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Grain Accumulation of Selenium Species in Rice (*Oryza sativa* L.)

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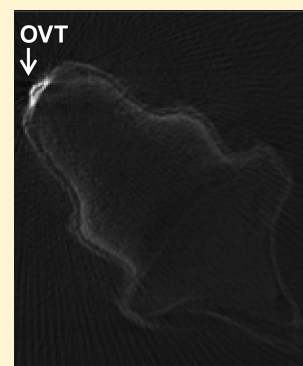
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S Supporting Information

ABSTRACT: Efficient Se biofortification programs require a thorough understanding of the accumulation and distribution of Se species within the rice grain. Therefore, the translocation of Se species to the filling grain and their spatial unloading were investigated. Se species were supplied via cut flag leaves of intact plants and excised panicle stems subjected to a \pm stem-girdling treatment during grain fill. Total Se concentrations in the flag leaves and grain were quantified by inductively coupled plasma mass spectrometry. Spatial accumulation was investigated using synchrotron X-ray fluorescence microtomography. Selenomethionine (SeMet) and selenomethylcysteine (SeMeSeCys) were transported to the grain more efficiently than selenite and selenate. SeMet and SeMeSeCys were translocated exclusively via the phloem, while inorganic Se was transported via both the phloem and xylem. For SeMet- and SeMeSeCys-fed grain, Se dispersed throughout the external grain layers and into the endosperm and, for SeMeSeCys, into the embryo. Selenite was retained at the point of grain entry. These results demonstrate that the organic Se species SeMet and SeMeSeCys are rapidly loaded into the phloem and transported to the grain far more efficiently than inorganic species. Organic Se species are distributed more readily, and extensively, throughout the grain than selenite.



INTRODUCTION

Se is an essential micronutrient in which up to 1 billion people worldwide are deficient,¹ causing a range of health disorders and potentially an increased risk of certain cancers.^{2–7} In a recent global survey of Se content in rice, concentrations were too low in the majority of samples to meet the nutritional requirements of populations depending on rice consumption for their dietary Se intake.⁵ There is, therefore, considerable interest in fortifying rice and other grain crops with Se through the application of Se fertilizers to crops or by genetically engineering cultivars to accumulate high concentrations of Se.⁸ Understanding the mechanisms of Se accumulation in the filling rice grain would help improve the efficiency of biofortification programs and direct the breeding of high Se rice cultivars.⁸

Organic Se species are better assimilated by the human body, and selenomethionine (SeMet) and selenomethylcysteine (SeMeSeCys) are more effective anticarcinogens than inorganic Se, with SeMeSeCys the most potent.^{2,3,9} Therefore, when the mechanisms of Se accumulation in the grain are investigated, Se speciation must be considered. Selenate and selenite are the main Se species that plants absorb from the soil,² although they can also take up organic species such as SeMet.^{10,11} While selenate and selenite are both water-soluble, selenite's affinity

for iron oxyhydroxides means that selenate is relatively more bioavailable to plant roots.⁸ The chemical similarity shared by selenate and sulfate enables selenate to enter the plant via sulfate transporters, although the affinity that sulfate transporters have for selenate appears to vary among plant species.⁸ Selenite uptake into the plant may occur via phosphate transporters.¹² Once in the plant root, selenate is widely thought to be transported via the sulfur assimilation pathway and ultimately converted to selenite.^{11,13} Selenite is converted to selenide by glutathione and, via a series of steps, is assimilated into organic Se species.^{2,3,13} The organic Se species selenocysteine (SeCys) and SeMet can be incorporated into proteins, replacing cysteine (Cys) and methionine (Met), respectively, which can result in toxicity in plants.^{10,14,15} SeMeSeCys, a nonprotein selenoamino acid, is produced by Se hyperaccumulators and to a lesser degree by other plants, thereby limiting Se toxicity.^{10,15} Within the plant, methylation of SeMet to dimethyl selenide, and of SeMeSeCys to dimethyl diselenide, can lead to substantial volatilization.^{2,8–10}

Received: October 31, 2011

Revised: March 21, 2012

Accepted: April 13, 2012

Published: April 13, 2012

A recent study reported that Se in the rice endosperm is predominantly organic while the bran layer contains both inorganic and organic Se.⁵ Analysis of the mature rice grain performed by Li et al.¹⁶ revealed grain Se to be primarily speciated as SeMet. Sun et al.¹⁷ analyzed the Se speciation and distribution in rice plants grown in a Se-rich environment and reported that Se concentrations in rice decreased in the order rice straw > bran > whole grain > polished rice > husk and that, within the grain, Se was concentrated in the bran layer, with concentrations almost twice those of the polished grain. Speciation of the mature grain revealed that Se was present primarily in organic forms, chiefly SeMet with lower concentrations of SeMeSeCys and SeCys.¹⁷

There remain significant knowledge gaps in our current understanding of how Se species accumulate in the filling rice grain and their relative mobility within the grain. Additionally, the relative contributions of the phloem and xylem transport pathways in their translocation from the shoot have yet to be established. This study, therefore, investigated the differential efficiency (i.e., the comparative readiness) with which important Se species are translocated and spatially unloaded into the rice grain together with the comparative contributions of phloem and xylem transport. Rice panicles were excised below the flag leaf node during grain development and exposed to a pulse of selenate, selenite, SeMet, or SeMeSeCys for 48 h. To evaluate the contributions of phloem and xylem transport in shoot to grain translocation of Se species, a stem-girdling treatment was applied. To investigate the retranslocation of these Se species from the flag leaves to the filling grain, selenate, selenite, SeMet, or SeMeSeCys was delivered to the filling rice grain via the cut flag leaf of intact plants. Total Se concentrations in rice grain samples were quantified by inductively coupled plasma mass spectrometry (ICP-MS). For those panicles treated with selenite, SeMet, or SeMeSeCys, via the excised panicle stems or the cut flag leaves of intact plants, the spatial unloading of Se into the fresh filling grain was examined by synchrotron X-ray fluorescence (XRF) microtomography.

