Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite

Hua-Fen Li[1,2], Steve P. McGrath[2] and Fang-Jie Zhao[2]

[1]College of Resources and Environmental Science, China Agricultural University, Beijing 100094, China; [2]Soil Science Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

Author for correspondence:
Fang-Jie Zhao
Tel: +44 1582 763133
Fax: +44 1582 760981
Email: Fangjie.Zhao@bbsrc.ac.uk

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Summary

- Selenite can be a dominant form of selenium (Se) in aerobic soils; however, unlike selenate, the mechanism of selenite uptake by plants remains unclear.
- Uptake, translocation and Se speciation in wheat (*Triticum aestivum*) supplied with selenate or selenite, or both, were investigated in hydroponic experiments. The kinetics of selenite influx was determined in short-term (30 min) experiments. Selenium speciation in the water-extractable fraction of roots and shoots was determined by HPLC-ICPMS.
- Plants absorbed similar amounts of Se within 1 d when supplied with selenite or selenate. Selenate and selenite uptake were enhanced in sulphur-starved and phosphorus-starved plants, respectively. Phosphate markedly increased K_m of the selenite influx. Selenate and selenite uptake were both metabolically dependent. Selenite was rapidly converted to organic forms in roots, with limited translocation to shoots. Selenomethionine, selenomethionine Se-oxide, Se-methyl-selenocysteine and several other unidentified Se species were detected in the root extracts and xylem sap from selenite-treated plants. Selenate was highly mobile in xylem transport, but little was assimilated to organic forms in 1 d. The presence of selenite decreased selenate uptake and xylem transport.
- Selenite uptake is an active process likely mediated, at least partly, by phosphate transporters. Selenite and selenate differ greatly in the ease of assimilation and xylem transport.

Key words: HPLC-ICPMS, selenate, selenite, selenium (Se), selenium speciation, uptake, xylem transport, wheat (*Triticum aestivum*).

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Introduction

Selenium (Se) is essential for humans and animals but has not been proven to be essential for plants (Terry *et al.*, 2000; Sors *et al.*, 2005). Low dietary intakes of Se by humans can cause health disorders, including oxidative stress-related conditions, reduced fertility and immune function, and an increased risk of cancers (Rayman, 2000, 2002; Whanger, 2004). It has been estimated that between 0.5 and 1 billion people globally may have inadequate intakes of Se, and these include populations in developed countries such as western Europe (Combs, 2001). Biofortification of Se in crops, through Se fertilization, which has been practised in Finland since the mid-1980s (Eurola *et al.*, 1991), breeding or genetic manipulation of crops with enhanced Se uptake, has been proposed as an effective way of increasing human Se intake (Lyons *et al.*, 2003; Broadley *et al.*, 2006). It is therefore important that the mechanisms responsible for, and the factors affecting, Se uptake by plants are understood in order to achieve more effective biofortification of Se in crops.

In aerobic soils, either selenate or selenite may be the dominant chemical species depending on the redox potential and pH.

Thermodynamic calculations show that selenate should be the predominant form in alkaline and well-oxidized soils (pe + pH > 15), and selenite in well-drained mineral soils with pH from acidic to neutral (7.5 < pe + pH < 15) (Elrashidi *et al.*, 1987). In a study of Se speciation in soil extracts, we found selenite to be the predominant species and selenate to be negligible in a number of UK arable soils (F.-J. Zhao *et al.*, unpublished). This suggests that selenite is the main form available for plant uptake in the soils studied, and challenges a common assumption that plants take up mainly selenate from aerobic soils.

The mechanism of selenate uptake by plants has been well established. Selenate is taken up by plant roots via the high-affinity sulphate transporters, although different plant species exhibit different selectivity for sulphate vs selenate (Bell *et al.*, 1992; Terry *et al.*, 2000; White *et al.*, 2004; Sors *et al.*, 2005). Sulphate competitively inhibits selenate uptake by barley roots (Leggett & Epstein, 1956). Selenate was used as a selection agent to isolate *Arabidopsis thaliana* mutants that have lesions in the *AtSultr1;2* gene, which encodes one of the high-affinity sulphate transporter genes and is highly expressed in the root cortex, root tips and lateral roots (Shibagaki *et al.*, 2002). This transporter is probably essential for both sulphate and selenate uptake into the root. By contrast, little is known about the mechanism involved in the uptake of selenite by plants. It has been suggested that selenite is taken up by plant roots through passive diffusion (Shrift & Ulrich, 1969; Arvy, 1993). Terry *et al.* (2000) concluded that there was no evidence that the uptake of selenite is mediated by membrane transporters. However, several studies have shown that selenite is readily taken up by plants at rates that are similar to or even faster than those for selenate (Arvy, 1993; Hopper & Parker, 1999; Zhang *et al.*, 2003). Furthermore, selenite uptake was found to be depressed by phosphate in long-term hydroponic experiments (Broyer *et al.*, 1972b; Hopper & Parker, 1999). These findings cannot be easily explained by a passive diffusion of selenite.

Apart from the difference in the mechanisms of uptake, selenate and selenite also differ in their mobility within plants. Selenate is easily distributed from roots to shoots, whereas selenite, or its metabolic products, tends to accumulate in roots (Asher *et al.*, 1977; Arvy, 1993; de Souza *et al.*, 1998; Zayed *et al.*, 1998). Under certain environmental conditions, both selenate and selenite may be present in the rhizosphere soil. It has not been investigated whether the two Se species interact during uptake and root-to-shoot translocation.

