Bangladesh J. Bot. **45**(4): 811-818, 2016 (September), Supplementary

DIFFERENCES IN SELENIUM ACCUMULATION IN GRAINS OF TWO RICE CULTIVARS

ZHOU XINBIN, ZHANG CHENGMING, WANG YEKUN AND XU WEIHONG*

College of Resources and Environment, Southwest University, Chongqing, Beibei-400716, China

Key words: Rice cultivars, Se species, Grains, Accumulation

Abstract

Two rice (*Oryza sativa* L.) cultivars, high-Se rice Xiushui 48 and low-Se rice S. Andrea, differed in their ability to accumulate Se in the grain as high as a three-fold during the grain filling period. Four Se species (Selenate, Selenite, Se-methyl-selenocysteine (SeMeSeCys), Selenomethionine (Se-Met) were supplied to individuals of the two cultivars via excised panicle stems subjected to a ± stem-girdling treatment and cut flag leaves. Results indicated that organic Se can be remobilized from the stems to the grains only through the phloem, but about 29.1 to 48.7% of inorganic Se can be transported to the grain through the phloem. The remaining inorganic Se is transported to the grain through the xylem. The cultivar Xiushui 48 was significantly more effective ($p = 0.05$) in transporting SeMeSeCys, Se-Met and selenate from stem to grains, and its ability to remobilize SeMeSeCys and Se-Met from flag leaves to grains is significantly more than that of S. Andrea. High-Se rice, Xiushui 48 can remobilize more Se from stems and flag leaves and transport it to grains more efficiently than the low-Se rice, S. Andrea.

Introduction

Rice is a very important crop in China, and improvement in the Se content of rice is considered as the main way to increase the intake of Se in the diet of the Chinese population. Organic Se is more easily absorbed by humans and Se-Met and SeMeCys are the most effective anti-cancer Se species (Combs 2001). Therefore, when the mechanisms of Se transfer and accumulation in the rice grains are investigated, Se speciation must be considered.

Selenite is the main species of Se in flooded rice fields (Masscheleyen *et al.* 1991); however, the oxidation of selenite to selenate in the rhizosphere may result in the uptake of selenate by rice as well. When rice plants absorb selenate, Se mainly exists in the roots, stems and xylem liquid, in contrast, selenite enters the root, it quickly metabolizes into amino acids, such as selenomethionine, Se-methyl-selenocysteine and very small amount of selenomethionine Se-oxide (SeOMet) (Li *et al.* 2008). Sun *et al.* (2010) collected rice from different areas of China, and came to the conclusion that Se-Met is the most prevalent species in rice grains, accounting for 82.9% of the total Se content, while SeMeSeCys and SeCys made up 6.2 and 2.8 - 6.3%, respectively. Bourgis *et al.* (1999) applied ^{35}S-Met as tracer material to supply leaves in the filling stage, and concluded that sulfur exists in the form of S-methyl-Met (SMM) in plants, and is mainly transported to grains through the phloem. Carey *et al.* (2012) have shown that selenite was retained at the point of grain entry; organic Se species *viz.,* SeMet and SeMeSeCys were loaded into phloem and transported to the grain.

However, the Se content of rice husk is low. Se in the flag leaf of high-Se cultivar, Xiushui 48, seems to be more easily remobilized than in low-Se cultivars (Zhou *et al.* 2008). So far, there are significant gaps in current understanding of whether a higher Se content of Se enriched rice is directly related to its high transport capacity. The excised panicle culture method was used to study differences between cultivars in Se transfer and remobilization in rice grains, with the aim of

*Author for correspondence: <zxbissas@163.com>.

investigating the differential efficiency of the translocation and transport of important Se species from stems and flag leaves to grains and comparative contributions of phloem and xylem transport.

