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Iron and Zinc Concentrations in Grain and Flour of Winter Wheat As Affected by Foliar Application

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Human deficiencies of iron (Fe) and zinc (Zn) are worldwide problems. Biofortification of wheat could reduce Fe and Zn deficiencies in societies that depend on wheat consumption. This study investigated the effects of foliar application of Fe with or without Zn on the concentrations of Fe and Zn in grain and especially in flour of three wheat cultivars. On average, grain Fe concentration was increased significantly from 29.5 mg kg^{-1} in the control to 37.8, 35.9, or 34.9 mg kg^{-1} by application of $FeSO_4$, ferric citrate plus $ZnSO_4$, or ferric citrate, respectively. As expected, grain Zn concentration was increased from 29.0 mg kg^{-1} in the control to 45.7 or 39.6 mg kg^{-1} by application of ferric citrate plus $ZnSO_4$ or a complex of micronutrients. Although the Fe and Zn concentrations in flour were inherently lower than in bran and shorts made by experimental mill, the concentrations in flour were simultaneously increased from 10.4 to 12.4 mg kg^{-1} for Fe and from 11.8 to 17.4 mg kg^{-1} for Zn by application of ferric citrate plus $ZnSO_4$. Importantly, Fe was peripherally localized within grain fractions and strictly limited to transport to endosperm, making it more difficult to increase the quantity of Fe in flour products by foliar Fe application, but the situation with Zn is promising because Zn is more readily transported to the endosperm than Fe. The current study increases the understanding of agronomic biofortification.

KEYWORDS: Foliar application; iron; zinc; wheat

INTRODUCTION

Iron (Fe) and zinc (Zn) deficiencies in humans are worldwide and prevalent problems leading to poor health in general and impaired development in women and children in particular (*1*). These deficiencies are two of the leading causes of illness and disease in low-income countries (*2*). More than three billion people are suffering from Fe and Zn deficiency, and the numbers are increasing (*2−4*). Iron and zinc deficiencies are also serious problems in China, where 245 million people suffer from Fe deficiency anemia and about 100 million people are affected by Zn deficiency (*5*).

Inadequate nutritional quality of agricultural products is the major reason for human micronutrient deficiencies, particularly in developing countries where products from cereal crops such as wheat and rice are staple foods (*4−7*). Apart from the inherently low concentration and the low bioavailability of micronutrients in cereal grain, concentrations of Fe, Zn, and other minerals are further reduced during milling (*4*, *7−9*).

Overcoming micronutrient deficiencies in humans is a tremendous challenge, and many reviews concerning Fe and Zn have been published in recent decades. Supplementation, food fortification, and dietary diversification were the main strategies in developed countries, but such strategies are less feasible in developing countries for economic or social reasons (*10*). Recently, scientists have emphasized "biofortification" as a new strategy to combat micronutrient deficiency. In biofortification, the staple food is enriched while the crop grows as a consequence of plant breeding, biotechnology, or application of fertilizer. Biofortification is potentially more sustainable, more economical, and more easily implemented than other strategies (*6*, *7*, *10*). Plant breeding and genetic engineering, however, are long-term goals, and breakthroughs in obtaining micronutrient-enriched genotypes have occurred slowly due to many factors such as the lack of target genes and the strong interaction between genotypes and environments (*6*, *11*, *12*). Thus, the traditional and efficient strategy of agronomic fortification (e.g., fertilizer application) is urgent and essential for improving micronutrient concentrations in wheat and other cereal grains in the short term (*6*, *12−14*). Wheat is one of the most popular cereal crops worldwide (*15*). It is the staple food for nearly half of the Chinese people, and >85% of wheat is consumed as flour-derived products in China (*16*). Therefore increasing Fe and Zn concentration and also bioavailability in wheat flour would be more meaningful and realistic to improve the nutritional health and well-being of people in China and other countries.

Although Zn application has been well studied for the correction of Zn deficiency in soil and for the increase in plant growth and yield, Zn application has seldom been studied with the goal of increasing Zn concentration in grains or other edible plant parts (*6*, *13*, *17*, *18*). Methods of Zn application such as soil application, foliar application, the use of Zn-enriched NPK

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fertilizers (e.g., Zn-enriched urea), and seed priming were summarized in a recent review (*6*). Due to the efficient mobility of Zn in wheat phloem (*19*, *20*), foliar application of Zn is thought to be the most efficient application method for improving Zn concentration in wheat grain and can increase grain Zn concentration up to 3- or 4-fold depending on soil status and climate conditions (*6*, *21*−*25*).

