Interactive effects of different inorganic As and Se species on their uptake and translocation by rice (*Oryza sativa* L.) seedlings

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Abstract There is a lack of information on the interactive relationship of absorption and transformation between two inorganic arsenic (As) species and two inorganic selenium (Se) species in rice grown under hydroponic condition. Interactive effects of inorganic As (As(III)) and (As(V)) and Se (Se(IV) and Se(VI)) species on their uptake, accumulation, and translocation in rice (*Oryza sativa* L.) seedlings were investigated in hydroponic culture. The results clearly showed the interactive effects of inorganic As and Se on their uptake by rice. The presence of Se reduced the sum of As species in the rice shoots regardless of Se speciation. If Se is present as Se(IV), then it is accompanied by a corresponding increase of the sum of As species, but if Se is present as Se(VI), then there is no change in the sum of As species in rice roots. These effects are observed regardless of initial As speciation. When the rice plants are exposed to Se(IV), the presence of As increases the sum of Se species in the roots, and decreases the sum of Se species in the corresponding shoots. This effect is more pronounced for As(III) than for As(V). There is no effect on Se during exposure to Se(VI). Co-existence of As also increased SeMet in rice roots.

Keywords Arsenic · Selenium · Interactive effect · Rice · Speciation · Translocation

Introduction

Arsenic (As), as a kind of carcinogen, is ubiquitous in the environment, which pose the risk to human health for millions of people worldwide (Meharg 2004). In many countries, rice (*Oryza sativa* L.) was the major source of As intake because rice is the staple food for many populations (Li et al. 2011; Rahman et al. 2008) and is a highly efficient As accumulator under anaerobic conditions in paddy soil. Selenium (Se) is an essential micronutrient and has important benefits for human within the appropriate content (Williams et al. 2009b). Cereals and cereal products such as rice are the main sources of dietary Se intake, so agronomic biofortification of food crops with Se fertilization is considered to be an effective way of improving Se intake for target populations (Hawkesford and Zhao 2007). However, the As contamination in paddy soil caused reduction of Se accumulation in rice grain (Williams et al. 2009a). Arsenic and Se are coupled in biogeochemical cycles (Couture et al. 2012) and in the way they appear in different geochemical strata (Edmonds and Francesconi 1987). As the ubiquitous elements, both inorganic As and Se co-exist in the rhizosphere soil. Therefore, interactive effects of As and Se would occur during uptake, assimilation, and transformation by/within plants. However, how As and Se affect uptake and translocation in plants with each other is still poorly understood.

In paddy soil, major As and Se are present as inorganic As, i.e., arsenite (As(III)) and arsenate (As(V)) and inorganic Se, i.e., selenite (Se(IV)) and selenate (Se(VI)), although tiny amount of organic As or Se are existed as well due to the microbial transformation. Normally, As(III) dominates in anaerobic environment such as flooded paddy soils, but As(V) is the main species in aerobic soils (Abedin et al. 2002; Onken and Hossner 1996). Selenium species in paddy soil depend on

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the pH. Selenite exists predominantly in well-drained mineral soils with pH from acidic to neutral ($7.5 < \text{pH} < 15$), like paddy soil, whereas Se(VI) is the predominant form in alkaline and well-oxidized soils ($\text{pH} > 15$; Elrashidi et al. 1987). Plants take up As(V) through the phosphate transporters because of their chemical analog (Meharg and Hartley-Whitaker 2002; Meharg and Macnair 1992). Li et al. (2008) demonstrated that Se(IV) can also be taken up by the phosphate transporters. It seems reasonable to speculate that the uptake of As(V) and Se(IV) by plant roots presents competitive inhibition with each other. Arsenite is transported into rice by aquaporins (Meharg and Jardine 2003), such as silicon transporters (Ma et al. 2008). Interestingly, the uptake of Se(IV) is mediated by a silicon (Si) influx transporter Lsi1 in rice as well (Zhao et al. 2010). Therefore, competition between As(III) and Se(IV) uptake is likely present through silicon transporters. However, so far, no systematic investigation is available about the interactive relationship between inorganic As (As(III) and As(V)) and inorganic Se (Se(IV) and Se(VI)) during uptake and translocation by/within plants.

