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Full Length Article

The Role of Selenium in Soil: Effect on the uptake and Translocation of Arsenic in Rice (*Oryza sativa*)

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Abstract

The contamination of paddy soils by arsenic (As) is a serious problem in rice production areas across the world. This study investigates the effect selenium (Se) has on the formation of iron plaques on the outside of rice roots and the accumulation of As in rice plants. A soil combined sand pot experiment having four concentrations of Se (0, 1, 5 and 10 mg kg^{-1}) and two concentrations of As (0 and 30 mg kg^{-1}) was designed for this investigation. The results indicate that higher concentrations of Se result in a significant decline in As recorded in rice plants. However, the concentration of As in plant roots show the opposite trend. The bio-concentration factor in stems and leaves, as an assessment index of metal accumulation, was 12-51 times greater than that recorded in brown rice, indicating that stems and leaves are the most important tissues for the accumulation of As, and not rice grains. In addition, Se can reduce the bio-concentration factor of As in leaves, stems, rice grains and rice husks, and Se can reduce the translocation factor of As. This mechanism can be ascribed to the higher amounts of Se that decreased the availability of As, increased the number of iron plaques outside the rice roots, and the glutathione concentration in the leaves. The addition of Se also significantly increased the concentration of selenomethionine (SeMet) in rice grains. Therefore, in an As contaminated cropland, fertilizer containing Se can be used to improve the Se nutrition of rice, reduce the accumulation of As in rice grains which are planting in As-contaminated soils, and thus reduce the potential harm of As to human health.

Keywords: Arsenic toxicity; Selenium; *Oryza sativa* L; Uptake; Translocation

Introduction

Arsenic (As), a carcinogen which potentially does not have a threshold, is regarded as one of the most serious elements to human health. Currently, daily consumption of As via crops exceeds set thresholds which may affect human health in the form of bladder and skin cancer, for example (Paul *et al*., 2004). Rice consumption, the staple food for more than three billion people in the world, is considered to be the main pathway of As exposure (Mondal and Polya, 2008). It is currently believed that As has higher bioavailability and mobility when rice fields are under waterlogged conditions; conditions which increase the absorption and accumulation of As in rice (Xu *et al*., 2008). The accumulation of As in rice grains is generally about ten times greater than that in other crops. Arsenic concentrations in rice grown in contaminated paddy fields in Bangladesh can reach 1.8 mg kg^{-1}, resulting in serious As exposure for the local population (Williams *et al*., 2007). The main species of As contaminated rice fields are dimethylarsinic acid (DMA) and sodium arsenate (Meharg and Rahman, 2003). With an increase of total As concentration, the content of DMA also increases (Syu *et al*., 2015). Thus, the amount of As uptake by rice is considered to be the main pathway for the transfer of As into the food chain, and a potential new disaster for the Southeast Asian population (Heikens *et al*., 2007). It is of high importance therefore to identify the mechanisms of As accumulation and explore solutions to reduce As accumulation in rice grains to secure food security.

Selenium (Se) is an essential trace mineral in humans and animals (Zeng, 2002). This element has many important physiological functions and plays an essential role in reducing the risk of cancer. However, there is a serious deficiency of Se in China; 72% of the country has different degrees of Se deficiency. Grain food, such as rice, is the main source of daily Se intake, therefore applying Se fertilizer to carry out biofortification with crops is considered to be the most effective way to improve Se intake of the target population (Hawkesford and Zhao, 2007).

