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Full Length Article

Selenium Speciation and Distribution in the Rhizosphere and Selenium Uptake of two Rice (*Oryza sativa*) Genotypes

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Abstract

This study was conducted to expound rhizosphere effects on chemical behavior of selenium (Se). The selenium-enriched and non-selenium-enriched rice (Xiushui 48 and Bing 9652) were studied by pot experiment. After 0.5 mg Se kg^{-1} was added, the rice was grown in the root box until ripening stage, and effects of interspecific root interactions of the two rice cultivars on Se chemical forms in different rhizosphere areas were evaluated using a sequential extraction procedure. Rhizosphere effect of different rice cultivars causes different chemical behaviors on selenium. The soluble Se content in rhizosphere soil and near rhizosphere soil of the two rice cultivars was significantly higher in tillering stage than ripening stage. The soluble Se content in rhizosphere soil and near rhizosphere soil of Xiushui 48 was higher than that of Bing 9652 in tillering and ripening stage. There were no significant differences in exchangeable Se content in rhizosphere of different rice cultivars between different distances from root chamber at the tillering stage of rice. However, in the ripening stage of rice, near-rhizosphere region of Xiushui 48 had higher microbial biomass than that of Bing 9652, and accordingly higher exchangeable Se content. At the tillering stage, there was no significant difference in organic matter-sulfide-bound and elemental (organic) Se in 5 mm within the rhizosphere. However, at the ripening stage, organic Se of Xiushui 48 root zone reduced by 5.7–11.4% in average compared to Bing 9652 in 5 mm within the rhizosphere. It indicated that organic Se with lower bioavailability in high-Se rice cultivar (Xiushui 48) root zone was converted to exchangeable Se with high bioavailability. Unlike low-Se rice cultivar (Bing 9652), Xiushui 48 had a lower root Se concentration and strong ability to transport Se to the grain. © 2018 Friends Science Publishers

Keywords: Rice; Rhizosphere; Selenium; Species

Introduction

Selenium (Se) is a micronutrient element essential for the human body, which is involved in *in vivo* oxidation resistance in the form of selenium protein (Rayman, 2012). Higher plasma selenium content has prevention and anti-cancer effects for certain cancers (such as breast cancer, lung cancer, esophageal cancer and gastric cancer, etc.,) (Cai *et al*., 2016). Lack of Se can easily cause local diseases, such as Keshan disease, Kashin-Beck disease, endemic liver cancer and colorectal cancer in Jiashan, Zhejiang (Jiang and Zhenzhi, 1992; Wang and Gao, 2001; Navarro-alarcon and Cabrera-vique, 2008). China's domestic Se census shows that 2/3 region from the northeast to southwest of China is internationally recognized as areas lacking Se and 72% of counties (cities) have low Se or Se deficiency. Thus, food crops grown on these Se-deficient soils have relatively low Se content (Chen *et al*., 2015). From the national perspective, content of brown rice Se is in the range of 15.6–69.0 μg kg^{-1}, with an average of only 32.27 μg kg^{-1}. The current staple food Se can hardly meet the human nutrition demand for Se. Selenium content in our residents' hair has decreased by 24–46% in average compared to the past 20 years, which is related to reduction in Se content of staple food (Li *et al*., 2014).

Increasing the content of Se in major grain crops (e.g., rice) is the most direct and effective way to improve human Se. The Se uptake and enrichment of rice depends not only on total Se content of the soil, but also on the occurrence form of Se in soil or soil solution (Fordyce *et al*., 2000). In drowned paddy fields (7.5 $<$pe + pH $<$15), selenite is the predominant form. Oxides and hydroxides in the soil have a strong adsorption of selenite, reducing its concentration in soil solution and thus affecting bioavailability of selenite (Elrashidi *et al*., 1989; Jayaweera and Biggar, 1996; Zawislanski and Zavarin, 1996). Therefore, in order to assess effectiveness of soil Se on rice, determination of soil Se speciation is more necessary than total Se, because the former determines mobility and bioavailability of Se.

