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Characterization of selenium accumulation of different rice genotypes in Chinese natural seleniferous soil

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Abstract: In this study, the difference of Se content in brown rice of different rice genotypes was evaluated on natural seleniferous soil. Firstly, the Se content of brown rice in 80 rice germplasm resources was determined, which ranged from 0.0249–0.1426 mg/kg, showing obvious differences. Next, two cultivars with a significant difference in Se content in brown rice, i.e., cv. Wuyangeng (high) and cv. IR68144 (low), were used to study the distribution pattern of Se in different organs. Moreover, the physiological mechanism of the Se content diversity in brown rice of the two cultivars was explored preliminarily. The results showed that the Se content of cv. Wuyangeng was 2–3 times higher compared to cv. IR68144. However, the Se contents of cv. IR68144 root and leaf were significantly higher than that of cv. Wuyangeng under both natural soil environment and artificial nutrient solution culture. Cv. IR68144 also had a stronger root Se accumulation coefficient and shoot Se transfer coefficient. Consequently, it can be inferred that the stronger Se transfer ability from stem to grain was the key reason for the higher Se content in brown rice cv. Wuyangeng than in cv. IR68144.

Keywords: selenium biofortification; rice cultivar; *Oryza sativa* L.; plant organ; limiting step

Selenium (Se) has many physiological functions on human body, such as anti-aging, anticancer and detoxification (Fairweather-Tait et al. 2011, Hatfield et al. 2014). It had been identified by the World Health Organization and China Medical Association as the third major micronutrient element for health care, but there is a general shortage of Se in the world (Williams et al. 2009). According to the report of the nutrition survey by the Chinese Nutrition Society, the intake of Se of Chinese adults was only 26.63 μg/days. It was far away from the Se intake of 40–240 μg/person/day recommended by the China Preventive Medicine Center and 50–200 μg/person/day proposed by the National Academy of Sciences of the United States.

Satisfying Se intake through daily diet is an important way to maintain residents' health. Rice is the staple food of 60% Chinese residents, and thus low Se content in rice has become an important factor affecting Se intake in China (Yang et al. 2010, Li et al. 2014). For instance, the nationwide sample survey showed that the problem of Se deficiency was widespread in the China's rice market (Chen et al. 2002, Zeng et al. 2008). Therefore, it was an ideal way to supplement Se by increasing Se intake from diet via Se-enriched rice produced from natural seleniferous soil (Wu et al. 2015).

It was quite rich in the natural seleniferous soil resources in the Jiangxi province, China. At present,

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4362 km^2 of seleniferous soil was found in the north of Jiangxi province and the Se content of the soil was moderate. Hence it was suitable for the development of natural Se-rich rice. However, it was found that the Se content of Se-rich rice produced by these seleniferous soils was mostly in the range of 0.04–0.10 mg/kg, which met the Chinese national standard of Se-rich rice, but could not reached the ideal level for Se supplement. Our previous investigations and studies had shown that the poor Se-enrichment ability of local rice cultivars was an important reason for this problem (data not shown).

The present study investigated the difference of the Se content in various rice genotypes and analysed the distribution characteristics of Se in different parts of rice, with the aim of screening the Se enriched rice germplasm resources and identifying the key step of Se absorption in rice with distinct Se accumulative ability. It is necessary to study the Se absorptive and transferring process in order to further reveal the limiting factor of molecular biological techniques to increase the Se content in rice grain.

MATERIAL AND METHODS

Field experiment with 80 rice cultivars. A total of 80 conventional rice (*Oryza sativa* L.) cultivars were tested. All materials were grown as mid-rice in 2016 at the natural seleniferous paddy field of the Fengcheng City, Jiangxi province. In the experiment, germinated seeds were sown in a seedling bed, and seedlings were transplanted to a paddy field 30 days later with single plant per hill. Each plot consisted of 6 rows with a 20 cm row space. Ten individuals were planted per row with a 15 cm space, and all plots were arranged in a completely randomized block design with 3 replications. Field management essentially followed the normal agricultural practices, with fertilizer applied (per hectare) as follows: 225 kg urea and 300 kg potassium dihydrogen phosphate as the basal fertilizer; 105 kg urea at the tillering stage; and 105 kg urea and 120 kg potassium chloride at the booting stage. In the mature period, 500 g rice grain was harvested from each plot and then dried to 13.5% moisture content. At last, brown rice was separated with a hulling machine (China Reserve Grain Management Group Co. Ltd., Beijing, China).