An investigation by Williams *et al*. (2009) into the content of Se in rice showed that the accumulation ability of

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Se in rice grown in Asia is better than in other continents. Zhao *et al*. (2010) identified that the absorption of selenite by rice mainly occurs by the silicon transporter Lsi1, and Ma *et al*. (2001) found that the silicon transporter in the NIP subgroup of aquaporins took part in As(III) absorption by rice, while the silicon transport mutant Lsi2 (OsNIP2;1) significantly reduced the absorption of As(III) in rice. Mutant rice plants which lacked the silicon transporter Lsi2 reduced the accumulation of As in soil, indicating that the silicon transporter of rice roots was also responsible for the transport of As(III) (Ma *et al*., 2001). This result suggests that competition may exist between selenite and the absorption of As(III) by rice. The interaction of Se and As also differs in different plants. For example, in Black Eyed Susan (*Thunbergia alata*) and Chinese brake fern (*Pteris vittata*), the content of As and its activity increased with an increase of Se (Bluemlein *et al*., 2009; Srivastava *et al*., 2009); the opposite was recorded in Chinese brake fern (*Pteris vittata*) and rice (*Oryza sativa*) with decreasing As content and activity (Feng *et al*., 2009; Dwivedi *et al*., 2010; Hu *et al*., 2014). At present, the reported conclusions of the effects of Se on As absorption by plants are not consistent. The results of previous investigations show that the effects of Se on the absorption of As and translocation in plants are still not clear and need further investigation and they were almost based on the seedling stage and hydroponic experiment, thus the effect of Se application in soil on As accumulation by mature rice has not been reported.

Due to the oxygenation of the rhizosphere of paddy rice (*O. sativa* L.), iron oxyhydroxide plaques form (abbreviated as "iron plaques") (Jiang *et al.*, 2009), which are able to absorb and fix As, therefore acting as an obstacle or buffer to As absorption (Liu *et al*., 2004). The application of Se in soil can affect the number of iron plaques outside the rice root; a low volume of Se can increase the amount of iron plaques whilst a high amount can inhibit the formation of iron plaques (Zhou *et al*., 2014). It is not currently known whether the application of Se in a soil would affect the absorption and accumulation of As by rice, or its effect on iron plaques during the whole growth stage.

Arsenic in rice fields mainly exists in the reduced state of arsenite, which can be combined with reduced glutathione (GSH) or phytochelatins to form As-GS or As-PCs complexes (Rakesh *et al*., 2010). If these complexes are combined in the leaves and roots, they can be bound to form peptide-thiol complexes, which can be transported to the vacuole membrane and then stored in the vacuole through active absorption pathways, thus reducing the toxicity of As (Dhankher *et al*., 2002). Although Se is a component of glutathione peroxidase, the relationship among Se and the formations of iron plaques, GSH and As accumulation in rice has not been previously reported. The purpose of this investigation therefore is to examine the relationship among these complexes. Arsenic and Se are coupled in biogeochemical cycles (Norton *et al*., 2010) with arsenic contaminated paddy fields resulting in a decrease of Se content in rice (Williams *et al*., 2009). Therefore, this study also aims to investigate these interactions under controlled conditions.

Materials and Methods

The variety of rice used in this experiment was Yuyou 32, and experimental soil was taken from a topsoil (0~25 cm) in a paddy field in Beibei, Chongqing, Southwest China. The paddy soil contained 0.23 mg kg^{-1} total Se, 4.81 μg kg^{-1} soluble Se, 2.36 mg kg^{-1} total As and 0.24 mg kg^{-1} HCl extractable As. Eight treatment plots were setup for the experiment combining two concentrations of As (Na_2HAsO_4, $7H_2O$) (0 mg As kg^{-1} represented as -As and 30 mg As kg^{-1} represented as +As, the national standard is 30 mg kg^{-1}), as well as four concentrations of Se (Na_2SeO_3) (0 mg Se kg^{-1} represented as -Se, 1, 5 and 10 mg Se kg^{-1} represented as +Se). Four replicate samples were undertaken. A rhizosphere bag device of soil-sand incorporation was designed to obtain rhizosphere and non-rhizosphere soils separately. The rhizosphere bags, made of nylon gauze (40 µm), were loaded with 500 g acid-washed quartz sand, and placed into pots containing 3 kg of dry soil. The collected soil was ground after being air-drying and passed through a 2 mm sieve mesh. The basal fertilizers, contained N 125 mg kg^{-1} as $(NH_2)_2CO$, P 80 mg kg^{-1} and K 125 mg kg^{-1} as KH_2PO_4 and K_2SO_4, were mixed with the processed soil to ensure normal growth of rice plants. The purpose of placing quartz sand in the rice growth chamber was to obtain a more complete rice root system coated with iron plaques.

