JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 2079–2084

Effect of Foliar Application of Zinc, Selenium, and Iron Fertilizers on Nutrients Concentration and Yield of Rice Grain in China

YONG FANG, LIN WANG, ZHIHONG XIN, LIYAN ZHAO, XINXIN AN, AND QIUHUI HU*

Key Laboratory of Food Processing and Quality Control, College of Food Science and Technology,
Nanjing Agricultural University, Nanjing 210095, People's Republic of China

---

Zn, Se, and Fe levels in 65 Chinese rice samples were investigated, and the results indicated that these micronutrients contents of rice products from different location varied considerably. The mean contents of Zn, Se and Fe in these rice samples were 21.5 ± 1.8, 0.020 ± 0.012, and 12.4 ± 4.3 mg kg^{-1}, respectively, which were too low to meet the micronutrient demands for the population feeding on the rice as staple. A field orthogonal experiment L_9 (3^4) was conducted on rice cultivar Wuyunjing 7, to evaluate the effect of Zn, Se, and Fe foliar fertilization on the concentration of these micronutrients, yield, and protein and ash content of rice grain. The results indicated that Zn and Se were the main variables influencing the Zn, Se, and Fe content of rice, and the optimal combination of fertilization for enhancing these micronutrients was 0.90 kg ha^{-1} Zn, 0.015 kg ha^{-1} Se, and 0.90 kg ha^{-1} Fe. Under the optimal application condition, Zn, Se, and Fe content of rice could be significantly increased by 36.7%, 194.1%, and 37.1%, respectively, compared with the control, without affecting grain yield and protein and ash content of rice products. Moreover, in the confirmation experiment on rice cultivar Ninggeng 1, the optimal fertilization could increase the Zn, Se, and Fe content of rice up to 17.4, 0.123, and 14.2 mg kg^{-1}, respectively.

KEYWORDS: Zinc; iron; selenium; rice; foliar application

INTRODUCTION

Micronutrients, such as zinc (Zn), selenium (Se), and iron (Fe) play an important role in human growth, development, and maintenance of the immune system (*1*). The Recommended Dietary Allowance (RDA) of Zn, Se, and Fe are respectively 15 mg, 70 μg, and 10 mg per day for man. However, micronutrient deficiencies affect more than half of the world's population, especially women and preschool children. Iron is one of the most important micronutrients, and approximately 2 billion people suffer from iron deficiency worldwide, which has often been claimed to be the predominant cause of anemia (*2*). Zinc deficiency is also considered to be quite common and affects newborn, children, pregnant women and old people (*3*). Selenium deficiency is another serious problem which causes Keshan and Kashin-Beck diseases in China (*4*).

Micronutrient malnutrition in human in developing countries is derived from deficiencies of these elements in staple food, such as rice or wheat in Asia and maize or sorghum in Africa. It is believed that increasing the micronutrient concentrations in these crops could increase the dietary intake of these elements in these regions significantly (*5, 6*). However, these plants contain "antinutrients" compounds, such as phytate, which are stored as phytate–mineral complex and limit the absorption of micronutrients especially zinc and iron in the digestive tract (*5*). Thus, it is very important to increase the amount of bioavailable micronutrient, which is dependent both on intake and absorption of micronutrients. Recently, many assessments of micronutrients bioavailability have been proposed, such as phytate/Zn or phytate/Fe molar ratios of cereals and legumes (*7*). Furthermore, in spite of the benefit of phytate for human health, many approaches were introduced to reduce it for improving mineral bioavailability of cereals and legumes, including molecular genetic modification, polishing, fermentation, soaking, and phytase treatment (*8–11*). For various reasons, however, none of these have been universally successful in solving micronutrient deficiencies in developing countries. Therefore, in our opinion, the best results of agronomic interventions should be used to aim for increased micronutrients mass fraction and further food processing methods should aim at decreasing phytate while even maintaining or increasing the density of minerals in crop grain.

Rice is one of the most important staple crops for more than half of the world population and, thus, is an important source of energy, vitamins, mineral elements, and rare amino acids for people feeding on the rice as staple. However, considerable genotypic differences were found in the concentration of Zn, Fe, and Se in rice grains in China (*5, 12*). Moreover, rice is mainly consumed

---

* To whom correspondence should be addressed. Fax (Tel.): 86-25-84399086. E-mail: qiuhuihu@hotmail.com.

10.1021/jf800150z CCC: $40.75

after polishing by the Chinese and rice products in general contain only low level of mineral elements, which are lost mostly during processing for food. In recent years, many research approaches have been chosen for fighting against micronutrients deficiencies in rice, for example, biofortification using breeding, genetic modification, and agricultural practices (*13–15*).