Selenate as analog of sulfate is taken up by plants from the soil via sulfate transporters in the roots (Hawkesford and Zhao 2007). The content of sulhydryl groups containing sulfur might be reduced in the presence of Se due to the competitive uptake of Se and sulfate by plants (Zhu et al. 2009). This might affect As accumulation in plants because, in rice, As is particularly present as thiol complex in thiol-containing amino acids (Lombi et al. 2009). There is significant evidence that exposure to As(V) caused the generation of reactive oxygen species, phytochelatins production, glutathione depletion, and lipid peroxidation (Hartley-whitaker et al. 2001). In contrast, Se acted as an antioxidant, inhibiting lipid peroxidation (reduced by 26–42 % in the fronds) via increased levels of thiols and glutathione (increased by 24 % in the fronds). Se activates plant protective mechanisms as an antioxidant, thereby alleviating oxidative stress (Srivastava et al. 2009). The oxidative damage due to As in plant was mitigated with Se application, which could be related to their accumulation with each other.

Until recently, only a few reports about the interactive effects of As and Se on plant uptake and accumulation were available and most of them are focused on terrestrial plants, such as Chinese brake fern (*Pteris vittata* L.; Feng et al. 2009; Srivastava et al. 2009), mung bean (*Phaseolus aureus* Roxb.; Malik et al. 2012), barley (*Hordeum vulgare*; Ebbs and Weinstein 2001), and alfalfa (Khattak et al. 1991). In addition, most of the present studies were focused on interaction of single inorganic As or Se species, such as As(V) and Se(IV) (Feng et al. 2009), As(V) and Se(VI) (Srivastava et al. 2009; Ebbs and Weinstein 2001). There is a lack of systematic information on the interaction between two inorganic As species and two inorganic Se species in higher plant such as rice grown under anaerobic condition. In addition, all related

previous researches did not investigated transformation of As and Se during uptake and accumulation, which is very important since the speciation of As and Se is crucial for evaluating As toxicity and Se nutrition in foods. The interaction between As and Se have not been systematically studied, therefore, in this study, we investigate the interactive effect of two inorganic As and Se species on their uptake and accumulation by rice plants grown in hydroponic solution.

Materials and methods

Rice seedling preparation and culture conditions

Rice (*O. sativa* L.) seeds were surface sterilized in 30 % H_2O_2 solution for 15 min and thoroughly washed with deionized water (Chen et al. 2008). The seeds were germinated in moist perlite. After 3 weeks, the seedlings were precultured in a 7 L plastic box with 1/3 strength nutrient solution for 1 week, and then the uniform seedlings were selected and transferred to poly(vinyl chloride) (PVC) pots (one plant per pot) containing 500 mL full-strength nutrient solution. The composition of the nutrient solution was: 5 mM NH_4NO_3 , 2 mM K_2SO_4 , 4 mM CaCl_2 , 1.5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.3 mM KH_2PO_4 , and 50 μM Fe(II)-EDTA, 10 μM H_3BO_3 , 1 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 5 μM $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.5 μM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, and 0.2 μM $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$. The pH of this solution was adjusted to 5.5 with KOH or HCl. The nutrient solution was renewed twice every week. Plants were grown in a controlled-environment growth room at a 14-h light period ($260\text{--}350 \mu\text{mol m}^{-2} \text{s}^{-1}$). The temperature was regulated to 28 °C during the daytime and 20 °C at night and the relative humidity was maintained at 70 %.