In the present study, we compared uptake, translocation and Se speciation in wheat supplied with selenate or selenite, and investigated whether the two Se species interact. A further objective was to examine the physiological mechanism of selenite uptake, which has so far remained unclear. We chose wheat as our test plant species because wheat and its products are a major source of Se intake by humans, and would be among the most effective crops for Se biofortification (Lyons *et al.*, 2003; Hawkesford & Zhao, 2007).

Materials and Methods

Plant material and culture conditions

Wheat (*Triticum aestivum* L. cv. Hereward) seeds were surface-sterilized in 0.5% (v/v) NaOCl for 15 min, rinsed and soaked in deionized water overnight at 20°C in the dark, and then germinated in 0.5 mM $CaCl_2$ solution. Wheat seedlings were precultured in a plastic container containing 30 l of a modified 1/5 strength Hoagland nutrient solution. The composition of the nutrient solution was: 1.0 mM KNO_3, 1.0 mM $Ca(NO_3)_2$, 0.457 mM $MgSO_4$, 0.1 mM KH_2PO_4, 1.0 μM $MnCl_2$, 3 μM H_3BO_3, 1 μM $(NH_4)_6Mo_7O_{24}$, 1 μM $ZnSO_4$, 0.2 μM $CuSO_4$ and 60 μM Fe(III)-EDTA. The pH of this solution was buffered at 6.0 with 2 mM MES (2-morpholinoethanesulphonic acid, pH adjusted with KOH). The solution was aerated continuously and renewed every week. Plants were grown in a controlled-environment growth chamber with the following conditions: 12 h photoperiod per d with a light intensity of 350 μmol m^{-2} s^{-1}, 20 : 16°C day : night temperatures, and relative humidity of 60–70%.

Comparison of selenite and selenate uptake and assimilation under normal, S-deficient and P-deficient conditions

Two-week-old seedlings were transferred to 1 l pots (two plants per pot) and treated with normal, –S or –P nutrient solutions for 1 wk. In the –S or –P treatment, $MgSO_4$, $ZnSO_4$, $CuSO_4$ or KH_2PO_4 were replaced by the corresponding chloride salts. One week later, seedlings were transferred to a normal nutrient solution plus either 10 μM selenite as Na_2SeO_3 or 10 μM selenate as Na_2SeO_4. Each treatment was replicated in three pots. After 24 h of Se treatment, shoots were cut at 2 cm above the roots. The cut surfaces were rinsed with deionized water and blotted dry, and xylem sap was collected with a pipette over the following 2 h. Roots were rinsed with deionized water three times and then soaked in 1 mM $CaSO_4$ solution for 15 min to remove Se in the root apoplast. Root and shoot samples were frozen in liquid nitrogen, and ground with liquid nitrogen to fine powder in a pestle and mortar. Subsamples were used for the determination of total Se and the chemical species of Se. Aliquots of nutrient solutions after plant growth were taken for the analysis of Se species.

Effect of phosphate on selenite uptake kinetics

Ten-day-old seedlings were transferred to 300 ml pots (two plants per pot) containing a normal nutrient solution. Two days later, seedling roots were rinsed with deionized water and then placed in a series of uptake solutions containing 0, 0.1, 0.5, 1.0, 5.0 or 10 μM selenite (Na_2SeO_3), with or without 0.1 mM phosphate. The composition of other nutrients was the same as that in the normal nutrient solution. Each treatment was

replicated in four pots. The uptake solutions were aerated vigorously. After 30 min, roots of plants were rinsed with deionized water and then transferred to an ice-cold desorption solution (1 mM $CaSO_4$, 2 mM MES, pH 6.0) for 15 min. Shoots and roots were then separated, weighed and used for the determination of Se concentrations. Three pots of seedlings were harvested before the selenite uptake experiment for the determination of the background concentration of Se in plants, which was found to be negligible.

Effect of a metabolic inhibitor on uptake of selenite and selenate

The effect of the metabolic inhibitor CCCP (carbonyl cyanide 3-chlorophenylhydrazone) on the uptake of selenite and selenate by wheat was investigated. Ten-day-old seedlings were transferred to 300 ml pots (two plants per pot) and grown with normal nutrient solution for 2 d. Seedlings were then treated with or without 1 μM CCCP, which was dissolved in ethanol and added to the nutrient solution with a final ethanol concentration of 0.01% (v/v). An additional control treatment of 0.01% (v/v) ethanol was included. One hour later, either 5 μM Na_2SeO_3 or 5 μM Na_2SeO_4 was added to the solutions. Each treatment was replicated in four pots. Plants were harvested after exposure to the Se treatments for 30 min. Root apoplastic Se was desorbed as already described. Shoots and roots were separated and their Se concentrations analysed.

Interactions between selenite and selenate

This experiment was conducted to investigate the relative uptake rates of selenite vs selenate, and whether the two Se species interact during uptake and assimilation by wheat plants. Fifteen-day-old seedlings were transferred to 1 l pots (two plants per pot) and grown for 2 d further in normal nutrient solution. Plants were then given 5 μM Na_2SeO_3, 5 μM Na_2SeO_4, or 5 μM of both Na_2SeO_3 and Na_2SeO_4. Each treatment was replicated in four pots. Twenty-four hours later, 1 ml nutrient solution was taken from each pot for the analysis of Se species, and xylem sap was collected for 2 h after cutting as described earlier. Roots were rinsed and desorbed of the apoplastic Se as described earlier. Roots and shoots were harvested.