Field experiment of cvs. Wuyangeng and IR68144. In the consecutive three years of 2015–2017, cv. Wuyangeng (Japonica rice) and cv. IR68144 (Indica rice) were planted as mid-rice in the Fengcheng City and Gao'an City, Jiangxi province, respectively. All of the plots distribution, field management and rice harvesting methods were the same as the field experiment with 80 rice cultivars. Soil fertility and Se content were measured at 10 cm depth in the test field before planting rice. A total of 4 points were obtained and the results were averaged (Table 1).

The rice plants of cvs. Wuyangeng and IR68144 were harvested at tillering stage (49 days after transplanting (DAT)), heading stage (79 DAT) and yellow maturity stage (107 DAT) at the Fengcheng test site in 2017. The whole growth period of the two rice cultivars was about 138 days. The plants were washed with tap water and rinsed with distilled water for 2 times, and then were placed in oven for 1 h at 100°C and dried at 60°C until constant weight. Afterwards, the roots, stems and leaves of the plants were cut separately, and brown rice and husk were prepared by a hulling machine.

Rice seedlings culture. Plump rice seeds of cvs. Wuyangeng and IR68144 were selected. After that, the seeds were soaked for 48 h in deionized water at room temperature and sprouted at 30°C for 24 h. Two hundred uniform germinating seeds were evenly and sparsely sown in a plastic basin (30 × 25 × 8 cm) filled with quartz sand. Ten days later, Yoshida nutrient solution (Yoshida et al. 1972) was added to the seedlings at about two leaf stage. The Yoshida nutrient solution contained 0, 0.1, 0.2, 0.5 and 1 mg/L Se, provided by $NaSeO_3$. All treatments were repeated 3 times. The pH was adjusted to 5.5 every 2 days with 1 mmol/L NaOH and 1 mmol/L HCl while evaporated water was replenished with deionized water every day, and also nutrient solution was replaced every 4 days. The seedlings were cultured

Table 1. Physical-chemical properties and total selenium (Se) content in the soil

Experimental site	Soil type	pH	Organic carbon (g/kg)	Cation exchange capacity ($cmol_+$/kg)	Se (mg/kg)
Fengcheng	Paddy soil	5.6 ± 0.1	35.1 ± 1.3	24.3 ± 0.8	0.56 ± 0.06
Gao'an		5.8 ± 0.1	27.3 ± 1.8	26.8 ± 0.6	0.42 ± 0.05

Data are mean ± standard deviation (*n* = 4)

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Table 2. Statistical analysis of brown rice selenium (Se) contents of 80 rice cultivars

Item	Maximum	Minimum	Average	Standard deviation	Coefficient of variation (%)	Skewness	Kurtosis
	(mg/kg)						
Se content	0.1426	0.0249	0.0655	0.0254	38.78	0.848	0.615

in growth chambers under a diurnal cycle of 12 h at 30°C for daytime and 12 h at 25°C for night. Humidity was 75%, and the light intensity at the plant tops was approximately 300 μm/m^2/s photosynthetic photon flux. The rice seedlings were harvested after 9 days treatment.

Determination. Rice organs, such as roots, stems, leaves, husks and brown rice, were crushed by high-speed grinder, then sifted through 60 meshes, after that sealed and stored for reserve. Samples were dried in a drying oven at 40°C until constant weight before test. The roots and shoots of the rice seedlings were determined as fresh. The content of Se in rice was determined by cultivar generation atomic fluorescence spectrometry according to GB 5009.93-2017 Determination of Selenium in Food, National Standards for Food Safety promulgated by China. A standard material of rice (GBW10045 (GSB-23), 0.053 mg/kg Se) and a blank were simultaneously digested with the test samples, with an average Se recovery between 90% and 95%.