Rice seeds were disinfected with 10% H_2O_2 (v/v) solution for 0.5 h and cleaned with deionized water, before being germinated in wet quartz sand at 25ºC. In a rhizosphere bag, seedlings with consistent growth were selected and cultured into a plastic container for 160 days. During the whole growth period, the soil was maintained under a flooded condition. The experiment was carried out in a plant culture room under controlled conditions: light conditions were set at 25ºC for 14 h and dark conditions were set at 20ºC for 10 h. A relative humidity of 60%~70% and a light intensity of 250 µmol m^{-2} s^{-1} were maintained throughout the experiment.

Sampling and Analysis

Before maturity, the penultimate fresh leaf of each plant was removed and used to determine the GSH content using Griffith's method (Griffith, 1980). After harvest, the plants were divided into roots, stems and grains, and the fresh roots were divided into two groups, with one group being used to extract iron plaques via DCB (dithionite-citrate-bicarbonate) (Zhou *et al*., 2007) and the other being conserved for further analysis.

Quartz sand retained in the root bag was extracted using 0.1 mol L^{-1} HCl (Hu *et al*., 2007). In the ACA (ascorbic-citrate-acetate) and HCl extracts, the method of Fan *et al*. (2010) was used to calculate the concentration of As in the rice plants. The concentration of As and Fe in solution were determined by ICP-AES and atomic fluorescence spectrophotometer, respectively.

During maturity of rice, fresh and dry plant samples were collected after determination of agronomic traits: fresh samples collected in the field were washed with distilled water, dried (including the removal of moisture), and preserved in liquid nitrogen to determine the morphological changes of Se in each part of the plants. After harvesting dry samples, all plant parts were quickly separated (roots, stems, leaves and grains), washed with distilled water, and then the moisture in each part was absorbed by using a gauze. The dry samples were placed in an envelope for 30 min. at 105℃ to deactivate the enzymes before being placed in an oven at 65℃ until a constant weight was achieved. The dry weight of each plant part was weighed and measured. The rice grains were shelled and the stalk and leaves were crushed for analysis. Finally, the concentrations of As and Se in the different parts of the rice plants were determined.

Extraction and determination of Se speciation was undertaken using the following method: frozen dried plant samples of about 0.2 g were placed in 15 mL centrifuge tubes with 10 mL 1:2 methanol water extracts (Casiot *et al*., 1999). The plant samples and extract solution were mixed using a vortex mixer and exposure to ultrasound for 20 min at room temperature. The extract was then centrifuged at 7500 * g for 30 min. The supernatant was collected using a liquid gun before being transferred to an eggplant bottle. The methanol was evaporated using a rotary evaporator (t = 40℃) and the supernatant was passed through a 0.22 μm microporous filter. HPLC-UV-HG-AFS spectrometry was used to analyze the samples (Beijing Titan Instruments Company) (Li *et al*., 2008).

Data Analysis

To identify the translocation pattern of As in rice in the different tissues, the indices as bio-concentration factor (BCF) and translocation factor (TF) were calculated (Singh *et al*., 2010).

An analysis of variance (ANOVA) was used to analyze the data for all parameters using SPSS version 13.0. The least significant difference (LSD) was used for examining the significance of different treatments ($P < 0.05$).

Results

Effect of Se on Rice Growth and as Uptake

In -Se treatments, the biomass of shoots and grains (P<0.05) both declined with an increase of As. In +Se treatments, the plant biomass (P<0.05) also recorded a decreasing trend with +As compared to those treatments without -As. Additionally, roots, shoots and grain biomass tended to increase with an increase of Se levels (0~5 mg kg^{-1}) (Fig. 1).