For wheat, less research has been done with Fe application than with Zn application, and very little research has considered how Fe application could increase Fe concentrations in wheat grain (*13*, *14*, *17*). Some work has shown that Fe has an intermediate level of mobility within the phloem (*26*) and that the loading of Fe into the phloem was likely limited by the availability of endogenous chelates (*27*). Furthermore, recent results showed that 77% of the total shoot Fe was translocated to wheat grain at maturity in a pot experiment, indicating good retranslocation of Fe from the shoot to the grain (*28*). Therefore, the increased active Fe concentration in shoot by foliar Fe application can be transported in phloem and even translocated to grain (*29*−*31*). It had been demonstrated that wheat grain Fe concentration was increased by foliar Fe application in arid climate conditions (*24*, *32*, *33*), but not in a humid climate (*34*). Generally, foliar application of complex micronutrients is superior to foliar application of a single micronutrient for yield increase (*17*, *24*, *32*, *33*, *35*); furthermore, the wheat grain Fe and Zn concentrations were increased simultaneously by foliar complex micronutrients (*24*, *32*, *33*). Therefore, foliar complex micronutrients seemed to be a promising agronomic strategy to enhance grain Fe and Zn.

However, few studies have focused on the effects of foliar application of micronutrients on the micronutrient distribution among the milling fractions (especially in flour) of wheat grain. Results from staining methods with dithizone (DTZ) demonstrated visually that Zn is predominantly localized in the embryo and aleurone of bread wheat grain (*21*, *36*), and a recent study showed that endosperm Zn concentrations as determined by a laser ablation (LA)-ICP-MS technique were increased significantly by foliar Zn application (*37*). Whereas nearly all of Fe is localized in the scutellum and aleurone of spelt grain stained by Perl's Prussian blue staining (*36*), it is doubtful whether foliar Fe application can increase endosperm Fe concentration because of the high chelating by enriched phytate in embryo and aleurone (*29*, *38*, *39*). Compared with staining and LA-ICP-MS methods, an experimental mill showed the advantage of separating the starchy endosperm and other fractions (embryo, aleurone, and outer layers) and then measuring the micronutrient concentrations of the separated fractions (*40*, *41*).

In brief, the first aim of this study was to investigate the effects of foliar applications of Fe, either alone or combined with Zn or other nutrients, on Fe and Zn concentrations in both grain and flour of winter wheat in the semiarid climate of the North China Plain. The second aim was to estimate the effects of the foliar applications on the potential bioavailability of Fe and Zn in grain and flour. The molar ratio of phytic acid (PA) to Fe or Zn, in most cases, is considered to be a predictor of bioavailability, and PA accounts for 60−80% of the total phosphorus (P) in grains and is positively correlated with total P (*40*, *42*, *43*). Therefore, total P instead of PA in the present study was used to calculate the molar ratios of P/Fe and P/Zn, which were used as predictors of the potential bioavailability of Fe and Zn in grain and flour.

MATERIALS AND METHODS

Field Locations. Two field experiments were carried out at the Dongbeiwang and Shangzhuang research stations of China Agricultural University (CAU) in Beijing during the 2006−2007 wheat cropping season. The soil at the two stations is typical calcareous alluvial soil of the North China Plain with a pH of 8.5 and 8.3 (water/soil, 2.5:1), total N of 0.39 and 0.69 g kg^{-1}, Olsen P of 8.2 and 11.0 mg kg^{-1}, and NH_4OAc-K of 141 and 78 mg kg^{-1} at Dongbeiwang and Shangzhuang, respectively. The concentrations of DTPA-extractable micronutrients in the soils of Dongbeiwang and Shangzhuang were 8.8 and 15.4 mg kg^{-1} for Fe and 3.1 and 0.6 mg kg^{-1} for Zn, respectively.

