



Full Length Article

Absorption, Translocation and Redistribution of Selenium Supplied at Different Growth Stages of Rice

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Abstract

Selenium (Se), as an essential micronutrient for humans is very importance for human health. In order to effectively improve the human Se intake, increasing the Se content in food intake rice is particularly important. The growth period is the most of importance for Se accumulated in grain of rice should be also confirmed. A sand-and solution-culture experiment was conducted to investigate the key process, as well as the key period resulting in the increase of Se in grain through Se supplied at different growth stages of rice. Xiushui 48 and Bing 9652 known as the high-Se and low-Se rice were grown in Kimura B nutrient solution and transferred to 1.5 mg L⁻¹ selenite treatment for only 7 days at four different stages: jointing stage, panicle initiation stage, flowering stage and milky stage then Se concentration in rice were measured at harvest. The accumulation and concentration of Se in shoot and grain was significant greater in the former two treatments before panicle initiation stage than in the latter two, in addition to the transfer coefficient and distribution coefficient have the similar trend. It indicates that the key growth period is in between jointing stage and panicle initiation stage. The grain transfer and distribution coefficient of Xiushui 48 in stem and grain was greater than Bing 9652. These findings confirmed that the Se in root and shoot of Xiushui 48 could be transferred and redistributed more than Bing 9652. Therefore, Se transferred from rood to shoot by xylem and redistributed from shoot to grain by phloem are the most vital processes to make the more Se content in grain of Xiushui 48 than Bing 9652. © 2017 Friends Science Publishers

Keywords: Accumulation; Se; Physiological process; Growth stage; Rice

Introduction

Selenium (Se) is an essential micronutrient element of human and animals, which has multiple functions in organisms to reduce the body of oxygen free radicals and maintain a healthy immune system thus reduce the risk of cancer (Legrain *et al.*, 2014; Cai *et al.*, 2016). According to the survey results of 13 provinces and cities the daily intake of Se in China is only 26.63 µg d⁻¹, while the recommended Se intake is 60 µg d⁻¹ (Chen *et al.*, 2002). Absolutely it cannot reach human Se nutritional needs. As the main staple in China, rice is an important source of Se nutrient intake. Increasing the grain Se content of rice through food chain is an important way to increase Se intake in human body (Lyons *et al.*, 2003). Improving Se content in crop via Se fertilizer is a commonly used method for Se biofortification, which has been applied in rice, wheat, corn and other major crops (Chilimba *et al.*, 2012; Boldrin *et al.*, 2013; Ducsay *et al.*, 2016). Eichgreatorex *et al.* (2007) showed that the utilization rate of Se in rice was only 5–30% and the remaining 70–95% Se retained in the soil, so the soil Se fertilizer may cause production cost waste and potential environmental risk (Broadley *et al.*, 2006). Therefore, it is of practical significance for the sustainable development of

agriculture to improve the utilization rate of Se in rice and improve the Se content in rice, which not only solves the current situation of people's lack of Se but also saves the cost and reduces the environmental pressure. Zhou *et al.* (2007) showed that the uptake of soil Se in the whole growing stage was non-constant, which means that the contribution of different growth stages to the accumulation of Se in rice was different. Through the study of the absorption and accumulation characteristics of Se in different growth stages of rice Zhang *et al.* (2017) considered that the jointing stage and booting stage are the key growing stages for the uptake and accumulation of Se in rice. The grain Se content of different rice varieties cultivated on the same land varied greatly for instance the Se content of high-Se Xiushui 48 differs three times from that of low-Se Bing 9652 (Zhang *et al.*, 2006a). Zhou *et al.* (2007) considered that the transport and distribution characteristics of the two varieties of rice after the absorption of Se are different. Zhang *et al.* (2006b) suggested that the process of Se transporting from the roots of rice to the shoot contributes more to the accumulation of Se in rice than that of in the roots of rice. According to Arvy (1982) mature leaves of bean could redistribute the Se carried in by the movement of flow through the xylem.

Leaves of rice have the same function (Carey *et al.*, 2012). The previous studies suggested that Cd loaded in xylem could transfer directly to phloem through a “node”, then accumulated into grain (Harris and Taylor, 2001; Uraguchi *et al.*, 2009) the transportation of Se might share the same method. Thus, it is needed to further researches that which process is the most efficient for Se accumulation in grain. So far, the effect and contribution of Se uptake of Se to the Se content of grain has not yet been clarified and the key stages of Se uptake and accumulation is very important to guide the scientific and effective application of Se fertilizer in theory and practice. On the other hand, the key physiological processes that cause differences in grain Se content between rice varieties are not yet clear, which is of great importance for understanding the difference mechanism between Se and non-Se in rice.

