Plant and Soil (2005) 276:359–367
DOI 10.1007/s11104-005-5691-9

Uptake of Selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms

P. Cartes[1], L. Gianfreda[2] & M.L. Mora[3,4]
[1]*Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Casilla 54-D, Temuco, Chile.* [2]*Dipartimento di Scienze del Suolo, della Pianta e dell'Ambiente, Università di Napoli, Federico II, Italy.* [3]*Departamento de Ciencias Químicas, Universidad de La Frontera, Casilla 54-D, Temuco, Chile.* [4]*Corresponding author**

Received 31 January 2005. Accepted in revised form 14 April 2005

Key words: GSH-Px, ryegrass, selenate, selenite, soil available Se, TBARS

Abstract

Selenium (Se) is an essential micronutrient for animal and human nutrition, but whether it is essential to plants remains controversial. However, there are increasing experimental evidences that indicate a protective role of Se against the oxidative stress in higher plants through Se-dependent glutathione peroxidase (GSH-Px) activity. The effects of the Se chemical forms, selenite and selenate, the rate of their application on shoot Se concentration and their influence on the antioxidative system of ryegrass (*Lolium perenne* cv. Aries), through the measurement of GSH-Px activity and lipid peroxidation, were evaluated in an Andisol of Southern Chile. Moreover, a soil–plant relationship for Se was determined and a simple method to extract available Se from acid soils is proposed. In a 55-day experiment ryegrass seeds were sown in pots and soil was treated with sodium selenite or sodium selenate (0–10 mg Se kg^{-1}). The results showed that the Se concentration in shoots increased with the application of both selenite and selenate. However, the highest shoot Se concentrations were obtained in selenate-treated plants. For both sources of Se, there was a significant positive correlation between the shoot Se concentration and the GSH-Px activity; and the Se-dependence of this enzymatic activity was related especially with the chemical form of applied Se rather than the Se concentration in plant tissues. Furthermore, the lipid peroxidation, as measured by Thiobarbituric Acid Reactive Substances (TBARS), decreased at low levels of shoot Se concentration, reaching the lowest level at approximately 20 mg Se kg^{-1} in plants and then increased steadily above this level. In addition, the acid extraction method used to evaluate available Se in soil showed a positive good correlation between soil Se and shoot Se concentrations irrespective of chemical form of Se applied.

Introduction

Some areas of North America, New Zealand, Australia, China, Finland and Sweden are Se-deficient (Gissel-Nielsen et al., 1984; Gupta and Gupta, 2000; Johnsson, 1992; Yläranta, 1985), which means that feed crops do not contain sufficient Se to meet animal requirements (Gupta and Watkinson, 1985). In Southern Chile, preliminary studies indicated that the range of Se content in forage was between 0.01 to 0.08 mg kg^{-1} and 83% of the samples had a Se concentration below the minimum dietary requirements for grazing cattle (Wittwer et al., 2002). According to a preliminary screening carried out by the Soil Service Laboratory of La Frontera University (Chile) in soils of the South of Chile amounts of Se range between 21 and 180 μg kg^{-1} soil.

* FAX No: 56-45-325053.
E-mail: mariluz@ufro.cl

The content of Se in plants is not correlated with low to moderate soil-Se content (Gissel-Nielsen et al., 1984) because several factors such as chemical Se forms, pH, the content of clay, Fe oxides and organic matter as well as competitive anions may influence the Se availability in soils (Gissel-Nielsen, 2002; Neal, 1990). Nevertheless, the Se content of plants grown on naturally acidic Se-deficient soils can be increased by adding Se to the soil in the form of either selenite or selenate (Yläranta 1983a, b, c). Moreover, Whelan and Barrow (1994) demonstrated that slow releasing Se fertilizers are adequate to long-term pasture areas where crop growth is restricted to cool wet winter months and there is very little soil moisture for plant growth during the summer, and Whelan et al. (1994a, b) found significant increases in live weight and wool production when Se fertilizers were applied to pastures grazed by sheep.

Se is an essential element for human and animal metabolism. It is a component of the enzyme glutathione peroxidase (GSH-Px; Ec 1.11.1.9), a selenoenzyme (Flohé et al., 1973; Rotruck et al., 1973), with an antioxidant activity capable of reducing peroxides. However, the role of Se as essential nutrient to plants remains controversial. Nevertheless, studies achieved in Finland on lettuce and ryegrass demonstrated that although Se is toxic at high concentrations, it can exert beneficial effects on plants at low concentrations. Thus, there are increasing evidences indicating a protective role of Se against the oxidative stress in higher plants through both the decrease of lipid peroxidation and the enhancement of GSH-Px activity (Hartikainen and Xue, 1999; Hartikainen et al., 2000; Pennanen et al., 2002; Xue and Hartikainen, 2000; Xue et al., 2001) and in the green algae *Chlamidomonas reinhardtii* (Takeda et al., 1993; Yokota et al., 1988). Studies on potato have suggested that Se is an antioxidant or it activates protective mechanisms, which can alleviate oxidative stress in the chloroplasts (Seppänen et al., 2003). Recently, Nowak et al. (2004) found large impacts of the applied Se on the activity of oxidoreductase enzymes in wheat plants. In particular, the lowest Se concentration (0.05 mmol kg^{-1} soil) positively affected the antioxidant defence in wheat plants, but higher concentrations provoked stress responses. Furthermore, Hatfield et al. (1992) have characterized a Se-cysteyl-tRNA from *Beta vulgaris* that contains the UGA anticodon as evidence of the incorporation of Se into Se-proteins, and Fu et al. (2002) probed that a UGA opal codon encodes selenocysteine in *Chlamidomonas reinhardtii* glutathione peroxidase. Other studies also confirm the occurrence of both selenocysteine-containing proteins and selenocysteine protein insertion machinery in *Chlamidomonas reinhardtii* (Novoselov et al., 2002; Rao et al., 2003), and Takeda et al. (2003) isolated a cDNA clone encoding a glutathione peroxidase-like protein from the halotolerant *Chlamidomonas* sp. W80.

