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Abstract: Biofortification of edible crops with selenium (Se) may represent an alternative system for providing selenium in the human diet. The aim of the present study was to provide insights into the ability of basil plants grown in hydroponics to take up Se from the growth substrate, and to study the effects of Se concentration on plant growth and Se accumulation. The addition of sodium selenate at the rates of 4, 8 and 12 mg Se L-1 to the nutrient solution induced a dose-dependent increase in the Se uptake rate. Se was absorbed by the roots and translocated to the above-ground organs and accumulated particularly in the leaves, without affecting the biomass production of the plants. Se concentration increased during seedling growth, was highest in the younger leaves, and then declined before or upon flowering. The results clearly highlight the potential of selenizing basil shoots through the addition of selenate to the nutrient solution. This study provides crucial information for assessing the appropriate Se dosage in order to obtain the desired Se content in leaves, and the best harvest time to obtain the highest leaf Se concentration in basil. The addition of selenate to the nutrient solution could be an efficient system for providing enriched basil plants.

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NATIONAL RESEARCH COUNCIL
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February 20, 2017

Dear Editor,

I would like to submit the manuscript entitled “Selenium uptake and partitioning by basil (*Ocimum basilicum* L) plants grown in hydroponics under different Se concentrations”, for publication in Scientia Horticulturae.

Selenium biofortification may provide alternative system to deliver selenium in human diet. The addition of selenium to the nutrient solution to fortify tissues of basil has not been yet investigated. The present study aimed to investigate whether basil can efficiently take up Se from the nutrient solution and accumulate in leaves, and to study the effects of Se concentration on plant growth and Se accumulation over the basil crop cycle.

This study provides crucial information for Se fortification of basil tissue and for assessing the best harvesting time in order to get the highest leaf Se concentration. Our results showed that the addition of selenate in the nutrient solution might be an efficient system for providing enriched basil plants.

The work submitted is original, unpublished and is not being considered for publication elsewhere.

The English has been revised by an English mother tongue professional editor.

I understand the objectives of the Journal and have formatted the manuscript to fit the style and needs of Scientia Horticulturae.

Yours sincerely,

Beatrice Pezzarossa

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HIGHLIGHTS

- Selenium added to basil plants grown in hydroponics did not affect biomass.
- Se partitioning was observed and the highest percentage accumulated in leaves.
- Se uptake rate increased during the first 27 days of treatment, then decreased.
- Selenium treatments induced a dose-dependent increase in Se uptake rate.
- Se addition to nutrient solution is an efficient tool for selenizing basil leaves.

Uptake and partitioning of selenium in basil (*Ocimum basilicum* L) plants grown in hydroponics

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Abstract

Biofortification of edible crops with selenium (Se) may represent an alternative system for providing selenium in the human diet. The aim of the present study was to provide insights into the ability of basil plants grown in hydroponics to take up Se from the growth substrate, and to study the effects of Se concentration on plant growth and Se accumulation. The addition of sodium selenate at the rates of 4, 8 and 12 mg Se L^{-1} to the nutrient solution induced a dose-dependent increase in the Se uptake rate. Se was absorbed by the roots and translocated to the above-ground organs and accumulated particularly in the leaves, without affecting the biomass production of the plants. Se concentration increased during seedling growth, was highest in the younger leaves, and then declined before or upon flowering. The results clearly highlight the potential of selenizing basil shoots through the addition of selenate to the nutrient solution. This study provides crucial information for assessing the appropriate Se dosage in order to obtain the desired Se content in leaves, and the best harvest

time to obtain the highest leaf Se concentration in basil. The addition of selenate to the nutrient solution could be an efficient system for providing enriched basil plants.

Key words: selenium fertilization; selenate; biofortification; selenium-enriched products

1 Introduction

The biofortification of edible crops with selenium (Se) may represent an alternative system for providing selenium in the human diet. Additional selenium intake in people with low status, without exceeding the toxic threshold, may have long-term health benefits, since Se is involved in metabolic processes such as thyroid hormone metabolism, antioxidant defence and immune function (Tapiero et al., 2003; Rayman, 2012).

The Food and Nutrition Board of the Institute of Medicine (USA) has proposed a Recommended Dietary Allowance (RDA) of 55 µg of Se per day^{-1} and a tolerable upper intake of 400 µg of Se per day^{-1} for adults (Institute of Medicine, 2000). A deficient Se status can be reversed by Se supplementation. Plants are the first link in the food chain, which ends with humans, therefore Se accumulation in plants may prevent Se deficiency in humans. The development of phytotechnologies for selenium biofortification requires a thorough understanding of the uptake, translocation and assimilation processes at the molecular, physiological and agronomic levels (Versini et al., 2016).