In this experiment, the effects of Se nutrient solution on the Se uptake, accumulation and distribution of high-Se and low-Se rice varieties were studied by different growing stages of rice. The aim of this paper is to identify the key growing stages of Se content in rice and to identify the differences in physiological characteristics of Se absorption, transport and distribution of different rice varieties, which is of great theoretical and practical significance for the enhancement of rice Se.

Materials and Methods

Plant Growth

The study was performed in the Plant Nutrition Lab of Southwest University in Chongqing, China. The tested varieties (provided by Institute of Soil Science, Chinese Academy of Sciences) were high-Se Xiushui 48 (grain Se content of $110 \mu\text{g kg}^{-1}$) and low-Se Bing 9652 (grain Se content of $32 \mu\text{g kg}^{-1}$) screened from 151 rice varieties in the previous study (Zhang *et al.*, 2006a). Sand culture experiment was applied the selected rice seeds were sterilized by 10% H_2O_2 for 15 min and rinsed with deionized water for three times then the seeds were sown on nylon net in plastic pots and cultured with 1/2 Kimura B nutrient solution for 15 days. In order to overcome the defect of hydroponic experiment, especially the biomass of the later stage would be too large to be fixed, in this study, sand and hydroponic culture were used. The seedlings with consistent growth were selected and planted into 2.5 kg of clean quartz sand (confirmed no Se after acid soaking and cleaning) in the plastic pots which the bottom left a number of small holes to absorb nutrient solution. Then these plastic pots were put in a big pot filled with Kimura B nutrient solution. The nutrient solution is formulated using the Kimura B nutrient solution (mg L^{-1}): $(\text{NH}_4)_2\text{SO}_4$ 48.2, MgSO_4 65.9, KH_2PO_4 24.8, KNO_3 18.5, K_2SO_4 15.9, $\text{Ca}(\text{NO}_3)_2$ 59.9, Fe-EDTA $20 \mu\text{mol L}^{-1}$, H_3BO_3 2.86, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.08, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.22, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 1.81 and $\text{H}_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}$ 0.09. Throughout the growing stage of

rice the nutrient solution was changed every 3 days. The pH was maintained at 5.5 by KOH. The greenhouse conditions were: 14 h daytime with a light intensity of $300 \mu\text{mol m}^{-2} \cdot \text{s}^{-1}$, daytime temperature of 25°C , night time temperature of 20°C , relative humidity of 67%.

Experimental Design

A total of 5 treatments were designed: The 4 supplemented the Se-containing Kimura B nutrient solution (Se provided by selenite) in jointing stage, panicle initiation stage, flowering stage and milky stage (10, 25, 40 and 80 days after transplanting), respectively and 1 supplemented non-Se Kimura B nutrient solution as control. To ensure that the Se content in rice can be detected at the mature stage in the 7-day short term, the Se concentration in Se-containing Kimura B nutrient was up to 1.5 mg L^{-1} . According to pre-trial, this concentration of Se does not affect the yield of rice. Each growing stage has the supplied of Se only for 7 days, then quartz sand was replaced and non-Se Kimura B nutrient solution was replaced to continue culture until the rice mature. Each treatment was repeated 4 times, while the nutrient solution was replaced every 3 days.

Sample Collected

Rice were harvested after maturing, and separated to roots, stems (sheaths and stems), leaves and grains, rinsed with distilled water and then dried in a 70°C dryer. Determination of biomass and Se content was made.

Sample Analysis and Quality Control

Weighed 0.30 g of the ground plant sample and place in a digestion tube, added 5 mL mixed acid (HNO_3 : HClO_4 =4: 1), soaked for 12 h after digestion. Added 2.5 mL mixed acid after digesting under 60°C for 2 h and 100 for 1 h, added 2.5 mL 1:1HCl after digesting for 2 h under 170°C to achieve colorless solution accompanied by white smoke, heated under 100°C to colorless and white smoke obtained a constant volume of 25 mL after cooling for determination. After solution cooling, the Se content in the solution was determined by atomic fluorescence spectrometry (AFS, AFS-920, Beijing Jitian Instrument Co., Ltd., Beijing, China) (Zhang *et al.*, 2006b).

