Selenium biofortification and antioxidant activity in lettuce plants fed with selenate and selenite

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ABSTRACT

Selenium is an important element associated with enhancement of antioxidant activity in plants, microorganisms, animals, and humans. In Brazil, the information on Se in agricultural crops is lacking, though there are indications that low levels of Se are consumed by the population. The experiment was conducted under greenhouse conditions with pots containing 3 l of nutritive solution in a completely randomized factorial design, with seven Se concentrations (0, 2, 4, 8, 16, 32, and 64 µmol/l) and two forms of Se (sodium selenate – Na_2SeO_4 and sodium selenite – Na_2SeO_3), with six replicates. The application of Se as selenate at low concentrations is more appropriate for lettuce biofortification because it favors shoot biomass growth and Se levels in the shoot biomass. Selenium in both forms had two effects on lettuce plant metabolism: at low doses it acted as an antioxidant and enhanced plant growth, whereas at higher levels it reduced yield.

Keywords: selenate; selenite; antioxidant enzymes; biofortification

Selenium (Se) is an important element for human and animal nutrition, due to its roles on a series of biochemical reactions enhancing antioxidant activity (Rayman 2002). In contrast, Se was not considered essential for plants (Terry et al. 2000, Kápolna et al. 2009). Nevertheless, several studies reported the beneficial effects of Se, because it increases the antioxidant activity in plants, leading to better plant yield (Hartikainen et al. 2000, Lyons et al. 2009).

Plants recycle Se within the food chain. Thus, biofortification of agricultural crops with Se, by means of adding Se along with fertilizers, is a useful technique to increase the consumption of Se by animals and man (Chen et al. 2002, Ríos et al. 2008, White and Broadley 2009, Broadley et al. 2010). Inorganic Se forms differ in terms of absorption and mobility within plants; selenate is more easily transported to shoots, while selenite tends to accumulate in plant roots (Zhang et al. 2003). For this reason, in some Se biofortification programs, use of selenate is recommended over selenite (Ríos et al. 2008).

The antioxidative effect of Se was related to an improved GSH-Px and SOD activity and a decreased lipid peroxidation in Se treated plants, like ryegrass (Hartikainen et al. 2000), lettuce (Xue et al. 2001) and soybean (Djanaguiraman et al. 2005). Moreover, Cartes et al. (2005) determined that selenite was more efficient that selenate in promoting enzymatic activity.

Even though there are reports of low Se consumption by the Brazilian population, studies on Se fortification of crop plants are scarce (Ferreira et al. 2002, Maihara et al. 2004). Since lettuce is the most consumed leafy plant in Brazil and many parts of the world (Luz et al. 2008, Li et al. 2010), this crop can be preferred in Se biofortification programs, as an efficient way of increasing intake of this element by the population. So in the present work the effect of the biofortification of different

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Se forms on the yield and antioxidant systems in lettuce plants has been studied.

MATERIALS AND METHODS

Plant materials and experimental design. Lettuce (*Lactuca sativa* L. cv. Vera) was planted in expanded polystyrene trays containing 128 compartments filled with vermiculite and irrigated with distilled water in the first five days. Seedlings were irrigated with the 0.5N Hoagland nutrient solution till 15 days. After that, seedlings with uniform seedling vigour were selected and transplanted to 3 l pots containing 0.5N Hoagland nutrient solution with different concentrations of Se.

The experimental design was a completely randomized factorial 7 × 2, in which the main factor was Se concentration in seven levels (0, 2, 4, 8, 16, 32 and 64 µmol/l) and the other factor was Se form (sodium selenate – Na_2SeO_4, and sodium selenite – $Na_2SeO_3 \cdot 5\ H_2O$, both from Sigma-Aldrich), with six replicates, totaling 84 plots. Each experimental unit was made up of one plant per pot.