Micronutrient uptake and transport to the edible parts of plants can be increased by fertilizer application to leaf. Foliar fertilization is one of the most effective and safest approaches to enrich essential micronutrients in crop grain. Leaf-applied substances can enter the leaf either by penetration of the cuticle or via the stomatal pathway. Foliar application is increasingly used to alleviate micronutrient deficiencies, and presently most of the studies have been focused on uptake and distribution of single micronutrient in fruit tree, corn, and wheat (*16–20*). In our previous study, Se contents of rice were significantly increased by foliar application of selenium-enriched fertilizer with selenate or selenite compared with no selenium treatment (*12*). Some other results of studies available showed that Zn applied as foliar spray had the effect on uptake of Zn and other micronutrients in plants (*16, 21*). In addition, Zn plays an important role in the activity of soluble starch synthetase for starch formation and increasing starch content (*22*), and application of Zn may increase yield of wheat and induce accumulation of lysine and methionine in the grain (*19*). However, minimal researches have been conducted on the effect of foliar application of mixed micronutrients on yield and their interactive translocation efficiently in rice grain.

In our study, the variations of Zn, Fe, and Se content of rice collected from the main growing regions in China were investigated, based on which two field experiments were conducted to evaluate the effect of Zn, Fe, and Se fertilization on their concentrations, yield, and protein and ash contents of rice grain to acquire a reasonable concentration of mixed micronutrients fertilizer for enriching micronutrients in rice grain.

MATERIALS AND METHODS

Collection of Rice Samples. Sixty-five rice samples of the most common species (about 1 kg per sample) were obtained from a supermarket or agricultural product market in the main growing provinces in China. The polished rice products widely consumed in these locations were reasonable to investigate the intake of Zn, Se, and Fe from rice products by the people of China.

Preparation of Foliar Fertilizers. The procedure was modified from the method described by Hu et al. (*23*). Lobster waste (20%), chicken excreta (30%), silkworm excreta (15%), pig excreta (34%), EM (effective microorganism) bacterium (1%, Hohai University Supplies, China), and water were mixed to ferment for about 4 weeks. After filtering, the filtrate was mixed with 1:1 distilled water as basal biofertilizer. Zinc sulfate, sodium selenite, and ferrous sulfate were added to the basal biofertilizer and well distributed, and then fermentation was allowed to continue for another 4 weeks to prepare for the different stock fertilizers of Zn, Se, and Fe. The concentrations of three kinds of final solutions were 100 g Zn L^{-1}, 20 g Se L^{-1}, and 60 g Fe L^{-1}. Subsequently, three kinds of stock fertilizers were stored in three plastic containers until application.

Field Experiment Design. In our study, two field experiments were conducted from May to November 2006 in Nanjing of Jiangsu province, southeast of China. The climate of the area is subtropical humid type with an average annual temperature of 16 °C and average annual rainfall of 1000 mm. Two experiments were exactly the same in design and implementation except for test sites and cultivars. The general properties of soils (0–25 cm) at the experimental sites (**Table 1**) were analyzed with the method described by Wang et al. (*24*). An orthogonal design L_9 (3^4) was conducted to optimize foliar fertilization of zinc, selenium, and iron on the micronutrients concentration of rice grain at the research farm of Jiangning district (N 31° 56′, E 118° 47′). The cultivar of rice used was Wuyunjing 7 (*Oryza sativa* L.). Factors and their levels were designed (**Table 2**). According to the orthogonal table, three kinds of stock fertilizers of Zn, Se, and Fe were mixed as combination solution for each treatment before application. The experiment was based on randomized factorial blocks design. Each treatment was done in triplicates, and each plot was 6.0 m × 4.5 m with an interrow spacing of 0.5 m (27 plots in total). The combination solutions of different treatments as foliar application were diluted with water (about 740 L ha^{-1}) and sprayed once to the test paddy during the heading stage of growth. The control was sprayed with distilled water only in the same volume. However, paddy transplanting, irrigation, and other treatments were carried out by standard farmers' practices. For validating the results of field experiment in Jiangning district, another field experiment was replicated at the research farm of Luhe district (N 32° 09′, E 119° 03′), in Nanjing of Jiangsu province in the same year. Ninggeng 1 (*Oryza sativa* L.) was grown as the test rice cultivar in the confirmation experiment. At physiological maturity, an area of 3 m^2 grains of each plot was hand harvested corresponding to their numbers in November 2006 for further determination of yield and 1000-grain weight. The grain yield was expressed at 14% moisture content.

Table 1. Soil Characteristics of Two Experimental Sites

locations	soil pH[a]	organic matter[b] (%)	available Fe[c] (mg kg^{-1})	available Zn (mg kg^{-1})	total Se (mg kg^{-1})
Jiangning	6.9	1.89	16.56	1.22	0.22
Luhe	7.2	2.23	9.71	0.97	0.31

[a] Soil weight/water volume ratio was 1:2. [b] Organic matter was analyzed by loss of weight on ignition. [c] Available Zn and Fe solutions were first obtained by the DTPA (diethylenetriaminepentaacetic acid) extracting method.

Table 2. Factors and Their Levels of the Orthogonal Experiment L_9 (3^4)[a]

	factors		
levels	A (Zn, kg ha^{-1})	B (Se, kg ha^{-1})	C (Fe, kg ha^{-1})
1	0	0	0
2	0.45	0.0075	0.90
3	0.90	0.015	1.80

[a] Different levels of the solutions were diluted with water from the stock fertilizers before application, and the spray rate was 740 L ha^{-1}.