The uptake and accumulation of Se in plants depend on the Se chemical form and concentration in the growing medium. Selenate treatment has been found to induce the highest concentration in shoots, followed by SeMet, and selenite treatment, whereas in roots, the highest Se concentrations were found when SeMet was provided, followed by selenite, and then selenate treatments (Lin, 2009). The chemical similarity between selenate and sulfate suggests that selenate is taken up actively by the sulfate transporters, whereas the

uptake of selenite by plants is passive and/or occurs by phosphate transporters (Broyer et al, 1972a; 1972b; Abrams et al., 1990; Terry et al., 2000; *Li* et al., 2008; Zhu et al., 2009). Selenate is readily translocated from roots to shoots, whereas most selenite remains in the roots (Hopper and Parker, 1999; *Li* et al., 2008; Zhu et al., 2009). In addition, Se distribution in plant organs is species specific, and depends on the stage of development and on the physiological conditions of the plant.

Studying the dynamics of Se plant uptake is crucial in controlling the Se content in plants and in reducing the risk of both Se toxicity and deficiency. Knowledge of the ability of selenium to be taken up and assimilated by plants is critical in providing insights into the appropriate Se dosage and the supplementation method in order to achieve the desired Se content in plant tissues. This would also improve the production of safe and effective Se-enriched products and decrease Se-deficiency in the human diet (Liu et al., 2016).

The selenium content in plants can be increased by soil and foliar application, by soaking seeds in Se solution before sowing, seed dressing, or hydroponic cultivation in a nutrient solution containing Se. The addition of selenium to the nutrient solution has been found to increase the Se concentration in lettuce (Rios et al., 2008; Malorgio et al., 2009; Smoleń et al., 2014), chicory (Malorgio et al., 2009), spinach (Ferrarese et al., 2012), and tomato (Pezzarossa et al., 2014) without decreasing the production and qualitative characteristics of the final product. Opposite results were obtained in lupin and sunflower (Ximénez-Embún et al., 2004) which showed a significant decrease in root and shoot dry matter when 1 mg of Se L^{-1} was added to the nutrient solution. In soy, alfalfa and lentil sprouts, as the Se concentration increased in the culture medium, the biomass of the plant decreased (Funes-Collado et al., 2013).

Basil (*Ocimum basilicum* L.), an herbaceous plant belonging to the family of Lamiaceae, is one of the most popular herbs used in the Mediterranean diet. The aromatic leaves, fresh or

dried, are highly valued due to their ability to enhance the flavour of food and to their antioxidant effects (De Masi et al., 2005; Barátová et al., 2016). Several studies deal with the enhancement of selenium content in basil plants through foliar fertilization (Hawrylak-Nowak , 2008; Kopsell et al., 2009; Oraghi Ardebili et al., 2015; Barátová et al., 2016; Mezeyová et al., 2016). The addition of selenium to the nutrient solution in order to fortify basil tissue has not yet been investigated.

The objectives of the present study were to investigate whether basil can efficiently take up Se from the nutrient solution and accumulate in leaves, and to study the effects of Se concentration on plant growth and Se accumulation over the basil crop cycle.

2 Materials and Methods

2.1 Plant material and growth conditions

The experiment was conducted from October 2015 to January 2016 at the Department of Agriculture, Food and Environment of the University of Pisa, Italy (lat. 43° 40' N) on basil (*Ocimum basilicum* L. cv Tigullio). The basil seeds were sown on October 16, 2015 in 254-cell plug-trays filled with perlite and vermiculite, and germinated in a growth chamber at 25°C. 21 days after sowing, seedlings were transferred to a heated greenhouse and placed into separate hydroponic systems, each consisting of a polystyrene tray floating in a 50 L plastic tank filled with nutrient solution. 16 plants were planted in each tank; the crop density was approximately 96 plants m^{-2} (on a ground area basis).

The nutrient solution contained 12.0 mM N-NO_3, 1.0 mM P-H_2PO_4, 2.44 mM S-SO_4, 4 mM Ca, 5 mM K, 2 mM Mg, 1 µM Cu, 40 µM Fe, 5 µM Mn, 1 µM Mo, 5 µM Zn. The pH and electrical conductivity (EC) values were 5.6 and 2.04 dSm^{-1} respectively, and were checked every 2 days. The nutrient solution was renewed once every two weeks and continuously aerated in order to maintain an oxygen content higher than 6.0 g m^{-3}.

Climatic parameters were continuously monitored by a weather station located inside the glasshouse. The minimum and mean air temperatures were 10°C and 16.8°C, respectively, and the relative humidity was 69.6%. Supplementary lighting was provided by high pressure sodium lamps (HPS, SON-T 400 W, Philips) for a constant day length of 9 hours. The cumulative and the daily mean global radiation were 267 and 3.5 MJ m^{-2} respectively.

2.2 Experimental plan

The treatments were arranged in a totally randomized design with four replicates, each consisting of a tank with 16 plants.

Seven days after transplanting, selenium, as sodium selenate (Na_2SeO_4), was added to the nutrient solution at rates of 0 (control), 4.0, 8.0 and 12.0 mg Se L^{-1}. Every two weeks the nutrient solution was replaced with fresh solution containing the same amount of Se, in order to maintain the same Se concentration throughout the experiment. Overall treatments lasted 69 days.