In order to verify the digestion process, the veracity and accuracy of the subsequent analysis, the tea standard sample (GBW07605, China Standard Research Center) and the blank and samples were digested at the same time Se recovery rate was 95–98% (Zhang *et al.*, 2006b).

Data Analysis

The Se transfer coefficient and distribution coefficient were calculated via following formula:

Se transfer coefficient = (Se content in the shoot/Se content in the root) \times 100% (Wan *et al.*, 2016).

Se distribution coefficient in shoot = (Se accumulation in the different organs in the shoot/Se accumulation in the shoot) × 100% (Zhang *et al.*, 2014)

Excel 2010 and SPSS 18.0 software were used for mapping and one-way analysis of variance and LSD (least significant difference method) statistical method was used for significant test.

Results

Effects of Se Supplied on Rice Biomass at Different Growing Stages

The Se concentration of 1.5 mg L⁻¹ is designed to ensure that the Se content in rice at the mature stage can be measured in the 7-day short term. From Table 1, it can be seen that the reduction of total biomass at different growing stages was not significant compared with the control group. The biomass of each rice organ at jointing stage and panicle initiation stage under the Se treatment was slightly lower than that of the control group, while almost no difference at flowering stage and mature stage. There was no significant difference in the Se content in rice under the two varieties of rice treatment. The results showed that the concentration of Se in the solution had little effect on the toxic effects of rice growth and development.

Effects of Se Supplied on Se Content in Different Parts of Rice at Different Growing Stages

Se supplied at different growing stages and the difference between high-Se and low-Se rice varieties can lead to significant differences in Se content in various organs of rice and there is a significant interaction between Se supplied stage and genotype (Table 2). The Se content in the root of high-Se Xiushui 48 with Se supplied at growing stage was significantly less than that of low-Se Bing 9652 but the Se content in the shoot and the organs of rice was opposite. In addition to the Se content in leaves of Xiushui 48 was slightly higher than that of Bing 9652 Se content in the other organs of Xiushui 48 was significantly higher than Bing 9652. The average Se content of the four growing stages with Se supplied in Xiushui 48 grains was 2.5 times of Bing 9652.

According to Table 2, the Se content in rice with Se supplied at flowering stage and milky stage is significantly lower than that at jointing stage and panicle initiation stage. The Se content in rice with Se supplied at milky stage is smaller than that of with Se supplied at flowering stage. The Se content of grains of Xiushui 48 and Bing 9652 with Se supplied at jointing stage was 3.3 and 3.6 times of that at milky stage, respectively. The Se content of roots of Xiushui 48 is the lowest when conducting Se supplied at jointing stage, while is the highest at panicle initiation stage; The Se content of roots of Bing 9652 is the lowest when conducting Se supplied at milky stage, while is the highest at flowering stage.

Effects of Se Supplied on Se Accumulation and Transfer in Different Parts of Rice at Different Growing Stages

The accumulation of Se in rice is the product of the Se content and the biomass of the organ. Seen from Table 3, Se supplied at different growing stages and the difference between high-Se and low-Se rice varieties can lead to highly significant difference of Se accumulation in rice plants and organs. There is a significant interaction between rice genotypes. The average Se accumulation of 48 Xiushui roots in 4 growing stages with Se supplied was significantly lower than that of Bing 9652. On the contrary, the average Se accumulation of 48 Xiushui roots in the shoot of rice (except leaves are slightly higher) was significantly higher than that of Bing 9652, where the difference of stems and grains was the most significant. The transfer coefficient of Se is the ratio of Se content in the shoot of rice to Se content in the roots, which can be used to reflect the ability of plants to transfer Se from roots to organs. The larger the value, the stronger the migration ability of Se in plants. The transfer coefficient of rice was also significantly different affected by the different growing stages with Se supplied or the differences in rice varieties (Table 4). The average transfer coefficients of stems, leaves and grains of Xiushui 48 in the 4 growing stages with Se supplied were all higher than that of Bing 9652, where the average transfer coefficient of grain is 4 times of Bing 9652. This shows that compared with the non-Se-rich variety Bing 9652 the Se-rich Xiushui 48 is more likely to transport Se out of the root for the shoot of rice and the grain, which is consistent with the difference in the accumulation of Se.