The role of Se as an antioxidant in ryegrass suggests that Se addition to the soil may improve the forage quality, by diminishing senescence and improving persistence of the Se-deficient pastures. Therefore, it is necessary to envisage right strategies for the management of Se as fertilizer and to develop adequate methodologies for the analysis of Se in both plants and soils, able to give reliable indications on the real content of Se in the forage. The aim of this study was to evaluate in an Andisol of the South of Chile the effects of both Se chemical forms at increasing rates of application on Se concentration in the shoots of *Lolium perenne* and their influence on the plant antioxidative system, through the measurement of GSH-Px activity and lipid peroxidation. To our knowledge the effect of different chemical forms of Se applied on both GSH-Px activity and lipid peroxidation have not been studied earlier. A soil–plant relationship for Se was determined and a simple method to extract available Se from acid soils is proposed, as well.

Material and methods

Greenhouse experiment

A greenhouse experiment was carried out with an Andisol (Vilcún Series of Southern Chile) never amended with Se fertilizers. The chemical properties of the soil are summarized in Table 1.

Soil pH was measured by potentiometry in a 1:2.5 (w/v) soil/distilled water suspension. Sulphur was extracted with $Ca(H_2PO_4)$ (Blackemore et al., 1987) and analyzed by turbidimetry

Table 1. Chemical properties of an Andisol of Vilcún Series

Parameter	Soil content
P (mg kg^{-1})	7.0
S (mg kg^{-1})	2.0
Total Se (mg kg^{-1})	0.68
pH (H_2O)	5.75
Organic matter (%)	16
K (cmol(+) kg^{-1})	0.79
Na (cmol(+) kg^{-1})	0.04
Ca (cmol(+) kg^{-1})	8.23
Mg (cmol(+) kg^{-1})	1.84
Al (cmol(+) kg^{-1})	0.20
Al saturation (%)	1.80

(Tabatabai, 1982). Phosphorus was extracted by the Olsen bicarbonate method and analyzed by the Murphy and Riley method (1962). Organic matter was estimated by wet digestion with a modified Walkley-Black procedure. Exchangeable cations (Ca, Mg, Na and K) were extracted with 1 M CH_3COONH_4 at pH 7.0 and analyzed by Flame Atomic Absorption Spectrophotometry (FAAS). Exchangeable aluminum was extracted with 1 M KCl and analyzed by FAAS. Total Se was determined by the methodology proposed by EPA (1996) and analyzed by using an Atomic Absorption Spectrophotometer-Graphite Furnace coupled (AAS-GF).

For the pot experiment, 1.5 kg soil samples were weighted and each soil sample was fertilized with 200 mg P kg^{-1} soil (as Triple superphosphate) and 50 mg S kg^{-1} soil (as Sulpomag). Sixty seeds per pot of *Lolium perenne* cv. Aries were sown and the soils were treated with solutions of sodium selenite (as $Na_2SeO_3{\cdot}5H_2O$, MERCK reagent) and sodium selenate (as Na_2SeO_4, SIGMA reagent) at rates of: 0, 0.1, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0 and 10.0 mg Se kg^{-1} soil. After germination, plants were thinned to 40 seedlings per pot and 50 mg N kg^{-1} soil (as Urea) was applied. During the growth period (55 days) the plants were watered daily with distilled water and one cut was harvested for chemical and biochemical analyses.

Plant analyses

The fresh weight (FW) of the shoot yields was registered and subsamples were dried at 65 °C for 48 h to determine both the dry weight (DW) and the Se concentration. The shoot samples were digested in an acid mixture (16 mL 65% HNO_3, 2 mL 70% $HClO_4$ and 2 mL 95% H_2SO_4). Se was separated by an ammonium pyrrolidine dithiocarbamate-methyl isobutyl ketone (APDC-MIBK) extraction system and the Se concentration was analyzed by using an AAS-GF at wavelength 196.1 nm and D_2 background correction (Kumpulainen et al., 1983). Two references samples, obtained from the Department of Applied Chemistry and Microbiology of Helsinki University-Finland, were included in each analytical run.