Pretreatment of Rice Samples. After grains were sampled from the field, husks were removed using a laboratory-scale dehulling machine (JLMZS, China). All the brown rice samples were polished for the same intervals (40 s) with a miller (JNMJ-11, China). The mill was thoroughly cleaned after each milling interval by brushing the bran and broken rice kernels from the screen and rotor. The resulting polished rice was ground using a Philips mill (HR2864, Netherlands), and passed through a 1 mm sieve. Subsequently, ground samples were oven-dried for approximately 5 h at 60 °C and subsamples of dried rice flour were packaged in plastic until analysis.

Determination of Zn, Se, and Fe. The procedures used to prepare and analyze samples for mineral content were similar to those described by Zhang and Liu (*25, 26*). The dry subsample was put into a Kjeldahl flask and digested with 10 mL of a mixture of concentrated HNO_3 and $HClO_4$ (v:v, 4:1) at 155–175 °C until the sample was completely mineralized. After cooling, 5 mL of 6 mol L^{-1} HCl was added to the digest to reduce Se^{6+} to Se^{4+} at 120 °C until the solution became colorless and clear, then diluted the remainder with ultrapure water. Blank digestions were also carried out in the same way.

The content of Se was determined with the dual-hydride generation atomic fluorescent spectrometry (AFS-3100, Beijing Kechuang Haiguang Instrument, China). The contents of Zn and Fe were determined by using graphite furnace atomic absorption spectroscopy (GFH-986, Beijing Puxi Instrument, China). Standards were prepared from stock standard solutions of zinc, iron, and selenium (China State Environmental Administration Supplies). The rate of recovery for the method was 92–106%.

Table 3. Zn, Se, and Fe Content of Polished Rice in the Main Growing Regions in China

growing regions[a]	no. of samples	content[b] (mg kg^{-1}) Zn	Se	Fe
Heilongjiang	8	20.2 ± 3.8	0.025 ± 0.021	10.5 ± 3.9
Anhui	7	21.2 ± 1.9	0.032 ± 0.032	11.1 ± 6.7
Jiangsu	7	19.0 ± 1.3	0.014 ± 0.008	8.6 ± 4.3
Sichuan	6	22.3 ± 4.5	0.013 ± 0.014	13.8 ± 2.7
Jiangxi	5	20.9 ± 2.5	0.023 ± 0.008	14.0 ± 4.4
Hunan	4	21.8 ± 1.3	0.049 ± 0.040	12.4 ± 8.9
Zhejiang	4	25.7 ± 6.1	0.013 ± 0.009	11.9 ± 1.4
Henan	3	23.3 ± 2.1	0.028 ± 0.021	25.6 ± 17.4
Hubei	3	21.8 ± 1.9	0.029 ± 0.014	10.7 ± 3.0
Jilin	3	21.4 ± 3.2	0.008 ± 0.002	7.8 ± 1.5
Fujian	3	19.7 ± 3.4	0.003 ± 0.003	11.4 ± 3.4
Guangdong	3	21.8 ± 2.2	0.006 ± 0.004	13.0 ± 4.5
Guangxi	3	22.7 ± 2.5	0.008 ± 0.001	10.3 ± 1.2
Hebei	3	22.8 ± 3.9	0.021 ± 0.001	15.6 ± 10.2
Shandong	3	18.2 ± 1.0	0.023 ± 0.020	8.6 ± 1.3
total/mean	65	21.5 ± 1.8	0.020 ± 0.012	12.4 ± 4.3

[a] Provinces listed in the table are main growing regions of rice in China. [b] Values are means of determinations ± standard deviation.

Nutritional Measurements. The total ash content of all samples was determined by incinerating the sample in an oven at 500–550 °C until the weight of the residue became constant (*27*). The accuracy of the ash content determinations was ±0.01–0.03%. Content of crude protein was calculated by total nitrogen multiplied by 5.95 after determining the nitrogen content of rice material using the Micro-Kjeldahl method (Foss 2300 Kjeltec Analyzer, Denmark).

Statistical Analysis. The data were presented as means ± standard deviations of determinations. Statistical analysis was performed using Student's *t*-test and one-way analysis of variance. Multiple comparisons of means were separated at $P < 0.05$ by the LSD (least significance difference) test. All computations were made by employing the statistical software (SAS, version 8.2).