As shown in Table 1, in the +As treatments, the concentration of As in the plant roots recorded the greatest concentrations (11.41~580.48 times greater than concentrations in other plant tissues). Results for the -As treatments recorded concentrations to be 3.22~89.48 times greater (Table 1). Furthermore, As concentrations were significantly reduced (P<0.05) when Se was supplied in the leaves in the -As treatment, and in brown rice and husks in both +As and -As treatments.

Moreover, BCF for leaves and stems were 12.3~60.4 times greater for brown rice and 5.7~20.9 greater for husks (Table 2), indicating that leaves and stems were the main area for As accumulation. Se addition significantly decreased BCF of As for the whole rice plant compared with the -Se treatments. In our research, TF of As was significantly lower with the addition of Se than without (Table 2), indicating that the addition of Se may reduce the transport of As from roots to the rice grains.

Effect of Se Application on GSH in Leaves and Iron Plaques outside the Rice Roots

The levels of Se and As in the soil affected the GSH content of the leaves (Fig. 2). In the -As treatment, the GSH content increased with an increase in the level of Se; the treatments of 1, 5 and 10 mg Se kg^{-1} significantly increased the GSH content of leaves (P<0.05). In the +As treatment, compared with the 0 and 1 mg Se kg^{-1} treatments, the treatments of 5 mg Se kg^{-1} and 10 mg Se kg^{-1} significantly increased the GSH content in the leaves.

In the -As treatment, the addition of Se significantly reduced manganese (Mn) in the iron plaque and increased Fe in the iron plaque on the root surface of the ACA extraction (Table 3). In the +As treatment, compared with the Se free treatment, the addition of Se resulted in higher Fe, Mn (P<0.05) and As (P<0.05) concentrations. The addition of As significantly increased As in the iron plaque (P<0.01). Compared to the -As treatments, the addition of As resulted in lower concentrations of Se in the iron plaque, however these results were not significant.

Effect of as Treatment on Grain Se Content and Morphology in Mature Rice

The supplication of Se in soil significantly increased the Se content in the rice grains, while the addition of As in the soil affected the content and distribution of Se in the rice grains. Under conditions with the addition of Se, the Se content in rice grains was slightly higher with the addition of As than those without the addition of As. However, there was no significant difference between these results. When the application of Se was 10 mg kg^{-1}, the content of Se in rice grains with the addition of As was significantly higher (1.4 times greater) than those treatments without the addition of As.

Table 1: The effect of Se application on concentrations of As in rice (mg/kg dry weight)

Se levels (mg/kg)	Roots		Leaves		Stems		Brown rice		Husk	
	-As	+As	-As	+As	-As	+As	-As	+As	-As	+As
0	17.7±2.5	142.3±11.2	5.8±0.5	11.4±1.3	3.8±0.9	12.1±2.4	0.31±0.03	1.75±0.34	0.66±0.17	2.95±0.34
1	15.6±1.6	140.5±10.3	5.6±0.4	10.8±1.4	3.2±1.1	11.8±2.5	0.21±0.05	1.60±0.35	0.56±0.15	2.84±0.35
5	18.5±1.5	152.4±12.8	5.5±0.3	9.4±1.5	3.2±0.7	11.0±2.6	0.18±0.06	1.20±0.36	0.42±0.11	2.65±0.36
10	18.8±2.1	165.6±9.8	5.4±0.6	8.2±1.6	3.1±1.1	10.5±2.7	0.16±0.05	1.16±0.37	0.33±0.08	2.55±0.37
Analysis of variance for As levels										
0	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
1	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
5	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
10	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
Se levels ×As	$P < 0.05$		$P < 0.05$		*ns*		$P < 0.05$		$P < 0.05$	

Values are means ± standard deviations; ns – not significant. Values followed by a different letter within a column indicate significance at P<0.05 (LSD test) for Se levels

Table 2: The effect of Se application on bioconcentration factor (BCF) and translocation factor of As in rice