■ EXPERIMENTAL METHODS

Plant Growth. For all experiments a quick flowering *Oryza sativa* L. cultivar (Italica Carolina) was utilized and grown to maturity with healthy panicles labeled at anthesis as described in Carey et al.¹⁸

Excised Stem Feeding of Selenium Species via \pm Stem-Girdled Panicles. Assessment of the comparative contributions of the phloem and xylem in the translocation of Se species from the shoot into the filling rice grain was conducted by subjecting panicles to a \pm stem-girdling treatment 24 h prior to their excision from the plant (details in the Supporting Information). Rice panicles were then excised below the flag leaf node and transferred into autoclaved hydroponic treatment solutions as described by Carey et al.¹⁸ As markers for phloem and xylem transport, respectively, rubidium (Rb) and strontium (Sr) were added to the treatment nutrient solutions at a final concentration of 1 mM.^{18,19} Rb is mobile in both the phloem and xylem^{19,20} but is rapidly transferred from the xylem into the phloem where it is highly mobile.^{19,20} Sr, conversely, is immobile in the phloem and translocated in the xylem.^{19–21} Rb and Sr have therefore been used as markers for phloem and xylem transport in previous studies on cereal crops such as wheat^{19,21–25} and barley.²⁶ Panicles were then transferred to a growth chamber with a 12 h

photoperiod, a day/night temperature of 28/23 °C, a relative humidity of 80/60, and a light intensity of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 48 h as described in Carey et al.¹⁸ For each treatment and control three replicate panicles were used. Treatment solutions were weighed at the beginning and end of the treatment period to generate a measure of total solution uptake by each panicle.

There were four Se treatments, 126.6 μM selenite, selenate, SeMeSeCys, or SeMet, and two sets of controls, zero exposure controls, which were not exposed to Se, Rb, or Sr, and Se controls, which were exposed to Rb and Sr, but not to Se. Following treatment, flag leaves were cut at the base of the leaf blade (above the ligule), and grains were selected from the top and middle regions of the panicle and manually dehusked. A subsample of the grains from each panicle was immediately chilled to 4 °C for the synchrotron analyses, and the remainder of the grains were oven-dried at 70 °C, together with the flag leaves, for ICP-MS analysis.

Leaf Feeding of Selenium Species. To investigate the remobilization of Se species from flag leaves to the rice grain during grain filling, rice panicles were exposed to 126.6 μM selenite, selenate, SeMeSeCys, or SeMet via the cut flag leaf of intact plants for 7 d. A sharp razor blade was used to remove the tip of the flag leaf lamina (approximately 2 cm), and this cut end was inserted into a treatment (Eppendorf) vial containing 126.6 μM selenite, selenate, SeMeSeCys, or SeMet. Rb and Sr were added as markers for phloem and xylem transport, respectively, at final concentrations of 1 mM. A fresh vial was applied every 24 h, as described by Carey et al.²⁷ As in the excised stem feeding experiment, there were two sets of controls, zero exposure controls, which were not exposed to Se, Rb, or Sr, and Se controls, which were exposed to Rb and Sr, but not to Se (please refer to the Supporting Information for details). The vials were weighed prior to application and upon removal to generate a measure of total solution uptake by each flag leaf. For each treatment and control, four replicate panicles were used. Plant material was then treated as for excised stem feeding.

Total Element Concentrations in the Flag Leaves and Grain. Total Se, Rb, and Sr concentrations were determined for digested samples by ICP-MS (7500 Agilent Technologies) as described by Sun et al.²⁸ Quality control measures were as described by Sun et al.²⁸ Oven-dried samples were microwave digested as described by Sun et al.²⁸ with 0.4 mL of nitric acid added to a \sim 0.04 g sample, which was left overnight to predigest, and 0.4 mL of hydrogen peroxide added immediately prior to microwave digestion.

Synchrotron XRF Microtomography. To examine the spatial unloading of Se into the fresh filling rice grain, synchrotron XRF microtomography was conducted on fresh rice grains that had been either pulsed with 126.6 μM selenite, SeMeSeCys, or SeMet via the excised panicle stem (subjected to a \pm stem-girdling treatment) for 48 h or fed via the cut flag leaf for 7 d. XRF microtomography took place at Geo-SoilEnviroCARS (sector 13) of the Advanced Photon Source, Argonne National Laboratory. Details (see the Supporting Information) were as described by Carey et al.¹⁸

Statistics. Data sets were analyzed using analysis of variance (ANOVA) and Tukey's pairwise comparisons. All statistics were conducted using Minitab v.15 (State College, PA). Where necessary, data sets were transformed to normalize the distribution.

RESULTS

Total Element Concentrations in the Grain and Flag Leaves for Excised Panicles \pm Stem-Girdling. Mean grain concentrations of Se, Rb, and Sr are reported in Figure 1,

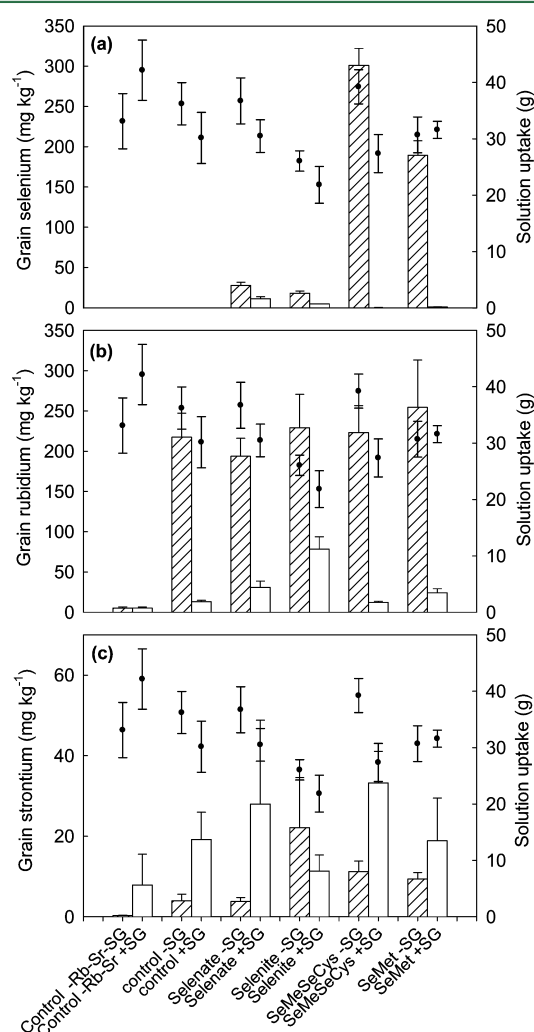


Figure 1. Mean total grain concentrations (bars) of (a) Se, (b) Rb, and (c) Sr in rice grain for panicles excised at 10 d postanthesis, subjected to a \pm stem-girdling (SG) treatment, and hydroponically fed (over a 48 h period) nutrient solution amended with 126.6 μ M selenate, selenite, SeMet, or SeMeSeCys, together with 1 mM Rb and Sr. Total solution uptake is also shown for each treatment (circles). Error bars represent \pm SE of three replicates.