RESULTS AND DISCUSSION

Zn, Se, and Fe Concentrations of Rice Samples from Different Growing Regions in China. Mean contents of Zn, Se, and Fe of 65 rice samples from different growing regions are shown in **Table 3**. The mean contents of Zn, Se, and Fe in polished rice were 21.5, 0.020, and 12.4 mg kg^{-1}, respectively. However, assuming a mean consumption of about 250 g of rice products, the contribution of these rice products to the mean Zn, Se, and Fe intake in China is estimated to be only about 5.4 mg, 5.0 μg, and 3.1 mg day^{-1} per person, which are far lower than the levels of RDA (15 mg, 70 μg, and 10 mg per day for man) for the population feeding on rice as staple (*28*). Moreover, the variations of Zn, Se, and Fe contents in rice products from different locations were considerable. The Zn concentration in these samples ranged from 18.2 to 25.7 mg kg^{-1}, while rice grown in Shandong had the lowest concentration of the provinces sampled. The mean content of Se was 0.020 mg kg^{-1} with range of 0.003–0.049 mg kg^{-1}, which was in accordance with our previous study (*12*). The lowest level of mean content of Se in the rice was found in Fujian province. However, the variation of Fe content (8.6–25.6 mg kg^{-1}) was broader than that of Zn content and the lowest level of Fe in rice was also in Shandong. All these results indicated that the considerable variation of Zn, Se, and Fe contents in rice products from different locations may not only be attributed to the rice genotype but also the processing and growing environment, including climate of the area, soil condition, and fertilizer management (*13, 29*). Therefore, it is important and feasible to find a new method to solve micronutrient deficiencies in rice through agronomic intervention or genetic selection.

Effect of Zn, Se, and Fe Fertilizers on Zn Content of Rice. The results of the orthogonal experiment and the analysis of variance (ANOVA) are presented in **Table 4**. Mean contents of Zn, Se, and Fe in polished rice at different levels and factors are shown in **Figure 1**. Zn and Fe foliar fertilizers played a vital role in the Zn content of rice ($P < 0.05$), whereas Se had no effect ($P > 0.05$, **Table 4**). Increasing the Zn concentration of foliar application could significantly enhance the Zn level in rice grain (**Figure 1A**). However, application of middle Fe level could increase to the highest Zn level in rice. These results are in agreement with the reports that foliar application of Zn or Fe could increase Zn level in wheat grain (*30*).

Effect of Zn, Se, and Fe Fertilizers on Se Content of Rice. Se fertilizer application had a significant effect on Se concentration of rice grain. *F* value of Se fertilizer was 50.81 ($P < 0.01$, **Table 4**), which was greater than the critical *F* value (6.01, $P = 0.01$). With increasing concentration of Se fertilizer, average Se content of rice was sharply increased from 0.035 to 0.103 mg kg^{-1} in our study (**Figure 1B**). The results indicated that the Se level of rice product could be controlled at the range of 0.06–0.28 mg kg^{-1}, meeting the quality requirement of Se-enriched rice commercial for the population in China. However, no prominent effects were observed of other two variables on Se content of rice in the field investigation ($P > 0.05$, **Table 4**), which suggested that foliar spray of Zn and Fe did not affect the uptake and transport of Se from leaf to grain in rice. The possible reason might be the different uptake metabolism of Se with Zn and Fe. Generally, Se metabolism parallels sulfate assimilation and Se compounds are formed as a result of the sulfate assimilatory pathway and sulfur amino acid-derived metabolism (*31*).

Effect of Zn, Se, and Fe Fertilizers on Fe Content of Rice. Zn fertilizer application had a significant effect on Fe concentrations of rice ($P < 0.01$, $F = 6.88$, **Table 4**). It was apparent that the Fe content of grain increased drastically when concentration of Zn fertilizer increased from 0 to 0.45 kg ha^{-1}, but decreased when Zn application increased from 0.45 to 0.90 kg ha^{-1} (**Figure 1C**). This result is in agreement with the study of wheat that a low concentration of 0.5% Zn solution spray could increase Fe concentration in wheat grain (*30*). However, no significant effects of the other two variables on Fe content of rice were found in the investigation ($P > 0.05$, **Table 4**). This result indicated that it was difficult to improve Fe nutrition of rice grain by Fe spray, which might be attributed to the loading genes of iron in the rice cultivar Wuyunjing and its limited mobility in the phloem (*13, 32*).

Selection of Optimum Spraying Fertilizers. The final results of orthogonal experiment L_9 (3^4) and the effects of those factors on Zn, Se, and Fe content of rice are shown in **Table 4** and **Figure 1**. Based on the magnitude order of *R* value, the effect of three factors on the Zn content of rice decreased in the following order: A (Zn) > C (Fe) > B (Se). The optimal condition for enhance Zn content of rice was determined as $A_3B_1C_2$. Similarly, for Se, the order of *R* value was as follows: B > C > A, and the optimal condition was $A_3B_3C_1$. From the *k* values of the variables, it was obvious that the potential optimization of fertilization for Fe content of rice was $A_2B_3C_2$. However, for comprehensive consideration of Zn, Se, and Fe contents of rice in our study, the highest levels of factor A and B and the middle level of factor C were chosen for the optimal combination. Accordingly, run 9 ($A_3B_3C_2$) could be the best treatment for foliar application at 0.9 kg ha^{-1} Zn, 0.015 kg ha^{-1} Se, and 0.9 kg ha^{-1} Fe, in which the average Zn, Se and Fe content of rice were 19.0 mg kg^{-1}, 0.100 mg kg^{-1}, and 8.5 mg kg^{-1}. Not surprisingly, the lowest nutritional concentration of

