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Establishment of Selenium Uptake and Species Distribution in Lupine, Indian Mustard, and Sunflower Plants

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Selenium has been recognized as essential for all mammals; therefore, its concentration level and speciation are of great concern. Plants are one of the main sources of selenium in the diet. Thus, inorganic selenium uptake and its transformation in different species were evaluated in Indian mustard (*Brassica juncea*), sunflower (*Helianthus annus*), and white lupine (*Lupinus albus*). More than 1.2 g kg^{-1} (dry matter) of Se was found in the aerial part of Indian mustard when growing on 1 mg L^{-1} of Se as Na_2SeO_4, and approximately half this amount was determined in the leaves of the lupine, which is still quite high. Selenomethionine was the main selenium-containing amino acid identified in most of the extracts by HPLC-ICP-MS. The higher values were 6.8 and 14.5 mg kg^{-1} (expressed as Se in dry matter) in the leaves of lupine and sunflower, respectively. This is of great importance because some authors have considered the combination of this enriched material with non-enriched food as a source of selenium supplementation.

KEYWORDS: Selenium; accumulation; speciation; lupine; Indian mustard; sunflower

INTRODUCTION

Although selenium (Se) in soils appears to be usually present in inorganic forms such as selenides, elemental Se, selenite, and selenate, it is common to find organic Se compounds metabolized by microorganisms. The oxidized forms such as SeO_4^{2-} and SeO_3^{2-} are more soluble and more easily absorbed by plants, whereas Se^{2-} and Se^0 are less available (*1*).

Plants show considerably different physiological responses to selenium (*2*). Some plant species have the ability to accumulate large amounts of selenium without showing symptoms of toxicity. These species have been applied for the bioremediation of Se-laden soils and water (*3*) in a natural process called phytoremediation (*4*), involving phytoextraction and phytovolatilization processes. However, most plants are Se-nonaccumulators, and the mechanisms in assimilation pathways are expected to differ between them and Se-accumulators. Selenium present in soil is taken up by plants, where it may be incorporated as inorganic forms (without further modifications), incorporated as Se-containing proteins, accumulated following the sulfur metabolic pathway being incorporated unspecifically into low and high molecular weight compounds, or taking part of several nonproteinic Se species (avoiding the incorporation of Se-amino acids into proteins) (*5*). Selenomethionine (SeMet) is one of the most important metabolites in the assimilation pathway. The formation of nonproteinic species such as γ-glutamyl-Se-methylselenocysteine, selenocystathionine, or mainly Se-methylselenocysteine has been suggested as the mechanism followed by Se-accumulators (*6*). Selenium tolerance seems to be related to the synthesis of these compounds, allowing plants to accumulate high amounts of selenium without symptoms of toxicity. Selenium has been recognized as an essential nutrient for animals and human beings (*7*). Its presence is necessary in several enzymes (*8*), and it plays an important role in anticarcinogenic activity and in the amelioration of the toxic effects of heavy metals such as mercury and cadmium (*9*, *10*). Deficiency diseases as well as toxicity may occur in human beings in a very narrow range of selenium intake (*1*), which depends on the source of the foodstuff. In this respect, plants can play a unique role inthe narrow margin between beneficial and harmful levels of selenium, because vegetables included in the diet are one of the main sources of selenium intake (*2*). Plants that accumulate Se may be used as a natural source of mineral supplements for both animals and human beings, especially in areas that are Se-deficient (*11*). In animal foods and plants selenium is generally present as Se-proteins containing selenoamino acids (*12*). Therefore, information on the speciation of selenium in plant tissues is of paramount importance.

Sensitive and selective analytical techniques are required in speciation analysis. Inductively coupled plasma mass spectrometry (ICP-MS) is widely used for this purpose, usually coupled with chromatographic systems. Among the difficulties that hinder speciation, sample preparation is still one of the most critical steps (*13*). The extraction process is not supposed to modify or alter the chemical form of the element or disturb the

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equilibrium between the various species present in the medium. The risk of inaccurate determination steadily increases when solid matrices are under study. The extraction efficiency must be considered in parallel to minimize changes in the nature of the species. Different extraction approaches have been applied to selenium extraction in biological and environmental samples (*9*, *14*), and they have been recently reviewed. Overall, enzymatic digestion is the most suitable sample treatment, not only from the recovery point of view but also because it minimizes the disturbance of the species. Protease extractions tend to be applied on biological samples, resulting in the cleavage of peptide bonds in proteins and thus also the release of amino acids (*15*).