The Se accumulation of two varieties of rice with Se supplied at different growing stages was basically the same: with the post-pone of growing stages with Se supplied the accumulation of Se increased first and then decreased (Table 3). The maximum value of Se accumulation in roots with Se supplied was at flowering stage; the maximum value of Se accumulation in shoot of rice with Se supplied was at panicle initiation stage and the minimum value was Se supplied at milky stage. Taking grains as an example, Xiushui 48 and Bing 9652 with Se supplied at panicle initiation stage were 4.1 and 3.2 times of Se supplied at milky stage, respectively.

From the rice grain Se transfer coefficient of two varieties of rice with Se supplied at different growing stages, Se supplied at jointing stage and panicle initiation stage are significantly higher, followed by flowering stage and milky stage (Table 4). The transfer coefficients of Xiushui 48 and Bing 9652 with Se supplied at jointing stage are 3.7 and 2.6 times of Se supplied at milky stage, respectively. The above shows that the Se transfer coefficients of Se supplied at panicle initiation stage and jointing stage were significantly higher than that of Se supplied at flowering stage and milky stage.

Table 1: Biomass (g pot⁻¹) of whole plant and each organ of rice treated with 1.5 mg L⁻¹ Se for 7 days at different growth stages

Cultivar	Treatment stage	Total	Rice				
			Root	Shoot	Shoot		
					Stem	Leaf	Grain
Xiushui 48	Control	93.9 a	10.9 a	83 a	29.4 a	36.7 a	16.9 a
	Jointing stage	80.9 a	9.3 a	71.6 a	27.3 a	27.5 a	16.8 a
	Panicle initiation stage	81.5 a	9.6 a	71.9 a	26.5 a	28.5 a	16.9 a
	Flowering stage	89.9 a	10.5 a	79.4 a	28 a	35.2 a	16.2 a
	Milky stage	93.1 a	10.6 a	82.5 a	29.4 a	36.6 a	16.5 a
Bing 9652	Control	92.0 a	9.3 a	82.7 a	29.5 a	36.5 a	16.7 a
	Jointing stage	79.0 a	8.6 a	70.4 a	26.5 a	27.3 a	16.6 a
	Panicle initiation stage	81.6 a	8.6 a	73.0 a	27.2 a	29.6 a	16.2 a
	Flowering stage	86.5 a	9.5 a	77 a	27.5 a	33.2 a	16.3 a
	Milky stage	89.7 a	9.6 a	80.1 a	29.1 a	34.6 a	16.4 a

Note: Values followed by the same letters within each column are not different significantly at the 0.05 probability level among treatments within cultivar. The same as below

Table 2: Se concentration (mg kg⁻¹) in each organ of rice plant treated with 1.5 mg L⁻¹ Se for 7 days at different growth stages

Cultivar	Treatment stage	Rice				
		Root	Shoot	Shoot		
				Stem	Leaf	Grain
Xiushui 48	Jointing stage	34.56 b	4.45 a	10.97 a	1.34 a	1.15 a
	Panicle initiation stage	43.24 a	4.69 a	10.21 a	1.23 a	1.43 a
	Flowering stage	42.12 a	3.43 b	8.45 b	0.76 b	0.84 b
	Milky stage	38.65 b	2.55 b	5.34 c	0.65 c	0.35 c
	Mean value	39.64	3.78	8.74	0.99	0.94
Bing 9652	Jointing stage	65.76 a	3.23 a	5.45 a	0.87 a	0.44 a
	Panicle initiation stage	65.45 a	2.65 b	5.36 a	0.86 a	0.48 a
	Flowering stage	75.56 a	2.43 b	4.89 b	0.54 b	0.34 b
	Milky stage	56.76 b	1.45 c	3.43 c	0.42 c	0.15 c
	Mean value	65.88	2.44	4.78	0.67	0.38
Significance analysis	Cultivar (C)	16.3**	17.5**	24.3**	3.6ns	24.3**
	Treatment (T)	35.3**	44.3**	34.5**	23.4*	25.7**
	C*T	31.9**	35.3**	31.2**	31.5**	23.7**

Note: * and ** denote significance at 0.05 and 0.01 probability level, respectively. Because the rice of control has not detected out Se, it is not display in the table. The same as below

Table 3: Se accumulation (μg pot⁻¹) in whole plant and each organ of rice plant treated with 1.5 mg L⁻¹ Se for 7 days at different growth stages

