

**Production of selenium-biofortified microgreens from
 selenium-enriched seeds of basil**

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Key Words:	antioxidants, dietary supplements, <i>Ocimum basilicum</i> , selenate

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11 9 **Abstract**

12 10 BACKGROUND: Microgreens (i.e. tender immature greens) are a popular alternative to sprouts (i.e.
13 11 germinating seeds) due to their higher content of vitamins, carotenoids and phenols, and lower content
14 12 of nitrates. Their nutritional value can be improved by biofortification, which increases micronutrient
15 13 levels during plant growth. Since selenium (Se) plays a significant role in antioxidant defense,
16 14 biofortification with Se is a good way to improve the nutritional quality of sprouts and microgreens.
17 15 This work studied the production of selenium-fortified microgreens from Se-enriched seeds of sweet
18 16 basil (*Ocimum basilicum* L.), which could be used as new beneficial dietary supplements.
19 17 RESULTS: Basil plants were grown in a nutrient solution, containing 0 (control), 4 or 8 mg Se L⁻¹ as
20 18 sodium selenate, to full maturity. Seeds accumulated a high amount of selenium and were then used
21 19 to produce microgreens. The germination index was higher in the seeds from Se-treated plants and
22 20 the microgreens resulted enriched in selenium. The antioxidant capacity of Se-fortified microgreens
23 21 was higher compared to the control.
24 22 CONCLUSIONS: The production of microgreens from Se-enriched seeds could be a good system to
25 23 obtain microgreens with a high nutritional value. Basil plants treated with selenium could be used to
26 24 produce both Se-fortified leaves and microgreens.

27 25
28 26 Key words: antioxidants; dietary supplements; *Ocimum basilicum*; selenate
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30 28 **1. Introduction**

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32 30 Vegetables may be harvested and eaten at any stage of development, including young shoots (sprouts,
33 31 microgreens). During seed germination, the efficiency of the enzymes involved in the decomposition
34 32 of proteins, carbohydrates and fatty acids increases ¹ and positively affects the nutritive value and
35 33 health properties of sprouts ². Sprouts are germinated, or partially germinated seeds, grown in the
36 34 dark, and eaten with their roots intact.
37 35 Microgreens are tender immature greens with two fully-developed cotyledon leaves and with or
38 36 without the emergence of a pair of first true leaves. They need light for their growth, are usually

harvested 7-21 days after germination, depending on the plant species, when they are 2.5-7.6 cm high, and are eaten without their roots³. Microgreens have much stronger flavor and taste than sprouts, and a broad range of leaf color, variety and shape which make them ideal to create an attractive combination of taste, texture, and colors⁴.

The nutritional value of vegetables can be improved by biofortification, which increases the micronutrient levels in crops during plant growth⁵. Enriched sprouts^{6, 7} and microgreens^{8, 9} are generally produced by adding the mineral element to the germination medium.

Biofortification with selenium (Se) is a good way to increase the Se content of fruit and vegetables, and thus the Se intake by humans in order to reach the Recommended Dietary Allowance (RDA) of 55 $\mu\text{g day}^{-1}$ ¹⁰. It is important not to exceed the toxic threshold of 400 μg of Se per day⁻¹, however if the Se intake remains below this level, it has a positive effect on long-term health due to the role of Se in antioxidant defense and in several biological processes^{11, 12}.

Experiments conducted on broccoli, radish, alfalfa and mung bean have shown that microgreens can be biofortified with iron⁹ and magnesium⁸, whereas the biofortification of microgreens with selenium has not yet been investigated.

Sweet basil (*Ocimum basilicum* L.) is an annual aromatic plant belonging to the Lamiaceae family cultivated for its edible leaves. Due to their antioxidant activity and their content of health-related substances, such as flavonoids, phenolics and essential oils, anti-bacteria and anti-mycotic substances¹³, basil leaves are also used to produce pharmaceuticals.

The present study investigated the possibility of producing Se-fortified microgreens from seeds of Se-enriched plants of sweet basil (*Ocimum basilicum* L.). Basil plants were therefore grown to maturity in a nutrient solution with selenium added in the form of sodium selenate, and the Se-enriched seeds obtained from these plants were used to produce microgreens. The absorption by plants and the translocation of the selenium accumulated in the seeds to the microgreens were evaluated, together with the effects of selenium on seed germination, biomass and antioxidant activity of the microgreens.