Se levels (mg/kg)	Leaves		Stems		Brown rice		Husk		Translocation factor	
	-As	+As	-As	+As	-As	+As	-As	+As	-As	+As
0	181.3±10.2	30.4±2.1	118.7±9.8	32.7±3.5	9.6±1.2	0.9±0.4	20.6±2.1	2.5±0.2	0.082±0.01	0.029±0.00
1	175.0±13.2	28.8±1.8	100.0±8.7	31.7±2.8	6.5±1.1	0.8±0.2	17.5±3.5	2.2±0.1	0.066±0.00	0.025±0.00
5	171.9±9.8	27.7±1.9	103.0±8.6	32.0±2.4	5.3±0.7	0.6±0.1	13.1±4.5	1.7±0.2	0.056±0.01	0.017±0.00
10	168.7±15.4	29.6±2.1	96.8±8.7	30.6±2.4	5.0±1.3	0.6±0.1	10.3±5.1	1.4±0.1	0.052±0.00	0.014±0.00
Analysis of variance for As levels										
0	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
1	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
5	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$		*ns*	
10	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
Se levels ×As	$P < 0.05$		$P < 0.05$		$P < 0.01$		$P < 0.05$		$P < 0.01$	

Values are means ± standard deviations; ns – not significant. Values followed by a different letter within a column indicate significance at P<0.05 (LSD test) for Se levels

Table 3: Effect of Selenium application on concentrations of ascorbic citrate acetic (ACA)-extractable Fe, Mn, As and Se in iron plaque outside roots in rice

Se levels (mg/kg)	Fe (g/kg)		Mn (mg/kg)		As (mg/kg)		Se (mg/kg)	
	-As	+As	-As	+As	-As	+As	-As	+As
0	65.7±3.5	63.3±3.2	117.8±48.6	90.2± 25.5	60.5±5.9	190.2±25.6	0.3±0.01	0.3±0.02
1	65.6±1.6	63.5±1.3	115.2±32.5	90.8± 15.4	53.2±9.1	211.7±32.5	1.1±0.08	1.0±0.05
5	88.5±1.5	85.4±1.8	96.5±14.3	88.4± 16.5	53.8±11.7	232.0±28.9	4.3±0.4	4.2±0.2
10	99.8±2.1	86.6±2.8	95.2±18.6	91.1±16.6	63.1±14.5	265.5±42.7	7.5±1.2	7.5±1.2
0	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
1	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
5	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
10	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
Se levels×As	$P < 0.05$		$P < 0.05$		*ns*		$P < 0.05$	

These results indicated that As could promote the absorption and accumulation of Se in rice grains under the condition of high Se application (Fig. 3).

For either the +As treatment or the -As treatment, the speciation of Se in grains mainly occurred as organic Se (SeMet). With an increasing concentration of Se, SeMet as the main component was also sequentially increased, while SeCys, accounting for a smaller proportion, sequentially decreased. In the speciation of inorganic Se with decreasing proportions, Se (VI) appeared with the Se treatment of 10 mg Se kg^{-1}, accounting for 3.4% (arsenic addition treatment) and 7.9% (As-free treatment) of total Se, respectively (Fig. 4).

Discussion

Selenium, an essential nutrient for animal and human growth, has also been identified as being beneficial for plant growth. Wu *et al*. (1998) exposed rice to different levels of Se in the form of sodium selenite and found that Se (0~20 mg kg^{-1}) promoted growth, increased grain weight, raised production and significantly improved the Se content of grain and straw. An excess of Se (≥ 30 mg kg^{-1}) was found to inhibit rice plant growth. Our results indicated that Se could increase the biomass of rice seedlings under As treatment.