Analysis of Se speciation

Aliquots of 0.4 g powdered plant materials were extracted with 20 ml ultrapure (> 18 MΩ) water under sonication for 30 min, filtered through a filter paper and then through a 0.45 μm filter. Samples of nutrient solution and xylem sap were diluted appropriately and filtered through 0.45 μm filters. Selenium speciation in nutrient solutions, xylem sap and plant extracts was determined using HPLC-ICP-MS (Agilent LC1100 series and Agilent ICP-MS 7500ce, Agilent Technologies, Santa Clara, CA, USA). Selenium species were separated by an anion-exchange column (Dionex AS14), fitted with a guard column (Dionex AG14). The injection volume was 50 μl per sample. The mobile phase was 6 mM Na_2CO_3 (pH 9.5), which was pumped through the column isocratically at 1 ml min^{-1}. The outlet of the separation column was connected via a polyetheretherketone (PEEK) tube to a concentric nebulizer and a water-jacketed cyclonic spray chamber of the ICP-MS. Signals at m/z 78 and 80 (Se) were collected with a dwell time of 500 ms. Possible polyatomic interference was removed by the Agilent Octopole Reaction System operating in the hydrogen gas mode (flow rate 4 ml min^{-1}). Other ICP-MS instrumental conditions were as follows: RF forward power, 1500 W; sample depth, 8 mm from the load coil; carrier gas flow rate, 0.9 l min^{-1}; spray chamber temperature, 2°C. Peaks were identified by comparison with the retention times of standard compounds. The Se standards (Na_2SeO_3, Na_2SeO_4, selenomethionine (SeMet), selenocysteine ($SeCys_2$), Se-methyl-selenocysteine (MeSeCys)) were obtained from Sigma (St Louis, MO, USA), and prepared in ultrapure (> 18 MΩ) water. Selenomethionine Se-oxide (SeOMet) was prepared by reacting SeMet with 3% (v/v) H_2O_2 under sonication for 1 h. This method was similar to that described by Larsen *et al.* (2004), and was found to convert SeMet to SeOMet quantitatively. The identified species of Se in the samples were quantified by external calibration curves with peak areas. Analysis of Se species was carried out immediately following sample collection or extraction and completed within 12 h. Samples that were analysed at the beginning of the run were repeated at the end of run; no changes in Se speciation were observed during this period of time.

Analysis of total Se

Powdered plant samples (*c.* 1 g fresh weight) were digested in 5 ml $HNO_3/HClO_4$ (85/15, v/v). Total Se concentrations in the digests and in the water extracts were determined by ICP-MS (Agilent 7500ce) operating in the hydrogen gas mode. Blanks and a certified reference material (tomato leaf material, NIST 1573a) were included in each batch of samples for quality control.

Statistical analysis

The significance of treatment effects were determined by analysis of variance. Least significant difference was used to compare treatment means.

Results

Comparison of selenite and selenate uptake and assimilation under normal, S-deficient and P-deficient conditions

In this experiment, the effects of P or S starvation on the uptake and assimilation of selenate and selenite in wheat were

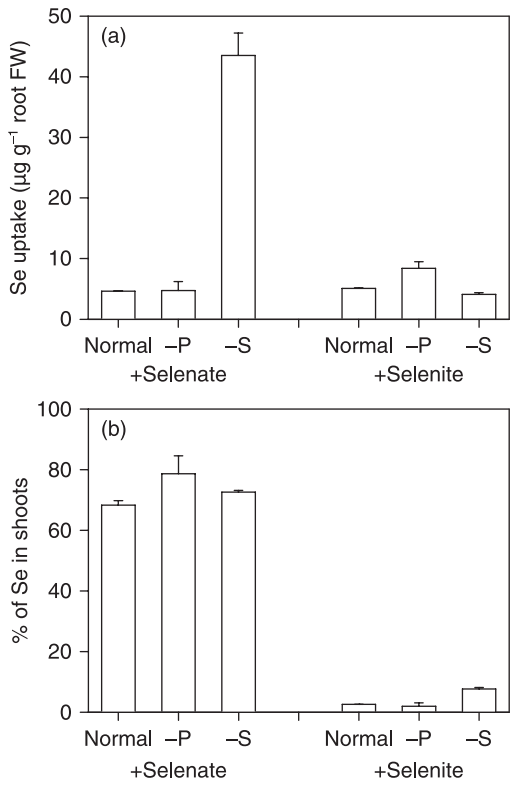

Fig. 1 Effect of sulphur (S) and phosphorus (P) starvation on the uptake of selenate or selenite by wheat (*Triticum aestivum*) (a) and the proportion of Se distributed to shoots (b). Selenate or selenite was supplied at 10 μM. Data are means + SE.

investigated. The treatments of P or S starvation were imposed before the exposure of plant roots to Se. During the period of Se exposure (24 h), a normal nutrient solution (i.e. containing both P and S) was supplied to all treatments. Root growth was more sensitive to P than to S starvation; root fresh weight was 43 and 16% smaller in the –P and –S treatments, respectively, than in the control (data not shown). Shoot growth was affected by P and S starvation to a similar extent, showing, on average, a 17% decrease in shoot biomass compared with the control.

There were highly significant ($P < 0.001$) effects of the P or S starvation treatments and interactions between these treatments and the Se species supplied on the total amount of Se taken up by wheat plants (Fig. 1a). The data shown in Fig. 1a are expressed on the basis of per unit root weight, so that the variation in root biomass as a result of P or S starvation was accounted for. In the plants with a normal nutrient status, the amount of Se taken up was similar between the selenite and selenate treatments. However, S- and P-starved plants showed different responses when supplied with selenate or selenite. Selenium uptake from the selenate treatment was enhanced 9.5-fold in the S-starved plants compared with the control, whereas S starvation had no significant effect on Se uptake from the selenite treatment. By contrast, P starvation had no effect on selenate uptake, but increased selenite uptake by 60% compared with the control.