Plant samplings were performed immediately after the selenium supplementation and then after 13, 27, 41, 55 and 69 days of treatment. At each sampling point, one plant per replicate was harvested. Leaves, stems, inflorescences, and roots were separated and the respective fresh weights (FW) were determined. The samples were oven dried at 50°C up to constant weight and the dry weight (DW) was recorded.

2.3 Selenium analysis

Total selenium content was determined at harvest in oven-dried ground leaf samples after digestion with nitric and perchloric acids and reduction by hydrochloric acid (*Zasoski* and *Burau*, 1977). The digests were analyzed by hydride generation atomic absorption spectrophotometry (Varian VGA 77). Glass tubes containing only the chemical reagents

were used as blanks for the analytical quality controls in order to constantly monitor for Se contamination in the chemical hood.

2.4 Data analysis

Selenium concentration in leaves was calculated on a dry weight basis.

Data of total dry weight were used to determine the Relative Growth Rate (RGR) as described by Hunt (1978) on the basis of the following formula:

$$RGR = \frac{(lnDW_2 - lnDW_1)}{(t_2 - t_1)} \quad (1)$$

where DW2 and DW1 are the total plant dry weight (g) recorded at times t_2 and t_1, respectively.

RGR is expressed as g DW g DW^{-1} day^{-1}.

The rate of Se uptake by plants was calculated from the differences in total Se content (K_{P1}, K_{P2}) multiplied by the differences in dry weight logarithm of the root (W_{R1}, W_{R2}), and divided by (T_2-T_1) (Pitman, 1972), as follows:

$$R_K = \frac{(K_{P2} - K_{P1}) \times (log_e W_{R2} - log_e W_{R1})}{(T_2 - T_1) \times (W_{R2} - W_{R1})} \quad (2)$$

Results were expressed as µg g_{root}^{-1} day^{-1}.

The translocation factor (TF) was calculated as the ratio of the Se concentration in the shoots to the Se concentration in the roots (Renkema et al., 2012).

Data were subjected to one-way ANOVA with Se treatment as variables, and mean values were separated by the least significant difference test (P <0.05). Statistical analysis was performed using Statgraphics Plus 5.1 (Manugistic, Rockville, MD).

A multiple non-linear regression was used to predict the Se uptake values as a function of the Se concentration in the nutrient solution (SN) and of the plant dry weight.

At the end of the experiment, the Bioaccumulation Index (BI) was calculated by dividing the Se concentration in the leaves by the Se concentration in the nutrient solution.

All data were tested for homogeneity of error variances using Levene's test (Glaser, 1983).

3 Results

3.1 Plant growth

The biomass of leaves, stem and roots increased steadily during the experiment (Fig. 1). The first inflorescences appeared after 55 days of treatment, and their biomass increased up to the end of the experiment (Table 1).

The addition of Se to the nutrient solution did not significantly affect the biomass production of leaves and inflorescences. Instead, all Se treatments were effective in reducing stem biomass at 27 and 55 days of treatment. Root biomass was reduced only by the highest selenium treatment, i.e. 12 mg Se L^{-1}, after 41 days of treatment (Fig. 1).

The Relative Growth Rate (RGR) of basil plants, calculated over the harvest intervals, showed a downward trend in all plants. The increased selenium concentration in the nutrient solution did not significantly affect the RGR. At the end of the experiment, the RGR was about 50% less than at the first sampling in all treatments (data not shown).

3.2 Se content and uptake

The addition of Se to the nutrient solution resulted in an increased Se concentration in all plant organs (Fig. 2). Increases in selenium concentrations in the nutrient solution resulted in higher Se concentrations in the plants.

In the first part of the experiment, the leaf Se concentration, expressed on a dry weight basis, increased, and then decreased until the end of the experiment. Plants treated with 8 and 12 mg Se L^{-1} reached the maximum leaf Se concentration earlier than plants treated with 4 mg Se L^{-1}, i.e at 27 and at 41 days of treatment, respectively (Fig. 2a).

The Se concentration in the stems increased until the first sampling, and then decreased in the plants treated with 4 and 12 mg Se L^{-1}, whereas in plants treated with 8 mg Se L^{-1} the concentration of selenium decreased after 41 days of treatment (Fig. 2b).

In the roots of plants treated with 8 and 12 mg Se L^{-1} the highest Se concentration was detected after 13 days of treatment, whereas when Se was added at the dose of 4 mg L^{-1} the highest Se concentration in roots was detected after 27 days of treatment (Fig. 2c).

Se in the inflorescences slightly increased until the end of the experiment, however the variance was not significant (Table 1).