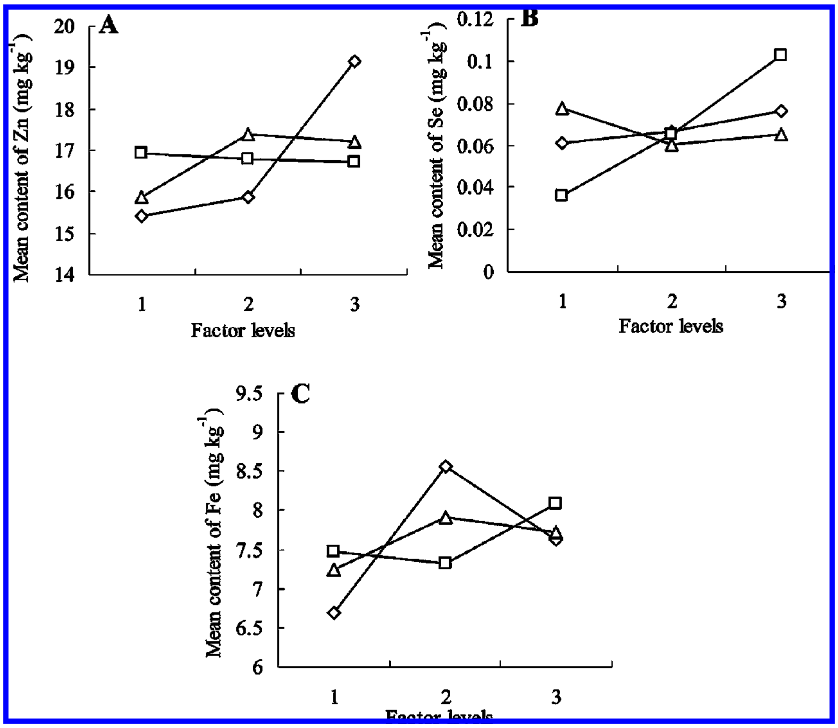

Figure 1. Effect of foliar application of Zn, Se, and Fe fertilizers on Zn (**A**), Se (**B**), and Fe (**C**) contents of rice grain in the Jiangning experiment: (–◇–) Zn fertilizer, (–□–) Se fertilizer, (–△–) Fe fertilizer.

rice (13.9 mg kg^{-1} Zn, 0.034 mg kg^{-1} Se, and 6.2 mg kg^{-1} Fe) was found in run 1 with no fertilizer spraying, in which contents of Zn and Fe were lower than average content of rice collected in our investigation before (**Table 4**). Under the optimum condition of fertilization, Zn, Se, and Fe content of rice could be increased by 36.7%, 194.1%, and 37.1%, respectively comparing with the control. In our confirmation experiment on rice cultivar Ninggeng 1 in Luhe district, the optimal fertilization could slightly increase Zn content of rice up to 19.4 mg kg^{-1}, while not significantly. The Se and Fe content of rice, however, were markedly increased up to 0.123 mg kg^{-1} ($P < 0.05$) and 14.2 mg kg^{-1} ($P < 0.01$), respectively, comparing with the control treatment (17.4 mg kg^{-1} Zn, 0.066 mg kg^{-1} Se, and 5.5 mg kg^{-1} Fe, **Table 5**). Therefore, the repeatability of the experiment for improving micronutrients of rice was good. Altogether, the results indicated that application of Zn, Se, and Fe mixed fertilizer as foliar spray could alleviate these micronutrients deficiency in rice grain.

Effect of Zn, Se, and Fe Fertilizers on Yield and Quality of Rice. The effect of Zn, Se, and Fe fertilizer on yield and quality of rice were evaluated (**Table 6**). The statistical analysis for protein and ash contents of rice showed significant differences ($P < 0.05$) regarding the fertilizer treatments used, while there were no significant effects of fertilizer application on the crop yield and 1000-grain weight ($P > 0.05$). The result of yield was in agreement with the previous studies that preharvest foliar application of mineral elements did not affect grain yield of crops (*12, 30*). As known, protein and ash contents are usually introduced to evaluate the nutritional value of cereal food, such as wheat, rice and legume (*19*). In our study, no treatments reduced the yield or quality of rice including the optimal treatment mentioned above (run 9); instead some treatments could significantly increase contents of protein and ash in rice grain. Maximum protein content obtained in run 4 and run 2 were 7.00% and 6.94%, which are markedly higher than run 1 (control treatment). With respect to ash content, maximum values were observed with run 7 (0.47%) and run 4 (0.44%).

Table 4. Zn, Se, and Fe Content of Polished Rice in the Orthogonal Experiment L_9 (3^4) in Jiangning