Sequential extraction procedure provides a powerful tool for assessing soil Se speciation and Se availability (Fujita *et al*., 2005). For example, Qu *et al*. (1997) performed an extraction scheme, which is the most widely applied method. It allows the division of the total Se content

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into five fractions: soluble, exchangeable and carbonate-bound, Fe-Mn oxide-bound, organic-sulfide-bound and residual fraction, among which soluble Se and exchangeable Se are considered as forms that can be used effectively by plant (Peng *et al*., 2016).

Rhizosphere soil is the region subjected to direct action by plant roots, which is very different from bulk soil in physical, chemical, biological nature (Mcgrath *et al*., 1997). Therefore, form, conversion and bioavailability of Se in rice rhizosphere may be significantly different from that in bulk soil. At present, only research results of dryland wheat are available, Se content of soil residue in wheat rhizosphere is about 10 percentage points lower than that of non-rhizosphere soil, and content of exchangeable, carbonate-bonded Se and Fe-Mn oxide-bound Se increase by 10 percentage points. Except residual Se, other forms of Se in wheat have significant positive correlation with grain Se content (Fu, 2011). The morphological changes of rhizosphere Se can affect Se uptake and accumulation of rice, and distribution characteristics of Se in rice rhizosphere has not been studied yet. Does the difference in morphological change of Se in rice rhizosphere cause difference in Se accumulation in grain between rice cultivars? This research aims to study content, distribution and change characteristic of Se of different forms in different Se-accumulating rice rhizosphere soils, explore the difference between behavioral characteristics of Se-enriched rice and non-Se-enriched cultivars in utilization of Se by rhizosphere, and thus understand the possible Se-enrichment mechanism of rice.

Materials and Methods

Experimental Design

A rhizosphere experiment was carried out in the artificial greenhouse in 2016. The rhizobox used was described by He *et al.* (2005). The dimension of the rhizobox was 150 × 150×240 (length×width×height in mm). The root box was divided into three parts with 300 mesh nylon net: root compartment (abbreviated as RC, the same below) (20 mm wide), near rhizosphere soil on both sides of root chamber. The near-rhizosphere soil was segmented into six different sub-regions with 1 mm interval by 300 mesh nylon net, respectively six different sub-regions of 1, 2, 3, 4, 5,> 5 mm according to the distance from the rhizosphere chamber. The experimental soil was 0–20 cm layer soil of neutral purple paddy soil in Beibei district of Chongqing, China. The basic physical and chemical properties of the soil are shown in Table 1, and total aluminum, iron content of the soil is 8.1 g kg^{-1} and 29.1 g kg^{-1}, respectively. The recovered soil was naturally air dried, passed through 1 mm sieve and thoroughly mixed. Selenium-containing soil was obtained by artificial addition of sodium selenite. The dry soil with 0.5 mg kg^{-1} Se was added, to be mixed evenly with 5 kg soil and loaded in pot. Each treatment was repeated 4 times. The experimental rice cultivars were Xiushui 48 (grain Se content was higher, at 112 μg kg^{-1}) and Bing 9652 (grain Se content was lower, at 32 μg kg^{-1}). The selected cultivars were screened through 151 field rice cultivars.

In the experiment, a Se level of 0.5 mg Se kg^{-1} was set, random arrangement was made in eight repeated times. The 5 kg experimental soil was mixed with $CO(NH_2)_2$ 0.75 g and KH_2PO_4 0.475 g, to be mixed evenly and loaded in the root box. Sodium selenite was used as exogenous Se, to be evenly mixed into the soil with urea and other fertilizers. The soil surface was flooded 2–3 cm during the whole rice growth period. Rice reached maturity after growth for 120 days, to be harvested according to different parts. The growth conditions of the culture chamber were 14 h/10 h day and night illumination time, light intensity of 300 μmol m^{-2} s^{-1}, temperature of 25±2℃, and relative humidity of 70–75%.