The fresh material was stored at −70 °C in order to evaluate the antioxidant effect of Se by means of glutathione peroxidase (GSH-Px, EC 1.11.1.9) activity. GSH-Px activity was measured according to a modified method of Flohé and Gunzler (1984) by using H_2O_2 as the substrate. The enzyme was extracted according to the protocol detailed by Hartikainen et al. (2000) modified by the inclusion of 1 mM EDTA and 1% poly(vinylpolypyrrolidone) as protease inhibitors. The enzyme activity was calculated as a decrease in GSH in the reaction time in comparison to a non enzymatic reaction and it was expressed on protein basis. The protein concentration in the enzyme extract was determined spectrophotometrically by the method of Bradford (1976).

In the fresh material, the thiobarbituric acid reactive substances (TBARS) were measured according to the method of Heath and Packer (1968) modified by Du and Bramlage (1992). In this modified procedure, the absorbance of the sample is measured at 532, 600 and 440 nm in order to correct for interference generated by TBARS-sugar complexes.

Analysis available Se of soil

At the end of the pot experiment, soil Se samples from each treatment were extracted by acid water (pH 4.5). The acid water (pH 4.5) was prepared for daily use by adding suitable drops of hydrochloric acid (HCl, p.a. Merck reagent). For the extraction, 5 g air dried-sieved soil was weighted into plastic bottle and 20 mL of deionised water pH 4.5 were added. The suspension

was shaken at 180 rpm for 30 min and filtered with filter paper Whatman N° 42. Then, aliquots of the filtered solution were taken for Se determination by AAS-GF at wavelength 196.1 nm and D_2 background correction.

Data analysis

Comparisons between averaged values from different treatments were made by means of the standard deviation of each determination and the Pearson correlation ($p < 0.01$) was used to test the relationship between two response variables.

Results

Dry matter yield and Se shoot concentration

Figure 1 (a and b) shows that shoot Se concentration in the control (0.07 ± 0.02 mg kg^{-1} DW) was significantly increased with the application of both selenite and selenate. The dissimilar response to the application of selenite and selenate became evident with all the Se dosages applied to the soil. Thus, at the lowest rate of Se application (0.1 mg kg^{-1} soil) Se shoot concentration was increased up to 0.28 ± 0.04 and 5.72 ± 0.2 mg kg^{-1} DW, in selenite- and selenate-treated plants, respectively, while at the highest rate of application (10 mg Se kg^{-1} soil) shoot Se concentration was about 4.17 ± 0.5 and 247 ± 13 mg Se kg^{-1} DW with the addition of selenite and selenate to the soil, respectively.

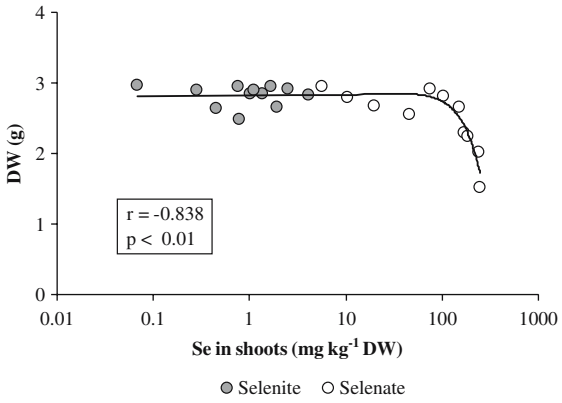

Figure 2. Pearson correlation between dry weight DW (g) and shoot Se concentration (mg kg^{-1} DW) in selenite and selenate-treated plants. Shoot Se concentration is plotted on a log scale in order to extend the *x*-axis to differentiate the responses to sodium selenite and sodium selenate.

Dry matter yield was not affected by Se addition as selenite. However, when the plants were treated with selenate, the growth of the plants was decreased, especially when shoot Se concentration was above 150 mg kg^{-1} DW, which was equivalent to the application of 4.0, 6.0, 8.0 and 10.0 mg Se kg^{-1} soil. Furthermore, a significant negative correlation ($r = -0.838$; $p < 0.01$) between Se in shoots and DW in selenite and selenate-treated plants was found as shown, on semi-log scale, in Figure 2. The decreases in DW were accompanied by selenosis symptoms, which