Materials and Methods

Seeds of two cultivars of *Oryza sativa* L. (Xiushui 48 and S. Andrea) were sterilized with 0.5% NaOCl for ten min, rinsed with and soaked in deionized water for overnight, and then placed on a nylon net floating on 0.5 mM $CaCl_2$ solution until germination occurs. After germination, seedlings were transferred 4 seedlings pot-1 to 10 L pots filled with the Kimura solution. The composition of Kimura nutrient solution was as follows: 0.40 mM $(NH_4)_2SO_4$, 0.09 mM K_2SO_4, 0.56 mM $MgSO_4$, 0.18 mM KH_2PO_4, 0.18 mM KNO_3, 0.37 mM $Ca(NO_3)_2$, 20 µM Fe-EDTA, 10 µM H_3BO_3, 1.0 µM $CuSO_4.5H_2O$, 1.0 µM $ZnSO_4.7H_2O$, 5.0 µM $MnCl_2.4H_2O$ and 0.5 µM $H_2MoO.4H_2O$. Deionized water was used throughout the growth period of rice. The solution was renewed every three days and the solution pH was adjusted daily to pH 5.5 using 0.1 M KOH or HCl (Zhou *et al*., 2008). The solution culture experiment was conducted in a growth glasshouse under a diurnal cycle of 14 hrs at 25ºC for daytime and 10 hrs at 20ºC for night. Relative humidity was 65%, and the light intensity at the plant tops was approximately 300 µmol/m^2/s of photosynthetic photon flux. Xiushui 48 and S. Andrea cultivars were grown separately in 24 pots. The rice cultivars were grown in a glasshouse until 10 days after flowering (95 days after germination).

Healthy panicles of Xiushui 48 and S. Andrea rice cultivars at flowering stage were selected. Stem-girdled panicles were subjected to a 30 sec jet of steam that was distributed on a 1 cm area of the stem between one and two cm below the panicle head, prior to their excision from the plant. This destroyed the phloem cells, preventing further phloem transport into the rice grain, while the xylem vessels remained functional. Se in grains can only be transported through xylem vessels. Therefore, the relative contribution of xylem and phloem to Se content was distinguished by the comparison of stem girdling (i.e., blocking phloem transport, only allowing xylem transport) and the control (i.e., functioning phloem and xylem transport). Panicle excision was conducted under low red light conditions to reduce evaporation and limit xylem air bubbles caused by cutting the stem base. Excised panicles were fixed in a 50 ml high silica glass tube with a nutrient solution (as described in Chen *et al*. 2007). Excised panicles were transferred to a growth glasshouse with a day light time of 14 hrs, light intensity of 400 µmol/m^2/s, and a day/night temperature of 25/20ºC. Twenty four hrs later, the excised panicles from the two rice cultivars were transferred and kept in a sterile, non-phosphorus nutrient solution with 100 µM selenite, selenate, Se-Met and SeMeSeCys for 72 hrs. The rice cultivars were treated with stem girdling (blocking phloem transport) and non stem girdling. Following treatment, flag leaves were cut at the base of the leaf blade, and grains were selected and manually dehusked. Se content of flag leaf and grain were measured. The samples specimens were dried at 70ºC, weighed, and then the Se content of the flag leaf and grain was measured.

To investigate the remobilization of Se species from flag leaves to rice grains during grain filling, two rice panicles were exposed to 100 µM selenite, selenate, Se-Met or SeMeSeCys as well via the cut leaf of intact plants for 10 days. The top of flag leaves (2 cm) was cut and inserted into bottles containing 100 µM selenite, selenate, Se-Met or SeMeSeCys. Each Se treatment repeated four times.

The Se content in rice samples was determined following the method described by Zhang *et al.* (2006) using an AF-610A atomic fluorescence spectrometer. Blanks and certified reference material (GSB-23 rice flour) were included in each batch of samples for quality control. The recovery for GSB-23 was 85 - 105%.

Data sets were analyzed using analysis of variance (ANOVA) tests and differences between treatment means were compared using the least significant difference (LSD) method at the 0.05 probability level. All statistics were conducted using SPSS 18.0.

Results and Discussion

For panicles that had not been stem-girdled, the Se content in rice grains with organic Se treatment (SeMeSeCys and Se-Met) was 13 to 14 times higher than that of inorganic Se (selenite and selenate). Stem-girdling significantly reduced the absorption of the four Se species by rice grains compared with the non-stem-gridling treatment (Fig. 1).