Soil and Plant Samples

At the middle stage of rice tillering, four root boxes were randomly selected from eight repetitions, rhizosphere soil (within root chamber), 1, 2, 3, 4, 5 mm thin near-rhizosphere soil and bulk soil (>5 mm) were selected. Each soil sample was divided into two parts, one for determination of microbial biomass carbon, and the other for continuous extraction of soil Se after dried in natural air. Finally, the remaining four root boxes were selected at mature stage for determination of Se content in soil and rice plants.

Cut the base of rice plants with scissor to divide the rice to shoot and root, to be rinsed with water three times, then rinsed with deionized water, wiped with filter paper, dried and weighed. All dried samples were digested with HNO_3-$HClO_4$, and then contents of Se in each sample were determined by atomic fluorescence spectrophotometry (He *et al*., 2005).

Sample Analysis and Quality Control

For determination of pH value of soil sample, 10 g dried soil (rhizosphere or non-rhizosphere soil) was dissolved in 25 mL deionized water, pH value of the solution was measured by pH electrode, and soil oxidation-reduction potential was determined by Perot electrode.

Soil microbial biomass carbon was measured by chloroform fumigation extraction method, and carbon content was measured by TOC analyzer (Vance *et al*., 1987).

All reagents were analytically pure. All plastic and glass instruments were soaked in 10% HNO_3 before use and washed with deionized water for standby use. For treatment of soil samples, the dried soil samples were ground, passed through a 100 mesh sieve, 1.00 g of soil samples were weighed and put in a clean 100 mL polyethylene centrifuge tube, to be added with extracting agent, followed by step-by-step extraction after vibration (see Table 2). Graded extraction of Se is as follows (Vance *et al*., 1987).

The elemental analysis quality of the sample is controlled by whole program blank value and recovery of standard, and the allowable deviation limit is 100 ± 10% of recovery rate of standard. Morphological analysis is limited within 10% of relative error between each form and the whole.

Statistical Analysis

The results are presented as the average of three replicates. The statistical software program used with SPSS version 18.0.

Table 1: Properties of the purple paddy soil

pH (1:2.5)	Organic matter (g kg^{-1})	Available P (mgkg^{-1})	Available K (mg kg^{-1})	Available SO_4-S (mg kg^{-1})	Total Se (mg kg^{-1})	Available Se (μg kg^{-1})	CEC[a] (cmol kg^{-1})
6.5	13.45	10.23	101.20	3.10	0.23	16.0	18.5

CEC[a]: cation exchange capacity

Table 2: The sequential extraction procedure of Se in soil

Se fractions extracted	Reagent per 1 g of soil	Shaking time and temperature
Ⅰ Soluble (Sol-Se)	10 mL of 0.25 M KCl	25℃, shaken for 1h
Ⅱ Exchangeable and bound to carbonates (Exc-Se)	10 mL 0.7 M KH_2PO_4 (pH=5.0)	25℃, shaken for 4h
Ⅲ Bound to Fe-Mnoxidates (FeMn-Se)	10 mL 2.5 M HCl	heated for 50 min in bathing with 90℃, shaken intermittently
Ⅳ Bound to organic-sulfide matter (OM-Se)	10 mL 5% $K_2S_2O_8$ 8 mL and（1：1）HNO_3 2 mL	heated for 3 h in bathing with 95℃,shaken intermittently
Ⅴ Residual (Res-Se)	Add 7.5 mL HNO_3 and $HClO_4$ (4:1),	heated to 150℃-165℃ for 2 h, until the solutions become clear

Results

Variation of Soluble Se Content in Proximal Root Zone of two Rice Cultivars

As shown in Fig. 1, soluble Se content shows such a trend: rhizosphere soil (RS)> near rhizosphere soil (1–5 mm)> bulk soil (5 mm), the closer to rhizosphere it is, the higher soluble Se content is. The soluble Se content in rhizosphere soil and near rhizosphere soil of the two rice cultivars was significantly higher in tillering stage than in mature stage. The soluble Se content in rhizosphere soil and near rhizosphere soil of Xiushui 48 was higher than that of Bing 9652 in tillering and mature stages.