2. Materials and methods

2.1 Plant material and growth conditions

The experiment was carried out from February to April at the Department of Agriculture, Food and Environment of the University of Pisa, Italy (lat. 43° 40' N) on basil plants (*Ocimum basilicum* L.). The basil seeds were sown in 254-cell plug-trays filled with perlite and vermiculite, and germinated in a growth chamber at 25°C. 5 days after sowing, seedlings were transferred to a heated greenhouse and after 15 more days transplanted into separate hydroponic systems, each consisting of a

polystyrene tray floating in a 10 L plastic tank filled with nutrient solution. The nutrient solution contained (mM) 12 N-NO₃, 1.0 P-H₂PO₄, 2.44 S-SO₄, 4 Ca, 5 K, 2 Mg, and (μM) 1 Cu, 40 Fe, 5 Mn, 1 Mo, 5 Zn. The pH and electrical conductivity (EC) values were 5.6 and 2.04 dS m⁻¹ respectively, and were checked every 2 days. The nutrient solution was renewed once every two weeks and continuously aerated 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 11.2 and 19.7 °C, respectively. The mean relative humidity was 60.1 %. Supplementary lighting was provided by high pressure sodium lamps (HPS, SON-T 400 W, Philips) for a constant day length of 9 hours, till the end of March. The daily mean and the cumulative global radiation were 6.7 and 676 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 one plant. One week after transplanting, selenium, as sodium selenate (Na₂SeO₄), was added to the nutrient solution at rates of 0 (control), 4 and 8 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 93 days. Plants were grown to seed maturity. At the end of the experiment, plants were harvested, divided in roots, stem, leaves, inflorescence, rachis and seeds, and the respective fresh weight (FW) were determined. The samples were oven dried at 50°C up to constant weight and the dry weight (DW) was recorded. The selenium content in the different plant parts was determined.

The seeds produced by plants were then sown in Petri dishes, and germinated.

2.3 Germination experiment

Ten seeds from each selenium treatment were germinated in 14 cm diameter Petri dishes. 10 g of quartz sand were poured into each Petri dish and water was added till saturation point. A Whatman filter was placed on the quartz sand and seeds were spread on the filter. The dishes were kept in the dark at the constant temperature of 25°C for 72 hours. At the end of the incubation period, the number of germinated seeds was counted and root length of each seedling was measured. The germination index was calculated multiplying the average number of germinated seeds by the average root length. The experiment was laid out in a completely randomized design in a factorial arrangement replicated three times.

2.4 Microgreens production

1 g of seeds from each selenium treatment was sowed in 14 cm diameter Petri dish filled with 10 g of quartz sand. A Whatman filter was placed on the quartz sand, saturated with distilled water and seeds were spread on the filter. The dishes were kept in the dark at 25°C for 4 days, and then in presence of light for 1 day. Microgreens were harvested 5 days after germination, and FW was recorded. Samples were oven dried at 50°C up to constant weight and DW was determined.

2.5 Antioxidant capacity

The total antioxidant capacity was determined in basil microgreens by using the ferric reducing ability of plasma (FRAP) assay, as adapted by Benzie and Strain (1996)¹⁴. 0.5 g of fresh material were grounded in 5 mL of 99 % (v/v) methanol, then the samples were sonicated and maintained at -18 °C for 24 h. Antioxidant capacity was measured using the ferric-reducing antioxidant power (Frap) method developed by Benzie and Strein (1996). 0.5 g of fresh leaf were extracted using methanol (10 mL). 0.1 mL of the methanol extract was added to 0.9 mL of Frap reagent, that consisted of 1 mol m⁻³ 2,4,6-tripyridyl-2-triazine (TPTZ) and 2 mol m⁻³ ferric chloride in 250 mol m⁻³ sodium acetate (pH 3.6) and mixed. The mixture was then kept at 20 °C for 4 minutes, and the absorbance was measured at 593 nm. Results were expressed as µmol of Fe(II) mg⁻¹ DW.