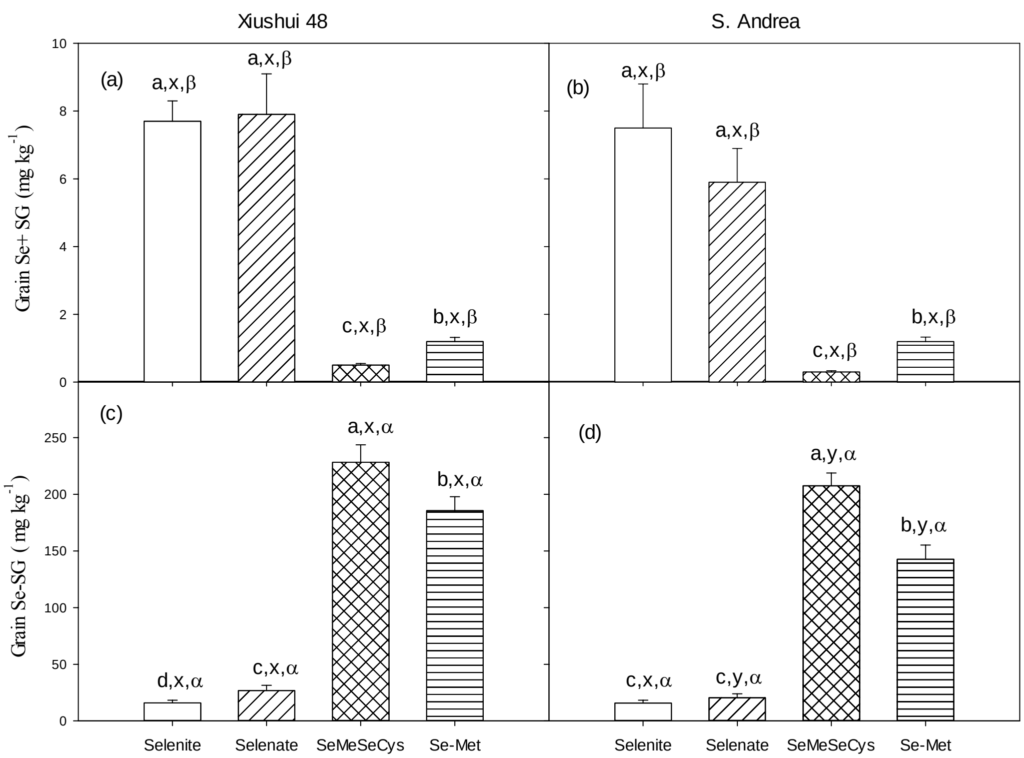

Fig. 1. Mean total grain Se concentrations of two rice cultivars of rice. Panicles excised at 10 days post-anthesis that were subjected to a ± stem-girdling treatment, and hydroponically fed a solution amended with 100 μM selenite, selenate, Se-Met, or SeMeSeCys for a 72 hrs. Data (means ± Sd, n = 4) with different letters denote significant differences between Se treatments in the same cultivar (a, b, c), between cultivars for the same Se treatment (x, y) and between stem-girdling treatment and non-stem-girdling treatment in the same cultivar under the same Se treatment (α, β) at $p < 0.05$. Abbreviations: Grain Se-SG: grain concentrations of Se in rice grain for panicles excised at 10 days post-anthesis subjected to non-stem-girdling treatment. Grain Se + SG: grain concentrations of Se in rice grain for panicles excised at 10 days post-anthesis subjected to stem-girdling treatment.

In rice threated with SeMeSeCys, stem-girdling reduced Se content by 99.8 - 99.9% as compared to non-stem-girdling. Similarly Se-Met, selenite and selenite reduced Se content by 99.2 - 99.4% compared to non-stem-girdling. In rice cultivated with selenite 70.3 - 70.9% and 51.3 to 51.9% reduced Se content by 70.3 - 70.9%, respectively compared to non-stem- girdling. The Se concentration in grains was greater for selenate and selenite stem-gridled panicles than for

SeMeSeCys and Se-Met stem-gridled panicles. Stem-girdling essentially stops the transport of organic Se into the grain, but some (29.1 - 48.7%) inorganic Se can be transported to the grain via the xylem (Fig. 1).