The supply of selenate vs selenite had a marked effect ($P < 0.001$) on the distribution of Se between roots and shoots (Fig. 1b). On average, 73% of the Se taken up from the selenate treatment had been distributed to the shoots after only 24 h exposure. This compares with only 4% in the plants from the selenite treatment. The distribution of Se from roots to shoots was not significantly affected by S or P starvation.

Selenium speciation was determined in the water extracts of root and shoot tissues, as well as in the xylem sap (Fig. 2). The extraction procedure extracted 63–70% of the total Se from the root and shoot tissues of the selenate-treated plants, but only 23–29% of the selenite-treated plants (data not shown). In the root and shoot extracts from the plants supplied with selenate, only selenate was detected (Fig. 2a,e). Similarly, selenate was by far the predominant species present in the xylem sap of the selenate-treated plants (Fig. 2c), although trace amounts of other Se species, including SeMet, SeOMet and an unidentified peak eluded at a retention time of 2.7 min ($RT_{2.7}$), were also detected. By contrast, the root extracts and xylem sap from the plants supplied with selenite contained a number of Se-containing species. Selenite, SeMet, SeOMet and MeSeCys were detected in the root extracts, while in the xylem sap, trace amounts of selenite, selenate, SeMet, SeOMet and MeSeCys were detected (Fig. 2b,d). In the root extracts, there were several unidentified Se compounds, most noticeably the first peak on the chromatogram ($RT_{2.7}$). Because of its early elution from the anion exchange column, close to the column void, this compound is likely to be a neutral or cationic species under the elution conditions (pH 9.5). This Se species was the most abundant in both the root extracts and the xylem sap from the selenite-treated plants. In the shoot extracts from the selenite-treated plants with a normal nutrient status, no Se was detectable (Fig. 2f).

Quantitative results for selenate, selenite, SeMet and SeOMet are shown in Tables 1 and 2 for the plant extracts and the xylem sap, respectively. In the selenate-treated plants, P starvation decreased the concentration of selenate in the root and shoot extracts by 56–63% ($P < 0.001$), whereas S starvation increased selenate concentration in roots and shoots by 5.4- and sevenfold ($P < 0.001$), respectively. In the selenite-treated plants, P starvation increased the concentrations of selenite and SeOMet in the root extracts by 88 and 153% ($P < 0.001$), respectively, but had no significant effect on SeMet concentration. Sulphur starvation decreased the selenite concentration in the root extracts from the selenite-treated plants by 48% ($P < 0.001$), but had no significant effect on SeMet or SeOMet concentration. In the S-starved plants supplied with selenite, a small amount of selenate was found in the shoot extract.

Very large concentrations of selenate were detected in the xylem sap from the selenate-treated plants (Table 2). These concentrations are 5.7–43.2 times higher than the selenate concentration supplied in the nutrient solution. Selenate