The bioavailability of Se as a nutrient or as a toxicant depends highly on the Se species present (*16*). More knowledge about the selective determination of a particular chemical species of selenium would be very useful to understand the biochemical and biogeochemical cycle of selenium from both biological and environmental points of view. In this respect, other problems should be solved such as the lack of commercially available standards (*17*).

This work evaluated the accumulation and transformation of selenium by different plant species grown hydroponically in selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) media. The plants selected for this study were a very well-known Se-accumulator, that is, Indian mustard (*18*); a nonaccumulator, that is, sunflower (*19*); and another plant species, white lupine, which to date has not been included in either of these categories. Lupine was chosen because it seems quite tolerant to other metals (*20*, *21*) and is widely distributed and used for animal feed, Spain being one of the richest countries in lupine flora (*22*). Speciation in the resulting plant tissues was carried out by high-performance liquid chromatography (HPLC)-ICP-MS after enzymatic digestion with protease XIV. The enzymatic hydrolysis procedure was chosen because it provides the highest values when Se species are released from solid biological samples (*17*, *23*), either bound or not bound to the protein.

EXPERIMENTAL PROCEDURES

Instrumentation. An atomic fluorescence spectrometer (Merlin, P. S. Analytical Ltd., Orpington, Kent, U.K.) equipped with a selenium-boosted discharge hollow cathode lamp (BDHCL, Photron, Victoria, Australia) with primary and boosted intensities of 25 mA was used.

An HP-4500 (Tokyo, Japan) inductively coupled plasma mass spectrometer fitted with a Babington nebulizer and a Scott double-pass spray chamber cooled by a Peltier system was used for selenium detection after chromatographic separation.

For the chromatographic experiments, a CM4500 HPLC pump (Milton Roy, Riviera Beach, FL) fitted with a six-port sample injection valve (model 7725i, Rheodyne) and a 100 μL injection loop was used. The separation was performed on a 250 $\times$ 4.1 mm i.d., 10 μm Hamilton PRP-X200 cationic exchange column (Hamilton, Reno, NV).

Reagents and Standards. All of the reagents were of analytical grade and were used without further purification. Selenomethionine (SeMet) and selenocystine ($SeCys_2$) (Sigma-Aldrich, Milwaukee, WI) were prepared in doubly distilled water. Hydrochloric acid (3%) was added to dissolve $SeCys_2$. The stock solutions containing 10 mg L^{-1} of selenium from each compound were stored in the dark at 4 °C. Working solutions were prepared daily by appropriate dilution in deionized water. For HG-AFS studies, 1% sodium borohydride solution in 0.3% sodium hydroxide (Merck) was prepared by dissolving $NaBH_4$ powder (Sigma-Aldrich) in water and filtered to eliminate turbidity. A 3 M hydrochloric acid solution was prepared by diluting the appropriate volume of concentrated HCl (Merck). For HPLC-ICP-MS studies, the mobile phase was 4 mM pyridine formate in 3% methanol. The eluent was prepared by diluting commercial pyridine (Merck) with distilled water and adjusting the pH to 2.8 with formic acid (Merck). HPLC-grade methanol was purchased from SDS (Barcelona, Spain). For the enzymatic extraction procedure, Tris-HCl buffer (pH 7.5) and the nonspecific protease *Streptomyces griseus* (protease XIV), both obtained from Fluka, were used to prepare the plant tissue samples.

The seeds were kindly provided by different suppliers: Sunflower seeds (*Helianthus annus* var. Peredovic) were obtained from Rocalba, S.A. (Zaragoza, Aragón, Spain), and white lupine (*Lupinus albus* var. Marta) and Indian mustard (*Brassica juncea*) seeds were supplied by the Agriculture and Trade Council of the Extremadura Autonomous Community Government (Cáceres, Extremadura, Spain). The reference material used was white clover (BCR-402) certified for total selenium content.