Cultivar	Treatment stage	Total	Root	Shoot	Shoot		
					Stem	Leaf	Grain
Panicle initiation stage	709.2 c	371.9 a	337.3 a	277.7 a	36.4 a	23.2 a	
Flowering stage	668.4 d	400.1 a	268.3 a	232.4 a	22.2 b	13.7 c	
Milky stage	554.6 e	371.0 a	183.6 b	155.4 b	22.5 b	5.7 d	
Mean value	629.2 d	351.4	277.8 a	233.6	28.8	15.4	
Bing 9652	Jointing stage	643.7 d	480.0 b	163.7 a	132.4 a	22.2 a	8.1 a
	Panicle initiation stage	802.9 b	628.3 a	174.6 a	142.0 a	24.5 a	8.1 a
	Flowering stage	954.8 a	793.4 a	161.4 a	136.9 a	19.0 b	5.5 b
	Milky stage	720.4 c	601.7 a	118.7 b	100.8 b	15.4 c	2.5 c
	Mean value	780.5	625.9	154.6	128.0	20.3	6.3
significance analysis	Cultivar (C)	34.5**	25.4**	26.3**	123.3**	6.7ns	32.2**
	Treatment (T)	43.2**	34.2**	32.5**	35.4**	33.3*	27.6**
	C*T	27.9**	26.4**	43.4**	42.2**	42.3**	42.1**

Effects of Se Supplied on Se Distribution Coefficient in Different Shoot of Rice at Different Growing Stages

The Se distribution coefficients in the shoot of rice was as follows: stem>leaf>grain, indicating that the accumulation of Se in the different growing stages of the shoot of rice was

mainly distributed in the stems, followed by leaves and the least distributed in the grains. It can be seen from Fig. 1 that the Se distribution coefficients of upper Xiushui 48 leaves with Se supplied at growing stages are less than that of Bing 9652 and the Se distribution coefficients of stems and grains of Xiushui 48 are larger than Bing 9652.

The average Se distribution coefficients of the stems and grains of Xiushui 48 with Se supplied were 84.1% and 5.5%, respectively in the 4 growing stages 82.8% and 4.0% for Bing 9652. This indicates that Xiushui 48 can transfer more Se from shoot to grain.

Discussion

Jointing stage and panicle initiation stage is the key stages to enhance the Se content in rice and the Se content with Se supplied at panicle initiation stage is greater than that of Se supplied at jointing stage (Table 2). The period between jointing stage and panicle initiation stage is the most rapid period for rice nutrition growth with strong metabolism of carbon and nitrogen and the fastest biomass accumulation (Zhang *et al.*, 2017), therefore, this stage is the key to the absorption of nutrients and water in rice and 50% of the nutrient accumulation in rice is completed at this stage (Guo *et al.*, 2002; Liu *et al.*, 2007) the absorbed and stored nutrients is the basis for later grain yield. For Se, from jointing stage to panicle initiation stage is also an important stage for its nutrient storage and most of the grain Se in late growth stage comes from the accumulation of Se stored in vegetative organs. The soil culture test results of Zhou *et al.* (2007) showed that the accumulation of Se in the vegetative growth stage accounted for 65–77% of the total Se accumulation, which also indicated that the vegetative growth period was the key stage of rice Se absorption. It can be seen from Table 3 that Se supplied at early growth period (jointing stage and panicle initiation stage) is more favorable for grain Se accumulation. Seen from the transfer coefficient of Se in shoot (the ability to transfer Se from the roots to shoot) the Se transfer coefficient of rice grain with Se supplied at jointing stage and panicle initiation stage was significantly higher than that of flowering stage and milky stage (Table 4). Only a small part of the Se accumulation in the shoot of Xiushui 48 and Bing 9652 is distributed to the grains after flowering stage (Fig. 1), indicating that the Se absorbed before the panicle initiation stage is more important for increasing the Se content in rice. The distribution and migration of nutrients in rice were closely related to the stages of rice growth and development. It is reported that the nutrients such as phosphorus and sulfur stored in vegetative organs in jointing stage and panicle initiation stage will later be supplied to the reproductive organs of rice to ensure its yield (Guo *et al.*, 2002). Similarly, the increase of Se accumulation in the vegetative organs of rice at this stage can ensure that more Se will be transferred to the reproductive organs (especially the grains) during the reproductive growth period and that the sufficient supply of Se in the early stages of rice production is very important for improving the Se content. To sum up, the period between jointing stage and panicle initiation stage is very important to improve the absorption and accumulation of grain Se content, which is very important to improve the grain Se content.