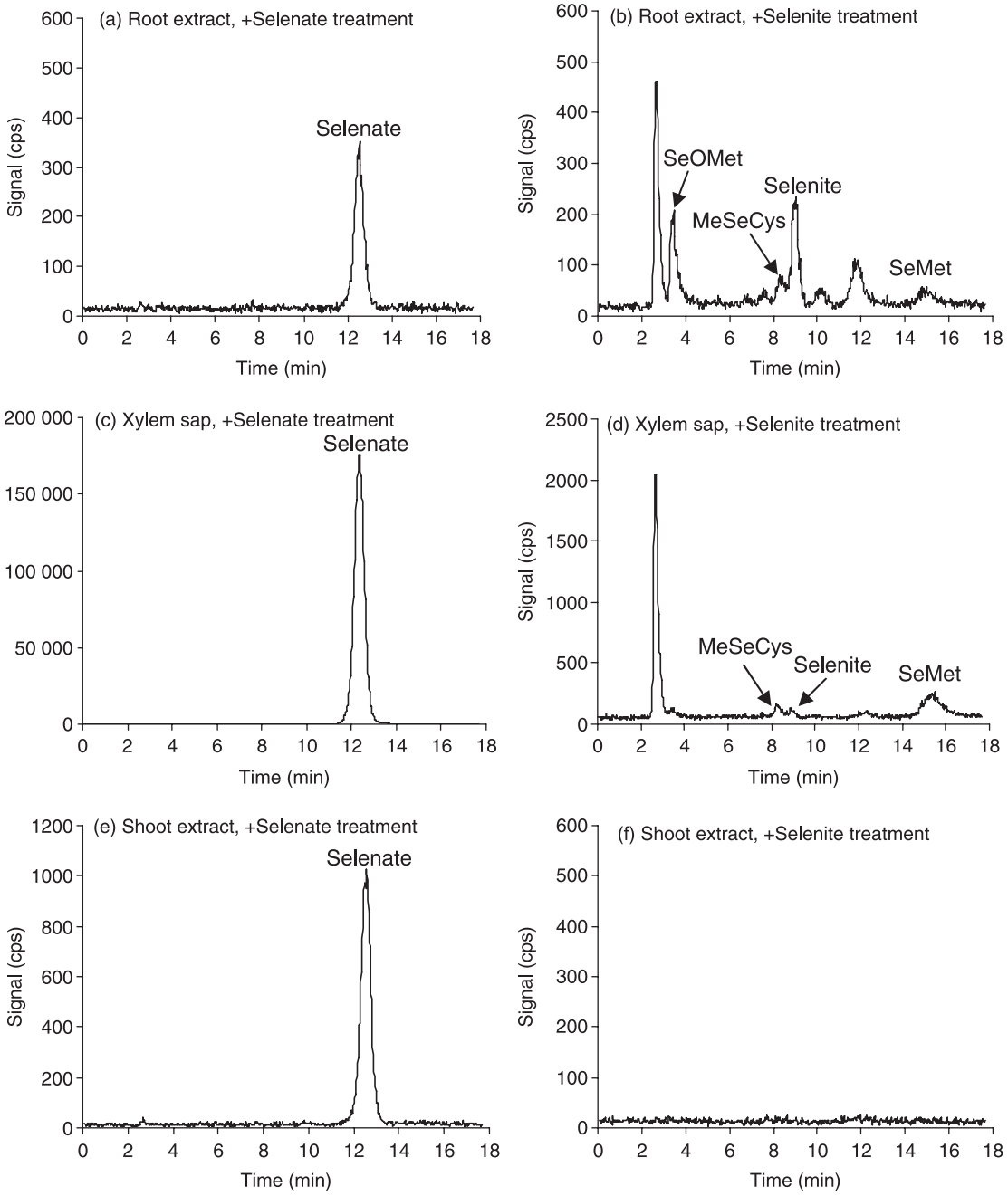

Fig. 2 Examples of chromatograms of selenium (Se) speciation in the water extracts of wheat (*Triticum aestivum*) roots and shoots or xylem sap, as determined by anion exchange HPLC-ICPMS. The signals (count per second, cps) are for *m/z* 80. Sample identities are shown on each panel. Note the difference in the *y*-axis scale. MeSeCys, Se-methyl-selenocysteine; SeMet, selenomethionine; SeOMet, selenomethionine Se-oxide.

concentrations decreased in the P-starved plants, but more than doubled in the S-starved plants. SeMet and SeOMet together accounted for < 0.5% of the Se in the xylem saps from the selenate-treated plants, and their concentrations were also affected by P- and S-deficiency in a similar way as that for selenate concentration. Selenium concentrations in the xylem saps of the selenite-treated plants were much lower than those of the selenate-treated plants; in the former Se speciation was dominated by the unidentified compound at $RT_{2.7}$. Small concentrations of selenate were found in the sap samples from the selenite-treated plants. Selenite was detected in only a single replicate sample of xylem sap from the S-starved plants. Selenomethionine and SeOMet were present in a similar concentration range as that in the xylem sap from the selenate-treated plants. Sulphur starvation increased the concentrations of selenate, SeMet and SeOMet, as well as the peak area of the $RT_{2.7}$.

Table 1 Effects of selenium (Se) species supplied and plant nutrient status on Se speciation in the water extracts from wheat (*Triticum aestivum*) roots and shoots

		Root (ng g^{-1} FW)				Shoot (ng g^{-1} FW)*
Se species supplied	Nutrient status	Selenate	Selenite	SeMet	SeOMet	Selenate
Selenate	Normal	1005 ± 19.6	0.0	0.0	0.0	3053 ± 149
	–P	370 ± 52.9	0.0	0.0	0.0	1336 ± 396
	–S	5461 ± 259	0.0	0.0	0.0	21307 ± 815
Selenite	Normal	0.0	157 ± 14.5	73.4 ± 12.3	111 ± 6.2	0.0
	–P	0.0	295 ± 47.9	65.4 ± 16.2	281 ± 22.6	0.0
	–S	0.0	81.4 ± 8.5	80.8 ± 10.8	103 ± 11.4	96.0 ± 6.3
ANOVA significance *P*						
Se species		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Nutrient status		< 0.001	< 0.001	0.722	< 0.001	< 0.001
Interaction		< 0.001	< 0.001	0.722	< 0.001	< 0.001

Values are means ± SE.
SeMet, selenomethionine; SeOMet, selenomethionine Se-oxide.
*Selenate was the only species detected in the shoot extracts.

Table 2 Effects of selenium (Se) species supplied and plant nutrient status on Se speciation in the xylem sap from wheat (*Triticum aestivum*) seedlings

Se species supplied	Nutrient status	Selenate (μg Se l^{-1})	Selenite (μg Se l^{-1})	SeMet (μg Se l^{-1})	SeOMet (μg Se l^{-1})
Selenate	Normal	14 564 ± 656	0.0	24.7 ± 16.7	8.2 ± 1.2
	–P	4482 ± 3082	0.0	4.8 ± 4.1	4.6 ± 1.2
	–S	34 146 ± 1816	0.0	98.5 ± 22.3	69.7 ± 7.0
Selenite	Normal	8.6 ± 1.2	0.0	35.3 ± 6.0	4.3 ± 1.1
	–P	40.1 ± 27.9	0.0	33.4 ± 13.8	6.7 ± 3.3
	–S	77.0 ± 1.1	1.4 ± 1.4	117.4 ± 13.3	36.7 ± 5.4
ANOVA significance *P*					
Se species		< 0.001	0.382	0.118	0.005
Nutrient status		< 0.001	0.463	< 0.001	< 0.001
Interaction		< 0.001	0.463	0.815	0.003

Values are means ± SE.
SeMet, selenomethionine; SeOMet, selenomethionine Se-oxide.

At the end of the experiment, Se speciation in the nutrient solutions was measured. There was no change in Se species in either the selenate or the selenite treatments.

Effect of phosphate on selenite uptake kinetics

The kinetics of selenite influx into wheat roots could be described satisfactorily by the Michaelis-Menten equation (Fig. 3). The presence of 100 μM phosphate in the uptake solution strongly suppressed the influx of selenite. The effect of P was found to be mainly on K_m, which was increased by more than threefold by the presence of phosphate, from 8.0 ± 0.9 to 26.3 ± 7.8 μM. V_{max} was decreased by the presence of phosphate only slightly, from 12.7 ± 0.7 to 8.6 ± 1.9 nmol g^{-1} root FW h^{-1}. These results suggest that phosphate competitively inhibits selenite influx by wheat roots.