Differences in Exchangeable Se Content of Proximal Root Zone between two Varieties of Rice

As shown in Fig. 2, exchangeable Se content of soil in the root zone of the two rice cultivars shows such a trend: bulk soil (5 mm)> near rhizosphere soil (1-5 mm)> rhizosphere soil (RS), the closer to root surface it is, the lower the exchangeable Se content is at the tillering stage of rice, there were no significant differences in exchangeable Se content in rhizosphere of different rice cultivars between different distances from root chamber. However, during the mature stage of rice, exchangeable Se content in root chamber soil and rhizosphere soil (1–4 mm) of Se-enriched Xiushui 48 was significantly higher than that of Bing 9652. There was no significant difference in exchangeable Se content of bulk soil (> 5 mm) between the two cultivars. Compared with the tillering stage, exchangeable Se content in the two cultivars was decreased in mature stage.

Changes in Fe-Mn-Boundand Organic Se in Proximal Root Zone of the two Rice cultivars

The organic bound Se content of soil in different domains of the two rice cultivars was significantly lower in mature stage and that in tillering stage. The organic Se content in Bing 9652 root zone in the range of RS to 5 mm was averagely 7.8–18.2% lower in mature stage than that in the tillering stage. The organic Se content in Xiushui 48 root zone in the range of RS to 5 mm was averagely 13.2–29.2% lower in mature stage than that in the tillering stage. There was no significant difference in organic Se content in the range of RS to 5 mm between the two cultivars at the tillering stage. At the mature stage, however, organic Se content in the range of RS to 5 mm was averagely 5.7–11.4% lower in Xiushui 48 than Bing 9652. There was no significant difference in organic Se content of bulk soil (> 5 mm) between the two rice cultivars. It can be seen from the Fig. 3 that there is no significant difference in Fe-Mn-bound Se in soil of different domains between the two cultivars at tillering stage and Fe-Mn-bound Se content in rhizosphere and near rhizosphere soil of Xiushui 48 is higher than that of Bing 9652 at the mature stage, but the difference between the two is insignificant. There was no significant difference in Fe-Mn-bound Se content in root zone (≧ 4 mm) soil between the two cultivars.

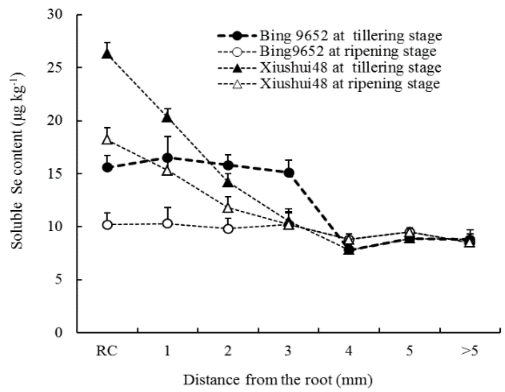

Fig. 1: The comparison of soluble selenium between rhizosphere soil and non-rhizosphere soil

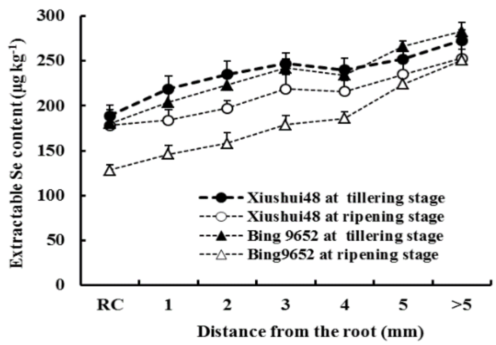

Fig. 2: The comparison of exchangeable and bound to carbonates selenium between rhizosphere soil and non-rhizosphere soil