Procedures. *Cultivation and Plant Pretreatment.* Prior to germination, the seeds were disinfected with diluted bleach (10%, v/v) for 10 min and carefully rinsed with distilled water. The sterilized seeds were germinated on sand moistened with deionized water. After germination of the seeds, the sand was moistened with 0.02 strength Hoagland's nutrient solution (*24*). The seedlings were transferred 5−7 days after germination to 1.5 L vessels containing a 0.1 strength Hoagland's nutrient solution, in which they were grown for 2 weeks. Air was continuously bubbled into the nutrient solution that was renewed every 3 days. Na_2SeO_3 and Na_2SeO_4 were independently supplied in the medium at two levels of concentration: 1 and 5 mg L^{-1}. Two pots were used per selenium source and concentration, each containing 12 plants. A control pot was also used as a blank with no selenium spike. After 2 weeks of treatment, the plants were harvested and carefully washed with distilled water. They were then divided into different parts: shoot and root in the case of Indian mustard; and leaves, stem, and root in the case of sunflower and lupine. The dry weight was recorded after ~48 h at 40 °C in a forced-air oven. Samples were finely ground with a pestle in an agate mortar and kept at 4 °C in high-density polyethylene (HDPE) bottles until the time of the analysis.

Selenium Species Determination. The selenium species were extracted after enzymatic hydrolysis with protease XIV following a method slightly modified by Moreno et al. (*12*, 25). A sample/enzyme (mass/mass) ratio of 10 was proved to be enough to reach the maximum yield in species; therefore, it was chosen for all of the experiments. The enzyme was added to ~50−100 mg of dried plant tissue and incubated in 3 mL of 0.1 M Tris-HCl buffer (pH 7.5) for 24 h at 37 °C. The resulting extract was centrifuged (model 5804, Eppendorf, Hamburg, Germany) at 14000*g* for 30 min. The supernatant was removed, and 2 mL of buffer was added to the residue and centrifuged again. Both fractions were mixed, and deionized water was added up to 10 mL. The solutions were passed through 10 kDa molecular cutoff filters (Millipore, Bedford, MA) while they were centrifuged at 7500*g* under controlled temperature (20 °C). The filtrates were appropriately diluted with Milli-Q water. The analyses were performed within 24 h after the last filtration step of the extraction procedure was completed. The selenium species were quantified using the standard addition method by ICP-MS after chromatographic separation by HPLC. The instrumental conditions are summarized in **Table 1**.

Total Selenium Determination. The plant tissues were digested with 2.5 mL of HNO_3 and 1 mL of H_2O_2 in a microwave oven (MSP 1000, CEM, Matheus, NC). The Se(VI) in 6 M HCl was reduced to Se(IV) on a hot plate at 95 °C for an hour. The enzymatic extract obtained from each sample was also mineralized in this way, before and after it was passed through the cutoff filters. The total selenium content was determined by a hydride generation atomic fluorescence spectrometer under the conditions summarized in **Table 2**.

RESULTS AND DISCUSSION

Total Selenium Accumulation. *Biomass Production and Tolerance toward Selenium.* Visual symptoms of selenium toxicity did not appear in mustard or lupine plants grown in the presence of selenium. However, a reduction in biomass yield was observed when lupines were exposed to 5 mg L^{-1} of sodium selenate or sodium selenite, showing biomass productions of 66 and 51%, respectively, compared to the dry weight of the

Table 1. Experimental Conditions for Se Determination by HPLC-ICP-MS

HPLC parameters	
analytical column	PRP-X200
eluent	4 mM pyridine formate solution, pH 2.8, 970 mL of H_2O + 30 mL of MeOH
eluent flow rate	1 mL min^{-1}
elution program	isocratic
injection volume	100 μL
ICP-MS conditions	
forward power	1300 W
plasma gas (Ar) flow rate	15 L min^{-1}
auxiliary gas (Ar) flow rate	1.3 L min^{-1}
carrier gas (Ar) flow rate	1.1 L min^{-1}
nebulizer type	Babington
spray chamber type	Scott double-pass
data acquisition mode	time-resolved analysis
monitorized isotopes	^{78}Se, ^{82}Se
integration time	100 ms

Table 2. Experimental Conditions for Total Se Determination by HG-AFS

hydride generation	
HCl concentration	3 M
HCl flow	8.5 mL min^{-1}
$NaBH_4$ concentration	1% (w/v)
$NaBH_4$ flow	4.0 mL min^{-1}
carrier argon	300 mL min^{-1}
drier argon	375 mL min^{-1}
auxiliary hydrogen	200 mL min^{-1}
detector	
primary lamp current	25 mA
boosted lamp current	25 mA