together with their respective solution uptake. Zero exposure controls were not exposed to Se, Rb, or Sr, while Se controls were exposed to Rb and Sr, but not to Se. Two-way ANOVA revealed that the Se and stem-girdling treatments had highly significant effects on the mean grain Se concentrations ($P < 0.001$ and $P < 0.001$, respectively, Figure 1a) and there was a highly significant interaction between treatments ($P < 0.001$). For panicles that had not been stem-girdled, the organic Se treatments, SeMeSeCys and SeMet, led to significantly higher grain Se concentrations (10–17 fold) than for panicles treated with inorganic Se, selenate, or selenite ($P < 0.001$ in all cases). Mean grain Se was not significantly different between SeMeSeCys- and SeMet-treated panicles or between selenate- and selenite-treated panicles.

Stem-girdling led to significant reductions for all four Se species compared with their non-stem-girdled counterparts. For panicles pulsed with SeMeSeCys, stem-girdling led to a 99.9% reduction in mean grain Se, and for panicles pulsed with SeMet, mean grain Se was 99.5% lower for stem-girdled panicles than for their non-stem-girdled counterparts. For selenate-treated panicles, stem-girdling led to a 60% reduction in mean grain Se concentration, and for panicles treated with selenite, there was a reduction of 70% in grain Se when stem-girdled. Grain Se concentrations were greater for selenate and selenite stem-girdled panicles than for SeMeSeCys and SeMet stem-girdled panicles. This could be due to a greater proportion of selenate and selenite, compared with organic Se, being transported in the xylem. Since the results presented here show that organic Se arrives in the grain solely via the phloem (Figure 1), stem-girdling essentially stops transport of these species into the grain, while for inorganic Se, some of which is transported to the grain via the xylem, a portion will still be transported into the grain in stem-girdled panicles.

The stem-girdling treatment had no significant effect on mean grain Se concentrations in either the zero exposure control or the Se control panicles. Zero exposure controls were not exposed to Se, Rb, or Sr, while Se controls were exposed to Rb and Sr, but not to Se. Details of background concentrations are reported in the Supporting Information.

With the exception of the selenite treatment, there were no significant differences in solution uptake between stem-girdled panicles and their non-stem-girdled counterparts for each Se treatment (Figure 1a). It should be noted that, despite the lack of statistical significance, there was a general trend of 20% lower solution uptake in stem-girdled panicles, with the exception of SeMet-treated panicles, which exhibited no difference (Figure 1a).

Two-way ANOVA revealed that Se and stem-girdling had a highly significant effect on grain concentrations of the phloem marker Rb (Figure 1b, $P < 0.001$), with stem-girdled panicles for all Se treatments resulting in significantly less Rb in the grain than their non-stem-girdled counterparts ($P < 0.001$ in all cases except for selenite, where $P = 0.036$).

Pairwise comparisons showed that, for non-stem-girdled panicles, grain Rb concentrations did not differ significantly among the Se treatments and controls, although all were significantly higher than that of the zero exposure control ($P < 0.001$), suggesting that Se treatments did not affect phloem transport. However, despite being significantly lower in grain Rb than their non-stem-girdled counterparts, stem-girdled selenite-treated panicles yielded significantly higher grain Rb concentrations (up to 6-fold higher) than all other stem-girdled Se treatments. As Rb transport in non-stem-girdled selenite-treated panicles is similar to that for other Se treatments, this suggests that, unlike the other Se species, selenite may have enhanced the xylem uptake of Rb in comparison with other treatments. There was a significant interaction between Se and stem-girdling treatments ($P < 0.001$). Stem-girdling treatments had no significant effect on mean grain Rb for zero exposure control panicles.

There was significant uptake of the xylem marker Sr into the rice grain for all Se treatments and Se controls compared to the zero exposure controls that were not pulsed with Sr (two-way ANOVA, $P < 0.001$, Figure 1c). Pairwise comparisons confirmed that differences between the Se treatments, including the Se control, were not significant, with the exception of stem-girdled SeMet-treated panicles, which yielded significantly

greater grain Sr than non-stem-girdled control panicles ($P = 0.0399$), and non-stem-girdled selenate-treated panicles, which led to significantly lower grain Sr than stem-girdled SeMeSeCys-treated panicles ($P = 0.0454$).

ANOVA determined that the stem-girdling treatment did have a significant effect on grain Sr concentrations; however, pairwise comparisons confirmed that there were no significant differences in grain Sr between stem-girdled panicles and their non-stem-girdled counterparts for each Se treatment. There was no significant interaction between the Se and stem-girdling treatments. The Sr data are variable, but there does appear to be a trend of increased grain Sr where panicles were stem-girdled, with the exception of selenite-treated panicles where the reverse appears to be true.

Mean flag leaf concentrations of Se, Rb, and Sr are reported in Figure 2, together with solution uptake. Two-way ANOVA revealed that the Se and stem-girdling treatments had highly significant effects on mean flag leaf Se concentrations ($P < 0.001$ and $P = 0.005$, respectively, Figure 2a) and there was a significant interaction between treatments ($P = 0.028$). Pairwise

comparisons confirmed that flag leaf Se concentrations were significantly higher for the four Se treatments than for the Se and zero exposure controls (with no significant differences between the two sets of controls). Flag leaf concentrations of Se were markedly different for the inorganic Se treatments, with inorganic Se treatments yielding flag leaf Se concentrations 30–150 times those of the two organic Se treatments. Mean flag leaf Se was significantly lower for all organic treatments than for all inorganic treatments, and there were no significant differences between the two organic treatments. Flag leaf Se was significantly lower in non-stem-girdled selenite-treated panicles compared with non-stem-girdled selenate-treated panicles.

Stem-girdling did not significantly affect flag leaf Se concentrations for panicles treated with organic Se or selenite. For panicles treated with selenate, however, stem-girdling led to a significant mean reduction of about 50% in mean flag leaf Se.

