

# Selenium species in the roots and shoots of chickpea plants treated with different concentrations of sodium selenite

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**Abstract** The trace element selenium has an essential role for human health. It is involved in redox center functions, and it is related to the immune system response. Legumes are among the main suppliers of selenium into the human food chain. Not only Se concentration as such but also more the chemical species of Se is of higher importance for successful Se supply to the human diet and its bioavailability. The current study was focused on the investigation of the Se species present in chickpea plants exposed to 0, 10, 25, 50, and 100  $\mu\text{M}$  selenite in short- and long-term treatment studies. The linear increase of total Se concentration could be linked to the increased concentrations of Se exposure. The selenium species (SeMet, SeCys, selenite, selenate, GPx) detected in varying concentrations in shoots and roots depend on the exposure's concentration and duration. The investigation showed that chickpea can accumulate Se in favorable concentrations and its transformation to bioavailable Se species may have positive impacts on human health and aid to implement Se into the diet.

**Keywords** Chickpea · Glutathione peroxidase · Selenium speciation · Selenocysteine · Selenomethionine · Selenate · Selenite

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## Introduction

The significant role of micronutrients for human health has been identified by complementary surveys. Specifically, the population in the developing countries is affected from micronutrient hunger, referring to irregular or no access to differentiated food and vitamin-packed groceries. This results in micronutrients (e.g., iron, zinc) and (or) vitamin deficiencies. But, also developed countries are confronted with deficiency syndromes. Micronutrient uptake depends on the soil concentrations, and people living in regions poor in soil micronutrients absorb them in inadequate quantities. The chemical form of the micronutrients in the soil and soil composition are factors important for micronutrient uptake in plants.

Selenium (Se) is chemically analogous to sulfur; it reacts with water and forms hydrogen selenide, oxides, and sulfides, too. After nature, Se is found in micro amounts in Se-containing ores, soils, and rocks. Selenium soil concentrations vary. Its action in soil depends on soil pH, soil redox potential, and soil composition. Selenium ions reach root surfaces via diffusion or mass flow where they are released through water transport. In plants, Se is believed to be taken up as  $\text{SeO}_4^{2-}$  using the same mediators like  $\text{SO}_4^{2-}$  in a competitive manner.

Selenium abundance in plants depends on Se concentration and species in the soil. In alkaline soils Se is present as soluble selenate, which can be easily assimilated and accumulated in plants. In acidic soils, Se is bound in iron(II) or iron(III) selenite and can be assimilated in plants only in limited amounts. Elemental Se is not bioavailable at all (Simonova and Pfannhauser 2008). According to Jezek et al. (2012), soil-Na-selenite fertilization is more environmentally compatible than Na-selenate fertilization. This could be explained by the lower Na-selenite mobility in the soil and its oxidation to selenate, which is the less-water-soluble species. Some researchers report about lower Se uptake rates compared to soil

Se application after direct leaf selenite application (Jezek et al. 2012). So far, selenite transport is not completely unraveled; however, it is supposed that phosphorus transporters may be involved in the process (Jezek et al. 2012).

Selenium uptake is different in Se assimilators and Se hyperaccumulators. Se assimilators are plant species growing on soils with very low Se content (e.g., fruits, vegetables, and cereals). On the other hand, Se hyperaccumulators could take up 100-fold higher Se concentrations in comparison to the Se assimilators, e.g., some of the *Astragalus*, *Haplopappus*, and *Stanleya*. Selenium is able to replace sulfur (S) in S metabolism and bothers its function. This property of Se has been described as nonessential in vascular plants; however, it may be essential for those species accumulating high Se concentrations (Jezek et al. 2012; Pilon-Smits et al. 2009). The hyperaccumulators defend themselves from negative effects of Se by storing it as Se-methylselenocysteine (MeSeCys, 80 %) or in proteins (20 %).

Se species distribution studies indicate that Se(VI) is transported apoplastically (by inert transport) without infiltration into the cell and without Se species transformation, but Se(IV) transport is symplastically (Se crosses the cell membrane), where it is reduced to Se amino acids (Bulska et al. 2006).

The focus of the current study was to assess of a possible fertilization effect of sodium selenite in shoots and roots of chickpea after short- and long-term exposures, as these aspects have been little studied. Chickpea (*Cicer arietinum* L.) is an important legume crop in the Mediterranean region, but its importance in the central part of Europe increases as well. Among legumes, chickpea accumulates the highest concentrations of Se in the pea, ranging between 104  $\mu\text{g kg}^{-1}$  fresh weight (FW) (Diaz-Alarcon et al. 1996) and 270  $\mu\text{g kg}^{-1}$  dry weight (DW) (Matos-Reyes et al. 2010). Despite its potential, only limited number of studies has been performed on the Se speciation in this legume crop (Poblaciones et al. 2014; Zhang et al. 2012; Thavarajah et al. 2008; Sugihara et al. 2004). So far, they are no publications on Se speciation in chickpea shoot and root, which are used as fodder. In order to gain new insights into Se speciation, extracts of chickpea shoot and root samples were analyzed using established techniques, such as inductively coupled plasma atomic emission spectrometer (ICP-AES) for total Se determination or high-pressure liquid chromatography (HPLC)-ICP-DRC-MS for Se speciation studies. ICP-DRC-MS was employed as a detector for speciation experiments as single Se species concentrations could have been low-concentrated and thus below the detection limit of ICP-AES. For checking comparability of the systems, total Se results from both analytical systems were compared and found to show no significant differences. Overall, these studies could contribute for winning knowledge about Se species transformation during their transport in plant tissues as well.