control plants (**Figure 1**). Indian mustard was especially tolerant to selenite addition. When this plant was grown in the presence of Na_2SeO_3 at both concentrations, no differences in biomass production were noticed. When Na_2SeO_4 was added to the medium, there was a reduction of 20% in biomass yield for both selenium concentrations evaluated in this study. With regard to sunflower, the dry matter of both shoot and root significantly decreased (~60%), even when only 1 mg L^{-1} of selenium as either Na_2SeO_3 or Na_2SeO_4 was added to the nutrient medium. When 5 mg L^{-1} of Se-modified Hoagland's solution was added, the dry weight did not reach 50% of the weight of the control plants. The results obtained indicate that both Indian mustard and lupine are quite tolerant to the presence of low levels of inorganic selenium, added as selenite or selenate, whereas sunflower seems to be the most sensitive plant to both selenium species.

Selenium Accumulation and Distribution. Once the tolerance was established, selenium accumulation and distribution were evaluated. The total selenium content was analyzed in each of the dried subsamples obtained from Indian mustard (root and shoot) and sunflower and lupine (root, stem, and leaves). The determination was performed by HG-AFS after following the steps summarized under Experimental Procedures. The accuracy of the results was evaluated by using a suitable reference material such as white clover (CRM-402). No significant differences were observed at a 95% confidence level between the certified value and that obtained experimentally; therefore, the proposed method is adequate for the determination of total selenium in plants. **Table 3** summarizes the results. As can be observed, the uptake of Se depends on the chemical form of the selenium added. For the three plant species tested, the selenate addition led to a higher accumulation rate than selenite. Other authors (*26*) confirm that enrichment procedures in Indian

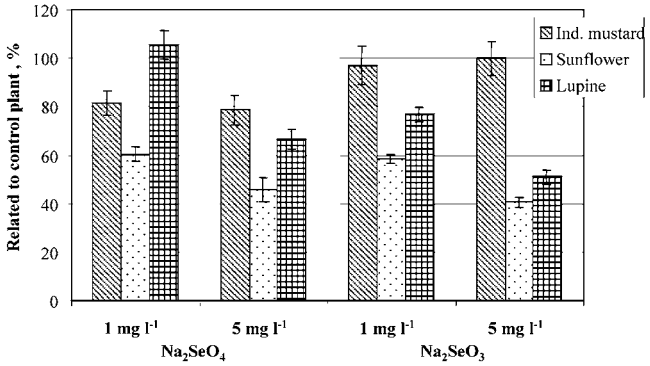

Figure 1. Effect of chemical selenium form and concentration added to the nutrient solution on biomass production (expressed as a percentage). The control plant represents 100% for each species.

Table 3. Total Selenium Concentration Found in Each Part of Mustard, Lupine, and Sunflower When the Growing Medium Was Independently Spiked with 1 or 5 mg L^{-1} of Selenium as Na_2SeO_3 or Na_2SeO_4 for 2 Weeks (Nutrient Solution Was Renewed Every 3 Days)

	total Se content[a] (mg kg^{-1})			
	when supplied with Na_2SeO_3		when supplied with Na_2SeO_4	
	1 mg L^{-1}	5 mg L^{-1}	1 mg L^{-1}	5 mg L^{-1}
mustard				
shoot	38 ± 5	58 ± 22	1230 ± 50	2081 ± 891
root	432 ± 21	605 ± 262	947 ± 30	3411 ± 2603
lupine				
leaves	50 ± 8	54 ± 28	631 ± 25	609 ± 12
stem	32 ± 2	105 ± 25	549 ± 12	465 ± 32
root	60 ± 1	721 ±185	866 ± 9	839 ± 532
sunflower				
leaves	11.7 ± 0.2	186 ± 15	73 ± 4	724 ± 187
stem	15 ± 5	89 ± 21	40 ± 6	143 ± 24
root	50 ± 8	572 ± 36	59 ± 3	164 ± 41

[a] Results are expressed as mean value ± standard deviation (n = 6) (three plant samples from each pot).

mustard with Na_2SeO_4 proved to be more effective in terms of selenium accumulation than with Na_2SeO_3. As explained elsewhere (*2*), there is enough evidence to show that the former anion is actively transported through the root membrane using the same channels and mechanisms as sulfate. However, there is no evidence for selenite uptake by membrane transporters.

Selenium concentration in the root was in general higher than the concentration found in the above-ground biomass of the plants. The concentration was in the same range of magnitude for all of the subsamples in all of the plants, except for Indian mustard when 1 mg L^{-1} of Na_2SeO_3 was supplied to the medium. In this case, the selenium found in the shoot was 10 times lower than in the root.