The total Se content in the plants, calculated as the product of Se concentration (mg Se g^{-1} DW) per dry weight (g), increased after 13 days of treatment at all of the Se rates added to the nutrient solution. The higher the amount of selenium added to the solution, the higher the amount of selenium accumulating in the plant (Fig. 3). In plants treated with 8 mg Se L^{-1} the total Se accumulated in plants increased until 41 days of treatment and then remained steady. In plants treated with 4 and 12 mg Se L^{-1} the Se content increased until the end of the experiment, however after 41 days of treatment, the increase was statistically significant only at the dose of 4 mg Se L^{-1}. The Se accumulated in plants treated with 4 mg Se L^{-1} reached the highest value at the end of the experiment (Fig. 3).

The highest Se content in plants treated with 4 or 8 mg Se L-1 was about 7% of the Se concentration in the nutrient solution, whereas in plants treated with 12 mg Se L^{-1} the highest Se content was about 6 %.

196 The highest percentage of selenium accumulated in the leaves, followed by the roots, stems
197 and inflorescences (Table 2). The percentage of Se in the stems ranged between 7.6% and
198 8.7% and did not show any differences among Se treatments. Instead, the percentage of Se in
199 the leaves, roots and inflorescences was directly affected by the Se concentration in the
200 nutrient solution (Table 2). By increasing the amount of selenium added to the solution, the
201 percentage of selenium significantly increased in the leaves and inflorescences, whereas it
202 significantly decreased in the roots (Table 2). As a result, the translocation factor increased
203 by increasing the Se content in the nutrient solution from 4 to 8 and 12 mg Se L^{-1} (Table 2).
204 The bioaccumulation index calculated at the end of the experiment was around 3 in all plants
205 treated, irrespective of the amount of selenium added to the nutrient solution. Se
206 accumulation in the leaves in fact increased proportionally to the selenium available in the
207 nutrient solution.
208 The addition of Se to the nutrient solution induced a dose-dependent increase in Se uptake
209 rate (Table 3) which showed the highest value when plants were treated with 12 mg Se L^{-1}.
210 In all treatments, the daily uptake increased during the first 27 days of treatment, and then
211 decreased over time.
212 Based on these results, we explored the relationship between the uptake of Se by the basil
213 plants, the Se concentration in the nutrient solution and the growth of plants. The results of a
214 multiple linear regression among these variables showed a higher variance at higher Se
215 uptake rate values compared to lower values. We thus decided to apply a multiple non-linear
216 regression, using a logarithmic transformation in order to stabilize the variance. The equation
217 of the fitted model was:
218

$$\log_{10} Se\ uptake\ rate = \log_{10}(10.09 + 5.38 \times [Se]SN - 2.42 \times total\ plant\ DW) \quad (3)$$

219 $(n=54;\ r^2=0.74)$

The results showed a relationship among Se uptake rate, Se concentration in the nutrient solution, and total DW. The regression reported in Fig. 4 was sufficient to explain 74 % of the experimental variability. The plot of residuals appears to exhibit homogeneity, normality, and independence, which means that the variance is normally distributed. Se uptake rate was positively related to the Se concentration of the nutrient solution, and negatively related to total plant dry weight. As plants grow, the total plant dry weight increases, thus the Se uptake rate per unit root decreases with plant age.

4 Discussion

Our results showed that selenium, added as sodium selenate to the nutrient solution at rates of 4, 8 and 12 mg Se L^{-1}, did not consistently affect the biomass production of basil plants. As a consequence, the RGR was unaffected by the addition of selenium. No negative effects on biomass production were found in lettuce plants (Smoleń et al., 2014) grown in nutrient solution enriched with 6.33 and 7.88 µM of Se (i.e. 0.5 and 1.5 mg Se L^{-1}), or in spinach plants (Ferrarese et al., 2012) treated with 2.6, 3.9 and 5.2 µM of Se (i.e. 0.21, 0.31 and 0.41 mg Se L^{-1}). In contrast, Hawrylak-Nowak et al. (2015). found a decrease in biomass and photosynthetic pigments in cucumber plants when selenate concentrations in the growth medium reached 80 µM (6.2 mg Se L^{-1}). The phytotoxicity effects of selenium are related to the interference with normal S metabolism (Mikkelsen and Wan, 1990; Pilon-Smits and Quinn, 2010) and result in leaf chlorosis and a decrease in protein synthesis and dry matter production (Mengel and Kirkby, 1987). Basil supplemented with selenium at concentrations ranging from 1 to 50 mg Se m^{-2} through foliar applications have not been shown to have any growth or biomass response (Oraghi Ardebili et al., 2015). In our experiment no evidence of toxic effects was detected at Se concentrations of 170 and 140 µg g^{-1}, respectively in leaves