Effect of a metabolic inhibitor on uptake of selenite and selenate

Uptake of selenate or selenite was measured after wheat roots were exposed to the two Se species (5 μM) for 30 min. Compared with the control treatment, addition of ethanol (0.01%) to the nutrient solution increased the uptake of selenite and selenate to a similar extent (16–18%) (Fig. 4). Compared with the ethanol treatment, addition of the metabolic inhibitor CCCP inhibited the uptake of selenate and selenite by 90 and 80%, respectively ($P < 0.01$).

Interactions between selenite and selenate

In the experiments described earlier, selenate and selenite were supplied to plants separately. Both Se species may be present

Table 3 Selenium (Se) uptake, distribution and Se speciation in the xylem sap from wheat (*Triticum aestivum*) plants supplied with 5 μM selenate, selenite or both for 24 h

			Xylem sap (μg Se l^{-1})		
Treatment	Se uptake (μg g^{-1} root FW)	% of Se in shoots	Selenate	Selenite	SeMet
Selenate	3.6 ± 0.5	89.3 ± 3.8	1474 ± 71.9	0.0	0.0
Selenite	3.0 ± 0.4	11.6 ± 2.3	1.2 ± 0.7	0.8 ± 0.5	6.3 ± 0.6
Selenate + selenite	5.5 ± 0.4	22.2 ± 1.1	540 ± 34.3	0.0	0.0
ANOVA significance *P*					
Se species	0.008	< 0.001	< 0.001	0.103	< 0.001

Values are means ± SE.
SeMet, selenomethionine.

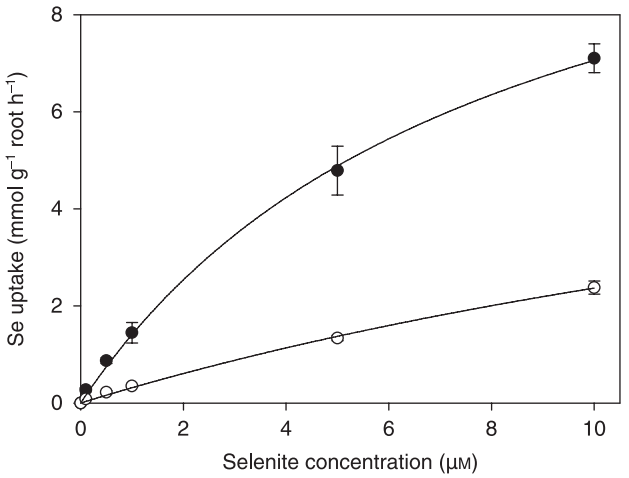

Fig. 3 Effect of phosphate (0.1 mM: closed circles, –P; open circles, +P) on the kinetics of selenite influx to wheat (*Triticum aestivum*) roots within 30 min. Data are means ± SE. The curves represent the fitted Michaelis-Menten kinetics.

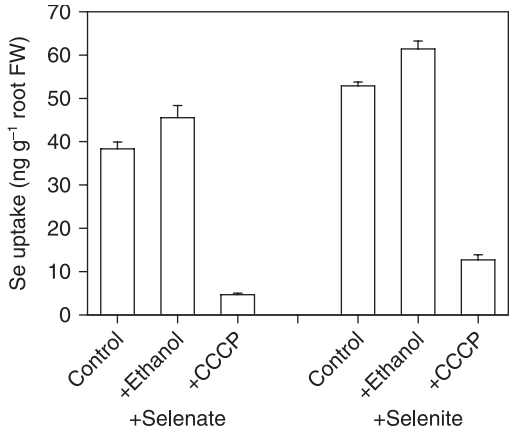

Fig. 4 Effect of the metabolic inhibitor CCCP (carbonyl cyanide 3-chlorophenylhydrazone) on the influx of selenate or selenite to wheat (*Triticum aestivum*) roots within 30 min. Selenate or selenite was supplied at 5 μM. Data are means + SE.

in aerobic soils. This experiment was therefore designed to test whether the two Se species interact during uptake and translocation in wheat. In the treatment that supplied both selenate and selenite at 5 μM, there was no significant ($P = 0.84$) change in the concentration of selenite in the nutrient solution after 24 h, whereas the selenate concentration increased significantly ($P < 0.05$), by 2.9%. Meanwhile, the volume of nutrient solution decreased by 5% as a result of transpiration. These results suggest that, in the presence of both Se species, selenite uptake was faster than selenate, and that their uptake rates were either similar (selenite) to or slower (selenate) than the transpiration rate.

Selenium uptake was not significantly different when selenate and selenite were supplied separately (Table 3). But when both species were added to the nutrient solution in equal concentrations, Se uptake was only 83% of the sum of the uptake when the two species were supplied separately, suggesting a nonadditive effect. A more striking difference was observed in the root-to-shoot distribution of Se. The efficiency of Se translocation, measured as the percentage of the total Se uptake that had been distributed to shoots, was approximately sevenfold greater in the selenate than in the selenite treatment. However, when both Se species were present in the nutrient solution, the efficiency of Se translocation (22%) was much smaller than the average (50%) of the two separate treatments. Furthermore, the concentration of selenate in the xylem sap from the two species' combined treatment was only 37% of that in the selenate-alone treatment. These results suggest that the supply of selenite inhibits translocation of selenate from roots to shoots. Similar to the Se speciation results from the first experiment, the unidentified Se compound $RT_{2.7}$ was the most abundant species in the xylem sap from the selenite-treated plants, accounting for 74% of the total peak area in the chromatogram. Small amounts of selenate, selenite and SeMet were found in the xylem sap, when selenite alone was supplied.