Two-way ANOVA showed that there was significant uptake of Rb into the exposed flag leaves, with Rb addition yielding significantly greater flag leaf Rb than that of the zero exposure controls ($P < 0.001$), and there were no significant differences in flag leaf Rb between the different Se treatments (Figure 2b). Stem-girdling had a significant effect on flag leaf Rb ($P = 0.007$); however, pairwise comparisons showed that no treatment combination actually yielded significant differences between stem-girdled and non-stem-girdled counterparts. This may be due to the large variation between replicates within each treatment as, with the exception of selenite, there is a clear trend of reduced Rb (20–30% less) in stem-girdled panicles exposed to Rb. There was no significant interaction between the Se and stem-girdling treatments.

There was a significant uptake of Sr into exposed flag leaves ($P < 0.001$, Figure 2c). The stem-girdling treatment had an overall significant effect, and there was a significant interaction between treatments ($P < 0.001$ and $P = 0.007$, respectively). Pairwise comparisons showed that stem-girdling led to a significant reduction in flag leaf Sr for SeMet-treated panicles ($P = 0.0334$) and for Se control panicles ($P = 0.0021$) with no significant effects for all other Se treatments. However, as with Rb concentrations, there is a trend of reduced concentrations (33–49% less) in stem-girdled panicles, with the exception of selenite. For the selenite treatment, flag leaf Sr concentrations were exceptionally low in non-stem-girdled panicles.

There was no significant difference in solution uptake for stem-girdled and non-stem-girdled panicles treated with SeMet, yet substantial, and significant, differences in flag leaf Sr, suggesting that the observed differences in Sr and Rb are not governed by solution uptake but rather by internal physiological/biochemical changes. The reduction in phloem transport into the developing grain, caused by stem-girdling, may result in an increased proportion of xylem flow being directed to the grain, reducing the proportion directed to the panicle flag leaf.

Total Selenium Concentrations in the Grain and Flag Leaves for Leaf-Fed Panicles. Total Se concentrations in grains and flag leaves, together with solution uptake, for plants pulsed with Se species via a cut flag leaf on intact plants are shown in parts a and b, respectively, of Figure 3. Zero exposure controls were not exposed to Se, Rb, or Sr, while Se controls were exposed to Rb and Sr, but not to Se. Se exposure had a highly significant effect on flag leaf Se concentrations ($P < 0.001$), with all Se treatments yielding significantly higher flag leaf Se than the controls. Organic Se treatments led to

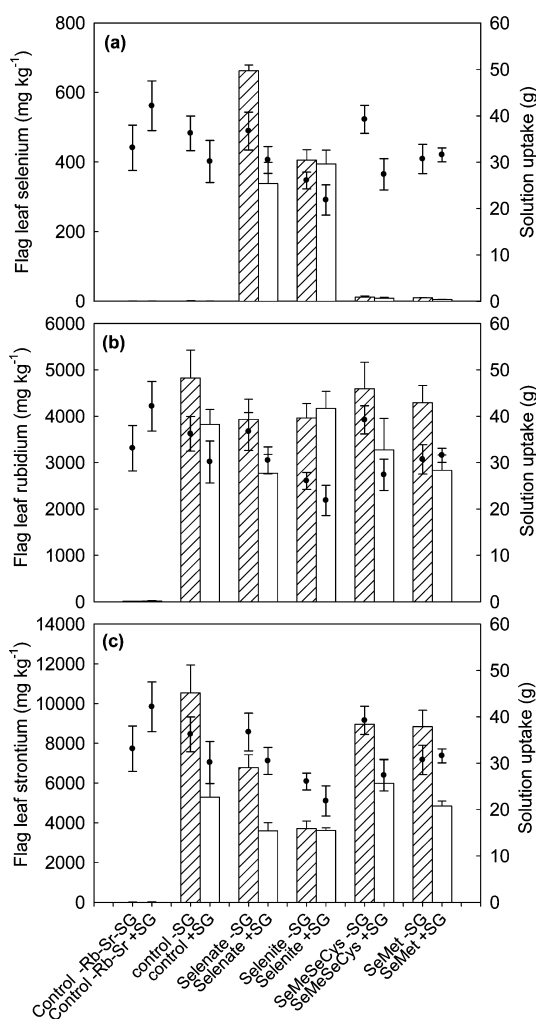


Figure 2. Mean total flag leaf concentrations (bars) of (a) Se, (b) Rb, and (c) Sr for panicles excised at 10 d postanthesis, subjected to a \pm stem-girdling (SG) treatment, and hydroponically fed (over a 48 h period) nutrient solution amended with 126.6 μ M selenate, selenite, SeMet, or SeMeSeCys, together with 1 mM Rb and Sr. Total solution uptake is also shown for each treatment (circles). Error bars represent \pm SE of three replicates.

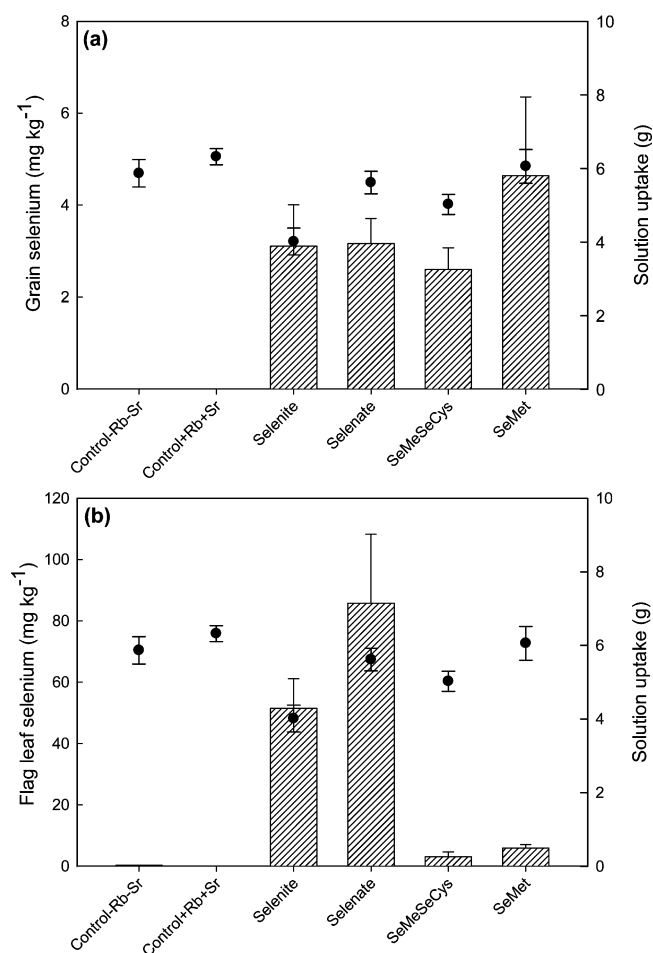


Figure 3. Mean total Se concentrations (bars) in the (a) grain and (b) flag leaf for rice fed 126.6 μ M selenate, selenite, SeMet, or SeMeSeCys, together with 1 mM Rb and Sr, via the flag leaf of intact plants for a 7 d period during grain fill. Total solution uptake is also shown for each treatment (circles). Error bars represent \pm SE of four replicates.