Arsenic and selenium treatments

For all treatments, inorganic As and Se were the As(III) as NaAsO_2 , As(V) as $\text{NaAsO}_4 \cdot 12\text{H}_2\text{O}$, Se(IV) as Na_2SeO_3 , and Se(VI) as Na_2SeO_4 . Four-week-old rice seedlings were planted in PVC pots (one plant per pot) containing 500 mL nutrient solution with eight treatments: T1, 1 μM As(III); T2, 1 μM As(V); T3, 1 μM Se(IV); T4, 1 μM Se(VI); T5, 1 μM As(III)+1 μM Se(IV); T6, 1 μM As(III)+1 μM Se(VI); T7, 1 μM As(V)+1 μM Se(IV); and T8, 1 μM As(V)+1 μM Se(VI). Each treatment was replicated in four pots. The culture medium was replaced by newly prepared solution every 24 h to avoid the speciation changes of As or Se species in the culture medium during the experiments. Two kinds of control were included in the experiment, the medium control in which the medium added the same concentration of As, Se, or their mixture but without rice seedling, and the plant control in

which the rice plants were grown in the same medium but without As or Se addition.

Sample preparation

After exposure to As, Se, or their mixture for 48 h, the rice plants were harvested. Shoots and roots were separated; the shoots were rinsed with deionized water, blotted dry, and then frozen in liquid nitrogen. For seedlings with As treatments, the roots were rinsed and dipped in an ice-cold desorption solution containing 0.5 mM $\text{Ca}(\text{NO}_3)_2$, 5 mM MES (pH 5.5), and 1 mM K_2HPO_4 for 15 min to remove adhered As (Abedin et al. 2002). For Se treatments, the roots were rinsed and dipped in an ice-cold desorption solution containing 0.5 mM $\text{Ca}(\text{NO}_3)_2$, 5 mM MES (pH 5.5), and 0.5 mM K_2SO_4 for 15 min to remove surface Se (Zhang et al. 2006). Roots were blotted dry and frozen in liquid nitrogen. Shoots and roots were ground in liquid nitrogen to fine powder with a mortar and pestle for As and Se sample preparation.

Analysis for As speciation

To analyze the As speciation in rice shoots and roots, 0.1 g finely ground shoot and root powder were weighed into a 50 mL volume centrifuge tube with the addition of 10 mL 1 % purity HNO_3 . After standing overnight at room temperature, the samples were extracted in a microwave digester (Mars, Matthews Inc., USA). The extraction program was set as follows: 55 °C for 10 min, 75 °C for 10 min, and 95 °C for 30 min with 5 min ramp time between each stage. A standard reference plant material (GBW07605 from the National Research Center for Standards in China) was included to verify the accuracy and precision of the extraction procedure and subsequent analysis. After extraction, the samples were centrifuged (5,000 rpm) for 10 min and the supernatants were filtered through 0.45 μm nitrocellulose filters (Sartorius, Goettingen, Germany) for analysis.

Arsenic speciation in shoot and root tissues was determined using high performance liquid chromatography (HPLC; Agilent Technologies 1100 series) coupled with inductively coupled plasma mass spectrometry (ICP-MS, 7500, Agilent Technologies) as described in published paper (Islam and Meharg 2008). Arsenic species, i.e., As(III), As(V), methylarsonic acid (MMA), and dimethylarsinic acid (DMA) were separated by a PRP-X100 anion-exchange column (250×4.1 mm, 10 μm ; Hamilton, UK) with the mobile phase consisted of 10 mM $(\text{NH}_4)_2\text{HPO}_4$ and 10 mM NH_4NO_3 , adjusted to pH 6.2 using HNO_3 at flow rate 1 $\text{mL}\cdot\text{min}^{-1}$. Arsenic species were identified and quantified by comparing with the retention times of standard compounds and the external calibration curves with peak areas.

Measurements of sample extracts were carried out within 12 h after extraction.