Treatments and Experimental Design. Three winter wheat (*Triticum aestivum* L.) cultivars were studied: Jingdong8 and Jing411 were studied at Dongbeiwang, and Nongda211 was studied at Shangzhuang. Jingdong8 and Jing411 are two predominant cultivars in the North China Plain, and Nongda211 was released recently by CAU. The seeding rate was 225 kg ha^{-1}, and the area of each plot was 20 m^2 (5 × 4 m). Phosphorus and potassium fertilizers at 60 kg of P_2O_5 ha^{-1} as superphosphate and 60 kg of K_2O ha^{-1} as potassium chloride were applied before sowing at both Dongbeiwang and Shangzhuang. All plots received 270 or 160 kg of N ha^{-1} as urea at Dongbeiwang or Shangzhuang, respectively. At Dongbeiwang, urea was applied in three split applications: the first split was applied at 60 kg of N ha^{-1} during land preparation, the second split was applied at 40 kg of N ha^{-1} at the turning-green stage (Feekes 4), and the third split was applied at 170 kg of N ha^{-1} at the jointing stage (Feekes 6). At Shangzhuang, urea was applied in two split applications: the first split was applied at 60 kg of N ha^{-1} during land preparation, and the second split was applied at 100 kg of N ha^{-1} at the jointing stage.

The experiments included five treatments: foliar application of deionized water (T_1, control), ferric citrate alone (T_2), $FeSO_4$ alone (T_3), ferric citrate plus $ZnSO_4$ (T_4), and a complex of micronutrients (T_5) (**Table 1**). The treatments were laid out in a randomized complete block design with four replicates for each combination of treatment and cultivar. Thus, there were 40 plots at Dongbeiwang (2 cultivars × 5 treatments × 4 replicates) and 20 plots at Shangzhuang (1 cultivar × 5 treatments × 4 replicates). Micronutrients were applied to the foliage three times: at the early heading stage (Feekes 10), at 10 days after flowering (Feekes 10.53), and at the milky ripe stage (Feekes 11.1). All solutions contained 0.01% (v/v) Tween as a surfactant and were applied to each plot at a rate equivalent to 1000 L ha^{-1} on cloudy days or after sunset with windless conditions. During these growth stages, most of the solution was captured by wheat plants. When the grain was mature, the wheat plants were harvested in a 2 m^2 area (2 × 1 m) in the center of each plot, and these plants were used to determine plant biomass and grain yield.

Sample Preparation and Nutrients Analysis. After the grain samples had been washed carefully and rapidly with deionized water, they were dried at 70 °C for 72 h. After a pre-experiment conducted to prepare wheat flour by grain of the Jing411 cultivar, grain samples of Jingdong8 and Nongda211 cultivars with 14% moisture were milled into flour, shorts (consisting of the fine bran particles, germ, and a small portion of floury endosperm particles as separated in the milling of flour), and bran by a Bühler experimental mill (MLU 220, Uzvil, Switzerland). Rate of flour extraction was 76% with 17% bran and 7% shorts, which is similar to the general rate of flour extraction in China. The method described by Tang et al. (*40*) and Shi et al. (*41*) was used to measure the nutrient concentrations in samples. All samples of grain and milling fractions were digested by using a HNO_3−H_2O_2 mixture in a microwave-accelerated reaction system (CEM, USA), and nutrient concentrations (Fe, Zn and P) were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES, OPTIMA 3300 DV). IPE556 (Wageningen University, The Netherlands) was used as reference material.

Statistical Analysis. The data were subjected to a separate analysis of variance (ANOVA) for each cultivar, and the least significant difference (LSD) at $p < 0.05$ was used to determine differences between treatment means. The Pearson correlation procedure and general line regression model was used to evaluate the relationship between grain Fe (Zn) and flour Fe (Zn). SAS software (SAS 8.0, USA) was used for all analyses.

RESULTS

Biomass and Grain Yield. Biomass and grain yield did not differ among the five treatments for all three cultivars (**Table 2**). Foliar application of $FeSO_4$ (T_3) reduced the thousand kernel weight and harvest index of Jing411 but not those of the other two cultivars.

Table 1. Five Fertilizer Treatments, Applied Three Times in Split Applications

treatment[a]	nutrient	fertilizer type	nutrient concentration (%) 1st and 2nd times	nutrient concentration (%) 3rd time	total Fe or Zn (g ha^{-1})
T_1		deionized water			
T_2	Fe	ferric citrate	0.67	0.50	1840
T_3	Fe	$FeSO_4 \cdot 7H_2O$	0.67	0.50	1840
T_4	Fe	ferric citrate	0.67	0.50	1840
	Zn	$ZnSO_4 \cdot 7H_2O$	0.67	0.50	1840
T_5	Fe	$FeSO_4 \cdot 7H_2O$	0.67	0.50	1840
	Zn	$ZnSO_4 \cdot 7H_2O$	0.67	0.50	1840
	Mn	$MnSO_4 \cdot H_2O$	0.02	0.02	
	B	H_3BO_3	0.02	0.02	
	N	$CO(NH_2)_2$	1.00	1.00	
	P	KH_2PO_4	2.30	2.30	
	K	KH_2PO_4	2.90	2.90	
		citrate	10.0	10.0	

[a] T_1, control; T_2, foliar application of ferric citrate; T_3, foliar application of $FeSO_4 \cdot 7H_2O$; T_4, foliar application of ferric citrate plus $ZnSO_4 \cdot 7H_2O$; T_5, foliar application of complex micronutrients.