significantly lower flag leaf Se (10–28-fold less) than the inorganic Se treatments ($P < 0.001$), with no significant difference between the two organic treatments or between the two inorganic treatments. All Se treatments led to significantly higher grain Se than the controls (one-way ANOVA, $P < 0.001$); however, there were no significant differences in grain Se between the Se treatments. One-way ANOVA conducted on flag leaf-to-grain translocation factors (grain/flag leaf Se) showed that, for organic Se treatments, translocation from the flag leaf to the grain was more than twice that for the inorganic Se treatments ($P = 0.002$). Concentrations of Rb and Sr, as markers for phloem and xylem transport, respectively, confirm that transport from the flag leaf into the developing grain occurred primarily via the phloem (see the text and Figure S1 of the Supporting Information for details).

Spatial Distribution of Selenium into the Grain. The synchrotron XRF microtomography images of virtual transverse sections scanned across the rice grain, and including the embryo, are shown in Figure 4. They illustrate the spatial distribution of Se in the developing grain for rice grains treated with 126.6 μ M selenite, SeMet, and SeMeSeCys via the excised panicle stem and via the cut flag leaf on intact plants. These images show considerably different patterns of Se distribution in the grain for the three Se treatments, and these species-specific patterns are retained whether delivery is via retranslocation from flag leaves or direct stem-to-grain transport. Selenium is very strongly localized in the ovular vascular trace (OVT), the site of nutrient influx into the grain, for the selenite-treated grains. Conversely, the images for the SeMet-treated grains show Se dispersed throughout the external layers with some possible migration into the endosperm; the SeMeSeCys-treated grains also display clear unloading into the pericarp/aleurone layer but with significant accumulation within the embryo.

DISCUSSION

Selenium species differ substantially in the efficiency with which they are translocated from the shoot to the filling grain. In the

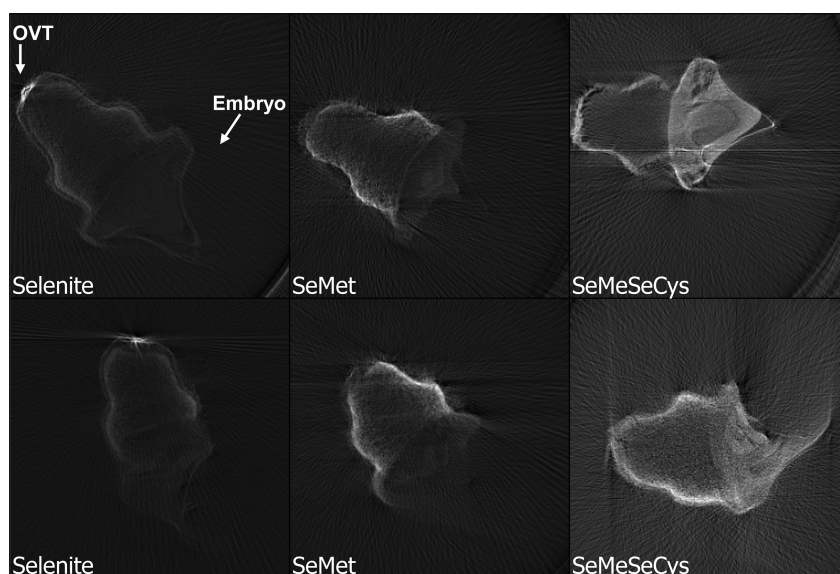


Figure 4. Synchrotron X-ray fluorescence microtomography images showing distributions of Se for virtual cross sections of fresh rice grain pulsed with 126 μ M selenite, SeMet, or SeMeSeCys through the excised panicle stem (top) or through the cut flag leaf on intact plants (bottom). OVT refers to the ovular vascular trace, the point of entry into the grain.

excised panicle experiment, exposure to SeMet or SeMeSeCys led to grain Se concentrations at least an order of magnitude greater than those from exposure to selenite or selenate, demonstrating that organic Se is transported through the stem to the filling rice grain far more efficiently than inorganic Se (Figure 1). Stem-girdling demonstrated that SeMet and SeMeSeCys were loaded into the phloem, with phloem transport essentially the sole pathway for their delivery to the rice grain, while xylem transport played a role in the shoot-to-grain translocation of inorganic Se. This may be explained by the incorporation of organic Se species into amino acids, although no analysis of amino acids was conducted in this study. There appeared to be a slight reduction in solution uptake for stem-girdled panicles; this reduction, however, is too small to account for the differences in grain Se observed for stem-girdled panicles. The trend of increased grain Sr (Figure 1) observed for stem-girdled panicles, with the exception of selenite-treated panicles, suggests that xylem flow to the grain may have increased where panicles were stem-girdled. Carey et al.^{18,27} found no significant effect on Sr xylem transport following stem-girdling, but this may also have been due to variability between replicates. It is, nonetheless, possible that interrupting the phloem pathway to the grain could result in a corresponding increase in xylem flow. The reduction in concentrations of Rb and Sr in the flag leaves of stem-girdled panicles (Figure 2) may support this theory, indicating that a greater proportion of xylem flow is directed to the grain where stem-girdling has interrupted phloem delivery to the panicle. Exposure to selenite appeared to affect the phloem and xylem transport pathways, with concentrations of Rb higher in stem-girdled panicles than for all other Se treatments and concentrations of Sr failing to follow the pattern exhibited by the other Se treatments and controls. This is likely to be a direct result of the toxicity of selenite to the panicles and is further supported by the lower uptake of the selenite treatment solution compared with all other treatments. Selenite has previously been reported to be toxic to plants, including rice, at concentrations lower than the 126.6 μM exposure used in this study²⁹ and has been demonstrated to be significantly more toxic to plants than selenate.³⁰