Analysis for Se speciation

For Se speciation in rice shoots and roots, the sample preparation and analysis were conducted as published paper (Sun et al. 2010) with minor modification. Briefly finely ground materials (0.1 g) were weighed into a 50 mL centrifuge tube, and protease K (20 mg), lipase VII (10 mg), and 5 mL of 30 mM Tris-HCl buffer solution (pH 7.5) were added. After tubes were constantly and gently homogenized on a water bath rotary shaker (60 rpm) at 37 °C in dark for 24 h, samples were centrifuged at 3,000 rpm for 30 min, and the supernatants were filtered through 0.45 μm filters for analysis. The supernatants were kept at −20 °C and measured within 24 h after preparation in order to minimize possible Se species changes during storage.

The Se species in the supernatants were determined by HPLC-ICP-MS (HPLC, Agilent Technologies 1100 series, ICP-MS, 7500, Agilent Technologies; Sun et al. 2010). Selenium species were separated by a precolumn and a Hamilton PRP X-100 anion exchange column (250×4.1 mm, 10 μm). The mobile phase was 5 mM ammonium citrate with pH 4.3 containing 2 % of methanol with a flow rate at 1 $\text{mL}\cdot\text{min}^{-1}$. Mix comprising standards of 50 $\mu\text{g}\cdot\text{L}^{-1}$ selenocystine (SeCys), Se-methyl-selenocysteine (MeSeCys), Se(IV), selenometnionine (SeMet), and Se(VI) were used to identify Se species by the retention times, and the quantities of Se species were calculated by the external calibration curves with peak areas measurements.

Statistical analysis

Data analysis was completed by SPSS software. One-way analysis of variance, which is based on the same assumptions as the *t* test, was used to identify significant differences ($P < 0.05$) between As or Se species concentration in rice shoots and roots by comparing different treatments such as As(III), As(III)+Se(IV), and As(III)+Se(VI).

Results

Measurement of As and Se in control samples

No changes in As or Se species were observed in the blanks without rice seedlings during the experiment (24 h), indicating that inorganic As and Se are stable in the culture medium within 24 h (data not shown). No As or Se species were

measurable in the rice tissues which were not exposed to As and/or Se (data not shown).

Inorganic Se suppressed the transfer of inorganic As from rice root to shoot

Both addition of Se(IV) and Se(VI) significantly ($P < 0.05$) decreased As(III), As(V) and the sum of As species concentration in rice shoots (Fig. 1). Comparing with As(III) exposure alone, Se(IV) addition decreased the concentrations of As(III), As(V), and the sum of As species in rice shoots by 61.0, 44.2, and 59.0 % respectively. The addition of Se(VI) decreased As(III), As(V) and the sum of As species levels of rice shoots by 33.2, 39.5, and 34.0 % respectively. As for As(V) treatments, the concentrations of As(III), As(V), and the sum of As species were 48.3, 42.9, and 46.8 % lower in As(V)+Se(IV) treatment, respectively, and 51.7, 61.9, and 54.4 % lower in As(V)+Se(VI) treatment respectively, than that in As(V) treatment alone. These results indicated that inorganic Se significantly decreased the transfer of inorganic As from root to shoot.

Selenite increased As accumulation in rice root

The supply of Se(IV) significantly ($P < 0.05$) increased As accumulation in roots comparing with As(III) or As(V) addition alone, respectively (Fig. 1), while no significant effects on As uptake and accumulation in roots were observed by supplying Se(VI). The concentrations of As(III), As(V) and sum

of As species in As(III)+Se(IV) treated roots were 3.7, 2.4, and 3.1 times, respectively, of those in As(III)-treated roots, and in As(V)+Se(IV)-treated roots, which were 1.4, 1.3, and 1.4 times, respectively, of those in As(V)-treated roots.