Data analysis. All samples were measured in 3 parallels and the results were averaged. The software Excel (Redmond, USA) was employed to process the initial experimental data and plotting. SPSS 19 software (Amund City, USA) was used to analyse the data.

RESULTS AND DISCUSSION

Exploration and utilization of Se-rich rice germplasm resources. The content of Se in brown rice of 80 rice cultivars was obviously different, and the range of variation was 0.0249–0.1426 mg/kg (Table 2). It was shown that the Se content of brown rice fit the normal distribution (Figure 1). Among the 80 selected rice cultivars, the Se contents of cvs. IR19735, Heishuai, Wuyangeng, Heizhan 96 and Yunanheixiannuo were the top five, which could be used as germplasms for breeding new cultivars of Se-rich rice.

It was an effective way to increase the Se content in rice through biofortification and improving Se intake by diet to alleviate Se deficiency for humans (Oliverira et al. 2015). Se enriched agricultural products can be obtained by supplement of Se from soil application and foliar spray (Zhou et al. 2007a, Boldrin et al. 2013). However, the utilization rate of Se in these method was only 5–30% (Eich-Greatorex et al. 2007), and Se content of rice was also difficult to control accurately. Additionally, improper operation can adversely affect the quality of agricultural products and long-term application may also cause environmental problems (Hartikainen 2005, Luo et al. 2011). Consequently, utilizing natural seleniferous soil was the ideal way to improve the Se content in rice. Although some areas suitable for producing natural Se-rich agricultural products were found in China, their yields were limited and it was difficult to fully meet human needs.

Rice was a kind of crop with low Se content, but the difference in Se accumulation ability among diverse genotypes was obvious (Jiang 2002, Zhang et al. 2006a). These studies implied that screening and utilization of Se enriched rice germplasm was an important way to improve the Se content in rice. In this study, the difference of Se content among cultivars was obvious which further verified the results of previous studies. On the other hand, the content of Se in most cultivars was higher than the Chinese national standard of Se-enriched rice (0.04 mg/kg), which also indicated the important role of soil Se

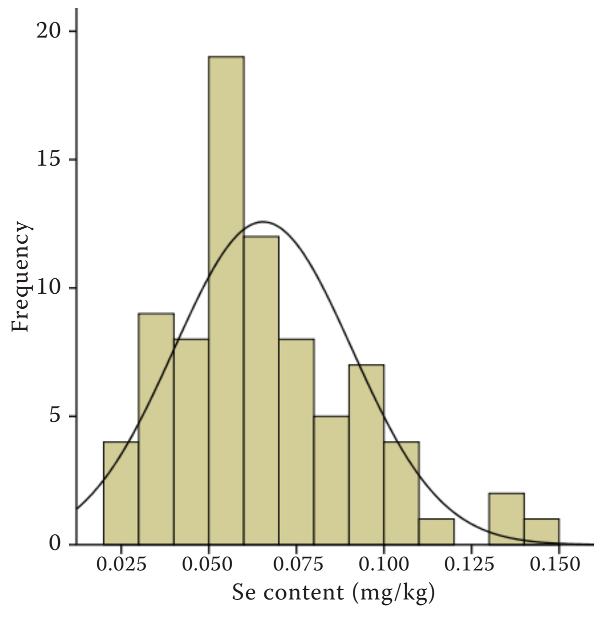

Figure 1. Selenium (Se) contents distribution of 80 brown rice cultivars

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Table 3. Selenium (Se) content in brown rice of cvs. IR68144 and Wuyangeng (mg/kg)

Cultivar	2015		2016		2017	
	Fengcheng	Gao'an	Fengcheng	Gao'an	Fengcheng	Gao'an
IR68144	0.078 ± 0.008	0.043 ± 0.005	0.068 ± 0.009	0.054 ± 0.007	0.081 ± 0.006	0.076 ± 0.009
Wuyangeng	0.132 ± 0.020	0.112 ± 0.013	0.186 ± 0.017	0.135 ± 0.010	0.157 ± 0.020	0.136 ± 0.014

Data are mean ± standad deviation (n = 3)

content. Accordingly, comprehensive utilization of natural seleniferous soil and rice germplasm resources with strong Se-accumulating ability was an ideal way to develop natural Se-rich rice products.