	factors			results[a]		
run	A	B	C	Zn (mg kg^{-1})	Se (mg kg^{-1})	Fe (mg kg^{-1})
1	1	1	1	13.9 ± 1.0	0.034 ± 0.002	6.2 ± 0.2
2	1	2	2	16.6 ± 0.1	0.048 ± 0.005	5.9 ± 0.3
3	1	3	3	15.7 ± 0.7	0.101 ± 0.019	8.0 ± 0.3
4	2	1	2	16.6 ± 0.9	0.035 ± 0.003	9.4 ± 0.2
5	2	2	3	15.6 ± 0.9	0.057 ± 0.003	8.4 ± 0.2
6	2	3	1	15.4 ± 0.2	0.107 ± 0.011	7.9 ± 0.2
7	3	1	3	20.3 ± 0.8	0.038 ± 0.008	6.8 ± 0.3
8	3	2	1	18.2 ± 0.1	0.091 ± 0.015	7.6 ± 0.1
9	3	3	2	19.0 ± 2.1	0.100 ± 0.011	8.5 ± 0.4
		Zn				
k_1[b]	15.4	16.9	15.9			
k_2	15.9	16.8	17.4			
k_3	19.2	16.7	17.2	$S_E = 29.25$		
R[c]	3.8	0.2	1.5			
F	23.28[f]	0.055	3.87[e]			
Q[d]	A_3	B_1	C_2			
		Se				
k_1	0.061	0.036	0.078			
k_2	0.066	0.065	0.061			
k_3	0.076	0.103	0.065	$S_E = 0.004$	$F_{0.05}(2, 18) = 3.55$	
R	0.015	0.067	0.017		$F_{0.01}(2, 18) = 6.01$	
F	2.58	50.81[f]	3.45			
Q	A_3	B_3	C_1			
		Fe				
k_1	6.7	7.5	7.2			
k_2	8.6	7.3	7.9			
k_3	7.6	8.1	7.7	$S_E = 20.58$		
R	1.9	0.8	0.7			
F	6.88[f]	1.38	0.99			
Q	A_2	B_3	C_2			

[a] Values are means of three determinations ± standard deviation. [b] k represents the average Se, Zn, and Fe content of polished rice (mg kg^{-1}). [c] R value means range between three average Se, Zn, and Fe content of each level. [d] Q represents the optimal level of three factors. [e] Means this factor has prominent effect on the result at 0.05 level. [f] Means this factor has higher prominent effect on the result at 0.01 level.

Table 5. Zn, Se, and Fe Contents of Polished Rice in Confirmation Experiment Conducted in Luhe

element content ($mg\ kg^{-1}$)	treatment		P value
	control	optimal fertilization	
Zn[a]	17.6 ± 0.8	19.4 ± 1.4	0.134
Se	0.066 ± 0.004	0.123 ± 0.023	0.0132
Fe	5.5 ± 1.4	14.2 ± 2.1	0.0038

[a] Values are means of determinations ± standard deviation.

Table 6. Effect of Foliar Application of Zn, Se, and Fe Fertilizers on Yield and Quality of Rice in Jiangning

run	yield[a] ($kg\ ha^{-1}$)	1000-grain wt (g)	protein (%)	ash (%)
1	8433	27.05	6.52 cd	0.36 bc
2	9056	27.12	6.94 ab	0.37 bc
3	8144	27.04	6.71 abcd	0.32 c
4	8522	27.41	7.00 a	0.44 b
5	8033	28.07	6.84 abc	0.31 c
6	8644	27.77	6.69 abcd	0.31 c
7	8233	26.96	6.61 bcd	0.47 a
8	8667	27.58	6.46 d	0.34 c
9	7922	27.23	6.56 cd	0.39 bc
LSD (0.05)	ns	ns	0.34	0.08

[a] Values were means of three determinations. Within each column, different letters indicated significant differences among the treatments at $P < 0.05$ in LSD test.

Possibly, run 4 was the preferable treatment for increasing protein and ash content in rice. Moreover, in the confirmation experiment on the rice cultivar Ninggeng 1, no significant difference of protein and ash content of rice was observed in the optimal fertilization compared with the control treatment (data not shown). Consequently, all of the results indicated that foliar sprays of mixed micronutrients did not reduce the protein content and mineral quality of rice grain in the study.

Rice is an important source of micronutrients including vitamins and minerals, most of which is located in the aleurone and the embryo. The primary depository for iron and zinc in cereals is the protein storage vacuole of the embryo and the aleurone, with the two minerals being stored together with phyate as a mineral salt in so-called globoids (*33*). In contrast, selenium which predominantly forms as selenomethionine in the protein storage, is quite evenly distributed in the different tissues of wheat grain (*34*). Thus, the processing of rice grain has only little effect on the selenium concentrations of polished rice products while there is great reduction in the content of zinc and iron. The longer the milling and extraction processes are conducted the more outer layers and their minerals are removed (*33*). In China, rice is mainly consumed after polishing and most of the rice products are polished rice. In the study, therefore, 65 common rice products consumed by the inhabitants in the rice growing regions were collected as polished rice in order to investigate the intakes of Zn, Se, and Fe from rice for the Chinese people. However, the mean contents of Zn, Se, and Fe in polished rice from different growing regions in our investigation were too low to meet the micronutrient demands for the population feeding on the rice as staple in China.