The translocation of the selenium present in the root to the above-ground plant section was highly dependent on the chemical form in which selenium was supplied. Selenium translocation from the root to the above-ground plant section was higher in selenate-supplied plants.

With regard to the above-ground plant section, selenium concentration was higher in Na_2SeO_4-growing plants than in plants enriched with Na_2SeO_3. In mustard, the shoot selenium concentration was ~1.2 g kg^{-1} dry matter (DM), whereas 40 and 73 mg kg^{-1} were found in the stem and leaves of the sunflower, respectively. In lupine shoots the accumulation was 10 times higher than the selenium amount found in sunflower and only 50% less compared to *B. juncea*. When Na_2SeO_3 was

spiked, the total selenium accumulation in the above-ground biomass for all of the subsamples ranged from 11.7 to 50 mg kg^{-1}, which were determined in sunflower leaves and in lupine leaves, respectively. As expected, the sunflower plants showed the lowest selenium accumulation.

As mentioned above, the similarity between SO_4^{2-} and SeO_4^{2-} appears to indicate that the assimilation pathways of both anions are the same. SO_4^{2-} contributes to a higher transpiration rate, and this increases the movement of Se through the xylem, helping the translocation (*19*).

When 5 mg L^{-1} of selenium was added to the nutrient solution, the mustard and lupine plants showed a moderate increase in the accumulation rate, which reached a maximum of 50% compared to the uptake when 1 mg L^{-1} was used. On the other hand, selenium concentration in sunflower increased from 5 to 10 times when Na_2SeO_3 and Na_2SeO_4 were supplied, respectively. This extremely high rate of uptake may be related to the nonspecific effects of membrane damage and the "breakthrough" of selenium to the root and shoot in more sensitive plants such as sunflower. Thus, to explore further the relationship between the addition of inorganic selenium and the production of Se species, our speciation analysis was focused on the treatment with 1 mg L^{-1} of selenium, avoiding the effects of metal toxicity in sunflower plants when higher amounts of selenium were applied to the nutrient medium.

From this study, it can be concluded that lupine plants possess the ability to accumulate a high concentration of selenium under the conditions tested, quite similar to the behavior shown by Indian mustard, a well-known selenium accumulator. Lupine may be a good candidate for selenium phytoremediation, especially in those areas where the commonly used Se-accumulator plants are not available or the environmental conditions are not suitable for their cultivation.

Se Species Determination: Selenium Transformation in Plants. After evaluation of the total selenium distribution in plants, its speciation was undertaken. Apart from total selenium uptake, different enrichment treatments usually provoke certain metabolic changes that determine the final product as well as its translocation and accumulation in different plant tissues. To date, very few Se speciation studies have been carried out in plants, most of them concerning white clover (*27*, *28*) and the well-known accumulator Indian mustard (*17*, *33*). Other samples include garlic (*29*, *33*) and broccoli (*30*). However, to our knowledge, no data have been reported about selenium accumulation and speciation in some kinds of crop plants such as lupine.

Enzymatic and nonenzymatic extraction treatments such as Tris-HCl (pH 8) and 1 M HCl were tested. The use of enzymes provided the maximum extraction yield. Thus, the selenium species were determined by the enzymatic treatment according to the procedure applied by Moreno et al. (*12*). Two different enzymes, subtilisin and protease XIV, in 0.1 M Tris buffer were evaluated to obtain the highest rate in hydrolyzed selenium species. Each enzyme was added in one or two steps requiring, in the case of the two-step addition, double the amount of enzyme and double the time of incubation (24 h × 2). No significant differences were found either between the types of enzyme or the incubation time; protease XIV applied to the sample in one step (24 h) was chosen for further experiments. This optimization was performed on a certified sample of white clover (BCR-402).

After enzymatic hydrolysis, the sample was centrifuged and the supernatant was removed. To enhance the cleanup of the sample prior to the chromatographic system, a portion was passed through the cutoff filters. The total selenium content was analyzed before and after the solution had been passed through the filters in order to ascertain whether there were selenium compounds retained in the filters. For most of the subsamples, selenium recoveries ranged from 90 to 99%, which indicates that the molecular weight of most of the selenium species extracted during the hydrolysis was <10 kDa. When the leaves and the root of the sunflower were analyzed, the recoveries ranged from 80 to 87% and from 55 to 58%, respectively, which means that the hydrolysis in the matrix corresponding to the nonaccumulator sunflower was not as effective in breaking down the peptides or proteins into smaller fractions as it was for the other samples.