Characteristics of Se accumulation in cvs. Wuyangeng and IR68144 during the whole growth period. It was found that the Se contents in brown rice of cvs. Wuyangeng and IR68144 were relatively stable in the three consecutive years (2015–2017) and two locations (Fengcheng and Gao'an). Furthermore, the Se content of cv. Wuyangeng was about 2–3 folds that of cv. IR68144 (Table 3). The results of the Se content in rice organs from the yellow ripening stage in the Fengcheng experimental site in 2017 showed that the content of Se in cv. Wuyangeng brown rice and husk was higher than that in cv. IR68144, but it was opposite in root, stem and leaf (Figure 2). Previous studies had shown that the distribution of Se in rice plants grown in seleniferous soil was root > stem and leaf > grain (Jiang et al. 2015), and in the upper part, it was straw > bran > polished rice > husk (Sun et al. 2010). In this study, the distribution of Se content in different parts of cv. Wuyangeng rice was leaf > brown rice > root > stem ≈ husk, and in cv. IR68144 it was leaf > root > stem > brown rice > husk (Figure 2). Although the distribution of the Se content was different between the two cultivars, both cultivars showed leaf > root > stem in plant vegetative organs and brown rice > husk in grains. Previous studies showed that the Se content in the rice root was the highest, but in this research, the Se content in the leaves of both rice cultivars was the highest. This may be related to the genetic background of the tested rice, and the Se content in the soil also affects the Se distribution (Zhou et al. 2007b). According to the distribution of Se content in various organs, Se in cv. IR68144 was mostly concentrated in leaves and roots, while Se in cv. Wuyangeng was more transferred to grains. Compared with cv. Wuyangeng, cv. IR68144 had higher root-stem transfer coefficient, but lower stem-leaf and stem-grain transfer coefficient (Figure 3). That is, the Se in the stem of cv. Wuyangeng had higher grain transfer ability.

Comparing the Se content change dynamics of the roots, stems and leaves of the two rice cultivars at tillering stage, heading stage and mature stage, it was found that the Se content of cv. IR68144 in the three growth stages was significantly higher than that of cv. Wuyangeng, and the dynamic change direction of Se content of the two cultivars was broadly consistent (Figure 4). From tillering to heading stage, Se content in roots decreased, whereas Se content in leaves increased rapidly (Figure 4), which indicated that a large amount of Se in roots was transferred to leaves. Heading to maturity stage was the period of rice grain growth and development, and also the main period for plants absorbing Se. About 65–77% Se of rice whole plant was absorbed and accumulated at this stage (Zhou et al. 2007b). During rice maturation, Se in roots and leaves was transported to grains through stems to achieve the purpose of the Se accumulating from vegetative organs to reproductive organs. From heading to mature stage, the Se content in roots and stems of the two cultivars increased, while the Se content in leaves remained stable (Figure 4). Therefore, it can be inferred that Se in rice grains may be mainly derived from root absorption and transferred from stem to grain when it was sufficient of Se in soil. Meanwhile, the leaf may act as a reservoir and replenish Se to the grain when the Se source of root was insufficient.