245 and roots. The tolerance of basil to selenium could be ascribed to the generally high content
246 of phenolic compounds in the leaves. Phenolics in fact could counteract the oxidative stress
247 due to high Se concentrations (Hartikainen et al., 2000; Sakihama et al., 2002).
248 The selenium added to the nutrient solution was absorbed by the roots, and translocated to
249 the above-ground organs, thus increasing the Se content in the different plant parts. Se
250 accumulated particularly in the leaves which is good because generally only the leaves are
251 consumed. These results are in agreement with studies on tomato by Pezzarossa et al. (1999),
252 and chicory (Stibilj et al., 2011). Opposite results have been found in mustard, sunflower and
253 lupin where the roots accumulated the highest amount of selenium (Ximénez-Embún et al.,
254 2004).
255 An increased Se concentration in leaf tissues when Se was added to the nutrient solution has
256 been observed in several species of leafy vegetables such as lettuce (Malorgio et al., 2009;
257 Smoleń et al., 2014), chicory (Malorgio et al., 2009), and spinach (Ferrarese et al., 2012).
258 In basil plants sprayed with 25 and 50 mg Se m^{-2} the concentration of Se in leaves was found
259 to be 2.1 and 6.1 mg kg^{-1} DW, respectively (Mezeyová et al., 2016). Kopsell et al. (2009)
260 reported that basil shoot Se concentrations increased linearly with increasing selenate-Se
261 concentrations from foliar sprays. The highest shoot Se concentration (22.9 µg g^{-1} DW) was
262 observed at 32 ml Se L^{-1} from foliar sprays. No data are currently available in the literature
263 concerning the effects of Se fertilization of basil through nutrient solutions.
264 In our experiment basil efficiently accumulated the Se absorbed by roots in the aerial parts,
265 and the Se concentration in leaves was even higher than results obtained by Kopsell et al.
266 (2009) when the same amount of selenium was applied by foliar spraying. Asher et al.
267 (1977) showed that when selenate was applied to severed tomato roots, the concentration of
268 selenate in the xylem exudates was 6–13 times higher than in the external medium. Since the
269 principal way of selenate translocation in plants is by xylem (Shrift and Ulrich, 1969), this

could explain the higher Se content detected when selenium was taken up by roots compared with foliar application.

These results clearly indicate the potential of selenizing basil shoots by adding selenate to the nutrient solution.

The growing trend in the total Se content accumulated in the plants was more dependent on the biomass, which increased throughout the experiment, than on the Se concentration, which reached the maximum values during the first part of the experiment and then decreased. Our results indicate that in order to obtain leaves with the highest Se concentration, the best harvest time is 4 weeks after treatment for plants treated with 8 and 12 mg Se L^{-1}, and after 6 weeks for plants treated with 4 mg Se L^{-1}. The trend in Se concentration in basil leaves is in agreement with previous studies on different vegetable species (Turakainen et al., 2004; White et al., 2007; Cappa et al., 2014; Harris et al., 2014).

Selenium concentrations generally increased to a maximum during seedling growth, were highest in the younger plant leaves, and then declined before or upon flowering, when Se moved from the leaves to the reproductive organs. The higher Se uptake during the first weeks of growth might be linked to leaf transpiration. In fact, transpiration increases during leaf expansion and stomata development, then decreases due to the stomatal control of transpiration (Wang et al., 2014). Although Se uptake is actively regulated (Breton and Surdin-Kerjan, 1977; Shibagaki et al., 2002), transpiration stimulates Se translocation through the xylem, reducing the Se concentration in root tissues and inducing a further Se uptake from the nutrient solution. The increase in Se concentration detected in basil inflorescences could be explained by the fact that selenium is easily redistributed through the phloem both as selenate and as the organic compounds, SeMet and SeMSeCys (Carey et al., 2012).

294 The reduction in the daily Se uptake after 27 days of treatment could be explained by a
295 reduced Se absorption due to root senescence, and/or with the dilution effect due to plant
296 growth (Zhang et al., 2014).
297 The different trend in leaf Se concentration seems to be related to the amount of Se in the
298 nutrient solution. Higher Se concentrations (8 and 12 mg L^{-1}) may induce a faster uptake and
299 translocation of Se to the leaves which more quickly reach the highest Se concentration.
300 Zhang et al. (2014) found that rice plants reached the highest Se content at the same time,
301 irrespective of the Se dose used for treatments.
302 Since basil is normally consumed as fresh leaves, Se concentration in the leaves was also
303 calculated on the fresh weight (FW) basis. At concentrations of 4, 8 and 12 mg Se L^{-1} in the
304 nutrient solution, the Se concentration in basil leaves was 2.8, 7.9 and 16.9 μg g^{-1} FW,
305 respectively. A daily consumption of 15 g of basil leaves (corresponding to the amount of
306 basil used to prepare the sauce for one portion of 'pasta al pesto') biofortified at 4, 8 and 12
307 mg Se L^{-1} would provide 42, 118 and 254 μg of Se, respectively. The consumption of 15 g of
308 basil leaves fortified at the higher doses of Se (8 and 12 mg Se L^{-1}) would provide a higher
309 amount of Se than the RDA (55 μg d^{-1}), although still under the daily toxic threshold (400 μg
310 d^{-1}). Instead, the consumption of leaves enriched with 4 mg Se L^{-1} would not lead to Se
311 toxicity, but could even provide Se supplementation.
312
313 **5 Conclusions**
314 This study provides in-depth knowledge of the Se uptake by basil and crucial information for
315 the Se fortification of basil tissue and for assessing the best harvest time in order to obtain
316 the highest leaf Se concentration. Our data suggest that the addition of selenium as sodium
317 selenate in the nutrient solution could be an efficient system for providing enriched basil
318 plants.