## Material and methods

### Chickpea cultivation

All chickpea treatments were performed under controlled greenhouse conditions in two experiments: short term (14 days) and long term (45 days). Chickpea seeds were germinated for 7 days before replanting them to the pots. The plants were grown under semi-hydroponic conditions in perlite. The greenhouse conditions were set to the following: 23 °C (day), 18 °C (night), and 65 % relative humidity. Micronutrient and macronutrient supply was provided by Murashige and Skoog nutrient solution (Murashige and Skoog 1962).

**Short-term experiment** For each sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) concentration, eight pots (10-cm diameter) were treated, each containing four plants. The plants were grown for 17 days in nutrient solution without sodium selenite before exposing them to different concentrations of 0, 10, 25, 50, and 100  $\mu\text{M}$  sodium selenite for 14 days.

**Long-term experiment** The experiment was performed with six pots (24-cm diameter), each containing four plants. The plants were pre-grown for 27 days before the start of the treatment with 0, 10, 50, and 100  $\mu\text{M}$  sodium selenite. The chickpea plants were exposed to Se for 45 days.

### Sample preparation

Selenium-exposed plants (“number” per Se exposure concentration/experimental setup) were pooled and rinsed with distilled water before drying for 5 days at 60 °C, until a constant DW was reached. Subsequently, the dry plant material was divided into roots and shoots and both subfractions were ground for 5 min each in a ball mill (SPEX Industries GmbH).

### Reagents and chemicals

Protease XIV from *Streptomyces griseus* (type XIV: bacterial, 4.4 units/mg solid), sodium selenite ( $\text{Na}_2\text{SeO}_3$ ), selenocysteine (SeCys), and glutathione peroxidase (EC 232-749-6)-bound selenium (SeGPx) were ordered from Sigma-Aldrich (Deisenhofen, Germany). The certified Se stock standards (1000 mg/L) are products of CPI International (Santa Rosa, CA, USA). Ammonium acetate ( $\text{NH}_4\text{OAc}$ ) and acetic acid (HOAc) were ordered from Merck (Darmstadt, Germany). Sodium selenite stock solutions were prepared at a concentration of 1000 mg Se/L by dissolving in Milli-Q water (Milli-Q system, Millipore, Bedford, MA, USA).

## Selenium determination

Aliquots of ground shoot and root samples (see above) were acid-digested (0.09–0.11-g sample in 1 mL concentrated sub-boiled distilled HNO<sub>3</sub>) in a digestion system (Seif, Unterschleissheim, Germany) during 10 h at 170 °C in drying oven (Memmert, Schwabach, Germany). During the period of the digestion, the system's pressure reached 10–12 bar. Then, the total volume of each sample was brought up to 10 mL with double-deionized water. Digested samples were used for Se concentration determination using an ICP-AES "Spectro Ciros Vision" (Spectro Analytical Instruments GmbH & Co. KG, Kleve, Germany). Sample introduction was carried out using a peristaltic pump (flow rate 0.8 mL min<sup>-1</sup>) connected to a SeaSpray nebulizer with a cyclone spray chamber. The measured spectral element line was as follows: Se 196.090 nm. The RF power was set to 1350 W, the plasma gas was 15 L Ar min<sup>-1</sup>, the auxiliary gas was 0.2 L Ar min<sup>-1</sup>, whereas the nebulizer gas was 600 mL Ar min<sup>-1</sup>. Every ten measurements, three blank determinations and a control determination of a certified Se standard were performed. Calculation of results was carried out on a computerized lab data management system, relating the sample measurements to calibration curves, blank determinations, control standards, and the weight of the digested sample. The measurements were checked by using certified standard reference materials from IRMM/EU.

## Selenium species extraction

Soluble Se species from chickpea shoots and roots were extracted by enzymatic hydrolysis (Vogrinčič et al. 2009). Eight milliliters of a solution containing 60 mg of enzyme protease XIV were added to 0.6-g ground chickpea samples. The mixture was shaken for 24 h in a water bath at 37 °C at 15,000g, followed by centrifugation for 60 min at 4 °C at 38,000g. The supernatant and the residue were separated by filtration through 0.45- and 0.22- $\mu$ m Millex GV filters (Millipore Co). Both extracted fractions were stored at -20 °C until total Se analysis by ICP-AES and Se speciation by HPLC-ICP-DRC-MS were performed.

## Selenium speciation

According to formerly established methods for biological matrices (Michalke and Berthele 2011; Solovyev et al. 2013), total Se and selenomethionine (SeMet), glutathione peroxidase (GPx), selenocysteine (SeCys), thioredoxin reductase (TrxR), selenite (SeIV), and selenate (SeVI) were determined in the chickpea samples using ion exchange chromatography (IEC) coupled with inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS). ICP-DRC-MS was employed for speciation experiments, as single Se species concentrations could have been low-concentrated and thus

below the detection limit of ICP-AES. The results of the total Se detected with ICP-DRC-MS were compared to the results from the ICP-AES analysis. No significant differences were seen, proving comparability of both analytical systems. For speciation, each extract was analyzed in duplicate. The sum of the quantified Se species was compared to the total Se determination, and the calculated recovery was in the range of 88–104 %.