In the field experiments, a kind of biofertilizer was used as basal fertilizer to increase the bioavailability of the added minerals chelated by organic matter after fermentation. EM bacterium employed in the fermentation contains about 80 species of microorganism including a predominant population of lactic acid bacterial (*Lactobacillus* sp.) and yeast (*Saccharomyces* sp.), and a small proportion of photosynthetic bacteria (*Rhodopseudomonas* sp.), actinomycetes, and fermenting fungi (*35*). The EM suspension was added to the animal manure to produce the biofertilizer, which is rich in microorganisms and organic matter that may significantly affect the chemistry of micronutrients in the solutions and enhance micronutrient uptake by plants. In our previous studies, the Se-enriched biofertilizer was successfully applied to enhance the Se content of rice, green tea, and soy bean (*36–38*). Whereas the basal biofertilizer used in the study also contains a low concentration of minerals and nitrogen (0.5 $mg\ L^{-1}$ for Zn, 18.0 $mg\ L^{-1}$ for Fe, 0.012 $mg\ L^{-1}$ for Se, and 0.58 $g\ L^{-1}$ for N). For eliminating the influence of minerals in the basal fertilizer, the amount of basal fertilizer for each treatment was controlled at about 6 $L\ ha^{-1}$ to make sure that the difference of each treatment was only the amount of the added minerals. Zn and Se fertilizers were the main variables influencing the Zn, Se, and Fe content of rice grains in the orthogonal experiment on the rice cultivar Wuyunjing, while it was difficult to improve the Fe nutrition of rice grain by Fe spray. The optimal fertilization of zinc, selenium, and iron mixed fertilizer could enhance these micronutrients of rice grain in both rice cultivars, but did not affect grain yield and protein content of rice grain. However, little is known about the physiological and biochemical competitive mechanisms of the micronutrients in plants. Moreover, numerous dietary and host factors in the diets inhibit the mineral bioavailability and play an important role in the mineral deficiencies. Therefore, further studies are needed, for (1) understanding the mechanisms of micronutrient uptake and transport to acquire more efficient accumulation of nutrients in rice grain, and (2) decreasing the inhibitors in rice such as phytate to improve the bioavailability of micronutrients for human nutrition.

LITERATURE CITED

(1) Shenkin, A. The key role of micronutrients. *Clin. Nutr.* **2006**, *25* (1), 1–13.

(2) Welch, R. M.; Graham, R. D. A new paradigm for world agriculture: meeting human needs: Productive, sustainable, nutritious. *Field Crops Res.* **1999**, *60* (1–2), 1–10.

(3) Salgueiro, M. J.; Zubillaga, M.; Lysionek, A.; Sarabia, M. I.; Caro, R.; De Paoli, T.; Hager, A.; Weill, R.; Boccio, J. Zinc as an essential micronutrient: a review. *Nutr. Res. (N.Y.)* **2000**, *20* (5), 737–755.

(4) Tan, J.; Zhu, W.; Wang, W.; Li, R.; Hou, S.; Wang, D.; Yang, L. Selenium in soil and endemic diseases in China. *Sci. Total Environ.* **2002**, *284* (1–3), 227–35.

(5) White, P. J.; Broadley, M. R. Biofortifying crops with essential mineral elements. *Trends Plant Sci.* **2005**, *10* (12), 586–593.

(6) Frossard, E.; Bucher, M.; Mahler, F.; Mozafar, A.; Hurrell, R. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *J. Sci. Food Agric.* **2000**, *80* (7), 861–879.

(7) Chan, S. S. L.; Ferguson, E. L.; Bailey, K.; Fahmida, U.; Harper, T. B.; Gibson, R. S. The concentrations of iron, calcium, zinc and phytate in cereals and legumes habitually consumed by infants living in East Lombok, Indonesia. *J. Food Compost. Anal.* **2007**, *20* (7), 609–617.

(8) Ren, X.; Liu, Q.; Fu, H.; Wu, D.; Shu, Q. Density alteration of nutrient elements in rice grains of a low phytate mutant. *Food Chem.* **2007**, *102* (4), 1400–1406.

(9) Badau, M. H.; Nkama, I.; Jideani, I. A. Phytic acid content and hydrochloric acid extractability of minerals in pearl millet as affected by germination time and cultivar. *Food Chem.* **2005**, *92* (3), 425–435.

(10) Lestienne, I.; Icard-Verniere, C.; Mouquet, C.; Picq, C.; Treche, S. Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chem.* **2005**, *89* (3), 421–425.

(11) Hemalatha, S.; Platel, K.; Srinivasan, K. Zinc and iron contents and their bioaccessibility in cereals and pulses consumed in India. *Food Chem.* **2007**, *102* (4), 1328–1336.

(12) Chen, L.; Yang, F.; Xu, J.; Hu, Y.; Hu, Q.; Zhang, Y.; Pan, G. Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. *J. Agric. Food Chem.* **2002**, *50* (18), 5128–5130.

(13) Poletti, S.; Gruissem, W.; Sautter, C. The nutritional fortification of cereals. *Curr. Opin. Biotechnol.* **2004**, *15* (2), 162–165.

(14) Welch, R. M. Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. *J. Nutr.* **2002**, *132* (3), 495S–499S.

(15) Welch, R. M.; Graham, R. D. Breeding crops for enhanced micronutrient content. *Plant Soil.* **2002**, *245* (1), 205–214.

(16) Kaya, C.; Higgs, D. Response of tomato (Lycopersicon esculentum L.) cultivars to foliar application of zinc when grown in sand culture at low zinc. *Sci. Hort.* **2002**, *93* (1), 53–64.

