effect in restricting nitrate accumulation in hydroponic lettuce (Lei *et al.* 2017). However, little is known about the relationship between Se accumulation and nitrate reduction in plants under different forms of exogenous Se.

Light is not only the driving force for photosynthesis but also serves as the transduction signal to regulate metabolism in plants (Bian *et al.* 2018a, b). Compared with light intensity and light duration, light spectra have more complex roles in regulating plant growth and development (Bian *et al.* 2015). To date, red and blue light emitting diodes (LEDs) have been proven to be the most efficient artificial light source for driving photosynthesis and are widely used in vegetable production (Hogewoning *et al.* 2010). Previous studies reported that the light spectral composition plays an important role in regulating the accumulation of mineral

elements, such as N, P and K in plants (Kosobrukhov *et al.* 1988; Almansa *et al.* 2017). However, few studies have focused on the effect of light spectra on Se uptake, translocation and accumulation under different forms of exogenous Se treatment.

In this study, we conducted a selenate concentration screening experiment and then comparatively investigated the effects of the light spectral composition and Se forms on the uptake, translocation and accumulation of Se, nitrate metabolism and photosynthetic performance of lettuce grown hydroponically. The main objectives of this study were to: (1) investigate the effect of the combination of light spectra and Se forms on the accumulation and distribution of Se and nitrate reduction and (2) investigate the relationship between the light spectral composition and the nitrate/Se content in lettuce under different forms of exogenous Se treatment. The results of this study are crucial to understanding and revealing the mechanisms that are responsible for Se uptake, distribution and toxicity in plants. Furthermore, the information from this study can provide guidance on producing high nutritional quality vegetables with safe Se concentrations.

2. Materials and methods

2.1. Plant materials and growth conditions

Lettuce seeds (*Lactuca sativa* L. var. Butterhead) were washed with distilled water and germinated at 25°C. After germination, these seeds were sown in sponge cubes (2.5 cm×2.5 cm×2.5 cm) with a density of one seed per sponge cube before being grown in an environment-controlled growth chamber. In the growth chamber, cool-white fluorescent light lamps (FL) were used as the growth light sources. The day/night temperature, relative air humidity, light intensity, photoperiod and CO₂ concentration were (25±1)/(18±1)°C, (75±5)%, 200 mmol m⁻² s⁻¹, 12 h and 400 mmol mol⁻¹, respectively. Every other day, half-strength Hoagland solution (Hoagland and Arnon 1950) was added from the bottom of the cubes to supply nutrition and water for plant growth. To investigate the influence of selenate concentration on lettuce growth, at the end of the dark period of day 21 (21 days after germination), similar size plant seedlings were transplanted into 25-L containers with six different concentrations of selenate (0, 1, 5, 10, 15 and 20 μmol L⁻¹) applied as sodium selenium (Na₂SeO₄) in Hoagland solution (pH (6.8±0.2), (1.9±0.1) dS m⁻¹). These plants were grown under FL for another 20 days.

2.2. Light treatment

In the main light experiment, the plants and previous growth

conditions were the same as above. At the end of the dark period of day 21, similar size seedlings were transplanted into 25-L containers with 0.5 $\mu\text{mol L}^{-1}$ selenite (Lei *et al.* 2017) or 10 $\mu\text{mol L}^{-1}$ selenate (the optimal concentration obtained from the selenate concentration screening experiment) in Hoagland solution. These plants were grown under different light spectra for 25 days. There were five different LED light treatments: monochromatic red LED light (660 nm; R), monochromatic blue LED light (450 nm; B), combined red and blue LED light with a red to blue ratio at 4 (R/B=4), 8 (R/B=8) and 12 (R/B=12), respectively. The plants exposed to FL were used as controls. The details of the light spectra from the light sources used in this study are summarized in Appendix A. The light intensity at the plant canopy was monitored every other day using a light intensity metre (LI-COR 2500, Lincoln, NE, USA). The light intensity was maintained at 200 $\text{mmol m}^{-2} \text{s}^{-1}$ by changing the distances between the light sources and plants. Other environmental factors were the same as those at the seedling stage. There were three replicates with a total of 48 plants per treatment. The nutrient solution with the same Se treatment was replaced every 5 days.

2.3. Plant growth measurements

At the end of the light period of 20 days (selenate concentration screening experiment) or 25 days (light and Se form study) after transplantation, 10 plants were randomly collected from each treatment. These plants were cut at the hypocotyls and weighed separately to obtain the fresh weight (FW) of the shoots and roots. These shoots and roots were dried in an oven at 105°C for 15 min and then at 75°C for 72 h to measure the dry weight (DW).

2.4. Gas exchange measurements

After a light treatment of 25 days, six plants (two plants per replicate, three replicates per treatment) were randomly selected from each treatment. The second fully expanded leaves from the top of the plants were used to monitor gas exchange using a portable photosynthetic apparatus (LiCor-6400, Lincoln, NE, USA). The light intensity, temperature and CO_2 in the leaf chamber of the LiCor-6400 were set at 200 $\text{mmol m}^{-2} \text{s}^{-1}$, 25°C, and 400 mmol mol^{-1} , respectively. The actinic light in the leaf chamber was supplied by the red/blue light source.