## Ion exchange chromatography

A Knauer 1100 Smartline inert Series gradient HPLC system was connected to an anion exchange AG 11 pre-column (50×4 mm ID)+ AS 11 analytical column (250×4 mm ID, Thermo (Dionex Idstein, Germany)) for species separation in a sample volume of 100  $\mu$ L. The mobile phases were as follows: eluent A: 10 mM Tris-HAc (TRIS-acetate buffer), pH 8.0, and eluent B: A + 500 mM NH<sub>4</sub>Ac (ammonium acetate), pH 8.0. Gradient elution was expressed as % eluent A: 0–3 min 100 %, 3–10 min 100–60 %, 10–23 min 60–45 %, 23–26 min 45–43 %, 26–28 min 43–0 %, 28–52 min 0 %, and 52–60 min 100 %. The flow rate was 0.70 mL min<sup>-1</sup>. For internal standardization, the column effluent was mixed with 1  $\mu$ g L<sup>-1</sup> rhodium (final concentration, total flow rate 0.75 mL min<sup>-1</sup>) and directed to ICP-MS.

## Peak identification

The peaks were identified according to Solovyev et al. (2013). When the peaks were matching to respective retention times, they were assigned to specific compounds. Since the used methods were developed for human matrices, retention-based Se species identification by only one method could lead to erroneous assignments. Therefore, we used additionally different combinations of chromatographic separations (2-D approaches), followed by on-line coupled ICP-DRC-MS detection: size exclusion chromatography (SEC).

## Size exclusion chromatography

SEC was used as the primary separation step to prepare mass-range-characterized SEC fractions for a 2-D approach for species identification. The Knauer 1100 Smartline HPLC system was connected to a column combination of 300×10-mm column, filled with TSK 55 S HW being in series with a column 250×10 mm, filled with TSK 40 S SW (both Tosoh Haas, Stuttgart, total separation range ca. 200–0.1 kDa). Tris-HAc (10 mM, pH 7.4) + 250 mM NH<sub>4</sub>Ac was used as the eluent at a flow rate of 0.75 mL min<sup>-1</sup>. Mass calibration was performed using protein standards of defined molecular mass. Retention times related to molecular masses followed the equations  $\ln(\text{kDa}) = -0.179 \times \text{RT} + 9.185$  ( $r^2 = 0.998$ ) for proteins and  $\ln(\text{Da}) = -0.315 \times \text{RT} + 16.606$  ( $r^2 = 0.986$ ) for low-molecular-

mass (LMM) compounds. For subsequent analysis, the SEC column effluent was fractionated by a Fraction Collector 100 (Pharmacia, Freiburg, Germany) and fractions were subject to either IEC-ICP-DRC-MS (see above) or affinity chromatography (AFC)-ICP-DRC-MS.

**Affinity chromatography**

Since specifically, a major unknown Se compound eluted at close to the retention time of selenoprotein P during IEC analysis, the AFC method aimed to explore the presence of this Se species in plant samples, but the method provided also information about GPx. Mass-characterized SEC fractions from the primary separation were injected on a heparin affinity column (Amersham, GE Healthcare Europe GmbH, Munich, Germany) and chromatographed by a linear 12 min lasting gradient of A=50 mM Tris, 10 mM NH<sub>4</sub>-acetate/acetic acid, pH 6.0, to buffer B = A but 800 mM NH<sub>4</sub>-acetate, pH 8.5, and remaining at 100 % B for further 8 min. The AFC column elution was monitored by ICP-DRC-MS.

Overall, Se species were analyzed (each monitored by ICP-DRC-MS) first by IEC, SEC, and AFC and second by combinations of SEC-IEC and SEC-AFC. Peak assignments were only positively done when each of the five systems proved the presence of a compound (in case of AFC: specific elution for selenoprotein P (SePP) and glutathione peroxidase (GPx), positive but nonspecific elution for other compounds).

**Results**

**Total Se accumulation**

Chickpea plants enriched with selenium did not exhibit any visual symptoms of selenium toxicity. Se content in chickpea plants after both short- and long-term Se exposure increased in shoots and roots polynomial after 14 days of exposure (Table 1). Selenium concentrations detected in chickpea controls (shoot and root) after 45 days of Se exposure correspond to the concentrations detected after 14 days of Se exposure (Table 1). Increased exposure concentrations led to 3-fold increase of Se content in long-term (LT)-treated plants in comparison to short-term (ST) Se-exposed plants. For example, shoot Se contents after 14 days were 6.3 mg kg<sup>-1</sup> Se (10 μM) and 18 mg kg<sup>-1</sup> Se (10 μM) after 45 days, respectively. Se concentrations detected after 50-μM Se exposure were 16 mg kg<sup>-1</sup> Se (14 days) and 51 mg kg<sup>-1</sup> (45 days), respectively. The highest Se content in shoots was detected after 45 days of Se exposure with 50 μM Se (Table 1).

During 45 days of exposure, Se concentration in chickpea roots increased less compared to Se concentration during 14 days. Although Se content in controls of roots in both experiments was equal, i.e., 0.35–0.38 mg kg<sup>-1</sup>, a significant

**Table 1** Se content description in chickpeas treated for 14 days (short term) and for 45 days (long term) with sodium selenite/total Se obtained by ICP-AES

Treatment	Total Se ICP-AES (mg kg <sup>-1</sup> )	Identified Se species (% of total Se)
Short term		
Shoot		
Control	0.41	95.9
10 μM Se	6.34	67.7
25 μM Se	13.05	57.4
50 μM Se	16.25	76.1
100 μM Se	16.13	93.3
Root		
Control	0.35	95.8
10 μM Se	16.62	93.9
25 μM Se	25.40	71.8
50 μM Se	39.61	82.5
100 μM Se	57.97	92.2
Long term		
Shoot		
Control	0.42	60.1
10 μM Se	18.00	95.3
50 μM Se	50.85	99.3
100 μM Se	29.50	89.1
Root		
Control	0.38	21.3
10 μM Se	11.18	83.8
50 μM Se	45.83	91.1
100 μM Se	36.06	98.8

Se recovery (expressed as %) is the percentage amount of identified Se species by standard matching in relation to the total Se obtained by ICP-AES

difference in Se content was detected after 100 μM of Se exposure: 36 mg kg<sup>-1</sup> (45 days) versus 58 mg kg<sup>-1</sup> (14 days), respectively (Table 1). The polynomial regression coefficient for selenium uptake roots after 45 days was *r*<sup>2</sup>=0.99.