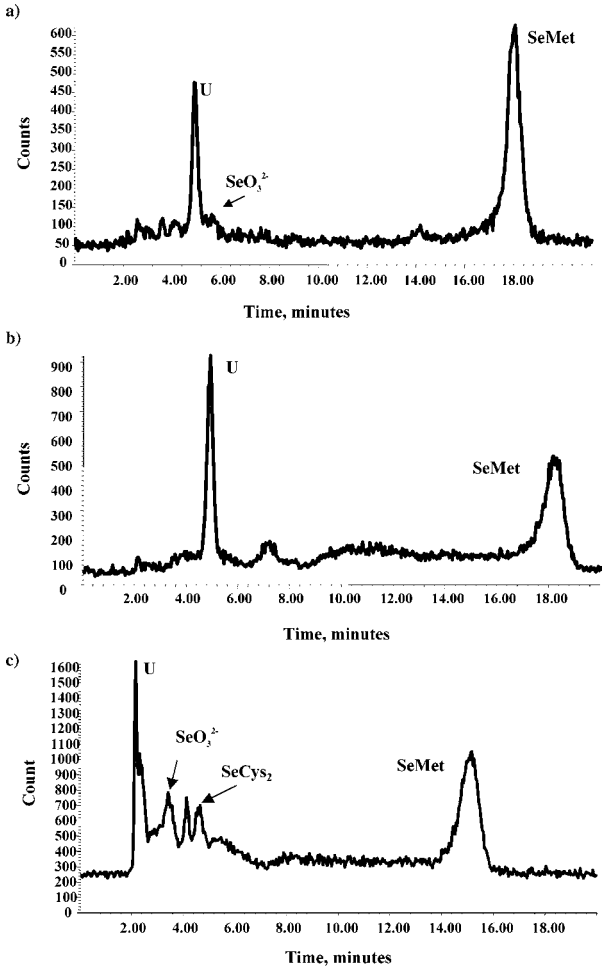

Figure 2. Typical chromatogram obtained at pH_{eluent} 2.8 from (**a**) the shoot of Indian mustard, (**b**) the leaves of sunflower, and (**c**) the stem of lupine after growing for 2 weeks in 1 mg L^{-1} of Na_2SeO_3 (U = unknown species).

For selenium speciation, the ultrafiltered hydrolyzed samples were injected into the chromatographic system. Under the chromatographic conditions compiled in **Table 1**, it was possible to identify several species, which included SeO_3^{2-}, SeO_4^{2-}, SeMet, and $SeCys_2$, within 40 min. Typical chromatograms of the subsamples obtained at pH 2.8 from Indian mustard, sunflower, and lupine plants grown on Na_2SeO_3 and Na_2SeO_4 are shown in **Figures 2** and **3**, respectively. Identification of the species was carried out using an alternative chromatographic condition (pH 4.7) or by spiking with the corresponding standards. The results obtained by both procedures were in good agreement. The selenium species were quantified using the standard addition method. A 10 μg L^{-1} selenium solution was used to evaluate the ICP-MS drift during the analyses. The