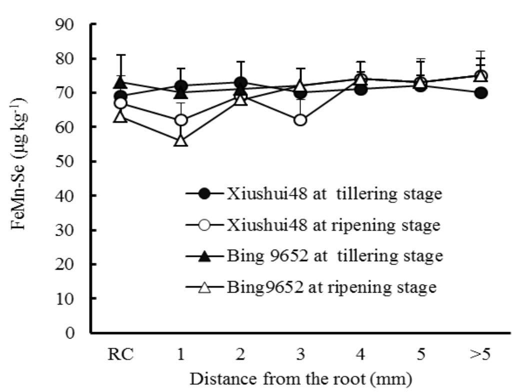

Fig. 3: The comparison of FeMn-Se between rhizosphere soil and non-rhizosphere soil

Dynamic Differences in Soil Microbial Biomass in Different Root Domains of Root Zone of the two Rice Cultivars

In order to further elucidate the effect of rice rhizosphere on Se species, the soil microbial biomass carbon of the two cultivars was higher in mature stage than in tillering stage. At the tillering stage of rice, soil microbial biomass carbon in rhizosphere soil and near rhizosphere soil was higher in Se-enriched Xiushui 48 than in Bing 9652. At the mature stage of the rice, microbial biomass of near-rhizosphere soil was significantly higher in Xiushui 48 than in Bing 9652, the former was increased by 21.8% compared with Bing 9625.

Rhizosphere Effect on Se uptake and Distribution in two Rice Cultivars

The various organs of the two rice cultivars have significantly different ability in Se accumulation at different growth stages (Fig. 4). There was no significant difference in root and stem Se content between two cultivars at tillering stage. The Se content in leaves of Xiushui 48 was significantly higher than that of Bing 9652. There were no significant differences in stem and leaf Se contents between the two cultivars at the mature stage, but there were significant differences in root and grain Se content. Compared with non-Se-enriched rice Bing 9652, Se content of Se-enriched Xiushui 48 was significantly lower, but its grain Se content was significantly higher.

Discussion

Our results confirm that rice rhizosphere process plays an important role in distribution of Se species in rhizosphere soil and Se uptake of rice. At the tillering and mature stages, contents of exchangeable Se and microbial biomass carbon in rhizosphere were significantly different between the two rice cultivars. In the rhizosphere and near rhizosphere region, Bing 9652 had lower rhizosphere exchangeable Se content and microbial biomass carbon content, while Xiushui 48 had higher exchangeable Se and microbial biomass carbon content (Fig. 2 and Fig. 5). Rhizosphere refers to the unique microecological environment formed by the interaction between plant roots and soil microorganisms. The rhizosphere is significantly different from soil in that a large number of biochemical processes occur in this area. These complex processes will affect soil chemistry characteristics, microbial composition and microbial activity. Rhizosphere bacteria may be involved in Se conversion in rhizosphere soil. Zhang *et al*. (2007) reported that when Se was added to the soil, rhizosphere bacteria could restore Se (VI) to Se (0), which resulted in conversion of soluble Se and exchangeable Se to organic Se and insoluble Se. On the other hand, microbial oxidation of Se has been reported, Se (0)→Se(Ⅳ) and Se(Ⅵ) (Dowdle and Oremland, 1998), which is expected to increase bioavailability of Se. That microbial biomass carbon content in the root zone of Xiushui 48 was significantly higher than that of Bing 9652 at the tillering stage and mature stage may be related to higher exchangeable Se content in root zone of Xiushui 48. Another most important factor affecting these changes is root exudates and shedding, such as organic acids (Fox and Comerford, 1990).