**Se speciation**

*Species identification*

The reliability of the peak assignment by matching the retention time was checked at the onset of the experiments. Table 2 sums up the Se species identification process.

Most compounds were identified by matching in different chromatographic systems and in 2-D combinations of these chromatographic approaches. However, when the regular IEC-ICP-DRC-MS system was used, a Se species peak could be detected in all analyzed extract samples, which seemingly could have been assigned to SePP when only the retention

**Table 2** Summary of the Se species identification process

Se compound	Found at respective retention time with			Status
	SEC	IEC (after SEC)	AFC (after SEC)	
SePP	No	Not specific, co-elution possible	No, very specific	Not present
GPx	Yes	Yes	Yes	Present
TrxR	Yes	Yes	Yes, but not specific	Present
SeMet	Yes	Yes	Yes, but not specific	Present
SeCys	Yes	Yes	Yes, but not specific	Present
SeIV	Yes	Yes	Yes, but not specific	Present
SeVI	Yes	Yes	Yes, but not specific	Present

time match by IEC would have been applied. However, SEC and especially AFC analysis clearly excluded the presence of SePP. The respective Se-containing compound is therefore considered as unknown Se species in this paper. Its Se amount varied considerably and reflects the difference of the Se content between total Se and identified Se compounds (Table 1). In future investigations, attempts will be performed to elucidate the nature of that Se species. The subsequent sections will focus only on identified Se species.

#### Short-term Se exposure (14 days)

Untreated chickpea shoots accumulated higher selenomethionine (SeMet) concentrations than roots. The highest SeMet concentrations were measured after 100- $\mu\text{M}$  ST Se exposure in shoots and after 50- $\mu\text{M}$  Se exposure in roots. The highest shoot/root ratio concerning their SeMet concentrations was 3:1 (Fig. 1).

The concentration increase during Se exposure resulted in a strong reduction of chickpea glutathione peroxidase (GPx) content in shoots (Fig. 1). After 100- $\mu\text{M}$  Se exposure, GPx concentration was only 10 % of the levels detected in the control shoots (15  $\mu\text{g kg}^{-1}$ ). The GPx content in roots decreased much less than in shoots. The highest GPx content was detected in the roots after 25- $\mu\text{M}$  ST Se exposure (Fig. 1).

Selenocysteine (SeCys) was detected in equal concentrations in the controls of chickpea shoot and root (126 and 122  $\mu\text{g kg}^{-1}$ , respectively). SeCys content was strongly reduced due to the increase of exposure concentrations in ST shoot (10, 25, 50  $\mu\text{M}$ ) (Fig. 1). Exposure to 10  $\mu\text{M}$  Se reduced the SeCys content in roots as well. All other Se treatments (25, 50, 100  $\mu\text{M}$  Se) led to declining SeCys content, in ST chickpea roots even more than 10-fold (Fig. 1).

In all ST shoot samples, thioredoxin reductase (TrxR) was below the limit of detection (LOD). Similar results were also obtained for ST root samples. Exceptions were the experiments with 10  $\mu\text{M}$  Se (1.2  $\mu\text{g kg}^{-1}$  TrxR) and 100  $\mu\text{M}$  Se (0.8  $\mu\text{g kg}^{-1}$  TrxR) (Fig. 1).

Selenite (SeIV) was detected in roots only after 25- $\mu\text{M}$  short-term Se exposure. Se exposure neither in chickpea shoot

nor in root led to further detectable SeIV contents. Selenate (SeVI) was detected in all samples except 25- $\mu\text{M}$  Se roots. SeVI content in ST shoot and root was equal (between 1 and 2  $\mu\text{g kg}^{-1}$ ) with exception of shoot and root controls, where values of 51 and 75  $\mu\text{g kg}^{-1}$ , respectively, were detected (Fig. 1).

#### Long-term Se exposure (45 days)

Selenomethionine (SeMet) contents of control chickpea shoots and roots after LT Se exposure were not comparable to those after ST Se exposure. SeMet was not detected in roots, but its shoot concentration accounted to 39  $\mu\text{g kg}^{-1}$ . The maximum SeMet content was detected after 10- $\mu\text{M}$  Se exposure in shoots and roots (Fig. 2). At 100  $\mu\text{M}$  Se, the SeMet content in shoots was reduced to the levels detected in the control.