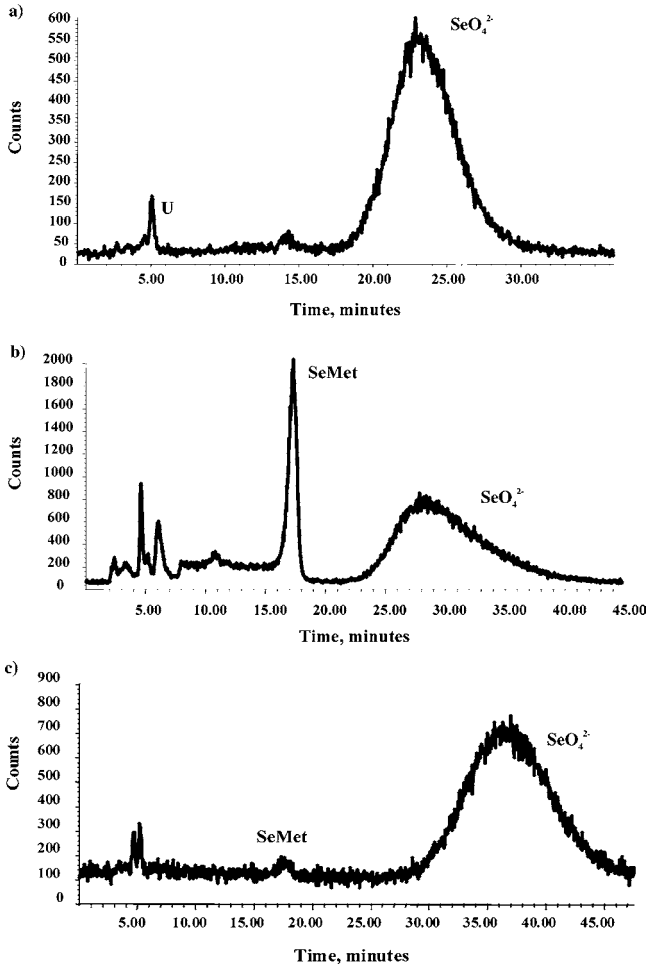

Figure 3. Typical chromatogram obtained at pH_{eluent} 2.8 from (**a**) the shoot of Indian mustard, (**b**) the leaves of sunflower, and (**c**) the stem of lupine after growing for 2 weeks in 1 mg L^{-1} of Na_2SeO_4 (U = unknown species).

differences in retention time of the Se species were due to the use of different PRP-X200 chromatographic columns during the whole study. All of the chromatograms were run until the elution of all the Se species. The representation of shorter chromatograms in **Figure 2** was due to the absence of SeO_4^{2-} in the extracts (previously checked in each of the samples). The results are summarized in **Table 4**. When plants were grown on the Na_2SeO_3-enriched medium, SeO_3^{2-}, $SeCys_2$, and SeMet were identified in the samples, whereas only SeO_4^{2-} and SeMet were found in the selenate-grown plants. For most of the plants, a selenium signal elutes at the beginning of the chromatogram, which did not match the standards available in the laboratory. As shown, SeMet was the main selenium-containing amino acid found in the extracts. When plants were grown in the presence of Na_2SeO_3, the production rate of this amino acid was higher than when plants were grown in the nutrient solution with Na_2SeO_4. In all of the Na_2SeO_3-enriched samples, the concentration of SeMet found was higher than the concentration of SeO_3^{2-} species. This conclusion was drawn from observing the ratio between SeMet and the total selenium content found in each subsample for both selenite- and selenate-grown plants.

The SeMet concentration was higher in the root than in the above-ground biomass. This agrees with the findings of authors (*31*) who indicate that inorganic selenium is mainly metabolized in the root, especially when selenite is added to the medium.

When the plants were grown in the Hoagland's solution modified with selenate, the ion SeO_4^{2-} was the main species found in the extract, being practically the only one in some subsamples such as the mustard shoot, the lupine leaves, or the sunflower stem. As can be observed, these samples correspond to the above-ground biomass of the plants and, as mentioned above, the similarity between SeO_4^{2-} and the ion SO_4^{2-} may explain how selenate is transported without further modifications through the xylem following the sulfur pathway. Lintschinger et al. (*32*) found that sunflower sprouts grown in selenate accumulated ~900 mg kg^{-1}, but almost 100% of the selenium was extracted with water and found to be nonmetabolized selenate. In our case, more mature plants were divided in different parts and no transformation of SeO_4^{2-} was observed in sunflower stem, in agreement with the results reported by Lintschinger et al. However, not only SeO_4^{2-} but also SeMet and some unidentified species were found in the leaves.

Kotrebai et al. (*33*) found that SeO_4^{2-} and SeMet (70 and 18%, respectively) were the main species in enzymatic extracts of Indian mustard treated with protease XIV. According to our results, we guess that Kotrebai's plants were grown in selenate-enriched medium, because selenium remains mainly as the SeO_4^{2-} form. However, in our plants no SeMet was detected. When selenite is added to the medium, most of the inorganic selenium is metabolized and no oxidation was observed. However, the recovery is very low, which may indicate that selenium is bound to nonproteinic structures that the enzyme is unable to cleave during the extraction step.