Se addition changed inorganic As distribution in rice roots and shoots

In all As-containing treatments, As presented as inorganic As species, while organic As, like MMA and DMA, were not detected in either rice shoots or roots. the percentages of As(III) to the sum of As species (70.8–88.6 %) were much higher than that of As(V) (11.4–29.2 %) in rice shoots, suggesting As(III) was the predominant As species in rice shoot (Table 1). A similar trend was observed by Meng et al. (2011), who reported that As(III) accounted for 90 % in the shoots in both transgenic and wild type rice treated with 10 μM As(III) for 4 weeks. In As(III)-treated plants, supplying Se(IV) significantly ($P < 0.05$) increased the percentage of As(III) in roots (68.6 %), thereby decreased percentage of As(V) in roots, whereas no significant differences were found in other treatments (As(III)+Se(IV), As(V)+Se(IV), and As(V)+Se(VI)) both in shoots and roots. Both addition of Se(IV) and Se(VI) significantly ($P < 0.05$) decreased the shoot-to-root As(III) and As(V) concentration ratio compared with As(III) or As(V) treatment alone, and the effect of Se(IV) supply on the shoot-to-root As concentration ratio was more significant than that of Se(VI) supply in the As(III)-treated plant (Table 1).

Fig. 1 Effect of inorganic Se on uptake of As(III) and As(V) by rice roots and shoots. Different letters above the bars in the same As species treatment indicate a significant difference at $P < 0.05$, $n = 4$. Samples were prepared and analyzed by HPLC-ICP-MS as described in “Materials and methods” section. Concentrations are from fresh weight

Effects of As(III) and As(V) on Se uptake, accumulation, and transfer by/within rice seedlings

Addition of As(III) or As(V) increased sum of Se species accumulation in rice roots in the presence of Se(IV) but no significant effect on its uptake and accumulation in the presence of Se(VI) (Table 2), causing the sum of Se species in the Se(IV) treatments was much higher (>10 times) than that in the Se(VI) treatments (Table 2). In the rice shoots, the sum of Se species concentration (2.2–4.7 mg kg^{-1}) in different Se(IV) treatments were much lower than that in their corresponding roots (15.32–25.90 mg kg^{-1}), while in different Se(VI) treatments, the sum of Se species concentrations in shoots (1.62–1.67 mg kg^{-1}) were similar to that in corresponding roots (1.20–1.43 mg kg^{-1} ; Table 2). In all Se(IV)-treated rice, the shoot-to-root sum of Se species ratio ranged between 0.08 and 0.31, but in all Se(VI)-treated plant, ranged between 1.14 and 1.35, indicating that most of Se remained in roots of plants exposed to Se(IV). In contrast, more percentage of Se was transported to shoots of plant treated with Se(VI). The results suggested that As suppressed Se transfer from root to shoot after exposure to Se(IV), but not Se(VI).

Table 1 Percentage of As(III) and As(V) in shoots and roots, and shoot to root As ratio. Different letters in column within the same As species treatment indicate a significant difference at $P<0.05$, $n=4$

Treatments	Shoot (%)		Root (%)		Shoot to root As concentration ratio	
	As(III)	As(V)	As(III)	As(V)	As(III)	As(V)
As(III)	87.6±1.82 a	12.4±1.82 a	56.2±2.14 a	43.8±2.14 a	0.461±0.01 a	0.082±0.01 a
As(III)+Se(IV)	87.0±1.40 a	13.0±1.40 a	68.6±2.30 b	31.4±2.30 b	0.041±0.01 c	0.017±0.00 c
As(III)+Se(VI)	88.6±1.05 a	11.4±1.05 a	62.3±2.39 ab	37.7±2.39 ab	0.226±0.01 b	0.048±0.00 b
As(V)	76.1±2.84 A	23.9±2.84 A	36.1±1.53 A	63.9±1.53 A	0.286±0.03 A	0.051±0.00 A
As(V)+Se(IV)	70.8±2.01 A	29.2±2.01 A	38.1±1.67 A	61.9±1.67 A	0.090±0.01 B	0.022±0.00 B
As(V)+Se(VI)	74.6±4.12 A	25.4±4.12 A	37.0±2.39 A	63.0±2.39 A	0.119±0.03 B	0.021±0.00 B