Table 3: The amount of organic acids and amino acids in two rice varieties

	Organic acids and Amino acids (mg g^{-1} fresh root)				
	Citric acid	Malic acid	Lactic acid	Succinic acid	Amino acids
Xiushui 48	1.85	1.03	0.45	1.18	2.62
Bing 9652	0.68	0.72	0	0.78	2.35

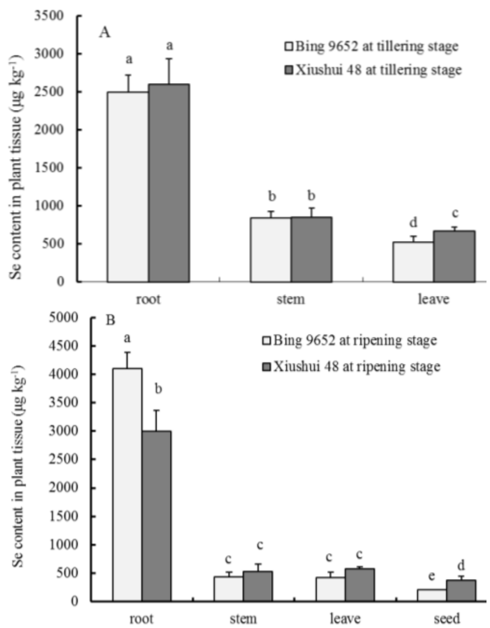

Fig. 4: Se uptake and partitioning in plant organs (root, stem and leave) of two rice plants, Bing 9652(A) and Xiushui 48 (B)

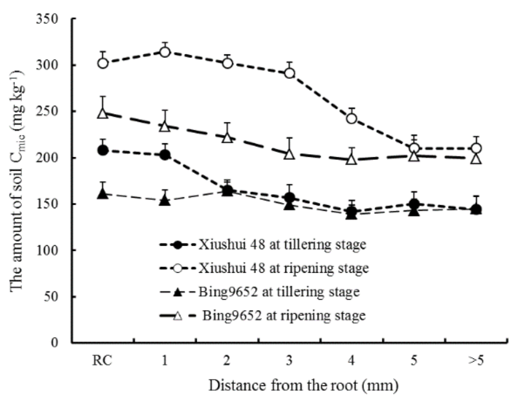

Fig. 5: Amount of microbial biomass carbon sampled at millimeter increments from the root compartment (RC) at tillering stage (TS) and ripening stage (RS) of two rice plants

The concentration of organic acids in rhizosphere low molecular weight can reach several millimoles (Dynes and Huang, 1997). Organic acids play an important role in enhancing ion availability due to its participation into ion exchange reaction of mineral surface. The amount of organic acids and amino acids secreted by the roots of Xiushui 48 was higher than that of Bing 9652 (Table 3). Experiments with pure culture system showed that citric acid, oxalic acid and other organic acid anions increased effectiveness of selenite by competing for adsorption sites of selenite anions in iron-aluminum oxide and goethite (Sharmasarkr and Vance, 1995; Zhou *et al*., 2007). On the other hand, low molecular weight organic acids can form a more stable complex with Fe^{3+}, which may destroy combination of ferric hydroxide and Se so that Se adsorbed on soil iron oxides can be released by organic acids, thus increasing Se solubility and mobility, potentially increasing crop uptake and transport of Se (Anderson and Mcmahon, 2001; Coppin *et al*., 2006).

Soil microflora structure, as the indicator of soil quality, shows the same trend in microbial biomass carbon and exchangeable Se in rhizosphere of the two rice cultivars (Fig. 2 and Fig. 4). Xiushui 48 had higher microbial biomass carbon content and exchangeable Se content in root zone. To determine whether the two have direct causal connections, we need to further identify whether number of Se oxide bacteria in rhizosphere microorganisms of Xiushui 48 was significantly higher than that of Bing 9652, so that exchangeable Se content in Xiushui 48 was significantly higher than that in Bing 9652. In addition, the root exudates provide the substrate for rhizosphere microorganisms, so distribution and difference of root exudates can change the microbial biomass and determine microflora structure to a certain extent. Our results confirmed that there were differences in rhizosphere microorganisms between Xiushui 48 and Bing 9652, and there were differences in root exudates of these two cultivars, as Xiushui 48 secreted more organic acids and amino acids than Bing 9652. Therefore, root microbial growth and flora changes caused by soil Se and root exudates need further study.