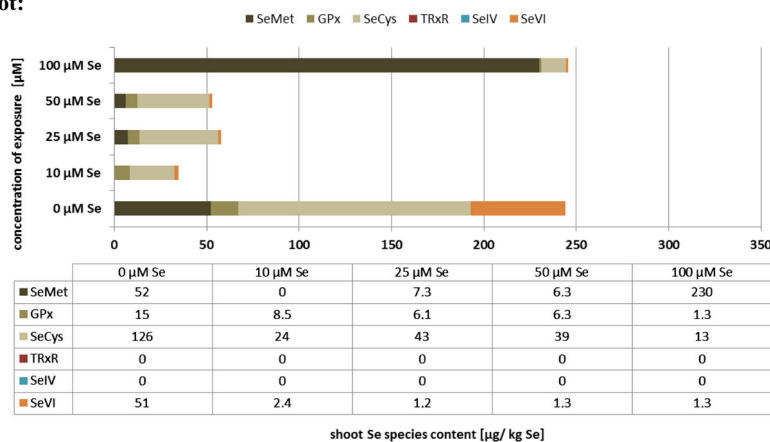
Glutathione peroxidase (GPx) content in LT chickpea shoots was reduced 4-fold compared to GPx content in the root control treatment. The increasing concentration of Se exposure (10–50  $\mu\text{M}$  Se) kept the GPx content in roots at the levels of controls. The highest GPx content in shoots was detected after 10  $\mu\text{M}$  Se (43  $\mu\text{g kg}^{-1}$ ), which was a 6-fold increase compared to controls. The treatment with the highest Se concentration (100  $\mu\text{M}$  Se) doubled the GPx content in chickpea roots and reduced the GPx content in shoots (Fig. 2).

In LT roots, selenocysteine (SeCys) was below the LOD for the concentrations of 0, 10, and 50  $\mu\text{M}$  Se. After 100- $\mu\text{M}$  Se exposure, 6.3  $\mu\text{g kg}^{-1}$  SeCys was detected. Contrary to this, SeCys content in chickpea shoot decreased in relation to the increase of Se exposure. SeCys content in shoot control was 17  $\mu\text{g kg}^{-1}$  and, after 100  $\mu\text{M}$  Se, only 2.5  $\mu\text{g kg}^{-1}$ , respectively (Fig. 2).

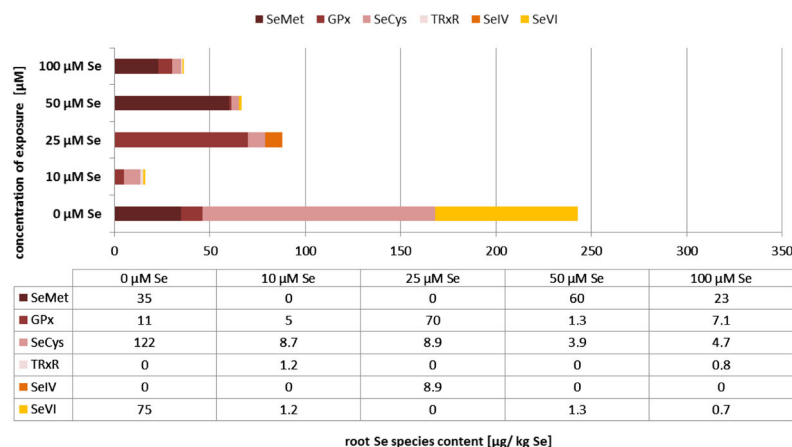
The thioredoxin reductase (TrxR) content in LT roots was comparable to SeCys content and was detected only after 100- $\mu\text{M}$  Se exposure. TrxR contented in LT shoots increased 10-fold from 1.3  $\mu\text{g kg}^{-1}$  (after 10  $\mu\text{M}$  Se) to 15  $\mu\text{g kg}^{-1}$  (after 50  $\mu\text{M}$  Se).

**Fig. 1** Se species ( $\mu\text{g kg}^{-1}$ ) detected after 14-day (short term) sodium selenite treatment. *GPx* glutathione peroxidase, *SeCys* Se cysteine, *SeMet* Se methionine, *TrxR* thioredoxin reductase, *SeIV* selenite, *SeVI* selenate

**Shoot:**



**Root:**



Selenite (SeIV) and selenate (SeVI) contents after long-term Se treatment were significantly affected in shoots and in roots. Neither SeIV nor SeVI was detected in controls of chickpea shoots and roots. SeIV was not detected after 10- and 100- $\mu\text{M Se}$  exposure in shoots and after 100- $\mu\text{M Se}$  exposure in the roots as well. In contrast, 6.7  $\mu\text{g kg}^{-1}$  SeVI (10  $\mu\text{M Se}$ ) and 3.8  $\mu\text{g kg}^{-1}$  SeVI (100  $\mu\text{M Se}$ ) were detected in LT-exposed shoots. The SeVI content in roots increased from 4.9 to 15  $\mu\text{g kg}^{-1}$  (10–50  $\mu\text{M Se}$ ) and reduced again after 100- $\mu\text{M Se}$  exposure—2.5  $\mu\text{g kg}^{-1}$  (Fig. 2).