Further studies are needed to better understand the physiological mechanism that regulates the Se uptake during the growth cycle of basil plants. Physiological measurements such as photosynthetic activity, transpiration rate and stomatal conductance, and studying the expression of the genes responsible for Se uptake and translocation could contribute to improving our knowledge.

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458 **Table 1.** Biomass (g DW plant^{-1}) and Se concentration (µg g^{-1} DW) of inflorescences of
459 basil plants after 55 and 69 days of treatment.

Se added (mg L^{-1})	Biomass of inflorescences (g DW plant^{-1})		Selenium concentration in inflorescences (µg g^{-1} DW)	
	Days of treatment			
	55	69	55	69
0	0.890	2.50	0.3 d	0.3 d
4	0.538	1.88	8.1 c	8.8 c
8	0.685	2.64	25.8 b	33.8 b
12	0.600	1.98	85.6 a	93.0 a
Significance				
Se concentration	ns	ns	***	***

460 Values followed by different letters in the same column differ significantly at 5% level by the LSD
461 test. Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.

462

463 **Table 2.** Proportion of selenium in different parts and Se translocation factor (shoot Se
464 concentration / root Se concentration) in basil plants subjected to different Se treatments.

	Selenium distribution in plants				
Se added (mg L^{-1})	leaf	stem	roots	inflorescences	Translocation Factor
4	53.4% b	8.6%	36.6% a	1.4% c	23% b
8	64.2% a	7.6%	24.6% b	3.6% b	53% a
12	65.2% a	8.7%	20.2% c	5.9% a	56% a
Significance					
Se concentration	***	ns	***	**	***

465 Values followed by different letters in the same column differ significantly at 5% level by the LSD
466 test. Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.
467

Table 3. Se uptake rate (µg Se g root^{-1} day^{-1}) in basil plants subjected to different Se treatments.

Se added (mg L^{-1})	Se uptake rate (µg Se g_{root}^{-1} day^{-1}) Days of treatment				
	13	27	41	55	69
0	0.27 d	0.22 d	0.16 d	0.15 d	0.06 d
4	22.9 c	31.1 c	24.8 c	14.5 c	10.4 c
8	49.6 b	59.6 b	58.5 b	31.1 b	23.5 b
12	67.4 a	130.3 a	117.9 a	59.8 a	32.3 a
Significance					
Se concentration	***	***	***	***	***

Values followed by different letters in the same column differ significantly at 5% level by the LSD test. Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.

Figure 1. Biomass production (g DW plant^{-1}) of leaves (A), stem (B), and roots (C) in basil plants subjected to different Se treatments. Values are means with standard errors (n=4).

Figure 2. Se concentration (µg g^{-1} DW) in leaves (A), stem (B), and roots (C) of basil plants subjected to different Se treatments. Values are means with standard errors (n=4).

Figure 3. Se content (mg plant^{-1}) in basil plants subjected to different Se treatments during the growth cycle. The statistical analysis was made separately for each sampling during the growth cycle. Values are means with standard errors (n=4).

Figure 4. Plot of residual values vs predicted values of log10(Se uptake rate), obtained with Equation 3.