2.5. Measurement of the Se concentration

The total Se concentration in the plant leaves and roots were determined as described in the method of Montes-Bayón *et al.* (2006) but with slight changes. Briefly, dried

tissue (0.25 g) was digested with 8 mL of HNO_3 and 2 mL of H_2O_2 at 180°C by microwave digestion until the extracts became clear. The extract solutions obtained were diluted with Milli-Q water up to 50 mL. The total Se concentration was measured by ICP-MS with an external calibration using internal standards (Ga, at 5 ng mL^{-1}). Each experiment was repeated three times.

2.6. Analysis of Se uptake, accumulation and translocation in the plants

The capacities for Se uptake and distribution in the lettuce were expressed as the root concentration factors (RCF) and translocation factors (TF) (Zhang *et al.* 2013; Hurtado *et al.* 2017).

The RCF was calculated in each experimental unit at harvest as follows:

$$\text{RCF} = \frac{\text{Se concentration in the roots}}{\text{The concentration of Se applied in the solution}} \quad (1)$$

The TF, which was the relationship between the Se concentration in roots and in the shoots, was calculated as follows:

$$\text{TF} = \frac{\text{Se concentration in the shoots}}{\text{Se concentration in the roots}} \quad (2)$$

2.7. Measurements of the nitrate content and total nitrogen content

The nitrate content in the lettuce leaves was spectrophotometrically determined as described in the method by Bian *et al.* (2016). The leaf samples (0.5 g) collected from the third fully expanded leaves were ground in liquid nitrogen and suspended in 10 mL of distilled water. The samples were boiled at 100°C for 30 min and then cooled with tap water. After filtration and dilution to 25 mL with distilled water, the extract (0.1 mL) was added to 0.4 mL of 5% (w/v) salicylic acid-concentrated sulfuric acid to react at room temperature for 20 min. The reaction was stopped by adding 9.5 mL of 8% (w/v) NaOH solution. The absorbance monitored at 410 nm was used to calculate the nitrate content with respect to its standard curve. The total nitrogen (N) content in leaves was determined as described in the method by Sorgonà *et al.* (2006).

2.8. Measurement of nitrogen assimilation enzyme activity

The third fully expanded youngest leaves were used to measure the nitrogen reduction enzyme activities. The nitrate reductase (NR, EC 1.6.6.6) activity was spectrophotometrically determined (Hageman and Reed 1980). The absorbance monitored at 540 nm was used

to calculate NR activity with respect to the NO_2^- standard curve. One unit of NR activity was defined as 1 nmol of NO_2^- formed per mg of protein per min.

The method described by Mendez and Vega (1981) was used to measure nitrite reductase (NiR, EC 1.6.6.4) activity. The absorbance was monitored at 540 nm and was used to calculate NiR activity. One unit of NiR activity in the plant leaves was expressed as 1 μmol of NO_2^- catalysed per mg of protein per min.

The glutamine synthetase (GS, EC 6.3.1.2) activity was determined by measuring the formation of glutamyl hydroxamate at 540 nm (Canovase *et al.* 1991). The reaction mixture containing 100 mmol L^{-1} Tris-HCl (pH=7.4), 4 mmol L^{-1} EDTA, 20 mmol L^{-1} MgSO_4 , 6 mmol L^{-1} NH_2OH and 12 mmol L^{-1} ATP was incubated at 26°C for 10 min. The activity of glutamate synthase (GOGAT, EC 1.4.1.13) was determined by detecting the oxidation of NADH in the supernatant at 340 nm (Chen and Cullimore 1988). The reaction buffer contained 0.3 mol L^{-1} NaCl, 50 mmol L^{-1} 2-oxoglutarate, and 50 mmol L^{-1} L-glutamine.

2.9. Statistical analysis

Two-way repeated-measure ANOVA was performed to compare the effects of the light spectral composition, Se forms and their interaction on the Se concentration, RCF and TF of Se, NO_3^- content and nitrogen metabolism enzyme activity in lettuce. Fisher's least significant difference (LSD) test was employed to determine the significant differences among the treatments at $P < 0.05$, when necessary. The relationships between the paired variables were determined by Pearson correlation analysis (two-tailed). The statistical analyses were conducted using SAS 8.1 Software (SAS Institute, Cary, NC, USA).

3. Results

3.1. Response of plant biomass to the exogenous selenate concentrations

The effects of the exogenous selenate concentration

on lettuce fresh weight, dry weight and root length are summarized in Table 1. Low selenate ($< 10 \text{ mmol L}^{-1}$) concentrations promoted lettuce growth (except root length). However, when the exogenous selenate doses were higher than 15 mmol L^{-1} , they led to significant decreases in the fresh weight (FW) and dry weight (DW) of the shoots and roots. Compared with the control (0 mmol L^{-1}), the highest FW and DW of the shoots and roots were obtained under the 10 mmol L^{-1} exogenous selenate treatment. These results indicate that 10 mmol L^{-1} selenate was the optimal concentration for the growth of hydroponic lettuce.