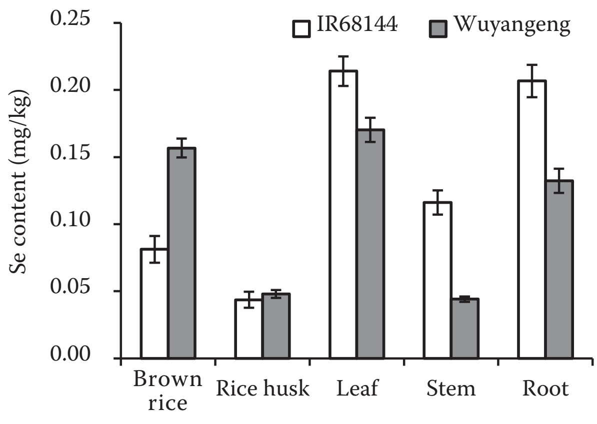

Figure 2. Selenium (Se) contents of rice organs of cvs. IR68144 and Wuyangeng at yellow ripening period

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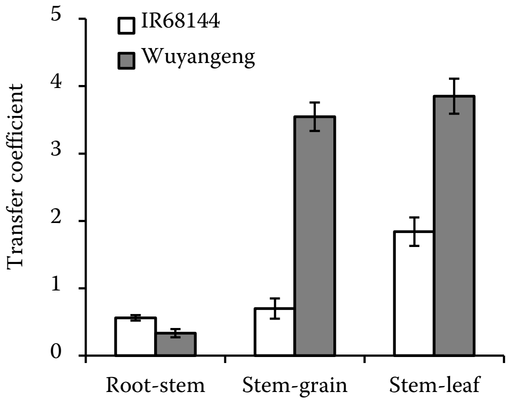

Figure 3. Selenium (Se) transfer coefficient of organs in mature rice plants

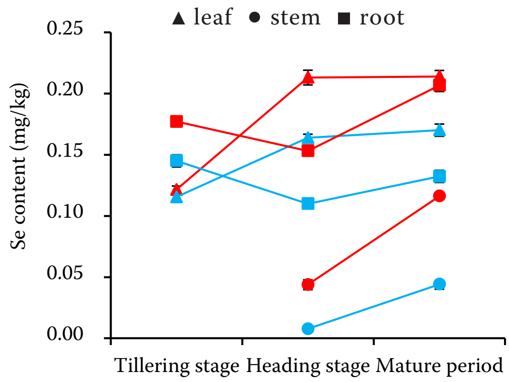

Figure 4. Change trends for selenium (Se) contents of rice organs from cvs. IR68144 (red) and Wuyangeng (blue) in tillering stage, heading stage and mature period

Characteristics of Se accumulation in rice seedlings of cvs. Wuyangeng and IR68144. With the rise of Se concentration in the nutrient solution, both the contents of Se in roots and shoots of cvs. IR68144 and Wuyangeng seedlings increased rapidly (Figure 5). However, the Se content in the root and shoot of cv. IR68144 was significantly higher than that of cv. Wuyangeng (Figure 5). The Se enrichment coefficient of cv. IR68144 root was higher than that of cv. Wuyangeng noticeably under all cultural conditions of diverse Se concentration in the nutrient solution (Figure 6a). It was illustrated that the ability of Se accumulation in cv. IR68144 root was distinctly stronger than that of cv. Wuyangeng. Nevertheless, their Se enrichment coefficient decreased gradually with the rise of Se concentration in the nutrient solution (Figure 6a). Mostly, the shoot transfer coefficient of cv. IR68144 was higher than that of cv. Wuyangeng (Figure 6b). Furthermore, there was no palpable correlation between the shoot transfer coefficient and the Se content of root (Figure 6b). This signified that the transfer of Se from root to shoot was not a restrictive step for Se accumulation in rice.

The Se content in cv. Wuyangeng grain was remarkably higher than that of cv. IR68144, but Se contents of cv. IR68144 vegetative organs, such as mature root, stem and leaf (in field condition), and seedling root and shoot (in hydroponic conditions), were higher than that of cv. Wuyangeng evidently (Figures 2 and 5). Hence, cv. IR68144 has a stronger ability to absorb Se in root and transfer Se to shoot. Nevertheless, cv. Wuyangeng has the ability to transport Se from stem to grain more efficiently. This may be the primary cause for accumulation of more Se in the grain of cv. Wuyangeng. It was significantly different from the results provided by other researcher that the root Se uptake ability of rice cultivar with high brown rice Se content was also strong (Zhang et al. 2006b). Consequently, the accumulation of Se in rice grain was a complex process, and there may be some differences in the limiting steps affecting the accumulation of Se in different rice cultivars, but the stronger ability of Se transfer from stem to grain may be an important option.