In plants treated with SeMeSeCys, Se-Met and selenate, in the non stem-girdling treatment, the Se accumulation capacity of Xiushui 48 was significantly higher than that of S. Andrea, but in the stem-girdling treatment no significantly differences were observed. This suggests that Xiushui 48 was significantly more effective ($p = 0.05$) in transporting SeMeSeCys, Se-Met and selenate from stems to grains than S. Andrea (Fig. 1).

Inorganic Se was transported more to the flag leaf than organic Se, and the inorganic Se concentration was 210 - 381 times higher than the organic Se concentration (Fig. 2). In the non-stem girdling treatment, selenate was transported more to the flag leaf than selenite, and stem-girdling caused a reduction in selenate. Se transport was reduced by about 50%, for selenite, stem-girdling caused no significant effect on Se transport to the flag leaf. This datum suggests that selenate may be transported to the flag leaf through both xylem and phloem, and selenite can only be transported to the flag leaf through xylem.

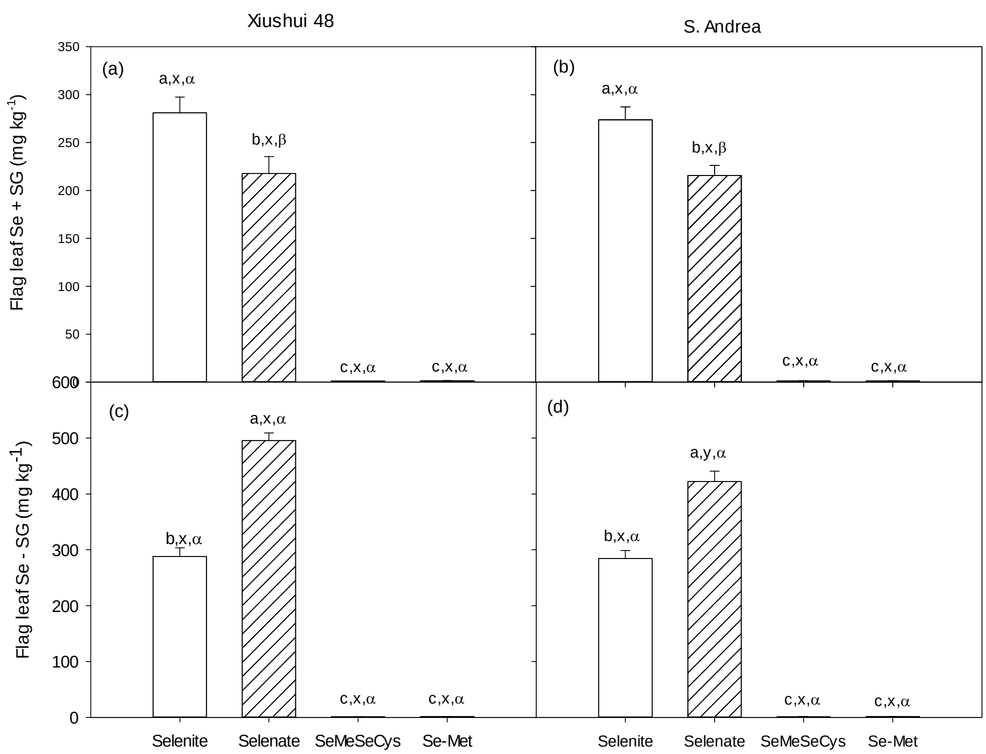

Fig. 2. Mean total flag leaf Se concentration of two rice cultivars. Denotations are same as Fig. 1.

There were no significant differences in the ability of the two rice cultivars to transport selenite from stems to flag leaves in either of the stem girdling treatments. For plants treated with non-stem girdling and selenate, the cultivar Xiushui 48 was found to be more effective ($p < 0.05$) to transport selenate than S. Andrea. However, after stem girdling, there was no significant difference between the ability of the cultivars to transport selenate. Rice excised panicles were able to weakly transport organic Se to flag leaves, and the stem girdling and non-stem girdling treatments had little effect on the transport of organic Se. There were no significant differences in the ability of the two rice cultivars to transport organic Se from stems to flag leaves.