2.6 Selenium analysis

Total selenium content was determined at harvest in leaf, stem, roots and seeds, and in the microgreens produced by the collected seeds. Samples were oven-dried at 50°C, grounded and digested with nitric and perchloric acids. After reduction by hydrochloric acid following Zasoski and Bureau (1977)¹⁵, the digests were analyzed by hydride generation atomic absorption spectrophotometry (Varian VGA 77). The Se reference standards, rice flour SRM 1567 (Se content 1.23 ± 0.09 mg kg⁻¹ with a recovery of 92%) was obtained from the U.S. National Institute of Standards and Technology. 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.

2.7 Data analysis

Data of biomass production, germination index and antioxidant activity were subjected to one-way ANOVA with Se treatment as variables. Data of Se content and concentration in different parts of basil plants were subject to two-way ANOVA. In all data the 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).

3. Results and discussion

The results showed that selenium added to the nutrient solution was absorbed by the roots and translocated to the aerial parts of the plant. The Se concentration, expressed as $\mu\text{g Se g}^{-1}\text{ DW}$, increased significantly by increasing the amount of Se added (Figure 1). A dose-dependent increase in Se concentration was detected both in the ground and in the aerial parts of the plants, except for the stems. These results are in agreement with previous studies conducted on basil by Puccinelli et al. (2017)¹⁶.

The Se concentration in the various parts of the basil plants followed the order of stem < rachis < seeds < leaves < roots. These results are in agreement with findings in mustard, sunflower and lupin by Ximénez-Embún et al. (2004)¹⁷. In contrast, in tomato¹⁸ and chicory¹⁹ the highest Se concentration was found in leaves.

Few data are available concerning the biofortification of basil through the addition of selenium to the nutrient solution¹⁶, whereas a few experiments have been conducted using Se foliar application^{20, 21}. The total selenium content, calculated as the product of Se concentration and plant dry weight, increased by increasing the amount of selenium added to the nutrient solution in all plant organs, except for the stems and seeds (Figure 2). Selenium accumulated mostly in the leaves, followed by roots, rachis, stem and seeds.

The basil yield, expressed as leaf fresh weight, and the biomass production, expressed as g DW per plant, were not affected by the addition of selenium to the nutrient solution, and plants did not show any toxicity symptoms. The average leaf fresh weight was 61.9 g plant^{-1} (data not shown).

Plant seeds from the Se-enrichment experiment were germinated in Petri dishes and at the appearance of the first true leaves, microgreens were harvested. Since microgreens are sprouts that are grown for a longer period of time and no data on Se biofortification of microgreens are available, the results of the current experiment are discussed on the basis of the results obtained in Se-enriched sprouts.

Seeds from plants treated with Se showed a significantly higher germination index than seeds from control plants, but no differences were detected among selenium treatments (Figure 3). Both the number of germinated seeds and the average length of roots were higher in the Se-enriched seedlings compared to the control (data not shown). No difference in the germination index was detected by Carlson et al. (1989)²² in sprouts from various plant species treated with concentrations of up to 32 Se mg L^{-1} . In contrast, Frias et al. (2009)²³ found a reduction in the germination index when lupin seeds were treated with selenite at concentrations ranging from 2 to 8 mg Se L^{-1} .

The biomass production, expressed as both the fresh and the dry weight, did not show significant differences among Se-enriched microgreens and microgreens produced from seeds of control plants (Table 1). Similarly, no toxic effects on sprouts biomass were detected by Zhang et al. (2012)⁶ when chickpea seeds were treated with Se concentrations of up to 50 mg L⁻¹.

Se concentration, expressed on a DW basis, was significantly higher in Se-enriched microgreens compared to the control, and was highest in seeds of plants treated with 8 mg Se L⁻¹. Se-enriched microgreens showed a higher Se concentration than leaves of Se-enriched mother plants (Table 1).

The antioxidant capacity was higher in the microgreens produced from the seeds of treated plants compared to microgreens produced from seeds of control plants (Figure 4), but no differences were detected between the different selenium treatments. The increased antioxidant capacity detected in the Se-enriched microgreens is in agreement with the results obtained in sprouts by Frias et al. (2009)²³. They found that when lupin seeds germinated with a Se concentration ranging from 2 to 8 mg L⁻¹ the antioxidant capacity of the sprouts increased. Piekarska et al. (2014)²⁴ found an increased antioxidant capacity only in Se-enriched mustard sprouts, but not in the sprouts of broccoli, cabbage and rye. The increased antioxidant capacity may depend on the ability of Se to increase the antioxidant defense of plants^{25, 26} by improving the glutathione peroxidase (GSH-Px) activity²⁷. The positive relationship between the Se concentration in plant tissues and GSH-Px activity suggests the presence of a Se-dependent GSH-Px²⁸.