Discussion

Mechanisms of selenate and selenite uptake

The fact that selenate is taken up via sulphate transporters has been well documented (reviewed by Terry *et al.*, 2000;

Sors *et al.*, 2005). An almost 10-fold increase in selenate uptake by the S-starved wheat plants (Fig. 1) is consistent with this mechanism; upon S starvation, plants up-regulate the expression of sulphate transporter genes in roots (Smith *et al.*, 1997; Buchner *et al.*, 2004), leading to greater uptake of selenate when it is present in the external medium.

Less well understood is the mechanism responsible for selenite uptake, even though this Se species can be a major species in aerobic soils (Elrashidi *et al.*, 1987). Selenite is a diprotic weak acid with pKa_1 and pKa_2 of 2.46 and 7.31, respectively, which means that selenite will exist primarily as $HSeO_3^-$ at pH values between 3 and 8 (Hopper & Parker, 1999). Unlike selenate, selenite uptake by tomato (*Lycopersicon esculentum*) and rice (*Oryza sativa*) was little affected by the presence of sulphate (Asher *et al.*, 1977; Zhang *et al.*, 2006). Sulphur starvation also had no significant effect on selenite uptake by wheat in the present study (Fig. 1). By contrast, two previous studies using hydroponic culture showed that a 10-fold increase in phosphate concentration led to decreases of selenite uptake by between 20 and 70% in ryegrass (*Lolium perenne*), strawberry clover (*Trifolium fragiferrum*), *Astragalus canadensis* and in the Se hyperaccumulator *Astragalus bisulcatus* (Broyer *et al.*, 1972b; Hopper & Parker, 1999), suggesting a role of the phosphate transport pathway in selenite uptake. This hypothesis is strongly supported by the results from the present study. First, P starvation, which is expected to up-regulate the expression of the phosphate transporter genes (Muchhal *et al.*, 1996; Muchhal & Raghothama, 1999), resulted in a 60% increase in selenite uptake by wheat, whereas S starvation had no significant effect (Fig. 1). Second, phosphate competitively inhibited selenite influx into wheat roots in the short-term (30 min) experiment, decreasing the affinity for selenite markedly (Fig. 3). The response of selenite uptake to P starvation was much smaller than that for the response of selenate uptake to S starvation. This difference may be interpreted by the following: different levels of gene up-regulation in response to P or S starvation; and/or the affinity of the phosphate transporters for selenite, which was relatively low compared with that of the sulphate transporters toward selenate. The second interpretation is certainly plausible in light of the strong effect of phosphate on the K_m of selenite influx. It is interesting to note that selenite also behaves rather similarly to phosphate in terms of its physicochemical adsorption in soils, whereas selenate shares similarity with sulphate (Barrow & Whelan, 1989).

Earlier literature suggested that selenite is taken up by plant roots through passive diffusion (Shrift & Ulrich, 1969; Arvy, 1993). This hypothesis is refuted by the results of the present study. The uptake of both selenate and selenite was highly sensitive to the metabolic inhibitor CCCP, with the latter being only slightly less sensitive than the former (Fig. 4). Both sulphate and phosphate are transported across the plasma membranes of root cells against their electrochemial gradients, and the uptake is driven by the co-transport with protons (Hawkesford *et al.*, 1993; Smith *et al.*, 2000). The same mechanisms are likely to be in operation for selenate and selenite. CCCP is an uncoupler of the oxidative phosphorylation and a protonophore, which causes a dissipation of the proton motive force across the membranes. This would explain its dramatic effect on the uptake of both selenate and selenite.

Earlier reports comparing the uptake rate of selenate and selenite were inconsistent. Shrift & Ulrich (1969) showed that selenite was taken up much more slowly than selenate in both Se-nonaccumulator and hyperaccumulator species of *Astragalus*. de Souza *et al.* (1998) and Zayed *et al.* (1998) also found that the uptake rate of selenate was two- to five-fold faster than that of selenite in *Brassica juncea*, *Brassica oleracea*, *Beta vulgaris* and rice. By contrast, Arvy (1993) found a similar uptake rate for the two Se species by bean plants (*Phaseolus vulgaris*) during a 3 h uptake experiment, whereas Broyer *et al.* (1972a) reported higher accumulation of selenite than selenate by the hyperaccumulator *Astragalus crotolariae* after 7–12 wk hydroponic culture, and Zhang *et al.* (2003) showed faster uptake of selenite than of selenate by soybean (*Glycin max*). These discrepancies are likely to be caused by different experimental conditions, especially the concentrations of sulphate and phosphate used in the culture solutions, as well as the different plant species studied. In our experiments, total uptake of selenite and selenate by wheat during 30 min or 24 h was similar under the normal nutrient conditions (0.46 mM sulphate and 0.1 mM phosphate) (Figs 1, 4; Table 3). Soil solutions typically contain about 0.5 mM sulphate and 2 μM phosphate (Barber, 1984; Hopper & Parker, 1999). High concentrations of phosphate used in most hydroponic experiments could have suppressed selenite uptake. In our experiment, when both selenate and selenite were present at 5 μM in the nutrient solution, more selenite than selenate appeared to have been taken up by wheat, as shown by the comparison between initial and final concentrations of the two Se species at the beginning and the end of the uptake experiment. These results show that selenite, when present in the soluble form, is at least as available as selenate for uptake by wheat. However, selenite is usually more strongly adsorbed by the soil solid phase (e.g. iron oxides/hydroxides), and thus less soluble than selenate in soil solutions (Gissel-Nielsen *et al.*, 1984; Barrow & Whelan, 1989).