Table 4. Selenium Species Concentration Found in Each Part of Mustard, Lupine, and Sunflower When the Growing Medium Was Spiked with 1 mg L^{-1} of Selenium as Na_2SeO_3 or Na_2SeO_4

	Se-species content[a] (mg kg^{-1})				
	when supplied with 1 mg L^{-1} of Na_2SeO_3			when supplied with 1 mg L^{-1} of Na_2SeO_4	
	SeO_3^{2-}	$SeCys_2$	SeMet	SeO_4^{2-}	SeMet
mustard					
shoot	1.21± 0.09 (3.18%)		4.8 ± 1.7 (12.65%)	1125 ± 115 (91.46%)	
root	21.2 ± 1.3 (4.91%)		28.0 ± 2.1 (6.48%)	292 ± 15 (30.86%)	25.8 ± 2.3 (2.72%)
lupine					
leaves	1.30 ± 0.42 (2.60%)	10.2 ± 1.2 (20.40%)		601 ± 25 (95.25%)	
stem	4.8 ± 1.9 (15.0%)	1.7 ± 0.2 (5.31%)	11.2 ± 3.6 (35%)	428 ± 14 (77.96%)	6.8 ± 1.1 (1.24%)
root	9.9 ± 5.4 (16.5%)		42.7 ± 4.2 (71.17%)	837 ± 23 (96.65%)	
sunflower					
leaves	0.38 ± 0.04 (3.25%)		1.83 ± 0.74 (15.64%)	25.2 ± 5.9 (34.52%)	14.5 ± 1.5 (19.86%)
stem	0.27 ± 0.12 (1.8%)		1.32 ± 0.64 (8.8%)	39.0 ± 5.7 (97.50%)	1.9 ± 0.9 (4.75%)
root	0.92 ± 0.03 (1.84%)		7.25 ± 0.29 (14.5%)	20 ± 11 (33.90%)	5.1 ± 0.1 (8.64%)

[a] Results are expressed as mean value ± standard deviation (n = 6). The figures in parentheses relate to the recovery (expressed as a percentage) of the total Se concentration previously determined.

Se-methylselenocysteine was identified in Indian mustard extracts when wild type and genetically modified plants grown in selenite solution were extracted with proteinase K (*26*). The production of this non-protein amino acid is believed to be the result of the mechanism of Se-detoxification in plants. Therefore, this compound could be among the unidentified species, especially in the case of Indian mustard.

The recovery of species in most of the samples grown in the Na_2SeO_4 medium ranged from 54 to 102% compared to the total selenium content, except for the root of the mustard and the root of the sunflower, where the sum of the species was 33.6 and 42.5%, respectively (**Table 4**). When the plants were grown in selenite, the sum of the identified species ranged from 10.6 to 23.0%, except for the stem and root of lupine, which reached higher values (55.3 and 87.7%, respectively). The low recoveries obtained in selenite-grown plants may indicate that SeO_3^{2-} is easily metabolized and incorporated into different Se compounds difficult to identify due to the lack of suitable standards (see **Table 4**).

The experiments show that the chemical form in which selenium is present in each part of the plant depends not only on the inorganic selenium source but also on the kind of plant analyzed. Selenate uses the same transporter as sulfate in getting across plant membranes. Once inside, this ion is either toxic to or cannot efficiently utilize ATP sulfurylase and the subsequent enzymes leading to cysteine and methionine synthesis. The reduction of selenate to selenite has also been pointed out as the rate-limiting step in selenate transformation (*2*). It therefore remains mainly as selenate in the various plant tissues, and it can readily move around the plant and, therefore, will accumulate in the above-ground biomass of the plant. Selenite, on the other hand, uses an unidentified mechanism to enter the cells of plants, which is much less efficient compared to selenate uptake. However, once inside the cells, selenite is able to use the sulfur enzymes leading to cysteine and methionine synthesis. This may be possible because selenite is at a lower oxidation state compared to sulfate. As a result, selenite will not move around the plant as readily as selenate and will accumulate more in the roots, where will be converted more easily to organic forms.

Differences with other authors concerning the selenium uptake and species found in the same plant type could be attributed to different environmental conditions during selenium accumulation. SeMet was the main amino acid produced by plants grown in inorganic selenium. This fact is very important because some authors have suggested the use of edible plants enhanced with essential elements and especially with the anticarcinogenic SeMet. In this respect, lupine and sunflower plants may be suitable because they are included in animal and human diets.

Although ICP-MS is a selective detector for selenium, identification techniques such as electrospray mass spectrometry (ESI-MS) or matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) are required to overcome the lack of suitable standards. These promising coupled techniques, after overcoming the current deficiency in sensitivity, will be helpful tools in the identification of not only the principal components of a sample (*33*) but also the rest of the components.

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