Due to their high concentration of active compounds, microgreens have a higher nutritional value and greater health benefits compared to mature leafy vegetables²⁹. The increased antioxidant capacity induced by Se treatments may improve the positive effect of microgreens on human health.

In order to calculate the intake of selenium by eating fresh Se-enriched microgreens, the Se content was calculated on a fresh weight (FW) basis. When plants were grown at concentration of 4 and 8 mg Se L⁻¹ in the nutrient solution, the Se concentration in Se-enriched microgreens was 8.5 and 15.5 µg g⁻¹ FW, respectively. Since the recommended dietary allowance (RDA) is 55 µg Se d⁻¹, the daily consumption of 6.5 or 3.5 g of microgreens enriched with 4 or 8 mg Se L⁻¹, respectively, could provide the amount of selenium necessary to meet the RDA. A consumption of 10 g of Se-enriched microgreens, would provide a higher amount of selenium than the RDA, but considerably below the toxic threshold (400 µg d⁻¹).

4. Conclusions

The results of the present study confirmed that selenium added as sodium selenate to the nutrient solution of basil plants grown in hydroponics is taken up by roots, and translocated to the aerial part where it accumulates mainly in the leaves. About 10% of selenium accumulated in the seeds, and then moved to the microgreens when Se-enriched seeds germinated. Enriched microgreens showed a

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2 208 higher content of selenium compared to the leaves of the treated plants. The presence of selenium in
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4 209 microgreens did not reduce the biomass production, and induced a high antioxidant capacity. This
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6 210 evidence suggests that the production of microgreens from Se-enriched seeds is feasible and
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8 211 represents a good system to obtain microgreens with a high nutritional and antioxidant value. Basil
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10 212 plants treated with selenium could be used to produce both Se-fortified leaves and microgreens. The
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12 213 selenium treatment would only need to be performed once, and growers would not need to treat the
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14 214 seeds.
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16 215 Further studies are necessary to evaluate the effects of the Se enrichment of mother plants on the
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18 216 content and activity of enzymatic and non-enzymatic antioxidative components, and on the sensory
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20 217 and nutritional properties of the microgreens obtained by Se-enriched seeds.
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Table 1. Biomass production and selenium (Se) concentration in microgreens produced from seeds of basil plants (mother plants) treated with different Se concentrations. 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.

Se added (mg L ⁻¹) to mother plants	Biomass production of microgreens		[Se] in microgreens
	g FW g ⁻¹ seeds	g DW g ⁻¹ seeds	µg Se g ⁻¹ DW
0	5.33	0.409	0.0 c
4	5.69	0.486	117 b
8	5.95	0.545	203 a
Significance			
Se	ns	ns	***

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Figure 1. Se concentration ($\mu\text{g g}^{-1}$ DW) in different parts of basil plants subjected to different Se treatments at harvest.

Verticals bars represent the mean \pm SE (standard error). Bars indicated by different letters within each selenium treatment are significantly different ($P<0.05$) by LSD test.

Figure 2. Se content (μg) in different parts of basil plants subjected to different Se treatments at harvest.

Verticals bars represent the mean \pm SE (standard error). Bars indicated by different letters within each selenium treatment are significantly different ($P<0.05$) by LSD test

Figure 3. Germination Index (GI) of basil seeds produced by plants treated with different Se concentrations. are means with standard errors ($n=4$).

Figure 4. Antioxidant capacity of microgreens produced from seeds of basil plants treated with different Se concentrations. Values are means with standard errors ($n=4$).