Treatment with selenite and selenate caused no significant differences in the Se content of the rice grains of the two cultivars. However, treatment with Se-Met led to a higher Se content in rice grain than other treatments (Fig. 3). When the rice cultivars were treated with Se-Met and SeMeSeCys, Xiushui 48 had significantly higher Se content than S. Andrea. Hence, Xiushui 48 was significantly more effective ($p = 0.05$) than S. Andrea in transporting Se-Met and SeMeSeCys from flag leaves to grains.

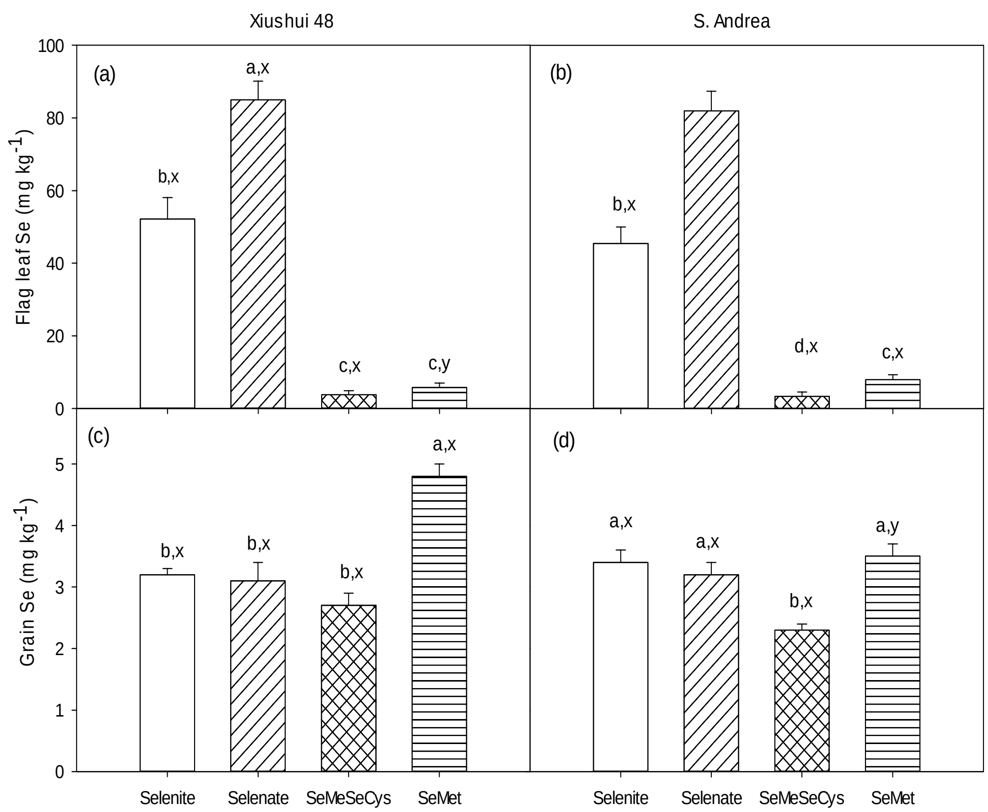

Fig. 3. Mean total Se concentration in the flag leaf (a, b) and grain (c, d) in two rice cultivars fed 100 μM selenite, selenate, Se-Met, or SeMeSeCys, via the flag leaf of intact plants for a 10 days during grain fill. Data (means ± Sd, n = 4) with different letters denote significant differences between Se treatments in the same cultivar (a, b, c), between cultivars for the same Se treatment (x, y) at $p < 0.05$.

The Se content of flag leaves with treated with inorganic Se was significantly higher than those treated with organic Se. Treatment with organic Se resulted in a (22.4 - 24.1-fold less) lower concentration of Se in the flag leaf than inorganic Se treatments ($p < 0.0001$). There was no significant difference between the two organic treatments (Fig. 3).

When treated with organic Se, the flag leaf to grain translocation factors (grain/flag leaf Se) was 17.0 - 20.8 times higher than with inorganic Se treatment (Table 1). Se can be absorbed by flag leaves and transported to grains mainly through the phloem. The ability of the two cultivars of rice transported significantly more organic Se than inorganic Se from flag leaf to grain. Xiushui 48 was able to transport more Se-Met and SeMeSeCys from flag leaves to grains than S. Andrea.