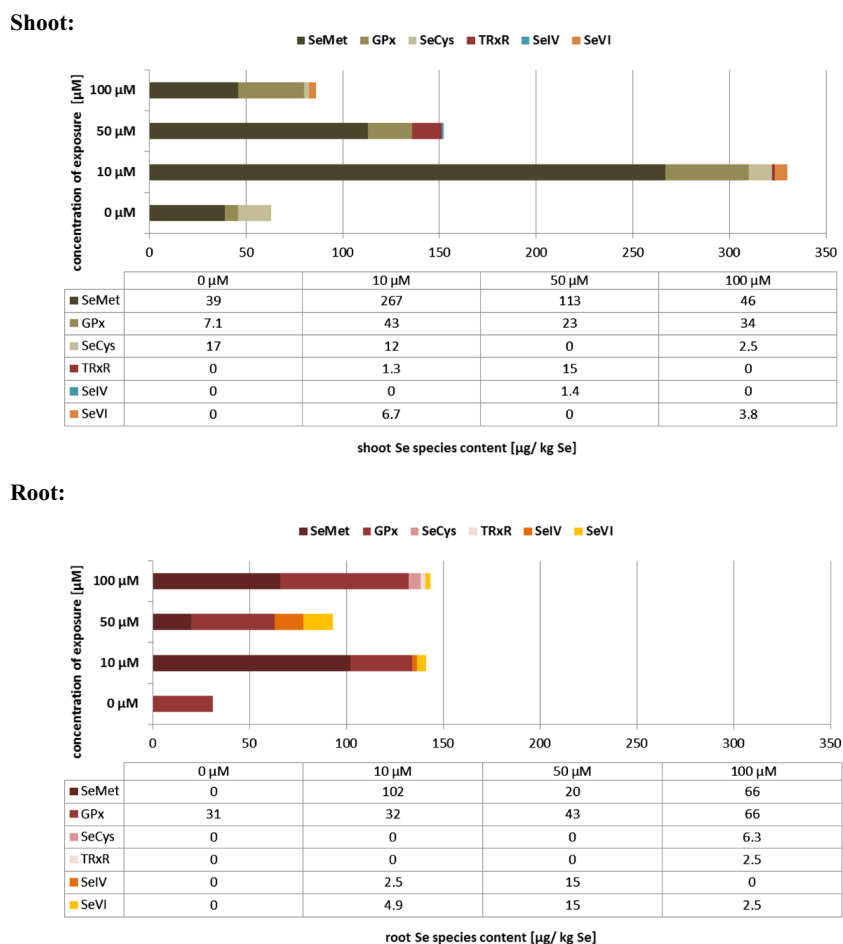
**Discussion**

Selenium is accepted to be a favorable nutrient, but it has not been shown to be essential for higher plants (Pilon-Smits et al. 2009; Hartikainen 2005; Lyons et al. 2009). In plants, Se uptake and accumulation might be influenced by the concentration of major anions (e.g.,  $\text{SO}_4^{2-}$ ). Root transport of  $\text{SO}_4^{2-}$  and  $\text{SeO}_4^{2-}$  of some plant species is assisted by a common cell membrane carrier, and the anions compete for their binding sites. In consequence, Se uptake is inhibited by external  $\text{SO}_4^{2-}$  (Germ et al.

2007; Grieve et al. 2001). Both forms, selenate ( $\text{SeO}_4^{2-}$ ) and selenite ( $\text{SeO}_3^{2-}$ ), are taken up by plants. Selenate uptake and distribution occur mainly in the form of sulfate-proton co-transport (Yoshimoto et al. 2003; Hawkesford 2003; Maruyama-Nakashita et al. 2004). Selenate reduction to selenite seems to be a limiting factor in the Se assimilation mechanism. Plants with affinity to selenate accumulate mainly selenate, while plants supplied with selenite accumulate organic Se species (de Souza et al. 1998). Selenate to selenite reduction occurs enzymatically in chloroplasts (Pilon-Smits et al. 2009). Nonenzymatic selenite reduction by reduced glutathione (GSH) is believed to be of significant importance as well.

It has been recognized that actively growing tissues contain highest amounts of Se (Kahakachchi et al. 2004). Plants accumulate more Se in shoot than in root tissues, but there are exceptions as well (Zayed et al. 1998). It has been shown that spinach, tea plants, and ryegrass accumulate Se in leaves (Zhu et al. 2004; Hu et al. 2003; Cartes et al. 2005). These findings do not correlate with the results presented in the current paper, where Se content in shoots is higher than in roots only after long-term (45 days) Se exposure. During short-term experiments (14 days), Se accumulation in the roots of chickpea was

**Fig. 2** Se species ( $\mu\text{g kg}^{-1}$ ) detected after 45-day (long term) sodium selenite treatment. *GPx* glutathione peroxidase, *SeCys* Se cysteine, *SeMet* Se methionine, *TrxR* thioredoxin reductase, *SeIV* selenite, *SeVI* selenate



more than twice higher than Se concentrations detected in shoots of the same plants. These differences may be a result of Se speciation. Selenium was applied to chickpea plants through the nutrient solution, under semi-hydroponic conditions. The excess of selenate over selenite in all shoot and root samples is associated with the Se uptake pathway of legumes.

Pilon-Smits et al. (2009) postulated that essential Se metabolism is a primitive trait that has been lost during evolution in higher plants. It has been shown that glutathione peroxidase (GPx) encodes a cysteine (Cys) instead of SeCys in the active site (Novoselov et al. 2002). Further, Pilon-Smits et al. (2009) did not exclude posttranslational conversion of serine into SeCys in specific plant species, which would result in a different selenoprotein production. Increased GPx content in Se-treated plants could be a consequence of the previously explained processes or of antioxidative triggers. Similar to the reports about ryegrass and soybean, the enhanced GPx concentration in the present study was determined in chickpea roots after short-term exposure and in shoots and roots after long-term Se exposure (Cartes et al. 2005; Djanaguiraman et al. 2005).