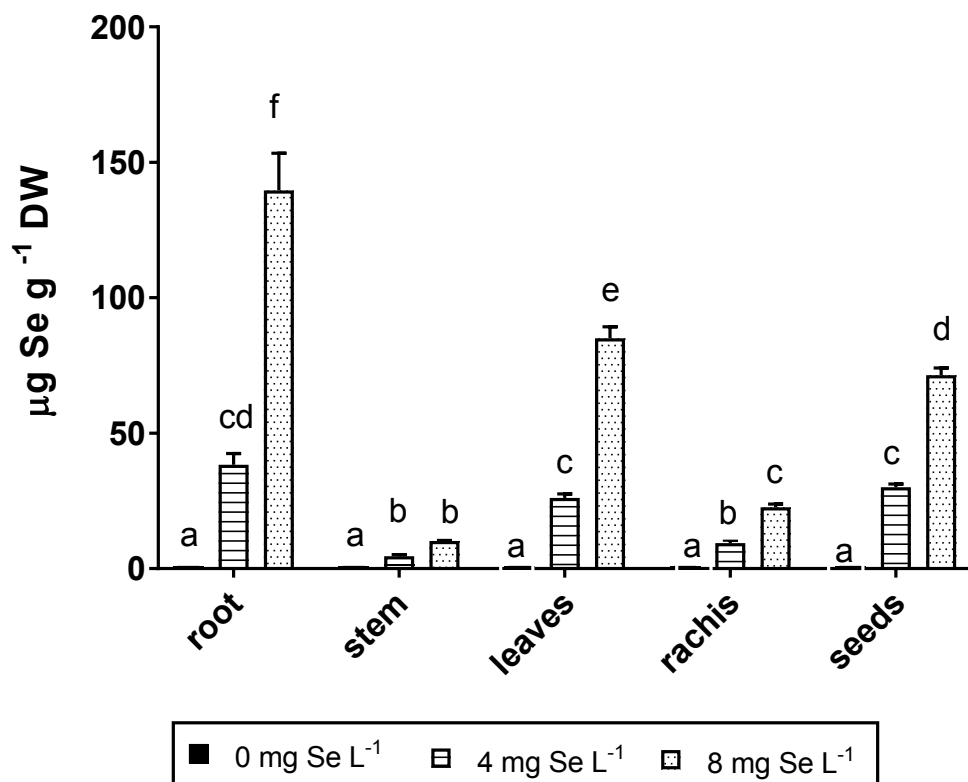


Figure 1

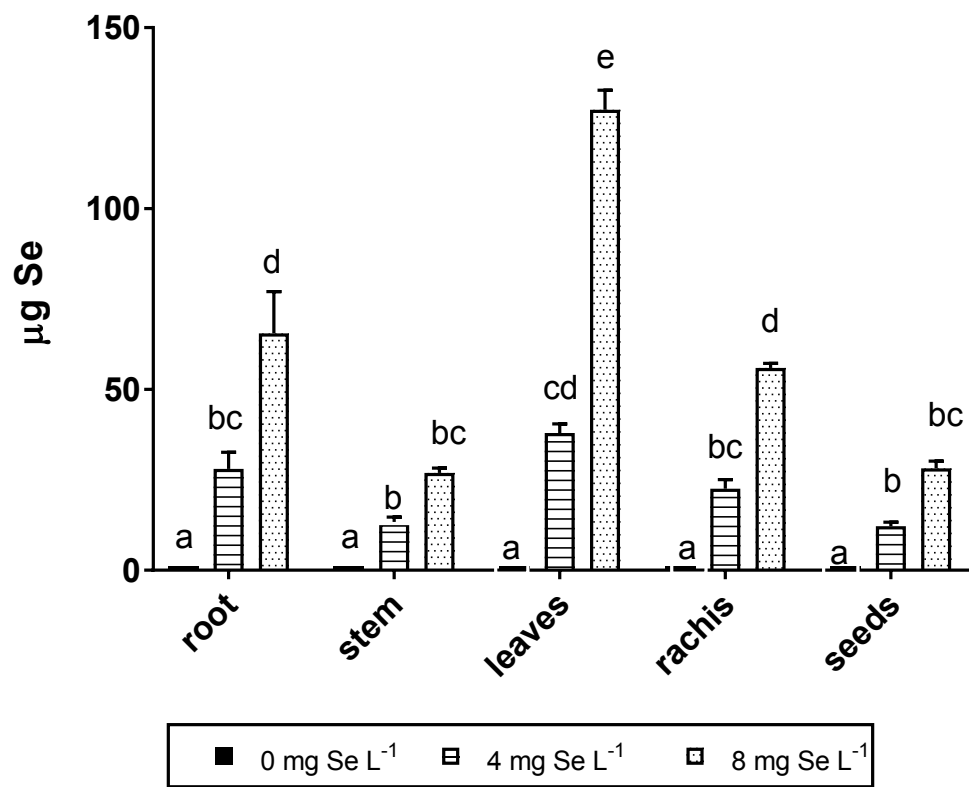


Figure 2