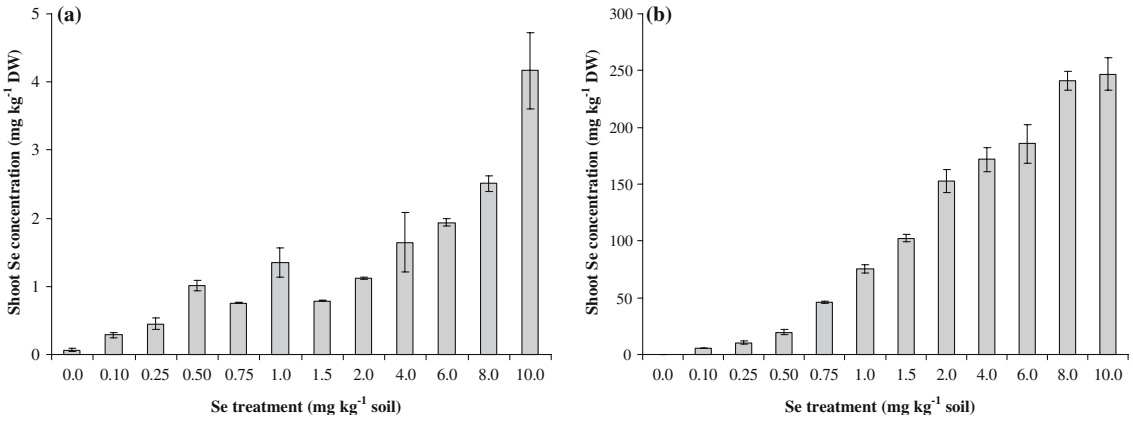

Figure 1. Se concentration in shoots of *Lolium perenne* cv. Aries at rates of application between 0 and 10 mg Se kg^{-1} soil with (a) Sodium selenite and (b) Sodium selenate.

were characterized by a general chlorosis of the leaves and necrosis at tips of the oldest leaves, particularly with the highest quantity of Se added to the soil. Thus, when 10 mg Se kg^{-1} soil were applied as selenate, DW was decreased by 48.7% as compared with the plants grown without Se.

GSH-Px activity and lipid peroxidation in plants

The application to the soil of both selenite and selenate increased GSH-Px activity in plants. However, the Se-dependent enzymatic activity became evident when shoot Se concentration and GSH-Px activity were intercorrelated for each Se source (Figure 3 a and b). Interestingly, both Se sources raised the GSH-Px activity, but when shoot Se concentrations and the enzymatic activity for both Se sources were plotted in a common graph, no correlation was found (data not shown). Thus, both selenite and selenate raised the GSH-Px activity in plants, but these increases were not related to the magnitude of increase of the total Se concentration in shoots when both Se sources were compared.

The addition of Se as selenite decreased the lipid peroxidation, monitored by TBARS, above 6 mg Se kg^{-1} soil, equivalent to a shoot Se concentration of 1.94 ± 0.06 mg Se kg^{-1} DW. When selenate was applied, it was observed a significant decrease in TBARS at rates of application between 0 and 0.5 mg Se kg^{-1} soil and, above this range lipid peroxidation steadily increased. Independently on the used Se source, the threshold for the decrease in TBARS corresponded to a shoot Se concentration of about 20 mg kg^{-1} DW (Figure 4).

Plant available Se in soil

Plant-available Se extracted by acid water (pH 4.5) at the end of the pot experiment showed the fertilization induced increase in soil Se to be dependent on the source applied (Figure 5a and b). As expected, selenate remained at least 100-fold more bioavailable than selenite at all the rates of Se added to the soil. On other hand, it is remarkable that a positive good correlation between available Se in soil and shoot Se concentration was found (Figure 6) when Se was applied as either selenite or selenate ($r = 0.945$; $p < 0.01$).

Discussion

Shoot Se concentration and antioxidant effect of Se in ryegrass

The role of Se as an antioxidant in plants has been previously reported in terms of increasing rates of Se application. However, the effect of different chemical forms of Se applied on both GSH-Px activity and lipid peroxidation have not been studied earlier. Moreover, a direct comparison of the effect of selenite and selenate on the antioxidant activity in higher plants, which define the threshold for Se toxicity in ryegrass have never been reported.

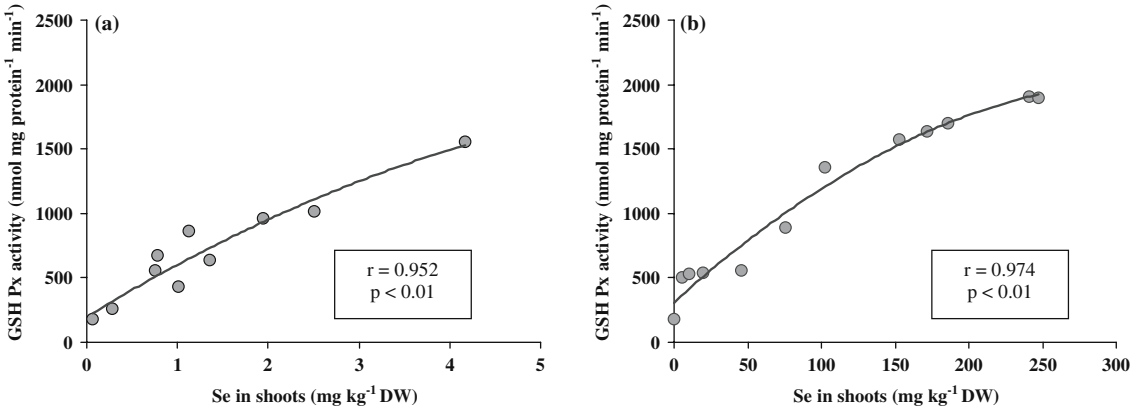

Figure 3. Pearson correlation between shoot Se concentration (mg kg^{-1} DW) and GSH-Px activity (nmol mg $protein^{-1}$ min^{-1}) in plants treated with (a) Sodium selenite and (b) Sodium selenate.