The leaf feeding experiment investigated the retranslocation of Se species from flag leaf stores to the rice grain during grain filling by feeding Se species directly into the panicle flag leaf of intact plants. The results demonstrate that, while all four Se species were retranslocated from flag leaf stores to the grain, retranslocation of organic Se species was more than twice that observed for inorganic Se species (Figure 3). Flag leaf Se was markedly lower for leaves exposed to organic Se treatments compared with those exposed to inorganic Se, despite similar uptake of treatment solutions. This may, in part, be explained by volatilization of organic Se from flag leaves, although volatilization was not specifically measured in this study. In planta methylation of SeMet to dimethyl selenide and of SeMeSeCys to dimethyl diselenide can lead to substantial Se volatilization, and rice plants have been shown to be particularly effective volatilizers of organic Se.¹⁰ In a study of Se volatilization rates from 15 crop species, rice, together with broccoli and cabbage, volatilized Se 20-fold faster than other plants.³¹ In a comparative study of Se volatilization rates under exposure to different Se species, rates of Se volatilization, from the shoots of rice plants exposed to SeMet, were 15-fold higher than from rice shoots exposed to selenate and 16-fold higher than from those exposed to selenite.³² It is also possible that a

large proportion of organic Se has been delivered to the plant roots. It has, for example, been demonstrated that plants accumulate volatile Se, absorbed via their leaves, within their root system.³³ Alternatively, organic Se may simply not have been taken up as effectively as inorganic Se into the leaf apoplast. The fate of Se, volatilization or translocation within the plant, warrants further investigation.

The retranslocation of Se from flag leaves to the grain observed in this study agrees with previous studies where leaf applications of Se have led to significant increases in rice grain Se.^{34–36} Hu et al.³⁴ reported a 10-fold increase in the Se content of the mature polished rice grain following a single foliar spray of 14–18 g of Se ha^{-1} applied at the heading stage. Chen et al.³⁵ measured a 25-fold increase in grain Se following a single foliar spray of 20 g ha^{-1} of inorganic Se after flowering, and Fang et al.³⁶ showed that foliar applications of 100 g ha^{-1} of selenite led to a 55-fold increase in rice grain Se, increasing the Se concentrations from 0.032 to 1.79 mg kg^{-1} . In the study by Chen et al.³⁵ foliar applications of selenate led to 36% greater grain Se than applications of selenite. In the study presented here there was no significant difference in grain Se between the selenate and selenite leaf-fed treatments; however, the leaf treatments in the study by Chen et al.³⁵ involved a single dose, via a surface spray, followed by harvest one month later, and the analysis was performed on the mature, rather than filling, grain.

The results of the flag leaf exposure to Se are in agreement with the findings of the excised stem feeding experiment, where organic species were translocated far more efficiently than inorganic species and were transported exclusively via the phloem. This is to be expected as the Rb and Sr concentrations for the flag leaf feeding experiment (Figure S1, Supporting Information) confirm that the primary transport pathway from the flag leaf to the filling grain is via the phloem, and organic Se species will, therefore, be more rapidly transported than inorganic species.

The significant retranslocation of Se from the fed flag leaves of intact plants to the filling grains, observed for both inorganic and organic Se but particularly efficient for organic species, suggests that Se that has accumulated within the plant throughout its growth will be rapidly transported to the panicle upon its development. This explains previous reports that Se accumulation within plants occurs primarily during early growth, with shoot concentrations decreasing before, or at the point of, anthesis.^{11,13} Furthermore, White et al.^{11,13} reported that the redistribution of Se throughout a plant is considered to occur via the phloem, as selenate and/or organic Se, and the findings reported here support this assertion.

Selenium Localization in the Grain. The synchrotron XRF microtomography images of Se distribution for the four Se treatments (Figure 4) show that, whether fed via the excised panicle stem or the flag leaf of intact plants, organic Se is more mobile within the grain than inorganic Se species. During grain fill, nutrients enter the grain via the OVT, which comprises both phloem and xylem elements, and cross the chalazal tissue into the nucellar epidermis, which encircles the grain, from where they are then transported, via the apoplast, radially inward into the filial tissue.³⁷ These images demonstrate that organic Se species SeMet and SeMeSeCys are more readily unloaded into the nucellar tissue and uploaded into the aleurone (and ultimately deposited in the endosperm). This interpretation does, however, assume that there has been no metabolism within the treated flag leaf and that the Se species

supplied to the grain is the primary form in which it reaches the grain. Interestingly, Se distribution for SeMet treatments appears similar to the distribution pattern of sulfur (S) observed in the mature rice grain analyzed by particle-induced X-ray emission (PIXE) in the study by Lombi et al.^{38,39} In a nanoscale secondary ion mass spectrometry (NanoSIMS) analysis of Se distribution in wheat grain, in which Se is also speciated primarily as organic Se, particularly SeMet, Moore et al.⁴⁰ demonstrated that the distribution of Se was coincident with that of S, being uniformly dispersed throughout the aleurone layer that surrounds the endosperm, and particularly concentrated in the proteinaceous area around the starch granules of the starchy endosperm cells. Se was also present in hot spots that surrounded the phytate granules of the aleurone, a pattern not matched by S, and the authors suggested that this could be a tolerance mechanism in wheat to restrict Se and limit toxic damage elsewhere in the grain.⁴⁰ The images presented in Figure 4 thus explain the findings of Williams et al.⁵ which showed Se in the endosperm of the mature rice grain to be predominantly present in organic form, with inorganic Se largely confined to the outer bran layer. Williams et al.⁵ reported that X-ray absorption near-edge spectroscopy (XANES) analysis of Se speciation in the separate fractions of the mature rice grain demonstrated that Se in the bran layer was present as an approximately 50:50 mixture of inorganic (53%) and organic (47%) species, while in the endosperm, conversely, only 5% of the Se was present as inorganic Se.