Throughout the experimental period, the nutritive solution underwent constant aeration and pH was monitored daily and kept at 6.0 ± 0.2 by addition of 0.1 mol/l NaOH or HCl. The solution was changed whenever the initial electrical conductivity dropped more than 30%. After 25 days of Se exposure, plants were harvested, and divided into shoots and roots.

During harvest, three replicates with five leaves each from the middle part of the plant were immediately wrapped in aluminum foil, submerged in liquid nitrogen and were stored in a freezer, at –80°C, for enzyme assay. The remaining plants (replicates) were dried in a forced-air drying oven at 65–70°C until constant mass of the root and shoot samples was achieved.

Antioxidant enzymes activity and lipid peroxidation measurement. In order to estimate the superoxide dismutase and catalase enzyme activities, frozen tissue was homogenized in a cooled 0.1 mol/l Tris-HCl buffer at pH 7.8 containing 1 mmol/l EDTA, 1 mmol/l dithiothreitol and 5 ml of 4% polyvinyl pyrrolidone per gram of fresh weight. The homogenate was filtered through a nylon mesh and centrifuged at 22 000 × g for 30 min at 4°C. The supernatant was used to measure enzyme activity.

Superoxide dismutase (EC 1.15.1.1) activity was assayed by monitoring photochemical inhibition of nitroblue tetrazolium (NBT) reduction (Beyer and Fridovich 1987). A 5 ml reaction mixture, containing 50 mmol/l Na_2CO_3 (pH 10.0), 13 mmol/l methionine, 0.025% (v/v) Triton X-100, 63 µmol/l NBT, 1.3 mmol/l riboflavin, and an appropriate quantity of enzyme extract was used. The reaction mixtures were illuminated for 15 min at photosynthetic photon flux density (PPFD) of 380 µmol/m^2/s. Non-illuminated mixtures were used to correct for background absorbance. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT reduction as monitored at 560 nm.

Catalase (EC 1.11.1.6) activity was tested by observing H_2O_2 consumption at 240 nm for 5 min (Rao et al. 1997). Reaction mixture (3 ml total volume) contained 25 mmol/l Tris-acetate buffer (pH 7.0), 0.8 mmol/l EDTA-Na, and 20 mmol/l H_2O_2, and the enzyme assay was carried out at 25°C.

For the malondialdehyde (MDA) assay, 0.5 g of lettuce leaf was homogenized in 5 ml of 50 mmol/l buffer solution (containing 0.07% $NaH_2PO_4 \cdot 2\ H_2O$ and 1.6% $Na_2HPO_4 \cdot 12\ H_2O$), ground with a cooled mortar and pestle, and centrifuged at 22 000 × g for 30 min (4°C). MDA concentration was calculated using the extinction coefficient of 155 mmol/l/cm (Fu and Huang 2001).

Selenium measurement. In this step, 500 mg of dried powder of plant materials was added with 10 ml of concentrated p.a. HNO_3 to Teflon PTFE flasks and digested at 0.76 MPa for 10 min in a microwave oven (CEM, model Mars 5, CEM Corporation, Matthews, NC, USA). After cooling to room temperature, the extract was filtered (Whatman number 40 filter) and diluted by adding 5 ml of bi-distilled water.

Total Se in the extracts was determined in a PerkinElmer Analyst 800 atomic absorption spectrophotometer with electrothermal atomization by (pyrolytic) graphite furnace with transversal heating and automatic sampler. A hollow cathode Se bulb was employed as a radiation source, operating at 6.0 mA, with a 196.0 nm wavelength, and a 2.0 nm gap. The matrix modifier consisted of a solution containing 0.005 mg Pd + 0.003 mg $Mg(NO_3)_2$; argon (95% pure) was used as an inert gas in the graphite furnace. In these conditions, pre-treatment temperature was 1300°C and an atomization temperature was 1900°C. Readings were carried out by peak absorption area, using a 5 s atomization time, taken out of the respective blanks of each sample digestion battery. Certified reference material (tomato leaf material, NIST 1573a) were included in each batch of samples for quality control, and the recovery percentages varied from 89.4 to 93.2%.