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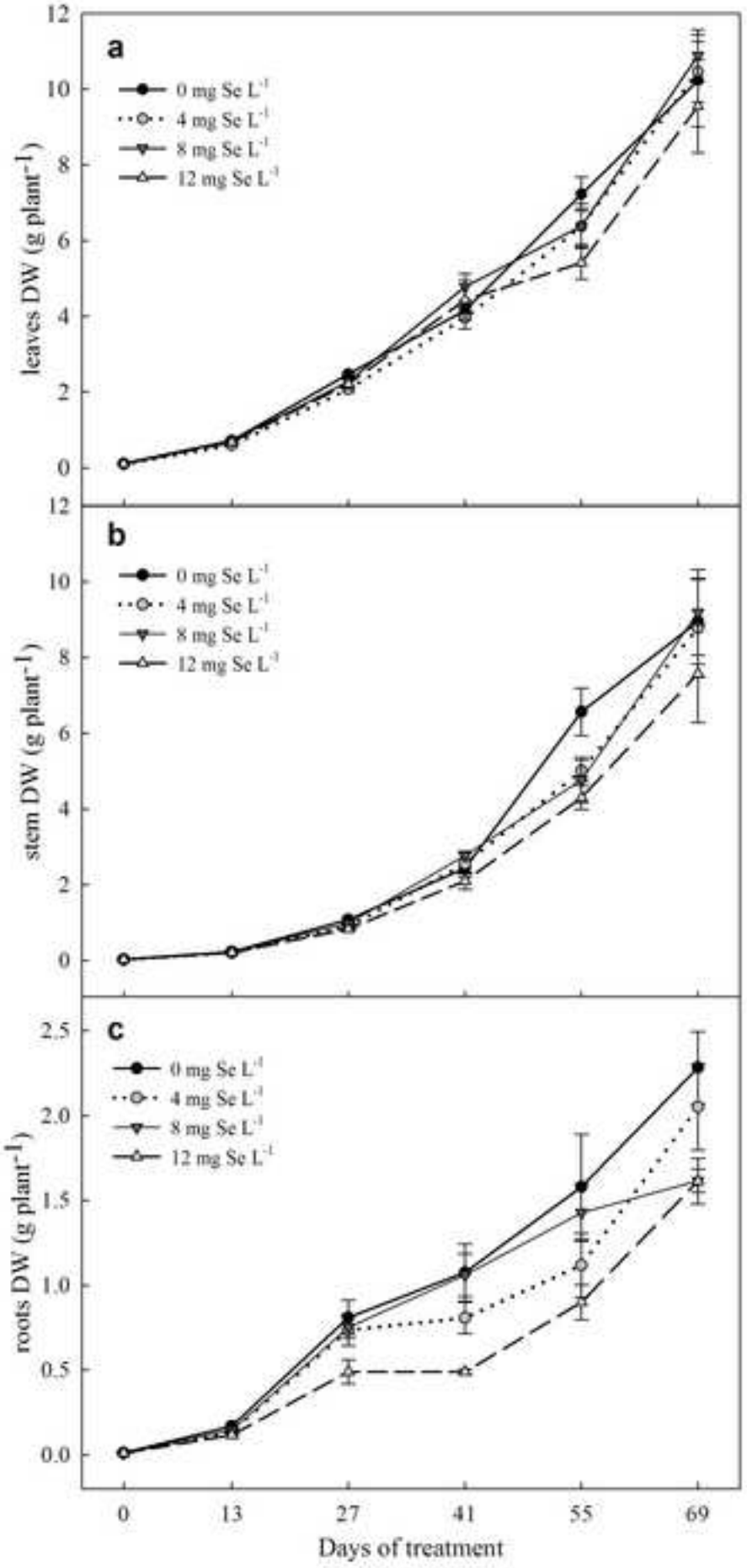

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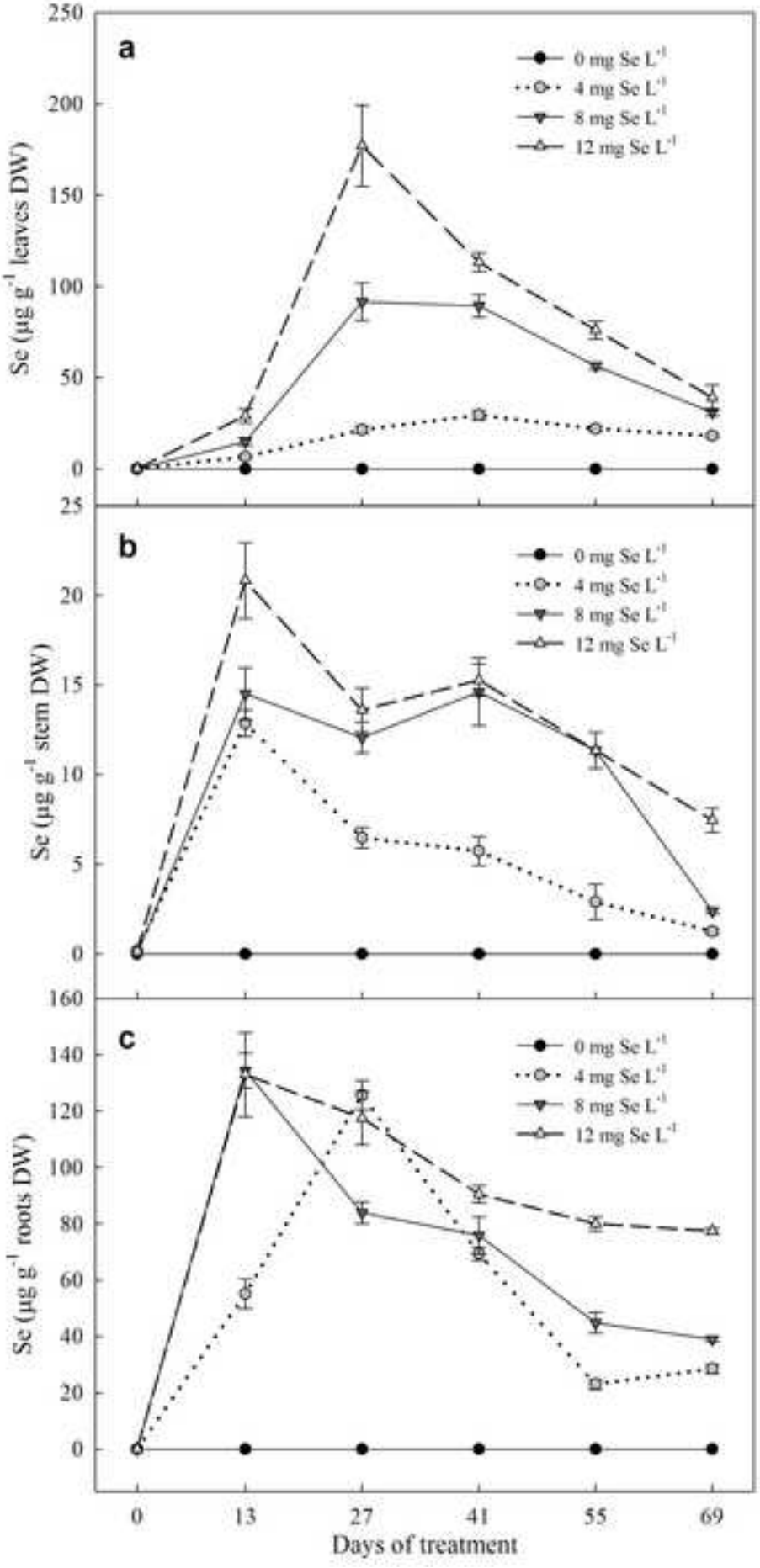