Effects of As(III) and As(V) on Se species in rice roots and shoots

In all Se(IV)-treated roots, apart from SeCys, MeSeCys, Se(IV), and SeMet, there were two kinds of unidentified Se compounds (named unknown 1 and 2), which were not observed in shoots (Table 2). In Se(IV)-treated roots, supply of As(III) significantly ($P<0.05$) increased the concentration of SeMet and unknown 1 in roots by 1.5- and 2.0-fold, resulting in the increase of sum of Se species by 1.7 times compared with Se(IV) treatment alone. Similar to As(III), supply of As(V) significantly ($P<0.05$) increased SeMet concentration by 1.6-fold compared with Se(IV) treatment alone. In Se(VI)-treated roots, only SeCys, SeMet, and Se(VI) were tested and Se(VI) was the predominant Se species, accounting for 53.1 % in Se(VI)+As(V) treatment, 54.2 % in Se(VI) treatment and 60.6 % in Se(VI)+As(III) treatment (Table 2). These results indicated that organic Se predominated in Se(IV)-treated roots

(accounted for 87.9–92.2 %), while inorganic Se (Se(VI)) (accounted for 53.1–60.6 %) predominated in Se(VI)-treated roots.

The supply of As(III) and As(V) significantly ($P<0.05$) decreased the shoot-to-root SeMet concentration ratio by 92.5 and 85.8 %, respectively, compared with Se(IV) treatment alone. In all Se(IV)-treated shoots, the effect of As(III) supply on SeMet concentration was more significant ($P<0.05$) than that of As(V). The concentrations of SeMet were decreased by 58.6 % by supplying As(III) and 17.5 % by supplying As(V), respectively, compared with Se(IV)-treated shoots (Table 2). The percentage of Se species in the sum of Se species of whole plant indicated that Se(VI) is easier to be translocated from root to shoot, while Se(IV) could not be transferred from root to shoot (Table 3). More SeMet existed in the shoots than roots, suggesting that SeMet is easily transferred from roots to shoots as well. The co-exposure of As with Se(IV) suppressed the SeMet transfer in comparison with Se(IV) treatment alone.

Table 2 Effects of As(III) and As(V) on uptake and assimilation of selenium species in rice roots and shoots (in milligram per kilogram FW)

Treatments		SeCys	MeSeCys	Se(IV)	SeMet	Unknow 1	Unknow 2	Se(VI)	Sum Se
Shoots	Se(IV)	0.32±0.09 a	0.24±0.03 a	ND	4.18±0.27 a	ND	ND	ND	4.74 a
	Se(IV)+As(III)	0.23±0.02 b	0.22±0.04 a	ND	1.73±0.11 b	ND	ND	ND	2.18 b
	Se(IV)+As(V)	0.18±0.01 c	0.18±0.02 a	ND	3.45±0.31 c	ND	ND	ND	3.81 a
	Se(VI)	0.09±0.01 A	ND	ND	0.38±0.03 A	ND	ND	1.15±0.13 A	1.62 A
	Se(VI)+As(III)	0.08±0.01 A	ND	ND	0.28±0.04 B	ND	ND	1.27±0.10 A	1.63 A
	Se(VI)+As(V)	0.07±0.01 A	ND	ND	0.43±0.05 A	ND	ND	1.17±0.02 A	1.67 A
Roots	Se(IV)	1.70±0.53 a	2.33±0.38 a	1.85±0.19 a	3.88±1.83 a	2.95±0.53 a	2.61±0.26 a	ND	15.32 a
	Se(IV)+As(III)	1.33±0.28 a	1.66±0.22 a	2.03±0.08 a	9.62±0.82 b	8.98±1.16 b	2.27±0.45 a	ND	25.90 b
	Se(IV)+As(V)	1.88±0.32 a	2.09±0.22 a	1.51±0.28 a	10.02±0.24 b	2.68±0.04 a	1.59±0.32 a	ND	19.77 a
	Se(VI)	0.20±0.01 A	ND	ND	0.35±0.03 A	ND	ND	0.65±0.12 A	1.20 A
	Se(VI)+As(III)	0.21±0.02 A	ND	ND	0.35±0.03 A	ND	ND	0.87±0.06 A	1.43 A
	Se(VI)+As(V)	0.18±0.01 A	ND	ND	0.41±0.01 A	ND	ND	0.67±0.12 A	1.26 A