3.2. Effect of the light spectra on plant growth under exogenous selenate and selenite

The growth of lettuce under different light spectra treated with exogenous selenate (10 mmol L^{-1}) or selenite (0.5 mmol L^{-1}) is summarized in Table 2. The growth of lettuce was significantly affected by the light spectral composition and exogenous Se forms. The biomass parameters of the plants under selenate were higher than those under selenite. Under the exogenous selenite treatment, the highest fresh weight (FW) and dry weight (DW) of the shoots and total biomass were obtained under R/B=8, while the highest root FW was observed under R/B=12. The shoot weight and total biomass under R/B=8, R/B=12 and R were significantly higher than those under FL. However, R/B=4 and B led to decreases in these parameters when compared with FL. When the plants were exposed to LED light and exogenous selenate, the FW and DW of the shoots, total biomass and shoot to root ratio showed increasing trends with an increasing red to blue ratio (R/B), reaching a peak at R/B=8, but R/B higher than 8 led to a decrease in these parameters. Compared with FL the plant growth under R/B=4–12 and R were significantly increased, but B led to substantial decreases in the studied parameters.

3.3. Response of the plant gas exchange parameters to the light spectra under exogenous selenate and selenite

Under the two forms of exogenous Se, net photosynthetic

Table 1 Fresh weight, dry weight and root length of lettuce under different Se (Na_2SeO_4) concentrations

Se (mmol L^{-1})	Shoot		Roots		
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Length (cm)
0	38.50±6.7 4 b	1.63±0.22 b	3.45±1.14 cd	0.07±0.02 c	14.3±1.66 a
1	39.26±6.32 b	1.79±0.53 b	4.06±0.39 bc	0.08±0.01 bc	13.38±1.03 a
5	43.44±5.24 ab	2.03±0.20 ab	5.33±1.36 ab	0.11±0.03 ab	17.28±4.40 a
10	52.87±6.67 a	2.46±0.32 a	6.90±1.87 a	0.13±0.02 a	16.26±1.44 a
15	44.73±6.09 ab	2.13±0.27 ab	5.91±1.15 a	0.11±0.02 ab	16.38±1.65 a
20	24.18±5.00 c	1.16±0.26 c	2.99±0.51 d	0.06±0.01 c	13.63±2.53 a

The different letters in the same column indicate significant differences among treatments at $P < 0.05$. Values represent mean±SE ($n=8$ or 10).

Table 2 The growth of lettuce under selenite and selenate and exposed to different red to blue light ratios

Treatment ¹⁾	Shoots		Roots		Total biomass		Shoot to root ratio (DW)
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	
Selenite							
FL	53.19 ±11.87 b	2.92±1.4 ab	5.46±1.89 bc	0.29±0.11 b	58.65±13.05 b	3.21±1.49 ab	9.95±1.70 b
B	3.40±2.18 d	0.62±0.19 c	3.93±1.89 c	0.17±0.11 c	8.33±3.93 d	0.80±0.21 c	4.44 ±0.96 d
R/B=4	32.70±11.51 c	1.78±0.55 b	7.99±2.35 ab	0.32±0.04 b	40.70±11.93 c	2.10±0.54 b	5.71±0.13 c
R/B=8	73.78±7.31 a	3.44±0.46 a	7.65±1.36 ab	0.54±0.20 a	81.43±6.30 a	3.98±0.65 a	6.64±1.30 c
R/B=12	64.14±5.82 ab	2.78±0.42 ab	8.95±2.52 a	0.36±0.14 b	73.09±5.94 a	3.14±0.31 ab	10.18±0.98 a
R	66.08±6.52 ab	3.43±0.75 a	7.65±1.29 ab	0.35±0.09 b	73.67±7.77 a	3.77±0.73 a	10.46±1.62 a
Selenate							
FL	37.58±15.22 c	2.42±0.78 c	4.86±2.12 c	0.29±0.11 bc	2.44±17.03 d	2.71 ±0.86 c	8.28±0.56 c
B	13.62±5.49 d	1.03±0.34 d	2.43±0.82 c	0.18±0.02 c	16.05±6.20 e	1.19±0.36 d	7.19±1.81 c
R/B=4	82.78±23.67 b	4.06±0.91 b	8.48±1.70 ab	0.45±0.12 a	91.26±14.70 c	4.51±0.98 b	9.30±1.07 bc
R/B=8	118.04±4.01 a	6.06±0.64 a	10.76±0.95 a	0.56±0.14 a	127.52±1.95 a	6.56±0.79 a	13.61±2.22 ab
R/B=12	103.86±10.68 a	5.37±1.19 a	7.50±1.53 b	0.40±0.03 ab	111.36±6.09 b	5.77±1.19 ab	13.61±2.22 a
R	66.33±11.37 b	4.06±0.74 b	10.04±2.09 ab	0.46±0.14 a	76.37±12.72 c	4.53±0.87 b	8.96±1.31 bc
Statistical analysis							
Se forms (Se)	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> =0.834	<i>P</i> =0.276	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001
Light spectra (L)	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.05
Se×L	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> =0.574	<i>P</i> =0.633	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> =0.126

¹⁾ Six different light spectra: fluorescent light (FL), monochromatic red LED light (R), monochromatic blue LED light (B), and mixed red and blue LED light with a red to blue light ratio at 4 (R/B=4), 8 (R/B=8), and 12 (R/B=12), respectively. Different letters in the same column indicate significant differences at *P*<0.05. Values are mean±SE (*n*=8 or 10).

rate (P_n) and stomatal conductance (G_s) of the plants exposed to different light spectra showed similar change tendencies. These parameters for plants under LED light (except P_n under R/B=12) were higher than those under FL. The highest P_n and G_s of the plants treated with exogenous selenite and selenate were both observed under R/B=8. Under the selenate treatment, the values of P_n and G_s for R/B=8 and R/B=4 were significantly higher than those under selenite (Fig. 1-A and B). The transpiration rate (T_r) of the plants treated with selenate was significantly higher than that under selenite. Under the selenate treatment, the T_r values under R/B=4 and R/B=8 were markedly higher than those under FL, but this parameter did not differ significantly between R/B=4 and R/B=8. In contrast, the T_r values of the plants treated with a combination of selenite and different light spectra of LED light were comparable to those with FL (Fig. 1-C).