Table 1. Flag leaf-to-grain translocation factors (grain/flag leaf Se) of two rice cultivars fed 100 μM selenite, selenate, Se-Met, or SeMeSeCys.

Different Se species	Grain/flag leaf Se	
	Xiushui 48	S. Andrea
Selenite	0.05 a	0.08 a
Selenate	0.04 a	0.04 a
SeMeSeCys	0.71 a	0.68 b
Se-Met	0.83 a	0.44 b

The different letters within a row indicate the significant differences according to LSD multiple comparison test ($p < 0.05$). Data are means of four replicates.

The mechanism of selenium accumulation in rice grains has important theoretical and practical significance in improving the selenium content in rice grains. Recent studies have indicated that rice endosperm contains organic Se, while the bran layer contains both inorganic and organic Se (Williams 2009, Sun *et al*. 2010). Se-Met is the major form of Se, followed SeMeSeCys, and a small part of SeCys. It is not yet clear how the different Se species are transported to grain. Treatment of the excised panicle with organic Se (Se-Met and SeMeSeCys) resulted in a higher Se content in the grain (Fig. 1), which showed that the transport efficiency of organic Se from the stem to the grain was much higher than that of inorganic Se. The stem girdling test confirmed that organic Se (Se-Met and SeMeSeCys) was transported to the grain only through the phloem, while inorganic Se was transported from the stem to the grain by both xylem (29.1 ~ 48.7%) and phloem (51.3 ~ 70.9%).

Treatment with organic Se increased the Se content in rice grains more than that with inorganic Se (Table 1 and Fig. 1). This indicates that during the grain filling stage, the ability of the panicle to transport organic Se was higher. The present results suggest that Xiushui 48 is more efficient to transport Se-Met, SeMeSeCys and selenate significantly than S. Andrea. Se-Met composed the main Se in rice and accounted for 82.9% of total Se. SeMeSeCys composed 6.2% of total Se and SeCys 2.8 - 6.3% of the total Se (Sun *et al*. 2010). The difference of the two rice cultivars in transporting Se-Met by phloem during maturity was an important reason for the differences in grain Se content.

Stem girdling significantly reduced selenate in stems and entering in the flag leaf, indicating that selenate was transported into the flag leaf through xylem and phloem. Stem girdling had no significant effect on the amount of selenite in the flag leaf, which indicated that selenite was transported into the flag leaf mainly through the xylem (Fig. 2). The absorption of Se through the flag leaf was similar to the absorption of Se through the excised panicle. The efficiency of organic Se transport to grains through the phloem was higher than that of inorganic Se. White *et al*. (2009) also reported that Se is mainly redistributed in plants through phloem.

The flag leaf is the functional leaf in rice grain filling at maturity. During the rice grain filling period the flag leaf is the most active assimilative organ and an important “source” of grain filling (Wang *et al*. 1997). Flag leaf Se may be an important "sink" and "source" to grain Se. Results show that significant transfer of Se in Xiushui 48 from flag leaf benefits the Se accumulation in grains (Zhou *et al*. 2008). The ability of the rice flag leaf to transport Se to the grain was assessed by the transfer factor (grain/flag leaf Se) as 17.0 - 20.8 times more than inorganic Se. The cultivar Xiushui 48 was able to transport Se-Met and SeMeSeCys to grains significantly more than S. Andrea.

Rice had different uptake and transport mechanisms of selenite and selenate. The mechanism for selenite is unknown, though may be transported by sulfate-transporter proteins and respiratory inhibitors can only inhibit 20% selenite absorption. Selenate is transported by active sulfate-transporter proteins (Anderson 1993, Terry *et al*. 2000). During the grain maturation process, the activity of high-affinity sulfate-transporter proteins of Xiushui 48 stems and flag leaves was possibly higher than that of S. Andrea. LeDuc *et al*. (2006) demonstrated that the (ATP sulfurylase) APS × (SeCys methyltransferase) SMT double transgenics accumulated almost eight times more MetSeCys than wild type plants and nearly twice as much as the SMT transgenics (LeDuc *et al*. 2006). However, the Se accumulation difference of the two rice cultivars included in present study is related to the activity of ATP sulfurylase and SeCys methyltransferase needs further research. To describe the precise molecular mechanism underlying the observed differences in these cultivars, further investigations using molecular techniques are needed.