Mean values ± SE. Different letters in column within the same Se species treatment indicate a significant difference at $P<0.05$, $n=4$

FW fresh weight, ND not detected

Table 3 The percentage of Se species in the sum Se of roots and shoots

	%	SeCys	MeSeCys	SeMet	Se(VI)	Se(IV)	Unknow 1	Unknow 2
Shoots	Se(IV)	3.92	3.12	53.31	–	–	–	–
	Se(IV)+As(III)	2.75	2.57	18.34	–	–	–	–
	Se(IV)+As(V)	2.18	2.21	42.33	–	–	–	–
	Se(VI)	5.19	–	21.62	60.29	–	–	–
	Se(VI)+As(III)	4.59	–	15.85	62.56	–	–	–
	Se(VI)+As(V)	4.11	–	21.84	61.89	–	–	–
Roots	Se(IV)	4.50	6.28	6.36	–	5.38	9.48	7.65
	Se(IV)+As(III)	3.43	4.19	25.35	–	4.55	33.74	5.10
	Se(IV)+As(V)	5.44	5.33	27.22	–	4.07	7.24	3.99
	Se(VI)	2.14	–	3.81	6.95	–	–	–
	Se(VI)+As(III)	2.38	–	4.00	10.62	–	–	–
	Se(VI)+As(V)	1.71	–	3.92	6.53	–	–	–

Discussion

The uptake of As or Se by rice root

Our results using co-exposure of different inorganic As and Se clearly show that inorganic As together with Se(IV), but not Se(VI), could improve uptake in rice root with each other (Fig. 1, Table 2). This conclusion is partly supported by other researcher (Feng et al. 2009), who reported that Se(IV) addition less than $2.5 \text{ mg} \cdot \text{L}^{-1}$ stimulated As(V) uptake in roots of As hyperaccumulator *P. vittata* L. To the best of our knowledge, it is the first systematic investigation of As and Se interactive relationship and the observation that Se(IV) and inorganic As (As(III) or As(V)) improved uptake in rice root with each other. In addition, Se(IV) have stronger interaction with As(III) than As(V) because the sum of As species in roots from As(III)+Se(IV) treatment is 3.1 times of that from As(III) treatment, higher than As(V)+Se(IV) treatment (1.4 times) comparing with its corresponding control (As(V) treatment) (Fig. 1), moreover, Se(IV) accumulation enhanced by 1.7 times by As(III) addition, higher than that by As(V) addition (1.3 times; Table 2).

It is generally accepted that the As(III) influx in rice root through silicon influx transporters OsNIP2;1 (Ma et al. 2008). Zhao et al. (2010) clearly showed that this transporter is permeable to Se(IV). It seemed that antagonistic interaction between As(III) and Se(IV) would exist because they share the same transporter (Si transporter). However, improvement rather than antagonism were observed for the uptake into root. The antagonistic interaction between As(III) and Se(IV) in rice was not observed by Zhao et al. (2010) either. The exact mechanisms on these are still unknown. Selenite as a diprotic weak acid is deprotonated in the environment with pH at 5.5 (culture medium utilized), and charged as HSeO_3^- (93 %) and SeO_3^{2-} (7 %; Zhao et al. 2010). However, As(III) is uncharged molecules of arsenous acid (As(OH)_3) at pH<8 (Ma et al. 2008). Silicon influx transporters belonging to aquaporins are