3.4. Effect of the light spectral composition on the Se concentration in lettuce under exogenous selenate and selenite

The accumulation of Se in the lettuce leaves and roots depended on the forms of exogenous Se and light spectra. The light spectra and Se forms had a significant interaction for the Se concentrations in the plant roots and leaves (Fig. 2). Compared with selenite, exogenous selenate led to substantial Se accumulation in the lettuce leaves and roots. After treatment with selenate, the Se concentrations in the leaves and roots were 33.65–66.13 times and 3.83–7.39

times higher, respectively, than those of the selenite-treated plants. Compared with FL, LED light exposure led to a significant decrease in the Se concentration in the leaves and roots. The lowest foliar Se concentrations in the plants treated with selenate and selenite were observed under monochromatic blue and red LED light, respectively (Fig. 2-A). However, the lowest concentration of Se in the roots was obtained under R/B=4 (Fig. 2-B).

3.5. Accumulation and translocation of Se in response to the light spectra and different forms of Se

The RCF and TF could be used to reflect the capacities for Se uptake, accumulation and translocation in response to the light spectra and exogenous Se. The RCF and TF of Se were significantly affected by the light spectral composition and applied forms of Se. Regardless of the concentration difference, the RCF for exogenous selenite was approximately 5 times higher and the TF was 6.8–11.9 times lower than that for exogenous selenate (Table 3). With regard to the selenate treatment, the values of RCF and TF in the plants exposed to red and blue LED were lower than those under FL. The RCF of Se under R/B=8 was markedly higher but the TF of Se was lower than that under the other LED light spectral treatments. Interestingly, these parameters for the plants under R/B=4 changed in an opposite direction compared to those for R/B=8, as shown by the lowest RCF and highest TF under R/B=4. These results indicate that R/B=8 was more efficient in promoting Se absorption, while R/B=4 was more effective in promoting Se

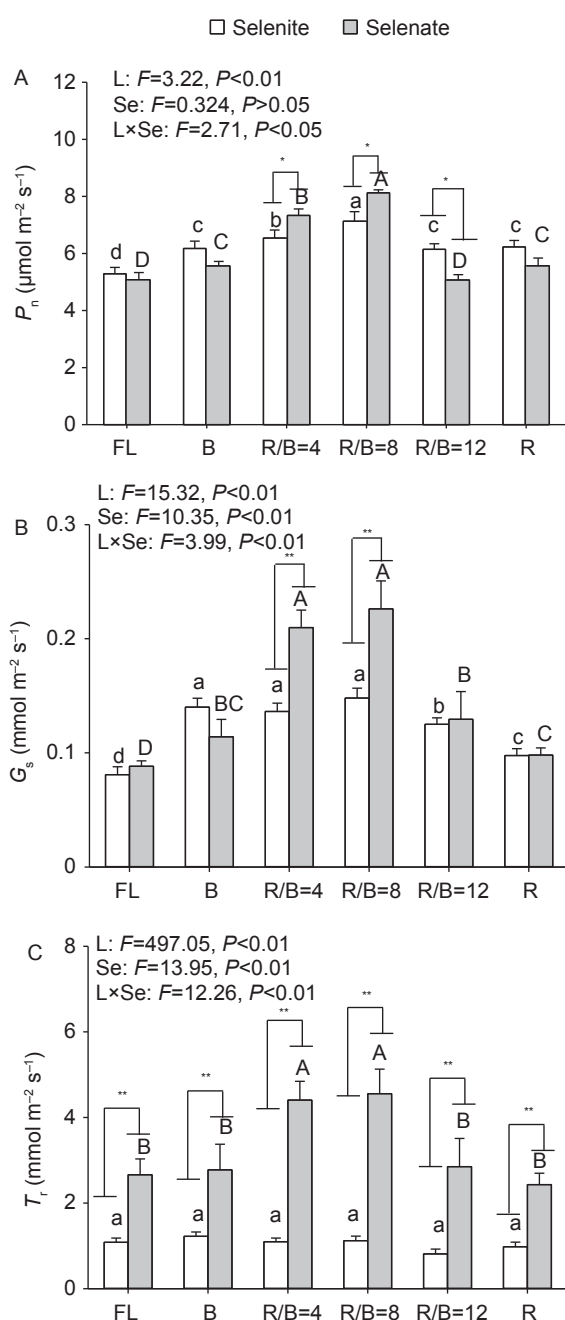


Fig. 1 The effect of light spectra on gas exchange parameters under exogenous selenate and selenite. A, net photosynthetic rate (P_n). B, stomatal conductance (G_s). C, transpiration rate (T_r). L, light spectra treatment; Se, exogenous selenite and selenite treatments. FL, fluorescent light; R, monochromatic red LED light; B, monochromatic blue LED light; R/B=4, R/B=8, and R/B=12, mixed red and blue LED light with a red to blue light ratio at 4, 8, and 12, respectively. Different letters in the same column indicate significant differences at $P<0.05$. * and ** indicate significant differences in each parameter between selenite and selenate treatment under the same light spectra at $P<0.05$ and $P<0.01$, respectively. Values are the mean±SE ($n=4$ or 6).