All data were subjected to a simple ANOVA at 95% confidence, using Sisvar 4.6 software (Build 6.1) and the graphs were done on Sigma Plot (version 11.0).

RESULTS AND DISCUSSION

Figure 1A shows that Se affected shoot biomass production, with an increase of 5.67 and 3.69% by selenate and selenite, respectively, at low concentrations of up to 8 and 4 µmol/l. However, further increase in Se concentrations reduced shoot biomass production. Our results are in agreement with Fargašová (2003) who observed an inhibitory effect of Se on the mustard growth. In general, the biomass production was higher when selenate was supplied to the nutrient solution than selenite (Figure 1A). Previous studies showed that selenate and selenite provide distinct responses in Se translocation (Zhang et al. 2003). Figure 1B shows that Se translocation was higher when supplied as selenate. When lettuce received lower Se concentrations (2, 4, and 8 µmol/l), approximately 70% of the element applied as selenate and 50% as selenite were found in the shoots. When the plants were treated with higher concentration of Se, there was a small reduction in translocation in both Se forms. The difference between selenate and selenite is due to the high affinity between sulfate transporters and selenate, which facilitates its absorption and translocation (Zhang et al. 2003). As for the selenite absorption processes, little is known (Rosen and Liu 2009). There are indications that selenite transport by cellular simplasm actively takes place and that phosphate transporters act in the process, at least partially (Hopper and Parker 1999, Li et al. 2008). In addition, when selenite is absorbed by plants, it is rapidly converted to organic forms of Se in roots, which have low mobility in xylem (Li et al. 2008).

Leaf Se concentration increased with an increase in Se concentration in the nutrient medium (Figure 1C). These results corroborate with those obtained in other works, which reported that increasing doses of Se in medium culture can cause a significant increase of Se content in agricultural crops (Ducsay et al. 2009, Broadley et al. 2010). Considering that Brazilian per capita consumption of fresh lettuce leaves is 33.3 g/day (Yuri et al. 2005), and that the recommended consumption of Se for adults is 50–70 µg/day (US Department of Agriculture 2001), the Se content obtained in this experiment with selenate without compromising production, met only ca. 5% of the recommended human Se intake