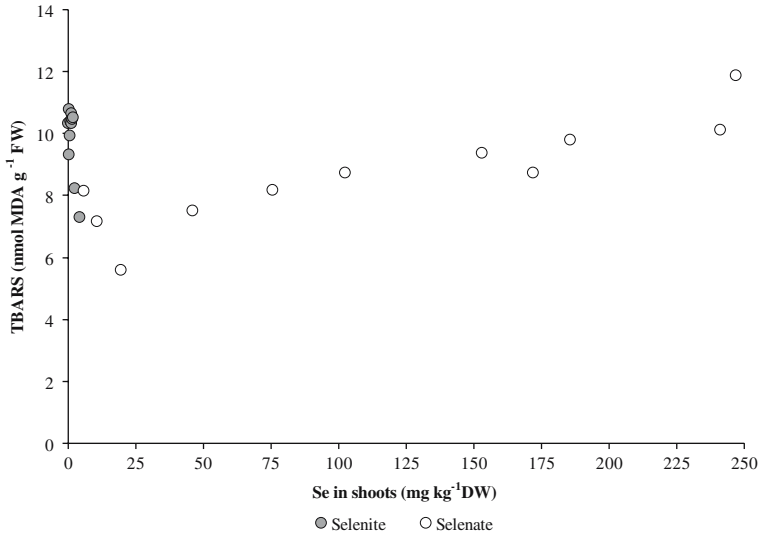

Figure 4. Relationship between lipid peroxidation (TBARS) and shoot Se concentration in selenite- and selenate-treated plants.

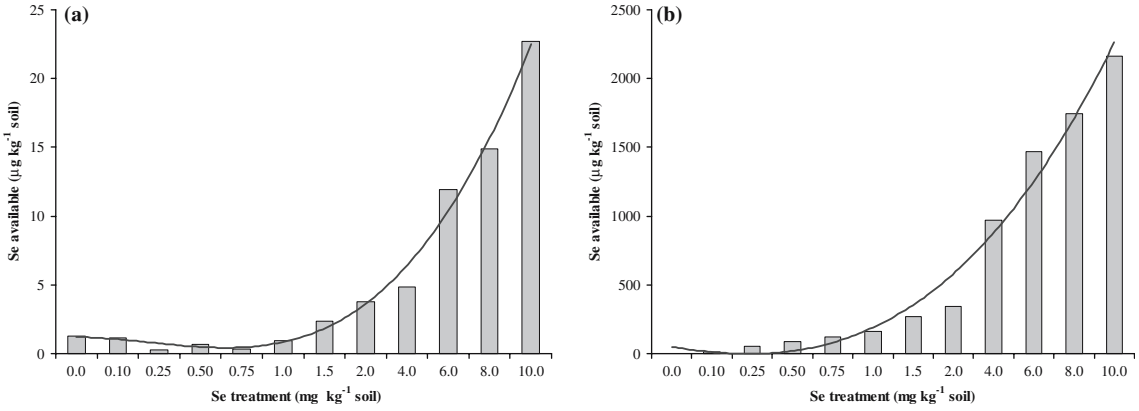

Figure 5. Se available in soil (μg kg^{-1} soil) at rates of application between 0 and 10 mg Se kg^{-1} soil with (a) Sodium selenite and (b) Sodium selenate.

The dissimilar response in shoot Se concentration to the application of selenite and selenate (Figure 1) is as expected. As demonstrated in sorption experiments in the same type of soil, selenite behaves like phosphate (Barrow et al., 2005) and therefore it is more stronger sorbed than selenate to the soil surfaces, thus becoming less bioavailable than selenate at equal rates of soil application. Furthermore, although selenite and selenate are uptaken quickly by plants roots, a much lower translocation occurs in selenite-treated plants than in selenate-treated plants (Arvy, 1993; Banuelos and Meek, 1989; de Souza et al., 1998; Hopper and Parker, 1999; Yläranta, 1990; Zayed et al., 1998).

The antioxidative effect of Se has been often related to an improved Se-dependent GSH-Px activity and a decreased lipid peroxidation in Se-treated plants, like ryegrass (Hartikainen et al., 1997, 2000), lettuce (Pennanen et al., 2002; Xue et al., 2001) and soybean (Djanaguiraman et al., 2005). The results shown in Figure 3a and b confirm that a raise in GSH-PX activity in response to the Se application occurred. It is noteworthy that, when both Se sources were compared, GSH-Px activity responded dissimilarly to the