transportation in lettuce plants. In contrast, the highest and lowest RCF of Se under exogenous selenite was observed in

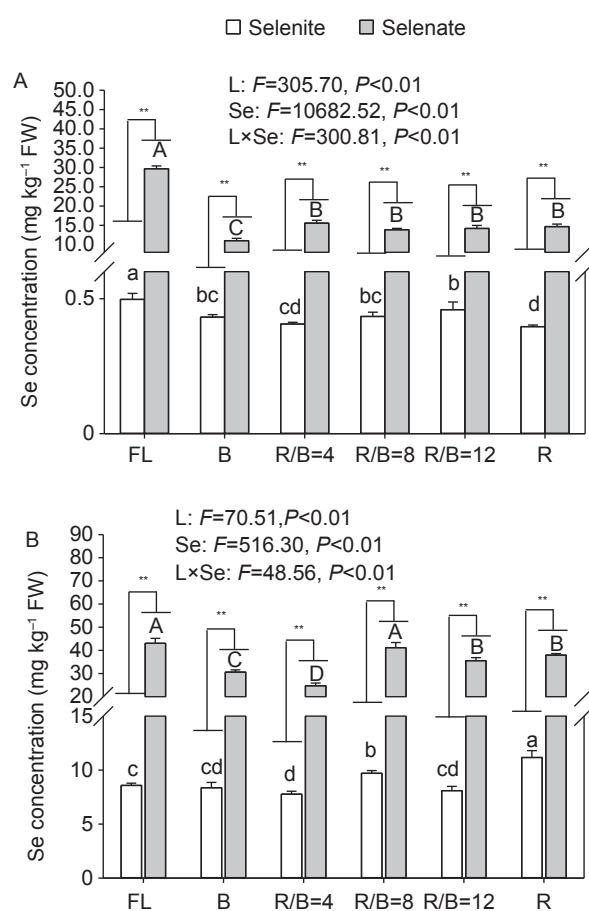


Fig. 2 Effect of the light spectra on the Se concentrate in lettuce leaves (A) and roots (B) under exogenous sodium selenate and sodium selenite. L, light spectra treatment; Se, exogenous selenite and selenite treatments. FL, fluorescent light; R, monochromatic red LED light; B, monochromatic blue LED light; R/B=4, R/B=8, and R/B=12, mixed red and blue LED light with a red to blue light ratio at 4, 8, and 12, respectively. Different letters in the same column indicate significant differences at $P<0.05$; * and ** indicate significant differences in each parameter between selenite and selenate treatment under the same light spectra at $P<0.05$ and $P<0.01$, respectively. Values are the mean±SE ($n=3$).

the plants exposed to R and R/B=4, respectively. However, the RCF under the other light treatments was comparable to that under the FL treatment. Compared with FL, red and blue LED light (except R/B=12) led to significant decreases in the TF of Se in the plants treated with exogenous selenite, and the lowest TF of Se was observed under R.

3.6. Effect of the light spectra and selenium forms on the nitrate content and nitrate metabolism enzyme activities

No significant difference was observed for the total nitrogen (N) content under different light spectra and exogenous Se treatments (Appendix B). However, the nitrate contents

and N assimilation enzyme activities in the lettuce leaves were markedly affected by the Se forms and light spectral composition (Table 4). The nitrate contents in the lettuce treated with selenate were lower than those under selenite, indicating that compared with selenite, exogenous selenate was more efficient in retarding nitrate accumulation in the lettuce plants. Relative to FL, the nitrate contents were significantly lower under red and blue LED light. Interestingly, regardless of the Se form, the lowest nitrate content was observed in the plants exposed to R/B=8.

NR activity was significantly affected by the light spectra,

applied Se forms and their interaction. The NR activity of the plants treated with selenate was higher than that under the selenite treatments. The highest and lowest NR activity were observed under R/B=8 and B, respectively, after exogenous selenate and selenite application. Unlike the changes in NR activity, NiR activity was mainly affected by the light spectral composition. The highest NiR activity was observed under R/B=8, while this parameter under the other light treatments was comparable to that under FL. The activities of GS and GOGAT under LED light (except R treatment) were higher than those under FL. The highest

Table 3 Effects of the light spectra on the root concentration factors (RCF) and translocation factor (TF) of Se in lettuce under selenite and selenate treatments