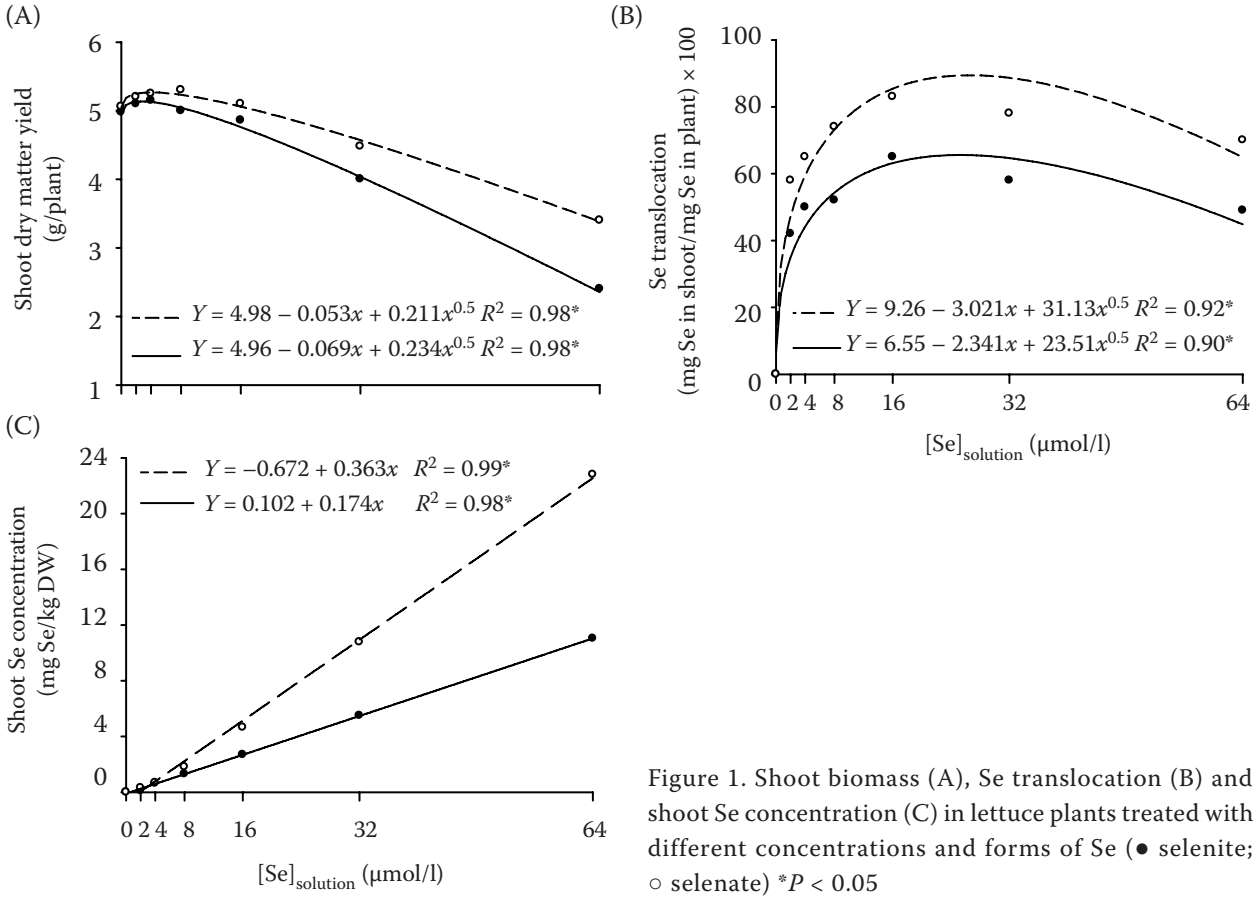

Figure 1. Shoot biomass (A), Se translocation (B) and shoot Se concentration (C) in lettuce plants treated with different concentrations and forms of Se (● selenite; ○ selenate) *$P < 0.05$