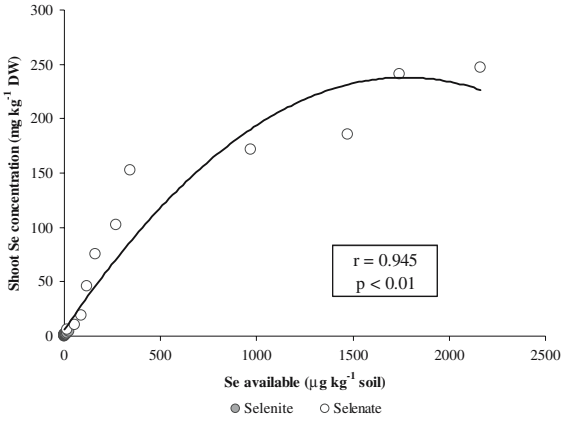

Figure 6. Pearson correlation between Se available in soil and shoot Se concentration (mg kg^{-1} DW) in selenite- and selenate-treated plants.

shoot Se concentration. However, this is the first report that compares the effect of two inorganic sources of Se on the GSH-Px activity. From the results it can be inferred that the Se-dependence of the GSH-Px activity must be related with the applied chemical forms of Se rather than with the Se concentration in plant tissues because they have different pathways of Se assimilation in higher plants (Terry et al., 2000). It can be expected that most of the applied Se as selenate remains as inorganic-Se in shoots, whereas most of the added selenite is incorporated as organic-Se, i.e., in Se-aminoacids and Se-proteins (de Souza et al., 1998; Zayed et al., 1998). As extensively reviewed by Stadtman (1980, 1991), GSH-Px enzymes are present in mammals and birds, and tentatively identified in a marine diatom, as soluble tetrameric proteins containing Se-cysteine residues, in plasma as a glycosylated form, and in membranes as a monomeric species that specifically reduces lipid peroxides. Although there are no concluding indications of the presence of a Se-dependent GSH-Px in higher plants, some molecular evidence of the machinery to synthesize Se-cysteine in the plant kingdom has been provided (Fu et al., 2002; Novoselov et al., 2002; Rao et al., 2003; Takeda et al., 2003). Moreover, Hatfield et al. (1992) identified UGA decoding Se-cysteyl-tRNA in sugar beet and, recently, in a study about the antioxidative properties of Se during photooxidative stress in potato, Seppänen et al. (2003) demonstrated the Se altered transcript accumulation of GSH-Px.

The level of lipid peroxidation was dependent on the shoot Se concentration rather than the chemical Se form supplied to plants (Figure 4). Thus, these results support the dual effect of Se on the antioxidative system in ryegrass, previously reported by Hartikainen et al. (2000): at low concentrations Se acted as an antioxidant, by reducing the accumulation of TBARS (Figure 4), whereas at shoot Se concentration above 20 mg kg^{-1} DW it acted as a pro-oxidant by increasing lipid peroxidation (Figure 4) and reducing the yields (Figure 2).

Soil Se determination and soil–plant Se relationship

As Andisols are acid soils with variable charge and high organic matter content, and in soil Se availability is governed by several physico-chemical factors (Gissel-Nielsen, 2002; Neal, 1990) the Se readily available to plants should be evaluated by a method able to simulate the acidity of rhizospheric soil. Data of Figure 5a and b show that the application of Se to soil (as selenite or selenate) raised steadily the concentration of available Se in soil and a good correlation was obtained between soil Se and shoot Se concentration (Figure 6). As respect to other digestion-extraction methods previously reported (Bujdoš et al., 2000; Fujii et al., 1988; Martens and Suárez, 1997; Tokunaga et al., 1991; Wang and Sippola, 1990), the extraction method here proposed appears a promising alternative for Se determination by AAS-GF because it is simple, less time-consuming, economically advantageous, and able to correlate soil Se with shoot Se concentration.

Acknowledgements

This work was supported by the FONDECYT project 1020934 and by the Grant projects Fundación Andes C-13755-28 and MECESUP FRO 0309. The authors especially thank Dr Helinä Hartikainen for hosting Dr Cartes between May and July of 2003 in the Department of Applied Chemistry and Microbiology of Helsinki University-Finland, and her help for the learning of the analytical techniques for the determination of plant Se concentration,

lipid peroxidation and GSH-Px activity. A sincere gratefulness to MSc Maija Ylinen, Dr Riitta Kivikari and Ms Salla Hartikainen for their technical support at Helsinki University. DiSSPA Contribution No 90.

References

Arvy M P 1993 Selenate and selenite uptake and translocation in bean plants (*Phaseolus vulgaris*). J. Exp. Bot. 44, 1083–1087.

Banuelos G S and Meek D W 1989 Selenium accumulation in selected vegetables. J. Plant Nutr. 12, 1251–1272.

Barrow J, Cartes P and Mora M L 2005 Modifications to the Freundlich equation to describe anion sorption over a large range and to describe competition between ions. Eur. J. Soil Sci. 0 (0): doi: 10.1111/ j.1365-2389.2005.00700.x.

Blakemore L C, Searle P L and Daly B K 1987 Methods for chemical analysis of soils. New Zealand Bureau Scientific Report Number 80. NZ Soil Bureau. Lower Hutt, New Zealand.