The chemical form in which Se is present in plant tissues is also pivotal since different forms lead to differences in Se

bioavailability and toxicity (Mazej et al. 2006). Selenium that might not be required in plants is incorporated into organic compounds using sulfur assimilation enzymes (Pilon-Smits et al. 2009). During that process, inorganic selenate is reduced and assimilated into organic Se, and SeCys is produced and incorporated into proteins replacing Cys, which leads to toxicity (Pilon-Smits et al. 2009). SeCys was also very well presented in the investigated chickpea samples exposed to selenite. However, bigger differences were detected concerning its concentrations depending on the exposure. SeCys concentrations were greater in the shoots compared to the roots after short-term Se exposure and decreased considerably in both, shoots and roots, following long-term exposure. Alternatively, SeCys can be converted to selenomethionine (SeMet), which also can be incorporated into proteins (Pilon-Smits et al. 2009) or to elemental Se and alanine (Pilon et al. 2003). If SeCys is methylated to methyl-SeCys, Se is not incorporated into proteins and can be accumulated (Neuhierl et al. 1999). It has to be considered that not all the Se is bioavailable and that organic Se forms, e.g., SeMet, are more bioavailable for humans than inorganic forms (Poblaciones et al. 2014). Broccoli accumulates high levels of Se in the form of SeMeSeCys (Lyi et al. 2005). Contrasting to numerous research publications

reporting about SeMet as the main Se species in buckwheat and pumpkin seeds, wheat, barley and rye (Srnkolj et al. 2005, 2006a, b; Stadlober et al. 2001), rice (Stadlober et al. 2001; Hart et al. 2011; Whanger 2002; Zhang et al. 2012), lentil grains (Thavarajah et al. 2008), or chickpea sprouts (Zhang et al. 2012), SeMet could not be detected in significant high concentrations in the chickpea shoots and roots of semi-hydroponically grown plants presented here. In soybean and kidney bean sprouts, Se was detected in the form of SeMeSeCys, with only a minor proportion in the form of SeMet and unidentified Se species (Sugihara et al. 2004).

An important object for discussion is also Se recovery. After short-term Se exposure, the Se recovery was equal in shoot and root controls (96 %). The lowest Se recovery in the short-term experiment was detected after 25- $\mu$ M Se exposure in shoots (57 %). The corresponding Se recovery in 25- $\mu$ M treated roots was 72 %. The results were different after long-term Se exposure. Selenium recovery in control shoots was 60 % and 3-fold higher compared to the Se recovery in control roots—21 %. Similar findings have not been described previously. The shift of recovered selenium could be related to the Se peaks, which were detected during the analysis but could not be identified yet. Most probably, the amount of the “X”-Se species depends on the increase of the Se dose and the prolongation of the exposure. Nevertheless, the identification of those unknown Se species is of high importance regarding legume Se speciation research.

Hart et al. (2011) described that 15 % of Se is usually present in species different from SeMet. Their explanation was that these Se forms often remained in the proportion of Se not recovered by HPLC. The levels of organic and bioavailable Se detected in the investigated samples differ significantly from patterns in other plants. It has also to be considered that our results do not concern chickpea seeds but are derived from experiments with shoots and roots of whole plants and hence offer deeper insight into Se speciation of so far not investigated plant tissues with excellent analytical tools. Further analysis will be necessary to unravel both, the nature and role of those Se species. Supplementary investigation of chickpea seeds, which are potential source of dietary Se, will elucidate their promising impact for human nutrition.

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**References**

Bulska E, Wysocka IA, Wierzbicka MH, Proost K, Janssens K, Falkenberg G (2006) *In vivo* investigation of the distribution and the local speciation of selenium in *Allium cepa* L. by means of microscopic X-ray absorption near-edge structure spectroscopy and confocal microscopic X-ray fluorescence analysis. *Anal Chem* 78:7616–7624

Cartes P, Gianfreda L, Mora ML (2005) Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. *Plant Soil* 276:359–367

de Souza MP, Pilon-Smits EAH, Lytle CM, Hwang S, Tai JC, Honma TSU, Yeh L, Terry N (1998) Rate-limiting steps in selenium volatilization by *Brassica juncea*. *Plant Physiol* 117:1487–1494

Diaz-Alarcon JP, Navarro-Alarcon M, Lopez-Garcia H, Lopez-Martinez MC (1996) Determination of selenium in cereals, legumes and dry fruits from southeastern Spain for calculation of daily dietary intake. *Sci Total Environ* 184:183–189

Djanaguiraman M, Durga Devi D, Shanker AK, Sheeba JA, Bangarusamy U (2005) Selenium—an antioxidative protectant in soybean during senescence. *Plant Soil* 272:77–86

Germ M, Stibilj V, Kreft I (2007) Metabolic importance of selenium for plants. *Eur J Plant Sci Biotechnol* 1:91–97

Grieve CM, Poss JA, Suarez DL, Dierig DA (2001) *Lesquerella* growth and selenium uptake affected by saline irrigation water composition. *Ind Crop Prod* 13:57–65

Hart DJ, Fairweather-Tait SJ, Broadley MR, Dickinson SJ, Foot I, Knott P (2011) Selenium concentration and speciation in biofortified flour and bread: retention of selenium during grain biofortification, processing and production of Se-enriched food. *Food Chem* 126:1771–1778

Hartikainen H (2005) Biogeochemistry of selenium and its impact on food chain quality and human health. *J Trace Elem Med Biol* 18: 309–318

Hawkesford MJ (2003) Transporter gene families in plants: the sulphate transporter gene family—redundancy or specialization? *Physiol Plant* 117:155–163

Hu QH, Xu J, Pang GX (2003) Effect of selenium on the yield and quality of green tea leaves harvested in early spring. *J Agric Food Chem* 51: 3379–3381

Jezeq P, Skarpa P, Losak T, Hlusek J, Juzl M, Elzner P (2012) Selenium—an important antioxidant in crops biofortification. *El-Missiry MA (Edt)*, InTech: 343–368

Kahakachchi C, Boakye HT, Uden PC, Tyson JF (2004) Chromatographic speciation of anionic and neutral selenium compounds in Se-accumulating *Brassica juncea* (Indian mustard) and in selenized yeast. *J Chromatogr A* 1054:303–312

Lyi SM, Heller LI, Rutzke M, Welch RM, Kochian LV, Li L (2005) Molecular and biochemical characterization of the selenocysteine Se-methyltransferase gene and Se-methylselenocysteine synthesis in broccoli. *Plant Physiol* 138:409–420