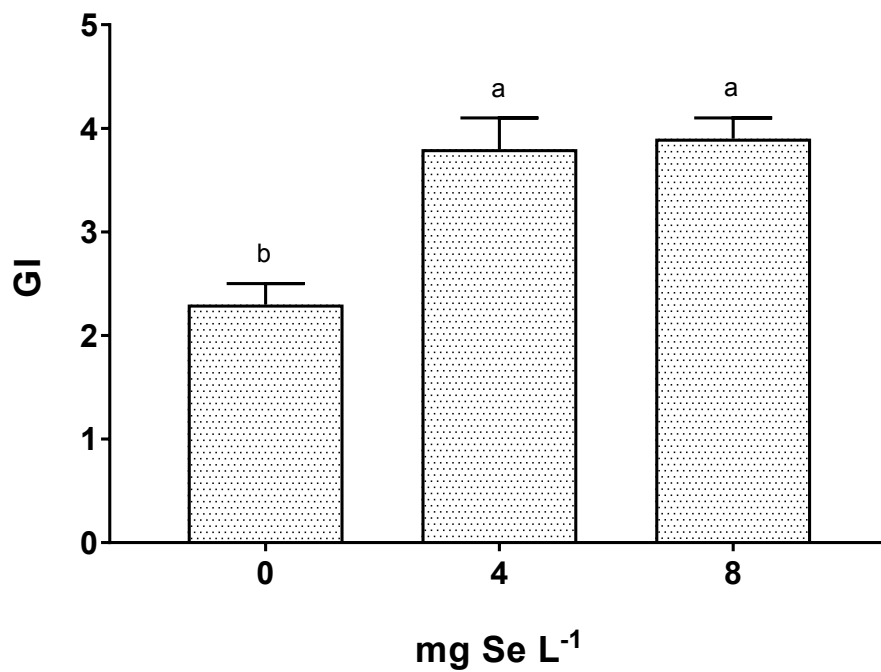


Figure 3

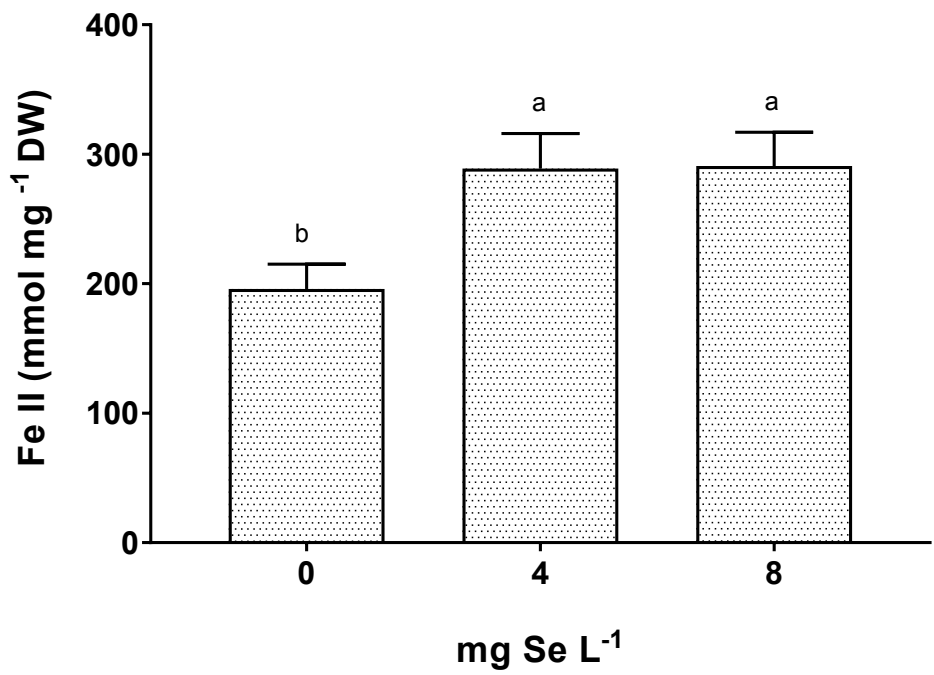


Figura 4