Organic Se can transfer into rice grains through the phloem, and inorganic Se is mainly transferred to the grain through the xylem. The more Se content in the cultivar Xiushui 48 was partly due to its ability to transport SeMeSeCys, Se-Met and selenate to the grain more efficiently than the cultivar S. Andrea. The two rice cultivars transported significantly more organic Se than inorganic Se from the flag leaf to the grain, yet Xiushui 48 was able to transport more Se than S. Andrea, thus Xiushui 48 had a higher final Se content in the rice grain.

Acknowledgements

This study was funded by the National Natural Science Foundation of China (No.31372141; 31672238; 31101610), The Fundamental Research Funds for Central Universities (2362015xk06) and China Agriculture Research System (Nycytx-25).

References

Anderson JW 1993. Selenium interactions in sulfur metabolism. *In:* Sulfur nutrition and assimilation in higher plants-regulatory, agricultural and environmental aspects. De Kok LJ (ed.). SPB Academic. The Netherlands. pp. 49-60.

Bourgis F, Roje S, Nuccio ML, Fisher DB, Mitchell CT, Li CJ, Herschbach C, Rennenberg H and Hanson A 1999. S-Methylmethionine plays a major role in phloem sulfur transport and is synthesized by a novel type of methyltransferase. Plant Cell **11**: 1485-1498.

Carey AM, Scheckel KG, Lombi E, Newville M, Choi Y, Norton GJ, Price AH and Meharg AA 2012. Grain accumulation of selenium species in rice (*Oryza sativa* L.). Int. J. Environ. Sci. Technol. **46**: 5557-5564.

Chen F, Wu F, Dong J, Vincze E, Zhang G, Wang F, Huang Y and Wei K 2007. Cadmium translocation and accumulation in developing barley grains. Planta. **227**: 223-232.

Carey AM, Scheckel KG, Lombi E, Newville M, Choi YS, Norton GJ, Price AH and Meharg AA 2012. Grain accumulation of selenium species in rice (*Oryza sativa* L.). Environ. Sci. Technol. **46**: 5557-5564.

Combs GF Jr 2001. Selenium in global food systems. Br. J. Nutr. **85**: 517-547.

LeDuc DL, Abdel Samie M, Montes-Bayón M, Wu CP, Reisinger SJ and Terry N 2006. Overexpressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phytoremediation traits in Indian mustard. Environ. Pollut. **144**: 70-76.

Li HF, McGrath SP and Zhao FJ 2008. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. New Phytol. **178**: 92-102.

Masschelyen PH, Delaune RD and Patrick WH Jr 1991. Biogeochemical behavior of selenium in anoxic soils and sediments: an equilibrium thermodynamics approach. J. Environ. Sci. Health **26**: 555-573.

Sun GX, Liu X, Williams PN and Zhu YG 2010. Distribution and translocation of selenium from soil to grain and its speciation in paddy rice (*Oryza sativa* L.). Environ. Sci. Technol. **44**: 6706-6711.

Terry N, Zayed AM, de Souza MP and Tarun AS 2000. Selenium in plants. Annu. Rev. Plant Phys. **51**: 401-432.

Wang QB, Wu JS and Zhao YF 1997. Research on the dynamics of Se uptake and accumulation in different peanut organs. Acta. Bot. Sin. **39**: 164-168.

White PJ and Broadley MR 2009. Biofortification of crops with seven mineral elements often lacking in human diets-Iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol. **182**: 49-84.

Williams PN, Lombi E, Sun GX, Scheckel K, Zhu YG, Feng X and Zhu J 2009. Selenium characterization in the global rice supply chain. Environ. Sci. Technolo. **43**: 6024-6030.

Zhang LH, Shi WM and Wang XC 2006. Difference in selenium accumulation in shoots of two rice cultivars. Pedosphere **16**: 646-653.

Zhou XB, Shi WM and Yang LZ 2008. Study on mechanisms of selenium accumulation in rice grains. Plant Nutr. Fert Sci. (in Chinese) **14**: 505-507.

(Manuscript received on 3 April, 2016; revised on 14 September, 2016)