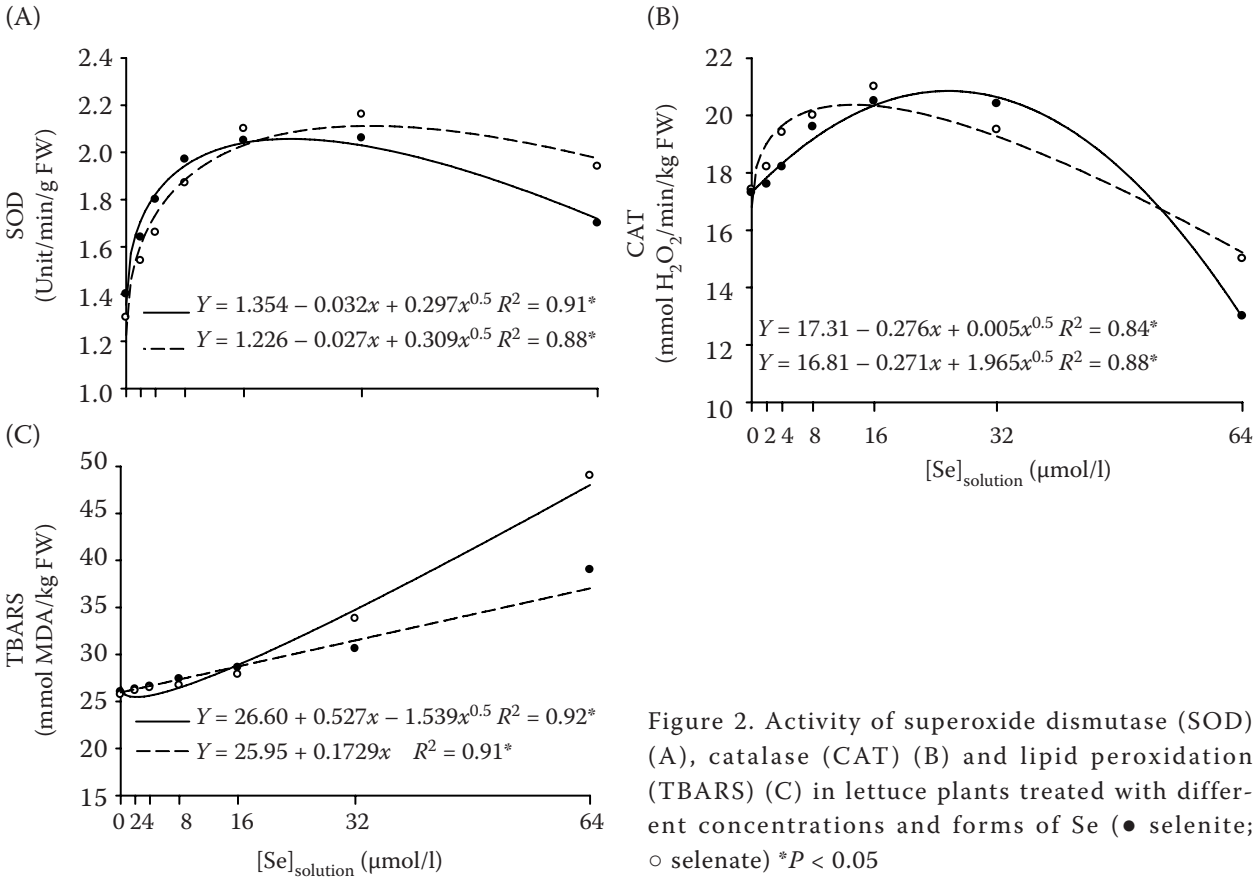

Figure 2. Activity of superoxide dismutase (SOD) (A), catalase (CAT) (B) and lipid peroxidation (TBARS) (C) in lettuce plants treated with different concentrations and forms of Se (● selenite; ○ selenate) *$P < 0.05$

(approximately 230 g FW/week and considering that lettuce is composed of 96% water). Thus, the present study suggests a need for further research on biofortification of different crops with Se, in order to meet ideal Se consumption by Brazilians. These results shown in Figure 1 are conform with the earlier result by Ríos et al. (2008).

Figure 2 shows changes in the activity of SOD, CAT and lipid peroxidation in lettuce leaves treated with an increasing concentrations of Se, either as selenate or selenite. SOD activity was maximum at Se concentrations of 32.9 and 22.1 µmol/l, for selenate and selenite, respectively (Figure 2A). As for CAT, the maximum activity was detected at concentrations of 12.3 and 20.8 µmol/l (Figure 2B). During oxidative stress, excess reactive oxygen species (ROS) production causes membrane damage that eventually leads to cell death (Das et al. 1992, Montillet et al. 2005). In our experiment, increased lipid peroxidation at higher Se concentrations indicated the occurrence of oxidative stress (Figure 2C). This might be one of the reasons for lowering lettuce production at higher Se concentrations (Figure 1A). To protect against ROS, plants have antioxidant enzymes such as SOD and CAT, as well as a wide array of non-enzymatic antioxidants (Mittler 2002).

Se induced increase in SOD and CAT activity at low concentrations, but probably enhanced lettuce leaf production through the enhanced activity of antioxidants. Similar results were reported in ryegrass (Hartikainen et al. 2000).

Our results indicate that for biofortification program with lettuce the application of Se as selenate at low concentrations would be more beneficial because it favors shoot biomass growth, Se translocation, and Se levels in the shoot biomass.

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