Table 4: Transfer coefficient (%) of Se in each organ of rice plant treated with 1.5 mg L⁻¹ Se for 7 days at different growth stages

Cultivar	Treatment stage	Shoot		
		Stem	Leaf	Grain
Xiushui 48	Jointing stage	31.7 a	3.9 a	3.3 a
	Panicle initiation stage	23.6 b	2.8 a	3.3 a
	Flowering stage	20.1 bc	1.8 b	2.0 b
	Milky stage	13.8 c	1.7 b	0.9 c
	Mean value	22.3	2.6	2.4
Bing 9652	Jointing stage	8.3 a	1.3 a	0.8 a
	Panicle initiation stage	8.2 a	1.3 a	0.7 a
	Flowering stage	6.5 b	0.7 b	0.4 b
	Milky stage	6.0 b	0.7 b	0.3 b
Significance analysis	Mean value	7.3	1.0	0.6
	Cultivar (C)	58.5**	57.3**	38.6**
	Treatment (T)	52.3**	43.1**	33.1**
	C×T	36.5**	29.6**	40.3**

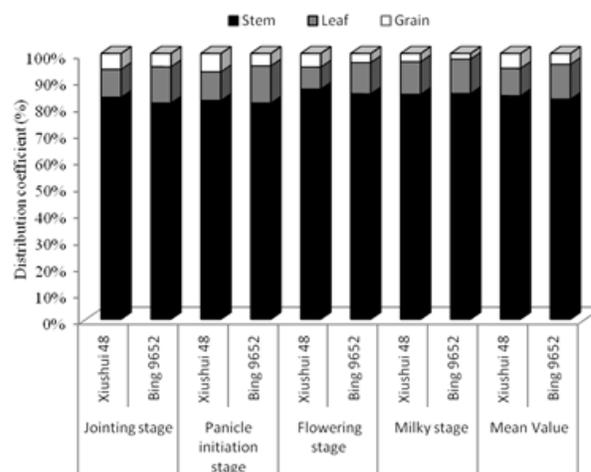


Fig 1: Distribution coefficient (%) of Se in each organ of shoot treated with 1.5 mg L⁻¹ Se for 7 days at different growth stages

In the practice of production, the application of soil Se fertilizer prior to the panicle initiation stage is more conducive to improve the Se content in rice meanwhile other agronomic biological measures will also improve the grain Se content.

Se accumulates from the soil to the grains through three physiological processes, namely the roots absorb Se from the soil solution, transport it from the roots to the shoot through the xylem and then transfer it from the shoot to the rice grains (Liu *et al.*, 2007; Carey *et al.*, 2012). The different transporting rate and quantity of Se may result in different Se content in rice. Therefore, it is very important to understand the effects of the three physiological processes on the accumulation of Se in rice and to understand the difference mechanism of grain accumulation in rice. From Table 3, it can be concluded that the accumulation of Se at different growing stages of high-Se Xiushui 48 was significantly lower than that of Bing 9652, indicating that