Treatment ¹⁾	RCF		TF	
	Selenite	Selenate	Selenite	Selenate
FL	217.33±5.12 c	54.54±2.69 a	0.058±0.001 a	0.69±0.02 a
B	211.83±12.39 b	38.77±1.22 c	0.051±0.002 b	0.36±0.03 d
R/B=4	196.72±3.69 c	31.28±1.49 d	0.052±0.003 b	0.63±0.03 b
R/B=8	245.77±3.69 b	52.17±2.78 a	0.045±0.002 c	0.37±0.01 e
R/B=12	204.91± 0.12 cd	45.00±1.65 b	0.057±0.005 ab	0.39±0.01 c
R	282.84±16.09 a	48.04±0.86 b	0.036±0.002 d	0.34±0.01 cd
Statistical analysis				
Se forms (Se)	<i>P</i> <0.001		<i>P</i> <0.001	
Light spectra (L)	<i>P</i> <0.001		<i>P</i> <0.001	
Se×L	<i>P</i> <0.001		<i>P</i> <0.001	

¹⁾ Six different light spectra: fluorescent light (FL), monochromatic red LED light (R), monochromatic blue LED light (B), and mixed red and blue LED light with a red to blue light ratio at 4 (R/B=4), 8 (R/B=8), and 12 (R/B=12), respectively. Different letters in the same column indicate significant differences at *P*<0.05. Values are mean±SE (*n*=3).

Table 4 Nitrate content and enzymatic activities of nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), and glutamate synthase (GOGAT) in lettuce exposed to different light spectra under exogenous selenite and selenate treatments

Treatment ¹⁾	Nitrate content (mg kg ⁻¹)	NR (μmol of NO ₂ ⁻ formed mg ⁻¹ protein min ⁻¹)	NiR (nmol of NO ₂ ⁻ catalyzed mg ⁻¹ protein min ⁻¹)	GS (μmol γ-glutamylhydroxamate g ⁻¹ protein min ⁻¹)	GOGAT (μmol NADH oxidized g ⁻¹ protein min ⁻¹)
Selenite					
FL	5 046.87±546.87 a	23.48±6.47 b	7.62±0.58 b	9.74±0.62 d	9.94±0.73 c
B	3 489.58±119.37 c	28.88±3.51 ab	9.67±1.22 b	18.69±1.23 a	16.22±0.38 a
R/B=4	1 921.88±620.09 d	32.02±3.71 a	7.80±1.63 b	19.73±1.24 a	9.07±0.68 c
R/B=8	828.12±206.69 e	32.03±3.38 a	12.86±0.53 a	19.97±1.67 a	11.42±0.24 b
R/B=12	3 972.08±332.01 b	23.79±2.17 b	8.50±0.21 b	13.81±0.43 b	13.39±2.26 b
R	3 661.46±636.29 bc	15.97±0.51 c	9.58±1.83 b	11.29±0.74 c	8.78±1.04 c
Selenate					
FL	3 744.79±238.67 a	30.22±1.68 b [*]	8.36±0.72 b	11.73±0.28 d [*]	12.65±1.42 d [*]
B	932.29±45.01 c	30.92±2.19 b	9.15±0.37 b	17.78±1.20 b	53.60±1.52 a [*]
R/B=4	802.08±236.68 c	32.45±3.58 b	8.41±0.37 b	15.73±0.35 c	17.17±1.28 c [*]
R/B=8	515.62±201.79 c	39.21±2.23 a [*]	13.32±0.21 a	21.53±0.70 a	19.91±1.72 b [*]
R/B=12	645.83±162.63 c	33.00±1.88 b [*]	8.69±0.56 b	17.82±0.61 b [*]	17.64±0.51 c [*]
R	2 677.08±325.90 b	23.02±1.28 c [*]	9.78±0.62 b	11.54±1.47 d	12.41±0.69 d [*]
Statistical analysis					
Se forms (Se)	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> =0.208	<i>P</i> =0.379	<i>P</i> <0.001
Light spectra (L)	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001
Se×L	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.05	<i>P</i> <0.01	<i>P</i> <0.001

¹⁾ Six different light spectra: fluorescent light (FL), monochromatic red LED light (R), monochromatic blue LED light (B), and mixed red and blue LED light with a red to blue light ratio at 4 (R/B=4), 8 (R/B=8), and 12 (R/B=12), respectively. The significant differences (*P*<0.05) in each parameter under different light treatments are indicated by different letters. ^{*} indicates significant differences in each parameter between selenite and selenate treatment under the same light spectra at *P*<0.05. Values are mean±SE (*n*=3).

GS activities were observed under R/B=8, while the GOGAT activities were the highest in the plants exposed to B for both the exogenous selenite and selenate treatments.

3.7. Relationships between the Se/nitrate content and the percentage of red light

Under the exogenous selenate and selenite treatments, the Se and nitrate contents in the lettuce leaves (except under monochromatic blue light treatment) were both negatively correlated with the percentage of red light from the light sources (Fig. 3-A and B). However, there was a significant linear relationship between the Se and nitrate content under exogenous selenate and selenite combined with different light spectral compositions (Fig. 3-C). These results indicate that the accumulation of Se and nitrate were regulated by the light spectral composition and that a higher ratio of red light was not conducive to Se and nitrate accumulation in lettuce.