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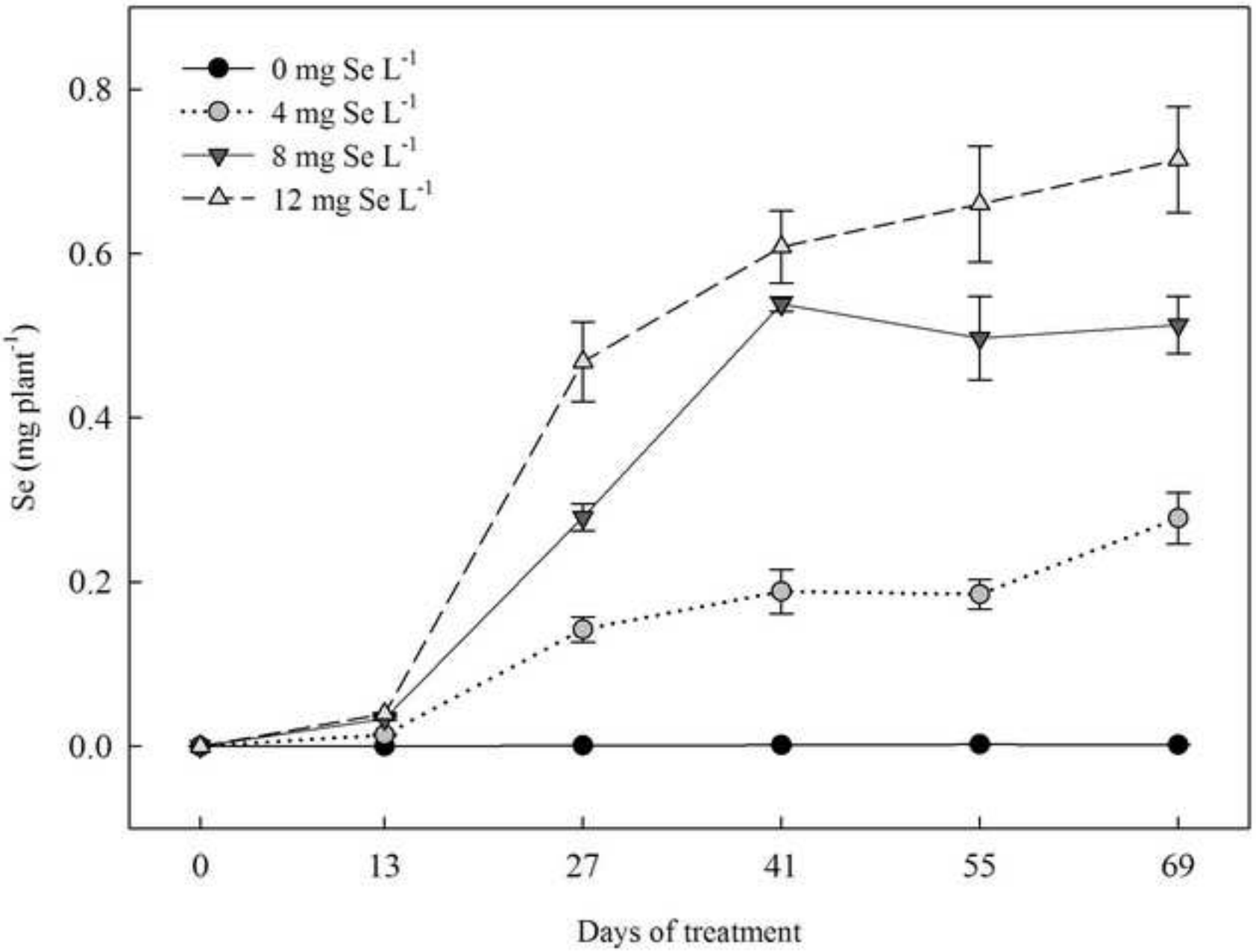

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