There was a genotype difference in Se absorption and accumulation of plants. The rice growth period affects absorption of Se in different organs, especially the roots and shoots. It was found that transport from roots to shoots was the main reason for grain Se enrichment. At the same time, some reports indicate that grain Se content of different cultivars depends on transport differences from stem to reproductive organ. In our experiments, the two rice cultivars were significantly different in Se of roots, stems, leaves and grains (Fig. 5). Rice Bing 9652 has high root Se content, while Xiushui 48 has high Se content in the shoots. Therefore, Xiushui 48 is a Se-rich cultivar, and its root mechanism promotes absorption of Se from the soil and transfers more Se to the shoots.

The organic bound Se is the main form of rhizosphere soil, followed by exchangeable Se. The organic bound Se content in the different root domains of the two cultivars is significantly higher at tillering stage than that at mature stage. At the tillering stage, there was no significant difference in organic bound Se and exchangeable Se content between the two cultivars in different areas from root surface. However, in the mature stage of rice, organic bound Se content in the rhizosphere and near rhizosphere soil was lower in Xiushui 48 than that in Bing 9652, while exchangeable Se content was significantly higher in Xiushui 48 than that in Bing 9652. This indicated that more amount of organic bound Se in rhizosphere and proximal root zone of Xiushui 48 was converted to exchangeable and soluble Se than Bing 9652. This corresponds to the previous rhizosphere biochemical process, and conversion of organic bound Se may have a great relationship with rhizosphere microorganisms. It has been reported that microorganisms in soil can promote mineralization of organic Se, so that it can be converted to effective state (Abrams *et al*., 2009). This form of Se is affected by soil microbial activity, because it controls process of synthesis and mineralization of soil organic matter. The distribution of Se between different components is a dynamic equilibrium process in which various forms of Se can be chemically or biochemically converted to establish a new dynamic equilibrium. Wang's results confirm that release of organic bound Se compensates for the Se taken away due to plant uptake in soil solutions, thereby increasing crop uptake of Se (Wang *et al*., 2012). This indicated that rhizosphere microbial biomass of Xiushui 48 was significantly higher than that of Bing 9652. Microorganisms play a very important role in conversion of bound Se in rhodosphere and proximal root zone to exchangeable Se, which made rhizosphere effective Se content of Xiushui 48 significantly higher than that of Bing 9652 in the later growth period, and root absorbing capacity of Xiushui 48 much higher than that of Bing 9652. Our previous results also confirmed that Se of Xiushui 48 was more easily transported to the shoots, which made its grain Se content higher (Zhou *et al*., 2014).

Conclusion

The rhizosphere effect of roots of different rice cultivars causes different chemical behaviors on Se. The soluble Se content in rhizosphere soil and near rhizosphere soil was significantly higher intillering stage than that in mature stage. The soluble Se content in rhizosphere soil and near rhizosphere soil in tillering and maturing stages was higher in Xiushui 48 than that in Bing 9652. The exchangeable Se and microbial biomass were determined by the distance from the root surface, showing positive correlation to a certain extent. In the mature stage of rice, near-rhizosphere area of Xiushui 48 had higher microbial biomass than that of Bing 9652, and accordingly, higher exchangeable Se content. After the careful study of the two cultivars, there remained many uncertainties about the rhizosphere process, as many factors may affect chemical behavior of rhizosphere Se. Therefore, there is need to further reveal dynamic complex mechanism of rhizosphere Se in order to better understand difference mechanism of Se absorption in rice cultivars.

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