4. Discussion

Se is an essential mineral element for both humans and animals and is mainly acquired from plants (Eiche *et al.* 2015; White 2016). Exogenous Se application can increase the concentration of Se in the edible parts of plants. However, Se has not been proven to be an essential mineral element for plants, and excessive Se can be toxic. In this study, the effect of selenate on lettuce growth was concentrate-dependent: low concentration ($<10 \mu\text{mol L}^{-1}$) promoting growth and high concentration ($>10 \mu\text{mol L}^{-1}$) inhibiting growth (Table 1). These results are consistent with those of previous studies showing that a small amount of Se is beneficial for plant growth, while excessive doses could induce a decrease in the photosynthetic capacity, ultimately leading to reduced biomass or even plant death (Van Hoewyk 2013; Hawrylak-Nowak *et al.* 2015). In the present study, the significant effect of the light spectra and the concomitantly marked interaction between light spectra and Se forms on most of the plant biomass parameters indicate that the light spectrum plays an important role in the process of Se regulation of plant growth. Under mixed red and blue LED light, the higher biomass of selenate-treated plants observed in our study is partly attributed to their high photosynthetic capacity, as shown by higher P_n under selenate than under selenite (Fig. 1-A). Similar results were also reported in wheat by Kaur and Sharma (2018).

Compared with other trace elements, Se is arguably one of the most interesting elements because of the very narrow window between deficiency and toxicity (Schiavon and Pilon-Smits 2017). An excessive intake of Se in the diet can also be harmful to human beings and animals

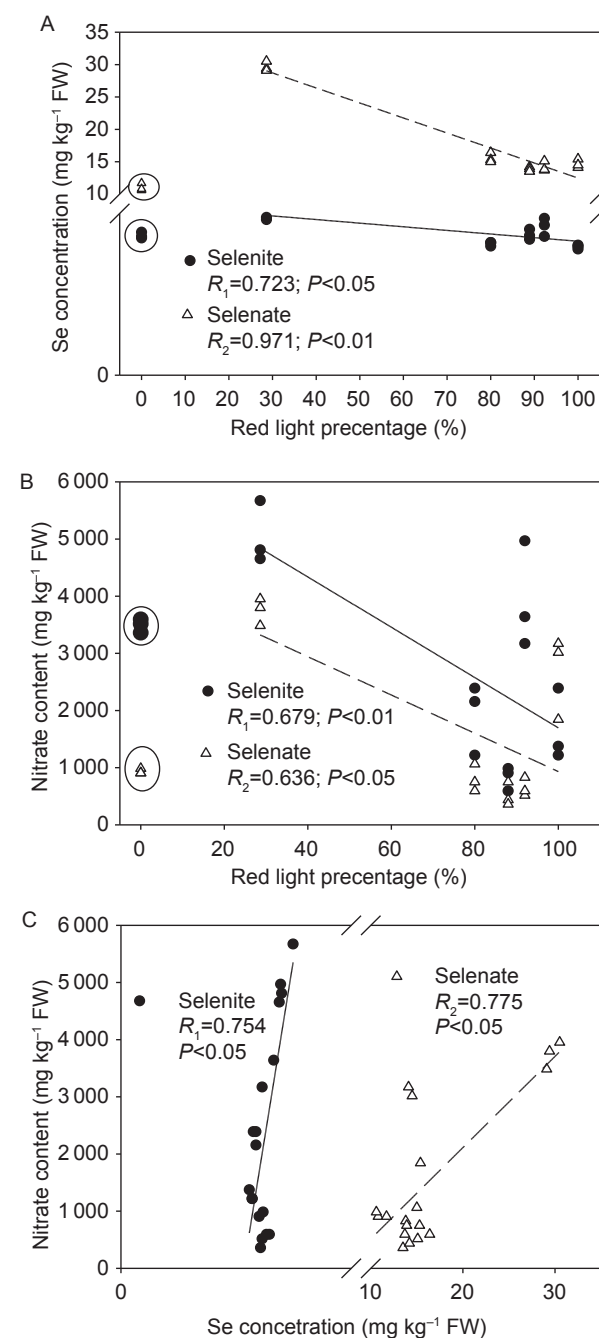


Fig. 3 Correlation analyses revealed links between the leaf Se concentration (A), nitrate content (B) and the percentage of red light of the light spectral composition, and between the Se concentration and nitrate content (C) in the plants treated with selenite and selenate, respectively. The data in the circles are the values of the Se concentration and nitrate content under monochromatic blue light.

(Fordyce 2013). The ability to control Se concentration at safe levels in the edible parts of plants is important, alongside promoting Se accumulation in plants. In plants, mineral element uptake and translocation are regulated by many factors. Light can strongly affect plant cell potentials

or fluxes of ions other than those associated only with energy (Clark 1981). Previous studies have found that the uptake capacity of mineral elements of plant tissue was affected by the light spectral composition (Withrow 1951; Kopsell and Sams 2013). Light spectra as transduction signals can trigger modifications of metabolism (Liu *et al.* 2004) and the uptake of macro and micronutrients in plants (Amoozgar *et al.* 2017). Red LED light may affect water absorption of plants leading to changes in the mineral element contents in leaves; however, blue LED light could alter intervening mechanisms *via* cryptochromes involved in the active uptake of elements. In our study, the concentrations of Se in the leaves and roots were significantly affected by the light spectra (Fig. 2), and the Se concentration in the leaves was negatively correlated with the percentage of red light from the light sources (Fig. 3-A). Hence, the present study confirms the potential for using light spectral technology with LED lighting to regulate Se concentration in plants.