(17) Elmer, P. A. G.; Spiers, T. M.; Wood, P. N. Effects of pre-harvest foliar calcium sprays on fruit calcium levels and brown rot of peaches. *Crop Prot.* **2007**, *26* (1), 11–18.

(18) Haslett, B. S.; Reid, R. J.; Rengel, Z. Zinc mobility in wheat: uptake and distribution of zinc applied to leaves or roots. *Ann. Bot.* **2001**, *87* (3), 379–386.

(19) Graham, R.; Senadhira, D.; Beebe, S.; Iglesias, C.; Monasterio, I. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field Crops Res.* **1999**, *60* (1–2), 57–80.

(20) Godsey, C. B.; Schmidt, J. P.; Schlegel, A. J.; Taylor, R. K.; Thompson, C. R.; Gehl, R. J. Correcting iron deficiency in corn with seed row-applied iron sulfate. *Agron. J.* **2003**, *95* (1), 160–166.

(21) Kaya, C.; Higgs, D. Inter-relationships between zinc nutrition, growth parameters, and nutrient physiology in a hydroponically grown tomato cultivar. *J. Plant Nutr.* **2001**, *24* (10), 1491–1503.

(22) Jyung, W. H.; Ehmann, A. Zinc nutrition and starch metabolism in phaseolus vulgaris L. *Plant Physiol.* **1975**, *55* (2), 414–420.

(23) Hu, Q.; Pan, G.; Zhu, J. Effect of fertilization on selenium content of tea and the nutritional function of Se-enriched tea in rats. *Plant Soil.* **2002**, *238* (1), 91–95.

(24) Wang, G.; Wang, Y.; Li, Y.; Cheng, H. Influences of alpine ecosystem responses to climatic change on soil properties on the Qinghai-Tibet Plateau, China. *Catena* **2007**, *70* (3), 506–514.

(25) Zhang, Y.; Pan, G.; Chen, J.; Hu, Q. Uptake and transport of selenite and selenate by soybean seedlings of two genotypes. *Plant Soil.* **2003**, *253* (2), 437–443.

(26) Liu, Z.; Wang, H.; Wang, X.; Zhang, G.; Chen, P.; Liu, D. Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron, and zinc contents in wheat (Triticum aestivum L.). *J. Cereal Sci.* **2006**, *44* (2), 212–219.

(27) *AOAC, Official Methods of Analysis;* 16th ed.; Association of Official Analytical Chemists: Washington, DC, 1995; pp 220–230.

(28) Welch, R. M.; Graham, R. D. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J. Exp. Bot.* **2004**, *55* (396), 353–64.

(29) Gregorio, G. B. Progress in breeding for trace minerals in staple crops. *J. Nutr.* **2002**, *132* (3), 500S–502S.

(30) Pol shekane Pahlavan, M. R.; Keykha, G.; Narouirad, M. R.; Koohkan, S. Effect of zinc, iron and manganese application on yield and nutritional elements concentration in wheat grain. In *Plant nutrition for food security, human health and environmental protection.*, Li, C. J., Welch, R., Oenema, O., Eds.; Tsinghua University Press: Beijing, China, 2005; pp 406–407.

(31) Sors, T. G.; Ellis, D. R.; Salt, D. E. Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynth. Res.* **2005**, *86* (3), 373–389.

(32) Graham, R. D.; Stangoulis, J. C. R. Trace element uptake and distribution in plants. *J. Nutr.* **2003**, *133* (5), 1502S–1505S.

(33) Brinch-Pedersen, H.; Borg, S.; Tauris, B.; Holm, P. B. Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. *J. Cereal Sci.* **2007**, *46* (3), 308–326.

(34) Hawkesford, M. J.; Zhao, F. Strategies for increasing the selenium content of wheat. *J. Cereal Sci.* **2007**, *46* (3), 282–292.

(35) Khaliq, A.; Abbasi, M. K.; Hussain, T. Effects of integrated use of organic and inorganic nutrient sources with effective microorganisms (EM) on seed cotton yield in Pakistan. *Bioresour. Technol.* **2006**, *97* (8), 967–972.

(36) Hu, Q.; Chen, L.; Xu, J.; Yang, F.; Zhang, Y.; Pan, G. Determination of selenium concentration in rice and the effect of foliar application of Se-enriched fertiliser or sodium selenite on the selenium content of rice. *J. Sci. Food Agric.* **2002**, *82* (8), 869–872.

(37) Hu, Q.; Xu, J.; Pang, G. Effect of selenium on the yield and quality of green tea leaves harvested in early spring. *J. Agric. Food Chem.* **2003**, *51* (11), 3379.

(38) Yang, F.; Chen, L.; Hu, Q.; Pan, G. Effect of the application of selenium on selenium content of soybean and its products. *Biol. Trace Elem. Res.* **2003**, *93* (1), 249–256.

Received for review October 2, 2007. Accepted January 23, 2008. This work was supported by the 111 Project of State Education Ministry of P. R. China (No. B07030) and by Nanjing Science and Technology Bureau under grant 071b241001.

JF800150Z