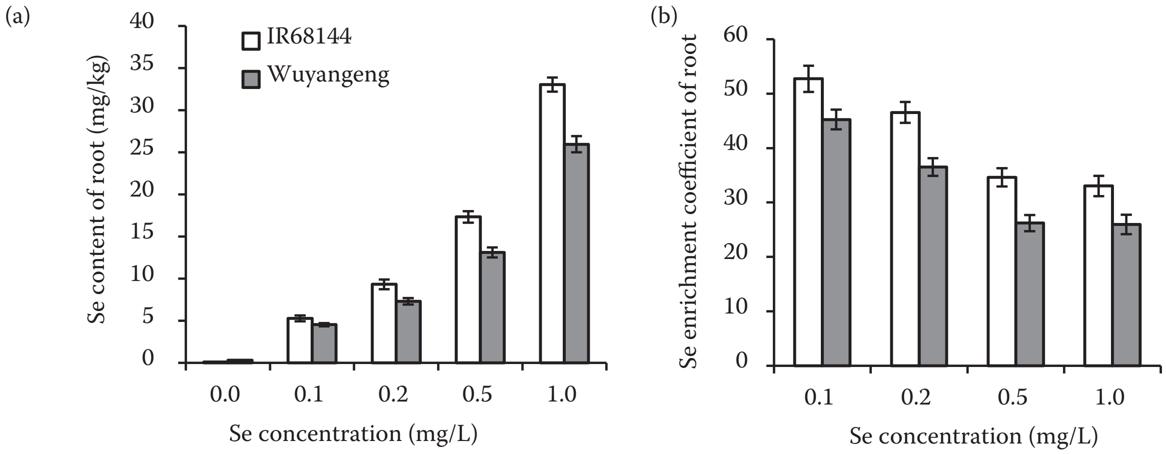

Figure 5. Selenium (Se) contents of rice (a) roots and (b) shoots from cvs. IR68144 and Wuyangeng in hydroponic culture

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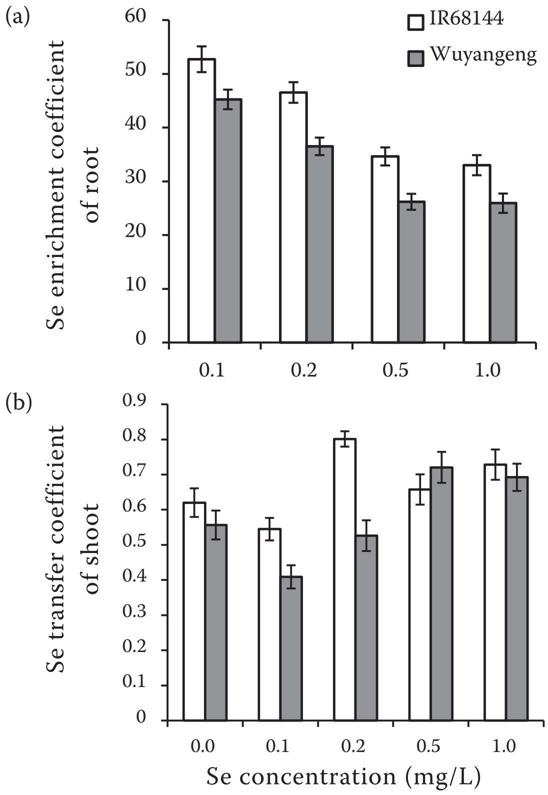

Figure 6. Selenium (Se) (a) enrichment coefficient from nutrient solution to root and (b) transfer coefficient from root to shoot

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