Bradford M 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Bujdoš M, Kubová J and Streško V 2000 Problems of selenium fractionation in soils rich in organic matter. Anal. Chim. Acta 408, 103–109.

de Souza M P, Pilon-Smits E A H, Lytle C M, Hwang S, Honma T S U, Yeh L and Terry N 1998 Rate-limiting steps in selenium assimilation and volatilization by Indian mustard. Plant Physiol. 117, 1487–1494.

Djanaguiraman M, Durga Devi D, Shanker A K, Sheeba J A and Bangarusamy U 2005. Selenium – an antioxidative protectant in soybean during senescence. Plant Soil 272, 77–86.

Du Z and Bramlage W 1992 Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. J. Agric. Food Chem. 40, 1566–1570.

EPA 1996 Method 3050B. Acid Digestion of Sediments, Sludges and Soils. En: SW-846 On Line. Test Methods for Evaluating Solid Wastes Physical/ Chemical Methods. http://www.epa.gov/epaoswer/hazwaste/test/3_series.

Flohé L and Günzler W A 1984 Assays of glutathione peroxidase. *In* Methods in Enzymology. Ed. L. Packer. Vol. 105, pp. 114–121. Academic Press, Inc., New York.

Flohé L, Günzler W A and Schock H H 1973 Glutathione peroxidase: A selenoenzyme. FEBS Lett. 32, 132–134.

Fu L -H, Wang X -F, Eyal Y, She Y -M, Donald L J, Standing K G and Ben-Hayyim G 2002 A selenoprotein in the plant kingdom. Mass spectrometry confirms that an opal codon (UGA) encodes selenocysteine in *Chlamidomonas reinhardtii* glutathione peroxidase. J. Biol. Chem. 277, 25983–25991.

Fujii R, Deverel S J and Hatfield D B 1988 Distribution of selenium in soils of agricultural fields, Western San Joaquin Valley, California. Soil Sci. Soc. Am. J. 52, 1274–1283.

Gissel-Nielsen G, Gupta U C, Lamand M and Westermarck T 1984 Selenium in soils and plants and its importance in livestock and human nutrition. Adv. Agron. 37, 397–460.

Gissel-Nielsen G 2002 Selenium. *In* Encyclopedia of Soil Science. Ed. R Lal. Pp. 1476. M. Dekker Inc. Schoool of Natural Resources. The Ohio State University Columbus, Ohio, USA.

Gupta U C and Gupta S C 2000 Selenium in soils and crops, its deficiencies in livestock and humans: Implications for management. Commun. Soil Sci. Plant Anal. 31, 1791–1807.

Gupta U C and Watkinson J H 1985 Agricultural significance of selenium. Outlook Agric. 14, 183–189.

Hartikainen H and Xue T 1999 The promotive effect of selenium on plant growth as triggered by ultraviolet irradiation. J. Environ. Qual. 28, 1372–1375.

Hartikainen H, Ekholm P, Piironen V, Xue T, Koivu T and Yli-Halla M 1997 Quality of the ryegrass and lettuce yields as affected by selenium fertilization. Agr. Food Sci. Fin. 6, 381–387.

Hartikainen H, Xue T and Piironen V 2000 Selenium as antioxidant and pro-oxidant in ryegrass. Plant Soil 225, 193–200.

Hatfield D, Choi I, Mischke S and Owens L 1992 Selenocysteil-tRNA UGA in *Beta vulgaris*, a higher plant, and in *Gliocladium virens*, a filamentous fungus. Biochem. Biophys. Res. Commun. 184, 254–259.

Heath R and Packer L 1968 Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–198.

Hopper J and Parker D 1999 Plant availability of selenite and selenate as influenced by competing ions phosphate and sulfate. Plant Soil. 210, 199–207.

Kumpulainen J, Raittila A M, Lehto J and Koivistoinen P 1983 Electrothermal atomic absorption spectrometric determination of selenium in foods and diets. J. Assoc. Anal. Chem. 66, 1129–1135.

Johnsson L 1992 Selenium in Swedish soils. Factors influencing soil content and plant uptake. Ph.D. Thesis. Department of Soil Sciences, Swedish University of Agricultural Sciences. Reports and Dissertations No. 10.

Martens D A and Suárez D L 1997 Selenium speciation of soil/sediment determined with sequential extractions and hydride generation atomic absorption spectrophotometry. Environ. Sci. Technol. 31, 133–139.

Murphy J and Riley J P 1962 A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.

Neal RH 1990 Selenium. *In* Heavy Metals in Soils. Ed. B J Alloway. Blackie and Son Ltd. 339 p.

Novoselov S, Rao M, Onoshko N, Zhi H, Kryukov G, Xiang Y, Weeks D, Hatfield D and Gladyshev V 2002 Selenoproteins and selenocysteine insertion system in the model plant cell system *Chlamidomonas reinhardtii*. EMBO J. 21, 3681–3693.

Nowak J, Kaklewski K and Ligocki M 2004 Influence of selenium on oxidoreductive enzymes activity in soil and plants. Soil Biol. Biochem. 36, 1553–1558.