Lyons GH, Genc Y, Soole K, Stangoulis JCR, Liu F, Graham RD (2009) Selenium increases seed production in *Brassica*. *Plant Soil* 318:73–80

Maruyama-Nakashita A, Nakamura Y, Yamaya T, Takahashi H (2004) A novel regulatory pathway of sulfate uptake in *Arabidopsis* roots: implication of CRE1/WOL/AHK4-mediated cytokinin-dependent regulation. *Plant J* 38:779–789

Matos-Reyes M, Cervera M, Campos R, de la Guardia M (2010) Total content of As, Sb, Se, Te and Bi in Spanish vegetables, cereals and pulses and estimation of the contribution of these foods to the Mediterranean daily intake of trace elements. *Food Chem* 122: 188–194

Mazej D, Falnoga I, Veber M, Stibilj V (2006) Determination of selenium species in plant leaves by HPLC-UV-HG-AFS. *Talanta* 68:558–568

Michalke B, Berthele A (2011) Contribution to selenium speciation in cerebrospinal fluid samples. *J Anal Atom Spectrom* 26:165–170

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissues cultures. *Physiol Plant* 15:473–497

Neuhierl B, Thanbichler M, Lottspeich F, Böck A (1999) A family of S-methylmethionine dependent thiol/selenol methyltransferases. Role in selenium tolerance and evolutionary relation. *J Biol Chem* 274: 5407–5414



- Novoselov SV, Rao M, Onoshko NV, Zhi H, Kryukov GV, Xiang Y, Weeks DP, Hatfield DL, Gladyshev VN (2002) Selenoproteins and selenocysteine insertion system in the model plant system, *Chlamydomonas reinhardtii*. EMBO J 21:3681–3693
- Pilon M, Owen JD, Garifullina GF, Kurihara T, Mihara H, Esaki N, Pilon-Smits EAH (2003) Enhanced selenium tolerance and accumulation in transgenic *Arabidopsis thaliana* expressing a mouse selenocysteine lyase. Plant Physiol 131:1250–1257
- Pilon-Smits EAH, Quinn CF, Tapken W, Malagoli M, Schiavon M (2009) Physiological functions of beneficial elements. Curr Opin Plant Biol 90:1310–1315
- Poblaciones MJ, Rodrigo S, Santamaria O, Chen Y, McGrath SP (2014) Selenium accumulation and speciation in biofortified chickpea (*Cicer arietinum* L.) under Mediterranean conditions. J Sci Food Agric 94:1101–1106
- Simonova A, Pfannhauser W (2008) Selen-Vorkommen, Wirkung und Versorgung. Ernährung/Nutrition 32:364–378
- Smrkolj P, Kreft I, Kapolna E, Stibilj V (2005) Selenium species determination in selenium enriched pumpkin (*Cucurbita pepo* L.) seeds by HPLC-UVHG- AFS. Anal Sci 21:1501–1504
- Smrkolj P, Germ M, Kreft I, Stibilj V (2006a) Respiratory potential and Se compounds in pea (*Pisum sativum* L.) plants grown from Se-enriched seeds. J Exp Bot 57:3595–3600
- Smrkolj P, Stibilj V, Kreft I, Germ M (2006b) Selenium species in buckwheat cultivated with foliar addition of Se(VI) and various levels of UV-B radiation. Food Chem 96:675–681
- Solovyev N, Berthele A, Michalke B (2013) Selenium speciation in paired serum and cerebrospinal fluid samples. Anal Bioanal Chem 405:1875–1884
- Stadlober M, Sager M, Irgolic KJ (2001) Effects of selenate supplemented fertilization on the selenium level of cereals: identification and quantification of selenium compounds by HPLC-ICP-MS. Food Chem 73:357–366
- Sugihara S, Kondo M, Chihara Y, Yuji M, Hattori H, Yoshida M (2004) Preparation of selenium enriched sprouts and identification of their selenium species by high performance liquid chromatography-inductively coupled plasma mass spectrometry. Biosci Biotechnol Biochem 68:193–199
- Thavarajah D, Ruszkowski J, Vandenberg A (2008) High potential for selenium biofortification of lentils (*Lens culinaris* L.). J Agric Food Chem 56:10747–10753
- Vogrinčič M, Cuderman P, Kreft I, Stibilj V (2009) Selenium and its species distribution in above-ground plant parts of selenium enriched buckwheat (*Fagopyrum esculentum* Moech). Anal Sci 25:1357–1363
- Whanger PD (2002) Selenocompounds in plants and animals and their biological significance. J Am Coll Nutr 21:223–232
- Yoshimoto N, Inoue E, Saito K, Yamaya T, Takahashi H (2003) Phloem-localizing sulfate transporter, Sultr1;3, mediates re-distribution of sulfur from source to sink organs in *Arabidopsis*. Plant Physiol 131:1511–1517
- Zayed A, Lytle CM, Terry N (1998) Accumulation and volatilization of different chemical species of selenium by plants. Planta 206:284–292
- Zhang L, Li Q, Yang X, Xia Z (2012) Effects of sodium selenite and germination on the sprouting of chickpeas (*Cicer arietinum* L.) and its content of selenium, formononetin and biochanin A in the sprouts. Biol Trace Elem Res 146:376–380
- Zhu YG, Huang YZ, Hu Y, Liu YX, Christie P (2004) Interactions between selenium and iodine uptake by spinach (*Spinacia oleracea* L.) in solution culture. Plant Soil 261:99–105