permeable to uncharged substrates such as silicic acid, arsenite, and glycerol (Zhao et al. 2010). It was reasonable that As(III) but not Se(IV) was permeable through Si influx transporters at pH 5.5. Some studies demonstrated that Se(IV) uptake is at least partly to be mediated by phosphate transporters based on evidence that phosphorus deficiency enhanced Se(IV) uptake in wheat (Li et al. 2008) or increasing phosphate levels decreased Se(IV) uptake in different plant species (Hopper and Parker 1999). Whereas As(V) is taken up through phosphate transporters because of its chemical analog of phosphate (Wu et al. 2011). Antagonistic interaction between As(V) and Se(IV) would be observed due to the share of phosphate transporters. However, the results herein showed that improvement, not antagonism between Se(IV) and As(III) or As(V) during uptake by rice roots. It will be interesting to investigate the possible mechanism in the future. As for Se(VI), which is indicated to be taken up into plants via the sulfate transporter as the chemical analog of sulfate ion (Terry et al. 2000; Zhu et al. 2009), no strong effects on As transport or As uptake by Se(VI) was obtained and vice versa (Fig. 1, Table 2).

The translocation of As or Se from rice root to shoot

Selenium, regardless of Se(IV) or Se(VI), supplying significantly ($P<0.05$) decreased As in rice shoots compared with the As(III) or As(V) treatment alone. This result indicated that the supply of inorganic Se, significantly decrease the translocation of As from roots to shoots (Table 1) and plays an antagonistic role in As translocation from roots to shoots. These findings are supported by other investigations in terrestrial plants. Malik et al. (2012) reported that the As-treated (10 μM) mung bean supplemented with 5 μM Se significantly reduced the As uptake by mung bean. Khattak et al. (1991) showed that Se strongly depressed shoot As concentration in alfalfa and Feng et al. (2009) observed Se application ($>2.5 \text{ mg l}^{-1}$) inhibited the transfer of As from the roots to

fronds in Chinese brake fern as well. Our result clearly demonstrated that supplying inorganic Se depress the As translocation in flooded plants. An efflux transporter, Lsi2, has been demonstrated to be responsible for the efflux of As(III) toward the xylem (Ma et al. 2008). No efflux transporter were identified for Se (Zhu et al. 2009), and Lsi2 has been demonstrated to be not involved in the Se transport (Zhao et al. 2010). Therefore, the antagonistic interaction observed between As and Se might not occur at the efflux transporter Lsi2. One possible explanation is that the supply of inorganic Se elevated the synthesis of As ligands (e.g., phytochelatin, GSH) in roots (Malik et al. 2012) which sequester As into vacuoles of roots.

The presence of As repress the Se(IV) translocation from root to shoot, but not Se(VI) (Table 2). The translocation of Se from root to shoot is dependent on the form of Se supplied. Se(VI) is transported much more easily than Se(IV) or organic Se (Terry et al. 2000; Zhu et al. 2009). Selenite is mostly assimilated in the roots, and rapidly converted to organic forms of Se and retained in the roots (Zayed et al. 1998; Zhu et al. 2009). In this study, the possible reason for As suppressing Se(IV) translocation is suppression of SeMet translocation from the roots to shoots in As+Se(IV) treatment compared with Se(VI) treatment (Table 3).

The exact mechanism for interactive relationship of As and Se in uptake and translocation in plants is still unclear. To the best of our knowledge, this is the first report about interactive relationship of inorganic As and Se on uptake, translocation by wetland plants. Herein, all results suggested the presence of inorganic Se decrease the As translocation from rice root to shoot, and probable decrease the As levels in edible part (grain), which would decrease the human health risk of As from rice consumption. The presence of inorganic As reduced the translocation of Se(IV), which is predominant Se species in paddy soil. This would cause the loss of nutrient elements in rice grain, which is supported by the fact that grain Se levels were exhibited a highly decline with increasing As content in paddy soil (Williams et al. 2009a). In the future, further investigation in paddy field would be performed for both mitigating As contamination and improving Se nutrition in rice grains.

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