The RCF indicates the root uptake potential, while the TF indicates the translocation potential of mineral elements, such as silver, selenium and arsenic, in plants (Stefanović *et al.* 2016). Compared with red and/or blue LED light, the higher RCF, TF and concentrations of Se in the plants under FL observed in the present study indicate that in addition to red and blue light, other light spectra may participate in and play a dominant role in regulating Se uptake and accumulation. To the best of our knowledge, this is the first study to demonstrate the potential role of light spectra in Se uptake, accumulation and translocation in plants.

In our study, the RCF of selenite-treated plants was almost 5-fold higher but the TF was approximately 10-fold lower than that of the plants under the selenate treatment (Table 3). This result indicates that the uptake Se was much easier to translocate throughout the plant body under the exogenous selenate treatment than under the selenite treatment, while selenite led to Se accumulation in the lettuce roots. This phenomenon could be explained by the different uptake mechanisms and different mobilities of selenite and selenate in plants. Selenate is taken up by plant roots *via* high-affinity sulphate transporters, while the selenite is taken up by plant roots through the process of passive diffusion (Sors *et al.* 2005; Li *et al.* 2008). When absorbed by roots, selenite is rapidly converted to organic forms with limited transferability, which leads to Se accumulation in the roots (Sors *et al.* 2005). In contrast, selenate has highly mobile in the xylem and is not easily assimilated into organic forms (Zayed *et al.* 1998). Furthermore, the translocation of mineral elements probably comprises two main steps: uptake and transfer (Amoozgar *et al.* 2017). The uptake of mineral elements from medium to xylem is energy-dependent. A higher photosynthetic capacity means

more photosynthetic energy could be used for mineral element absorption. Thus, in current study, the differences of Se concentrations in roots may also lie in the different P_n caused by LED light spectra. The transfer of mineral elements from xylem to leaves is driven by transpiration. Leaf transpiration is not only one of the most important driving forces for mineral element translocation in plants but can also promote the uptake of minerals through water transport (Taiz and Zeiger 2010). In the present study, the higher concentration of Se in the roots and leaves as well as the higher TF of Se under the exogenous selenate treatment might be explained by the increase in leaf transpiration rates after selenate application (Fig. 1-C).

In nature, nitrate is an important nitrogen source for plant growth and development. To match the demand for growth and development, plants can integrate potential signals of internal nitrogen status to regulate nitrate uptake and assimilation. Aslam *et al.* (1990) reported that an exogenous Se application using selenate and selenite led to a decline in the reduction of nitrate in barley. However, in our previous study, we found that exogenous selenite had a positive effect on nitrate metabolism in hydroponic lettuce (Lei *et al.* 2017). To reveal the mechanism of Se in regulating nitrate assimilation, we comparatively investigated the effect of Se forms on the nitrate content and metabolism enzyme activity under different light spectra. Compared with the selenite treatment, the lower nitrate content and concomitantly higher activities of NR and GOGAT of selenate-treated plants indicate that selenate was more efficient in promoting nitrate *via* increasing the activities of NR and GOGAT (Table 4). Contrary to our finding in the present study, Rios *et al.* (2010) reported that compared with selenate, exogenous selenite was more efficient in reducing the foliar nitrate content of soil-grown lettuce by enhancing the activities of N metabolism enzymes. The difference between the data reported by Rios *et al.* (2010) and the results in our study may be due to the different application concentrations of Se and the different experimental conditions. Compared with other light treatments, the lower activities of NR and GOGAT in leaves of selenium-treated plants under R might result from the lower Se translation in lettuce after R exposure, as shown by the high RCF and concomitantly low TF. In our study, there was a significant interaction effect between the Se forms and light spectra on the activities of N metabolism enzyme (Table 4). The results of this present study can provide the necessary information not only for solving the problem of Se deficiency in Se-poor areas but also for providing a potential strategy for controlling Se concentration in crops in seleniferous areas. In addition, the presence of a lower nitrate content would enhance

potential health benefits.

In plants, the uptake, translocation and accumulation of Se depend on the Se forms, plant species and other environmental conditions (e.g., sulfate status and light condition) (White *et al.* 2004; White 2016). Many related genes, such as *SULTR1;2*, *SULTR2;1*, *APS* and *SMT*, have been shown to be involved in the processes of Se uptake, distribution and assimilation (Zhao *et al.* 2017; El Mehdawi *et al.* 2018). Light, as an important transduction signal, regulates plant growth and development, triggering related gene expression. Therefore, the regulatory genes and their function in Se metabolism and transformation in plants under different light spectra should be investigated further.

5. Conclusion

The uptake and distribution of Se and the reduction in nitrate in hydroponic lettuce were regulated by the forms of exogenous Se and light spectral composition. The light spectra and Se forms had significant interactions for Se accumulation and nitrate reduction in the lettuce leaves. Red light had a negative effect on foliar Se and nitrate accumulation. Exogenous Se applied as selenate and selenite increased nitrate metabolism, with selenate inducing these physiological processes more strongly than selenite by promoting higher NR and GOGAT activities. Therefore, our study confirms the potential for using light spectra to regulate the Se concentration and nitrate content of natural crops in order to maintain human health.

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Appendices associated with this paper can be available on <http://www.ChinaAgriSci.com/V2/En/appendix.htm>

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