Pennanen A, Xue T and Hartikainen H 2002 Protective role of selenium in plant subjected to severe UV irradiation stress. J. Appl. Bot. 76, 66–76.

Rao M, Carlson B, Novoselov S, Weeks D, Gladyshev V and Hatfield D 2003 *Chlamidomonas reinhardtii* selenocysteine $tRNA^{[Ser]Sec}$. RNA 9, 923–930.

Rotruck J T, Pope A L, Ganther H E, Swanson A B, Hafeman D G and Hoekstra W G 1973 Selenium: Biochemical role as a component of glutathione peroxidase. Science 179, 588–590.

Seppänen M, Turakainen M and Hartikainen H 2003 Selenium effects on oxidative stress in potato. Plant Sci. 165, 311–319.

Stadtman T 1980 Selenium-dependent enzymes. Ann. Rev. Biochem. 49, 93–110.

Stadtman T 1991 Biosintesis and function of selenocysteine-containing enzyme. J. Biol. Chem. 266, 16257–16260.

Tabatabai M A 1982 Sulfur. *In* Methods of Soil Analysis. Part 2-Chemical and Microbiological Properties, Eds. A L Page R H Miller and D R. Keeney. pp. 501–538. American Society of Agronomy, Madison, WI.

Takeda T, Nakano Y and Shigeoka S 1993 Effects of selenite, CO_2 and illumination on the induction of selenium-dependent glutathione peroxidase in *Chlamidomonas reinhardtii*. Plant Sci. 94, 81–88.

Takeda T, Miyao K, Tamoi M, Kanaboshi H, Miyasaka H and Shigeoka S 2003 Molecular characterization of glutathione peroxidase-like protein in halotolerant *Chlamidomonas* sp. W80. Physiol. Plant 117, 467–475.

Terry N, Zayed A M, de Souza M P and Tarun A S 2000 Selenium in higher plants. Ann. Rev. Plant. Phys. Plant Mol. Biol. 51, 401–432.

Tokunaga T K, Lipton D S, Benson S M, Yee A W, Oldfather J M, Duckart E C, Johannis P W and Halvorsen K E 1991 Soil selenium fractionation, depth profiles and time trends in a vegetated upland at Kesterson Reservoir. Water Air Soil Pollut. 57–58, 31–41.

Wang D and Sippola J 1990 Selenium in soil extracts and plants determined by fluorometry. Ann. Agric. Fenn. 29, 151–156.

Whelan B R and Barrow N J 1994 Slow-release selenium fertilizers to correct selenium deficiency in grazing sheep in Western Australia. Fert. Res. 38, 183–188.

Whelan B R, Peter D W and Barrow N J 1994a Selenium fertilizers for pasture grazed by sheep I Selenium concentrations in whole blood and plasma. Aust. J. Agric. Res. 45, 863–875.

Whelan B R, Barrow N J and Peter D W 1994b Selenium fertilizers for pasture grazed by sheep II Wool and live weight responses to selenium. Aust. J. Agric. Res. 45, 877–887.

Wittwer A, Araneda P, Ceballos A, Contreras P, Andaur M and Böhwald H 2002 Actividad de glutatión peroxidasa (GSH-Px) en sangre de bovinos a pastoreo de la IX Región, Chile y su relación con la concentración de selenio en el forraje. Arch. Med. Vet. 34, 49–57.

Xue T and Hartikainen H 2000 Association of antioxidative enzymes with the synergic effect of selenium and UV irradiation in enhancing plant growth. Agric. Food Sci. Fin. 9, 177–186.

Xue T, Hartikainen H and Piironen V 2001 Antioxidative and growth-promoting effect of selenium on senescing lettuce. Plant Soil 237, 55–61.

Yläranta T 1983a Effect of added selenite and selenate on the selenium content of Italian ryegrass (*Lolium multiflorum*) in different soils. Ann. Agric. Fenn. 22, 139–151.

Yläranta T. 1983b Effect of liming and sulphate on the selenium content of Italian rye grass (*Lolium multiflorum*) in different soils. Ann. Agric. Fenn. 22, 152–163.

Yläranta T. 1983c Effect of applied selenite and selenate on the selenium content of barley (*Hordeum vulgare*). Ann. Agric. Fenn. 22, 164–174.

Yläranta T 1985 Increasing the selenium content of cereals and grass crops in Finland. Academic dissertation. Faculty of Agriculture and Forestry, University of Helsinki, 72p.

Yläranta T 1990 Effects of the addition of sulphate and phosphate on the leaching of selenite and selenate in acidic soils. Ann. Agric. Fenn. 30, 311–319.

Yokota A, Shigeoka S, Onishi T and Kitaoka S 1988 Selenium as inducer of glutathione peroxidase in low-CO_2-grown *Chlamidomonas reinhardtii*. Plant Physiol. 86, 649–651.

Zayed A, Lytle C M and Terry N 1998 Accumulation and volatilization of different chemical species of selenium by plants. Planta 206, 284–292.

Section editor: A.C. Borstlap