A number of studies have now reported that Se speciation in the rice grain is predominantly organic.^{5,16,17,36,41,42} Fang et al.³⁶ found that 87% of grain Se was present as SeMet following foliar application of inorganic Se, while Zhao et al.⁴² reported that Se was present exclusively as SeMet in a Se-enriched rice sample. Li et al.¹⁶ reported that the rice grain was predominately speciated as SeMet and, furthermore, that soil fertilization with selenate or selenite, and water management treatments, did not alter the percentage speciated as SeMet, i.e., did not change the conversion of inorganic Se species to SeMet.¹⁶ In the study by Sun et al.¹⁷ speciation of the mature grain revealed that Se was speciated primarily as SeMet with lower concentrations of SeMeSeCys and SeCys.¹⁷

Considering the high rates of assimilation of inorganic Se to SeMet observed in a number of studies, it might be expected that the spatial unloading pattern for selenite-fed rice would be similar to that of SeMet-fed rice. Although speciation analysis was not performed in this study and it cannot be discounted that some metabolism of Se may have occurred within experimental plants, the vastly different patterns of Se distribution suggest that the majority of selenite has not yet been converted to SeMet. It should be noted that, in contrast to the single dose foliar applications or soil treatments discussed, this study involves continual delivery of a particular species for 48 h via excised panicle stems or for 7 d where applied to the cut flag leaf on intact plants, thus bypassing the root system. Any converted SeMet will be swamped by the input of selenite, thereby allowing us to clearly observe the spatial unloading for selenite.

■ ASSOCIATED CONTENT

■ Supporting Information

Further details of the methods used for stem-girdling, panicle excision, leaf feeding of selenium species, and synchrotron XRF microtomography and detailed results for total Rb and Sr in the grain and flag leaves for leaf-fed panicles and the relevant figure,

Figure S1. This material is available free of charge via the Internet at <http://pubs.acs.org/>

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Notes

This manuscript has not been subjected to USEPA review and, therefore, does not necessarily reflect the views of the Agency. No official product endorsement should be inferred. The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was partly funded by a Biological and Biochemical Science Research Council - Doctorate Training Grant (BBSRC-DTG). Portions of this work were performed at GeoSoilEnviroCARS (sector 13), at the Advanced Photon Source (APS), Argonne National Laboratory. GeoSoilEnviroCARS is supported by the National Science Foundation—Earth Sciences (Grant EAR-0622171) and Department of Energy—Geosciences (Grant DE-FG02-94ER14466). Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract DE-AC02-06CH11357. The United States Environmental Protection Agency (USEPA), through its Office of Research and Development, funded and managed a portion of the research.

■ REFERENCES

- (1) Combs, G. F., Jr. Selenium in global food systems. *Br. J. Nutr.* **2001**, *85*, 517–547.
- (2) Ellis, D. R.; Salt, D. E. Plants, selenium and human health. *Curr. Opin. Plant Biol.* **2003**, *6*, 273–279.
- (3) Whanger, P. D. Selenium and its relationship to cancer: An update. *Br. J. Nutr.* **2004**, *91*, 11–28.
- (4) Rayman, M. P. Food-chain selenium and human health: Emphasis on intake. *Br. J. Nutr.* **2008a**, *100*, 254–268.
- (5) Williams, P. N.; Lombi, E.; Sun, G. X.; Scheckel, K.; Zhu, Y. G.; Feng, X.; Zhu, J.; Carey, A. M.; Adomako, E.; Lawgali, Y.; Deacon, C.; Meharg, A. A. Selenium characterization in the global rice supply chain. *Environ. Sci. Technol.* **2009**, *43*, 6024–6030.
- (6) Clark, L. C.; Combs, G. F., Jr.; Turnbull, B. W.; Slate, E. H.; Chalker, D. K.; Chow, J.; Davis, L. S.; Glover, R. A.; Graham, G. F.; Gross, E. G.; Krongrad, A.; Leshner, J. L., Jr.; Park, H. K.; Sanders, B. B., Jr.; Smith, C. L.; Taylor, J. R. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: A randomized controlled trial. *JAMA, J. Am. Med. Assoc.* **1996**, *276*, 1957–1963.
- (7) Tsubura, A.; Lai, Y. C.; Kuwata, M.; Uehara, N.; Yoshizawa, K. Anticancer effects of garlic and garlic-derived compounds for breast cancer control. *Anti-Cancer Agents Med. Chem.* **2011**, *11*, 249–253.
- (8) Zhu, Y. G.; Pilon-Smits, E. A. H.; Zhao, F. J.; Williams, P. N.; Meharg, A. A. Selenium in higher plants: Understanding mechanisms for biofortification and phytoremediation. *Trends Plant Sci.* **2009**, *14*, 436–442.
- (9) Rayman, M. P.; Infante, H. G.; Sargent, M. Food-chain selenium and human health: Spotlight on speciation. *Br. J. Nutr.* **2008b**, *100*, 238–253.
- (10) Terry, N.; Zayed, A. M.; De Souza, M. P.; Tarun, A. S. Selenium in higher plants. *Annu. Rev. Plant Biol.* **2000**, *51*, 401–432.
- (11) White, P. J.; Broadley, M. R. Biofortification of crops with seven mineral elements often lacking in human diets—Iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytol.* **2009**, *182*, 49–84.