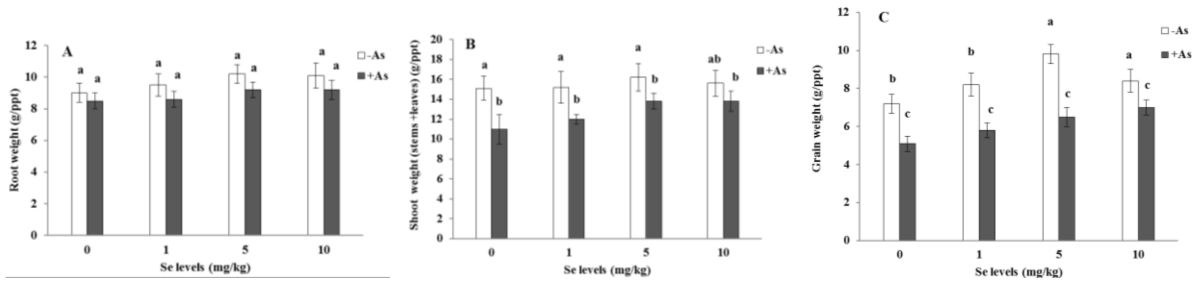

Fig. 1: The effect of Se on rice biomass. Different upper-case letters indicate significant differences at $P<0.05$ (LSD test) for Se levels and As levels

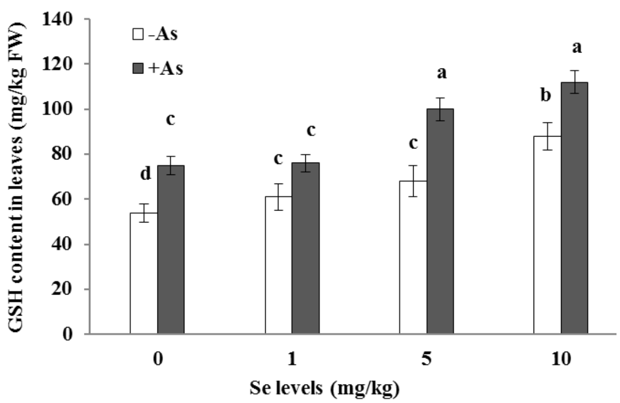

Fig. 2: The effect of Se on glutathione (GSH) content in the penultimate leaves of rice. Different upper-case letters indicate significant difference at $P < 0.05$ (LSD test) for Se levels and As levels

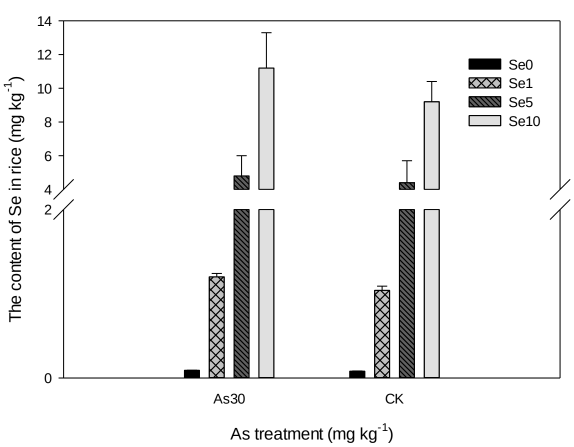

Fig. 3: The effect of As on the selenium content of rice grains

Although Se plays an important role in alleviating heavy metal stress and preventing heavy metal absorption in plants, its mechanism is not clear. In treatments with increased levels of As, the addition of Se (IV) significantly reduced the accumulation of As in the plant roots, and decreased the absorption and accumulation of As in plant stems, leaves and grain. Se(IV) significantly inhibited the translocation of As from roots to shoots (Table 1 and 2). What is the interaction mechanisms between Se and As in rice plant? There might be several reasons following.