the absorption capacity of high-Se Xiushui 48 was less than that of low-Se Bing 9652. This also suggests that the difference between the two varieties in the Se content in rice is not due to differences in the ability of the roots to absorb Se from the soil. The results showed that the Se content and Se accumulation in Xiushui 48 grains were about 2.5 times that of Bing 9652 and the transfer coefficient of each organ was almost 3 times that of Bing 9652 and the transfer coefficient of grain was even 4 times (Tables 3 and 4). The key to the high Se content in Xiushui 48 grains lies in the post two physiological processes, ie Se being transferred from the roots to the shoot through the xylem and then transported from the shoot to the rice grains. Zhou *et al.* (2014) found that the Se concentration in the xylem of high-Se rice was twice as high as that of the low-Se rice varieties but the Se content of the two grains was different by three times, indicating that the Se being transferred from the roots to the shoot is not the only reason for the difference in Se accumulation in different rice varieties. The experiment of Carey *et al.* (2012) showed that leaves of rice are the most active organs of assimilation and an important source for grain enrichment. Therefore, the flag leaves are an important Se source for the accumulation of Se in shoot and grains and the Se is transferred mainly through the phloem to the grains. The results showed that the distribution coefficients of Se in the leaves of Xiushui 48 and Bing 9652 were 10.3 and 13.1%, respectively and 5.5 and 4.0% in the grains, respectively (Fig. 1), indicating that the Se in Xiushui 48 leaves is more easily transported into the grains. Therefore, the re-transport process of rice Se from leaf to grain through phloem is also the key physiological process to determine the accumulation of Se in rice. In summary, the main reason for the difference in Se accumulation in high-Se Xiushui 48 and low-Se Bing 9652 grains is that in the latter two physiological processes, Se is transported from the roots to the shoot through the xylem and re-transported from the shoot to the grains through the phloem. Some proteins in rice are responsible for the above two Se transport physiological processes. Studies have shown that silicon transport protein (OsNIP 2; 1) regulates the selenite uptake and transport to the shoot (Zhao *et al.*, 2010). The selenite in rice is absorbed by the phosphate transporter (OsPT2) (Zhang *et al.*, 2014a). The high-affinity sulfur transport protein is mainly responsible for the transport of sulfur in the plant. Sulfur and Se belong to the same main family and have similar transport routes. Terry *et al.* (2000) reported that excessive expression encoding high-affinity sulfur transport protein increased the Se accumulation of Indian mustard twice and this implies that high-affinity sulfur transport protein regulates the accumulation of selenate in plants. From the above, we can conclude that the activity and expression quantity of silicon transport protein and high-affinity sulfur transport protein in the stems and flag leaves of high-Se Xiushui 48 may be higher than that of low-Se Bing 9652 in the process of grain maturation but the scientific hypothesis needs to be further validated by trial. In general, inorganic Se enters rice and is assimilated into

organic Se in the roots (Zhou *et al.*, 2010). Leduc *et al.* (2006) confirmed that the double transgenic ATP thiolase and Se cysteine methyltransferase plants absorb Se nine times more than that of wild types. Then, if the activity and expression quantity of the activity and expression quantity in low-Se Xiushui 48 were significantly higher than those in low-Se rice Bing 9652, so far unclear. In conclusion, the molecular mechanism of the accumulation of Se in two varieties of rice is the focus of the next step.

Jointing stage and panicle initiation stage are the most key stages to enhance the Se content in rice and it is beneficial to increase Se fertilizer or to improve the bio-availability of soil Se via agronomic measures in this period, which is of great significance to formulate the practice plan of high-Se rice production. Leaf is an important “source” of Se accumulation in grains then the delay in leaf senescence can improve the transfer of leaf nutrients to the grain. Studies have shown that delaying leaf senescence significantly increased the redistribution of several elements such as nitrogen, iron, copper, manganese and zinc from vegetative organs to rice grains (Sheehy *et al.*, 2004; Gamett and Graham, 2005; Yan *et al.*, 2010). This study shows that the transfer coefficient of Se in Xiushui 48 leaves is almost three times that of Bing 9652 (Table 4). The fact that Xiushui 48 has a higher transfer coefficient of grain Se may be related to its slower leaf senescence rate, which needs further verification. In the practice, many agronomic factors such as nitrogen, potash fertilizer application and appropriate water management measures can delay leaf senescence (Li *et al.*, 2012; Zhou *et al.*, 2013). At the same time, the increase of light intensity at the late growth stage of rice will cause the leaf area of rice to increase and delay the senescence of leaves (Gamett and Graham, 2005), which will make the accumulation of Se in rice grains more favorable for redistribution of leaf Se. Then the nitrogen fertilizer, potash fertilizer operations, appropriate water management and supplied of light and other measures at the growing stage of rice may delay the leaf senescence, so that more leaf Se elements are redistributed into the grains, thereby increasing the grain Se content.

Conclusion

The studies led to conclusion that the period between jointing stage and panicle initiation stage is the key period to improve the Se content in rice and the Se content of rice with Se supplied at panicle initiation stage is greater than that at jointing stage. And we also indicated that the process of Se absorption by roots is not the key process causing differences in grain Se content. The physiological process of rice Se being transported from the root to the shoot and the distribution of Se from the flag leaf to the grain are the key physiological processes determining the accumulation level of grain Se and also the root cause of the difference between high-Se and low-Se rice varieties. Thus, it is conducive to improve the Se content in rice by increasing Se fertilizer or

taking agronomic measures to improve the bioavailability of soil Se from jointing stage to panicle initiation stage. By applying appropriate agronomic measures to delay the senescence of leaves can reallocate more Se into the grains so as to improve the Se content in grains, which is of great theoretical and practical significance for the biological enhancement of Se in rice.

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