- (12) Li, H. F.; McGrath, S. P.; Zhao, F. J. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. *New Phytol.* **2008**, *178*, 92–102.
- (13) White, P. J.; Broadley, M. R.; Bowen, H. C.; Johnson, S. E. Selenium and its relationship with sulfur. In *Sulfur in Plants—An Ecological Perspective*; Hawkesford, M. J., De Kok, L. J., Eds.; Springer: Dordrecht, The Netherlands, 2007; Vol. 6, pp 225–252.
- (14) Brown, T. A.; Shrift, A. Exclusion of selenium from proteins of selenium-tolerant *astragalus* species. *Plant Physiol.* **1981**, *67*, 1051–3.
- (15) White, P. J.; Bowen, H. C.; Parmaguru, P.; Fritz, M.; Spracklen, W. P.; Spiby, R. E.; Meachan, M. C.; Mead, A.; Harriman, M.; Trueman, L. J. Interactions between selenium and sulphur nutrition in *Arabidopsis thaliana*. *J. Exp. Bot.* **2004**, *55*, 1927–1937.
- (16) Li, H. F.; Lombi, E.; Stroud, J. L.; McGrath, S. P.; Zhao, F. J. Selenium speciation in soil and rice: Influence of water management and Se fertilization. *J. Sci. Food Agric.* **2010**, *58*, 11837–11843.
- (17) Sun, G. X.; Liu, X.; Williams, P. N.; Zhu, Y. G. Distribution and translocation of selenium from soil to grain and its speciation in paddy rice (*Oryza sativa* L.). *Environ. Sci. Technol.* **2010**, *44*, 6706–6711.
- (18) Carey, A. M.; Scheckel, K. G.; Lombi, E.; Newville, M.; Choi, Y.; Norton, G. J.; Charnock, J. M.; Feldmann, J.; Price, A. H.; Meharg, A. A. Grain unloading of arsenic species in rice. *Plant Physiol.* **2010**, *152*, 309–319.
- (19) Kuppelwieser, H.; Feller, U. Transport of Rb and Sr to the ear in mature, excised shoots of wheat—Effects of temperature and stem length on Rb removal from the xylem. *Plant Soil* **1991**, *132*, 281–288.
- (20) Feller, U. Transfer of rubidium from the xylem to the phloem in wheat internodes. *J. Plant Physiol.* **1989**, *133*, 764–767.
- (21) Schenk, D.; Feller, U. Rubidium export from individual leaves of maturing wheat. *J. Plant Physiol.* **1990**, *137*, 175–179.
- (22) Herren, T.; Feller, U. Influence of increased zinc levels on phloem transport in wheat shoots. *J. Plant Physiol.* **1997**, *150*, 228–231.
- (23) Herren, T.; Feller, U. Transport of cadmium via xylem and phloem in maturing wheat shoots: Comparison with the translocation of zinc, strontium and rubidium. *Ann. Bot.* **1997**, *80*, 623–628.
- (24) Zeller, S.; Feller, U. Redistribution of cobalt and nickel in detached wheat shoots: Effects of steam-girdling and of cobalt and nickel supply. *Biol. Plant.* **1998**, *41*, 427–434.
- (25) Zeller, S.; Feller, U. Long-distance transport of cobalt and nickel in maturing wheat. *Eur. J. Agron.* **1999**, *10*, 91–98.
- (26) Chen, F.; Wu, F.; Dong, J.; Vincze, E.; Zhang, G.; Wang, F.; Huang, Y.; Wei, K. Cadmium translocation and accumulation in developing barley grains. *Planta* **2007**, *227*, 223–232.
- (27) Carey, A. M.; Norton, G. J.; Deacon, C.; Scheckel, K. G.; Lombi, E.; Punshon, T.; Guerinot, M. L.; Lanzirrotti, A.; Newville, M.; Choi, Y.; Price, A. H.; Meharg, A. A. Phloem transport of arsenic species from flag leaf to grain during grain filling. *New Phytol.* **2011**, *192*, 87–98.
- (28) Sun, G. X.; Williams, P. N.; Carey, A. M.; Zhu, Y. G.; Deacon, C.; Raab, A.; Feldmann, J.; Islam, R. M.; Meharg, A. A. Inorganic As in rice bran and its products are an order of magnitude higher than in bulk grain. *Environ. Sci. Technol.* **2008**, *42*, 7542–7546.
- (29) Zhang, Y.; Pan, G.; Chen, J.; Hu, Q. Uptake and transport of selenite and selenate by soybean seedlings of two genotypes. *Plant Soil* **2003**, *253*, 437–443.
- (30) Smith, G. S.; Watkinson, J. H. Selenium toxicity in perennial ryegrass and white clover. *New Phytol.* **1984**, *97*, 557–564.
- (31) Terry, N.; Carlson, C.; Raab, T. K.; Zayed, A. M. Rates of selenium volatilization among crop species. *J. Environ. Qual.* **1992**, *21*, 341–344.
- (32) Zayed, A.; Lytle, C. M.; Terry, N. Accumulation and volatilization of different chemical species of selenium by plants. *Planta* **1998**, *206*, 284–292.
- (33) Zieve, R.; Peterson, P. J. The accumulation and assimilation of dimethylselenide by four plant species. *Planta* **1984**, *160*, 180–184.
- (34) Hu, Q.; Chen, L.; Xu, J.; Zhang, Y.; Pan, G. Determination of selenium concentration in rice and the effect of foliar application of Se-enriched fertiliser or sodium selenite on the selenium content of rice. *J. Sci. Food Agric.* **2002**, *82*, 869–872.
- (35) Chen, L.; Yang, F.; Xu, J.; Hu, Y.; Hu, Q.; Zhang, Y.; Pan, G. Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. *J. Agric. Food Chem.* **2002**, *50*, 5128–5130.
- (36) Fang, Y.; Zhang, Y.; Catron, B.; Chan, Q.; Hu, Q.; Caruso, J. A. Identification of selenium compounds using HPLC-ICPMS and nano-ESI-MS in selenium-enriched rice via foliar application. *J. Anal. At. Spectrom.* **2009**, *24*, 1657–1664.
- (37) Krishnan, S.; Dayanandan, P. Structural and histochemical studies on grain-filling in the caryopsis of rice (*Oryza sativa* L.). *J. Biosci.* **2003**, *28*, 455–469.
- (38) Lombi, E.; Scheckel, K. G.; Pallon, J.; Carey, A.-M.; Zhu, Y.-G.; Meharg, A. A. Speciation and distribution of As and localization of nutrients in rice grains. *New Phytol.* **2009**, *184*, 193–201.
- (39) Lombi, E.; Scheckel, K. G.; Kempson, I. M. In situ analysis of metal(loid)s in plants: State of the art and artefacts. *Environ. Exp. Bot.* **2011**, *72*, 3–17.
- (40) Moore, K. L.; Schröder, M.; Lombi, E.; Zhao, F. J.; McGrath, S. P.; Hawkesford, M. J.; Shewry, P. R.; Grovenor, C. R. NanoSIMS analysis of arsenic and selenium in cereal grain. *New Phytol.* **2010**, *185*, 434–45.
- (41) Beilstein, M. A.; Whanger, P. D.; Yang, G. Q. Chemical forms of selenium in corn and rice grown in a high selenium area of China. *Biomed. Environ. Sci.* **1991**, *4*, 392–398.
- (42) Zhao, Y.; Zheng, J.; Yang, M.; Yang, G.; Wu, Y.; Fu, F. Speciation analysis of selenium in rice samples by using capillary electrophoresis-inductively coupled plasma mass spectrometry. *Talanta* **2011**, *84*, 983–988.