Firstly, from a physiological standpoint, in an experiment using mung beans, Malik *et al*. (2012) found that Se can reduce the absorption of As. We also report that Se reduced As absorption (Table 1). The main mechanism was identified as the low dose of Se (2.5 μM) which increased membrane lipid peroxidation and enhanced the activities of peroxidase, sueroxide dismutase, catalase and ascorbate peroxidase. The low Se dose also enhanced the activity of methallothioneins, mercaptans and glutathione-S-transferase compared with the control treatment, indicating that Se improved the detoxification ability of mung beans to alleviate As stress (Malik *et al*., 2012). Further research is needed if the mechanism of rice is the same with mung bean. But the previous findings in *P. vitata* (Srivastava *et al*., 2009) is contrary to our study that the increasing of Se would increase the As content in plant, it might be related to the plant species. Our results show that inorganic Se could inhibit the translocation of As in plants under flooded conditions as earlier reported by Feng et al. (2009) in Chinese brake fern and Khattak *et al*. (1991) in the shoots of alfalfa. Se can also activate phytochelatin synthase (PC) and increase the precursor of PC synthesis, making plants produce more PC and form more complexes of heavy metal-PC (Feng and Wei, 2012). Although its movement in plants is not easy, Se prevents the translocation of As in rice from roots to shoots. The supply of Se can also lead to the formation of rice GSHs, which can cause As to form As-PCs or As-GS complexes (Rakesh *et al*., 2010). As-PCs or As-Gs complexes can then be adsorbed by vacuole which further reduces the translocation of As from roots and leaves to grain (Zhao *et al*., 2010). Our results show that 5 mg Se kg^{-1} and 10 mg Se kg^{-1} significantly increased the GSH content of rice leaves (Fig. 2). This increase may also be due to Se supplied increasing the As ligands compound (e.g., phytochelatins, GSH) in leaves which sequestrate As into vacuoles in the leaves.

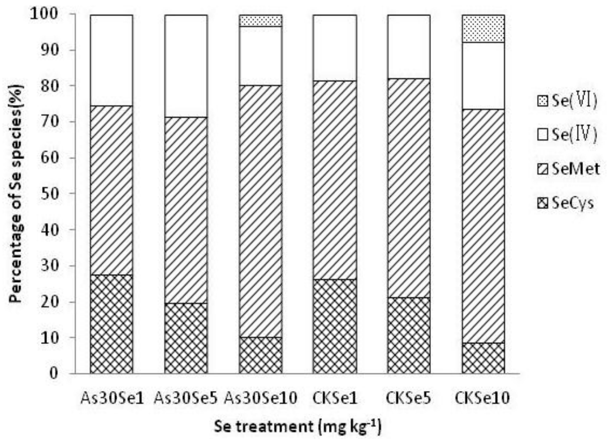

Fig. 4: The effect of Se on the percentage of Se species in rice

Secondly, it is widely recognized that silicon influx transporters OsNIP2; 1 can load As(III) into rice roots (Ma *et al*., 2008). Zhao *et al* (2010) found that OsNIP2; 1 is also in charge of Se(IV) absorption by rice, indicating that an antagonistic effect exists between the absorptions of As(III) and Se(IV) by rice, for the same shared transporter (Si transporter). However, our experiment confirmed that in the -As treatment, Se(IV) increased the accumulation of As in rice roots, and no antagonism was observed. The adverse interaction between Se(IV) and As(III) in rice was also not displayed by Zhao *et al*. (2010). The exact mechanism is not very clear and it may be related to the speciation of As and Se under different pH levels. Sodium selenite is a diprotic weak acid and $HSeO_3^-$ and SeO_3^{2-} account for 93% and 7% (Zhao *et al*., 2010), respectively with a pH of 5.5, while As(III) exists only in one form $As(OH_3)$ when pH is less than 8 (Ma *et al*., 2008). Silicon influx transporters, as a type of aquaporins, are able to penetrate into silicon acid, arsenite, and glycerol (Zhao *et al*., 2010), resulting in silicon influx transporters preferentially absorbing As(III) rather than Se(IV) within the lower pH range, such as pH 5.5. Some investigations confirmed that partial absorption of Se(IV) by rice is regulated by phosphate transporters due to the absorption of Se(IV) by wheat, which increased under phosphorus deficient conditions (Li *et al*., 2008). Hopper and Parker (1999) found that increasing the level of phosphate reduced Se(IV) uptake of plants, while Se(IV) increases the As(III) uptake of rice (Hu *et al*., 2014). This phenomenon is very interesting, and its specific mechanism needs further study.

Thirdly, excessive Se supply can reduce the availability of As by forming insoluble As_2Se_3 or FeAsSe. Under the condition of anaerobic reduction, Se(IV) can be reduced to Se(-II), and arsenic mainly exists as As(III), which can form insoluble As_2Se_3. An environment with high levels of Fe(II) may result in the co-precipitation of Se, As and Fe to form FeAsSe. Therefore, due to the above results, an increase in the supply of Se can significantly limit the uptake and accumulation of As in As-contaminated paddy soils.