Selenium speciation in wheat plants

A large array of Se-containing compounds exists in plants (Whanger, 2002). Recently, various techniques of HPLC-ICP-MS and electrospray ionization mass spectrometry (ESI-MS) have been applied to the analysis of Se speciation in plants, especially vegetables, with the aim of understanding the Se nutritional value to humans (Kahakachchi *et al.*, 2004; Shah *et al.*, 2004; Cankur *et al.*, 2006; Mazej *et al.*, 2006;

Montes-Bayon *et al.*, 2006; Polatajko *et al.*, 2006). These studies have identified a number of Se-containing compounds, although some eluded peaks remain unidentified. Little information is available on Se speciation in the vegetative tissues of crop plants. The method used in our study (anion exchange HPLC-ICP-MS) allowed us to quantify inorganic Se species (selenate and selenite) as well as a number of organic Se species that are water-soluble. As with other similar studies, the method used in the present study was limited by the availability of standard Se compounds. Nevertheless, the results (Fig. 2, Tables 1–3) showed that, in the selenate-treated wheat plants, selenate remained by far the most dominant species in roots, shoots and xylem sap, with little assimilation into organic forms within 1 d of feeding. By contrast, when plants were fed selenite, it was rapidly converted to organic forms in roots, and the majority (> 70%) of Se in roots was not extractable with water, presumably having already been incorporated into proteins or other water-insoluble forms. Similarly, Ximénez-Embún *et al.* (2004) obtained a low recovery of Se in selenite-treated plants even after hydrolysis with protease. Only small amounts of selenite were detected in the root extracts and in the xylem sap. The organic forms identified included SeMet, SeOMet and MeSeCys, although these compounds were present at relatively small concentrations. Kahakachchi *et al.* (2004) reported 5.5 and 51% of the Se in roots and shoots, respectively, of selenite-treated *Brassica juncea* as SeOMet. Although SeOMet was detected in our study, it was only a minor species. This difference may be because the exposure to Se was much shorter (1 d) in our experiments than in the study of Kahakachchi *et al.* (2004) (40 d). Furthermore, they used a protease to hydrolyse the samples before the determination of Se speciation, whereas we measured water-soluble Se species only.

The most abundant Se species in the root extracts and the xylem sap of the selenite-treated plants was an unidentified compound ($RT_{2.7}$), possibly of a neutral or cationic species. Using radioactive ^{75}Se and column chromatography and electrophoresis, Asher *et al.* (1977) also found an unidentified Se compound to be dominant in the xylem sap from the selenite-treated tomato plants, which they speculated was selenotrisulphide. However, selenotrisulphide is unstable under alkaline conditions, such as in the conditions used in our HPLC procedure. The identity of this major Se compound remains to be elucidated. Overall, our results show that selenite was assimilated rapidly in wheat roots, whereas selenate was hardly assimilated within 1 d. The results are in agreement with previous studies showing that the reduction of selenate to selenite is the rate-limiting step in selenate metabolism in plants (de Souza *et al.*, 1998; Sors *et al.*, 2005).

Selenium translocation from roots to shoots

The most striking difference between selenate and selenite lies in the translocation from roots to shoots. After 24 h exposure, 70–90% of the selenate taken up by wheat had been distributed to shoots, compared with < 12% in the selenite-treated plants (Fig. 1, Table 3). This finding is in agreement with previous reports on a range of other plant species (Asher *et al.*, 1977; Arvy, 1993; de Souza *et al.*, 1998; Zayed *et al.*, 1998; Hopper & Parker, 1999; Ximénez-Embún *et al.*, 2004). Selenate was extremely mobile in xylem transport, with its concentration in the xylem sap being five to 43 times higher than in the external solution. Similar results were found in a study of the xylem transport of Se in tomato (Asher *et al.*, 1977). In comparison, little selenite was transported in xylem, probably because it was readily converted to organic forms in roots. Although some organic Se species, such as SeMet, MeSeCys and the unidentified compound at $RT_{2.7}$, were transported in the xylem sap of the selenite-treated plants, their concentrations were far smaller than the selenate concentration in the selenate-treated plants. Small amounts of selenate were detected in the xylem sap and some of the shoot extracts of the selenite-treated plants, particularly in the S-starved plants. This is likely because of the small amount of selenate present as an impurity in the selenite compound, which would be preferentially transported in the xylem to shoots, particularly in the S-starved plants. An alternative explanation, but less plausible because of the response pattern to S starvation, is an oxidation of selenite to selenate by plants (Asher *et al.*, 1977).

Interestingly, when both selenite and selenate were present in the nutrient solution, the selenate concentration in the xylem sap and Se translocation to shoots were both suppressed significantly compared with the selenate treatment alone (Table 3). This effect was unlikely to be the result of Se toxicity, because the concentrations used in our experiment (up to 10 μM) were far below that reported to cause toxicity in wheat (*c.* 125 μM) (Lyons *et al.*, 2005). It appears that selenite can inhibit uptake and, especially, xylem transport of selenate.

In conclusion, the present study has provided strong physiological evidence that selenite can be taken up by the phosphate transporters in wheat roots. Selenite uptake is a metabolically-dependent active process, and the rate of uptake by wheat was found to be similar to that of selenate. Selenite is rapidly assimilated into organic forms in roots, and has a low mobility in xylem transport. By contrast, selenate is not readily assimilated into organic forms, but is highly mobile in xylem transport. Furthermore, selenite appears to inhibit the transport of selenate in the xylem.

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