Fourthly, under As stress, a high supply of Se (5 mg kg^{-1} and 10 mg kg^{-1}) can increase the formation of iron plaques outside the rice root (Table 3). Investigations have shown that the application of sulfur in soil can promote the formation of iron plaques on rice roots (Hu *et al*., 2007). Under flooded conditions, soil forms a strong reducing environment where SO_4^{2-} can be reduced to S^{2-}; Fe^{3+} can be reduced to Fe^{2+} by S^{2-}; and MnO_2 can be reduced to Mn^{2+} (Murase and Kimura, 1997). These changes can increase the concentrations of Fe^{2+} and Mn^{2+} in the soil to promote the formation of iron plaques on the root surface. In the same main group, Se and sulfur have similar chemical properties. Se^{2-} formed under waterlogged conditions has a stronger reducing ability, and it can reduce high valence iron and manganese in the soil to increase the concentrations of Fe^{2+} and Mn^{2+}. This occurrence is beneficial to the migration of iron and manganese to the surface of rice roots, thus promoting the formation of iron plaques on the root surface. In addition, iron oxide and iron plaques on the roots have high affinity to SeO_3^{2-} (Zhou and Shi, 2007). The absorption of SeO_3^{2-} reduces the contact probability between iron bacteria and $Fe(OH)_3$, which may block iron plaque dissolution of $Fe(OH)_3$ to Fe^{2+} induced by iron bacteria (Qu *et al*., 2003). This can further increase the formation of iron plaques on the root surface of rice. Iron plaques outside the rice roots may be a buffer or a barrier to the uptake of As; As in roots has a significant positive correlation with As in iron plaques (Liu *et al*., 2004). Our results suggest that iron plaques may be used as a buffer against the uptake of As by rice roots. Moreover, after the supply of Se, rice has lower BCF and TF (Table 2), which indicates that Se inhibited the transport of As from plant roots to shoots. These results show that a higher amount of Se can increase the formation of iron plaques on roots, causing the immobilization of As in roots to reduce the transport of As from roots to shoots, and then to the rice grains. Further investigations are needed explore the specific mechanism whereby Se(IV) treatment significantly decreases the concentration of As and its distribution coefficient in rice shoots.

Our results showed that with a high concentration of Se, As promotes the absorption and accumulation of Se by rice grains. With an increase in concentration of Se, SeMet as the main component also increased (Fig. 3 and 4). Zayed *et al*. (1998) showed that selenite can be largely absorbed by roots and rapidly transformed into organic species which accumulates in roots. In our investigation, As increased the translocation of Se(IV), probably due to an increase of SeMet transportation from rice roots to its shoots in Se(IV) +As compared to Se(VI) (Table 3 and 4). The mechanism of interaction of Se and As in the pathway from roots to shoots in rice is still undefined. We believe this is the first report on the interaction between As and Se uptake, translocation and accumulation in rice during the growth period. Therefore, the application of the appropriate volume of sodium selenite fertilizer in paddy fields not only improves the Se content of rice grains, it also reduces the translocation of As from roots to stems. This reduction will reduce As concentrations in the edible parts of rice and therefore reduce the level of arsenic exposure, as well as improving dietary selenium uptake levels.

Conclusion

Results from our investigation show that an increase in the concentration of Se resulted in a significant (P<0.05) decrease in the concentration of As in rice leaves (not significant in the -As treatment), husks and grains in both +As and -As treatments. An increase in Se reduced the bio-concentration factor of As and the translocation coefficient of rice leaves, stems, grains and husks. Se could reduce the translocation of As from soil to roots and then to grains. A higher concentration of Se increases the formation of iron plaques outside rice roots and the glutathione content in rice leaves, and decreases the bioavailability of arsenic in soil. The application of Se in soil also significantly increases the concentration of Se in grains and the content of SeMet in grains.

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