Table 2. Biomass, Grain Yield, Thousand Kernel Weight (TKW), and Harvest Index of Winter Wheat As Affected by Foliar Application of Fertilizers[a]

location	cultivar	treatment[b]	biomass (t ha^{-1})	yield[c] (t ha^{-1})	TKW (g)	harvest index (%)
Dongbeiwang	Jing411	T_1	10.7	5.4	37.1	44
		T_2	11.5	5.7	35.5	43
		T_3	11.8	5.3	33.7	39
		T_4	11.3	5.4	36.3	41
		T_5	11.9	5.8	37.7	42
		$LSD_{0.05}$	NS[d]	NS	2.2	3
	Jingdong8	T_1	10.7	5.3	44.6	43
		T_2	11.0	5.3	44.5	42
		T_3	11.2	5.3	43.0	41
		T_4	10.5	5.2	43.3	43
		T_5	11.4	5.6	44.8	43
		$LSD_{0.05}$	NS	NS	NS	NS
Shangzhuang	Nongda211	T_1	10.1	5.1	38.6	44
		T_2	10.2	4.9	37.4	42
		T_3	11.0	5.1	36.3	41
		T_4	10.4	5.0	39.3	42
		T_5	11.6	5.6	35.4	42
		$LSD_{0.05}$	NS	NS	NS	NS

[a] Values are means of four replicate plots. [b] T_1, control; T_2, foliar application of ferric citrate; T_3, foliar application of $FeSO_4 \cdot 7H_2O$; T_4, foliar application of ferric citrate plus $ZnSO_4 \cdot 7H_2O$; T_5, foliar application of complex micronutrients. [c] Yield, grain yield with 13% H_2O. [d] NS, no significant difference.

Grain Fe and Zn Concentrations and Contents. The concentrations of Fe and Zn in grain were significantly increased by foliar application of micronutrients at both locations (**Table 3**). Compared to the control, foliar application of micronutrients increased grain Fe concentration by 7−36% and increased grain Zn concentration by 28−68% depending on the cultivar. Averaged across cultivars, grain Fe concentrations were increased from 29.5 mg kg^{-1} in the control to 37.8 mg kg^{-1} by application of $FeSO_4$, to 35.9 mg kg^{-1} by application of ferric citrate plus $ZnSO_4$, and to 34.9 mg kg^{-1} by ferric citrate; these represented increases of 28, 22, and 18%, respectively. Foliar application of micronutrients containing $ZnSO_4$ (T_4 and T_5) significantly improved grain Zn concentration in all tested cultivars. Averaged across cultivars, grain Zn concentration was increased from 29.0 mg kg^{-1} in the control to 45.7 mg kg^{-1} by application of ferric citrate plus $ZnSO_4$ and to 39.6 mg kg^{-1} by application of a complex of micronutrients; these represented increases of 58 and 37%, respectively.

The contents of Fe and Zn in grain of all cultivars were also significantly increased by foliar application of Fe (T_2, T_3, T_4 and T_5) and Zn (T_4 and T_5) with the exception of Fe in treatment T_4 in Jingdong8 cultivar (**Table 3**).

Distribution of Fe and Zn in Milling Fractions of Wheat Grain. The cultivars Jingdong8 and Nonngda211 were used to investigate the distribution of Fe and Zn within the wheat fractions as affected by foliar application of micronutrients (**Table 4**). Iron and zinc concentrations were lowest in flour, highest in bran, and intermediate in shorts. Averaged across both cultivars and all treatments including the control, the Fe concentration in the bran was 150.5 mg kg^{-1}, which was almost 13-fold greater than the Fe concentration in flour (**Table 4**). Averaged across both cultivars and all treatments including the control, the Zn concentration in bran was 111.1 mg kg^{-1}, which was 8-fold greater than the Zn concentration in flour (**Table 4**).

The results also showed that flour Fe and Zn concentrations were positively correlated with grain Fe and Zn concentrations (**Figure 1**). The slope of the regressive line between grain Fe and flour Fe is lower than that between grain Zn and flour Zn, which indicates the lower response of flour Fe to grain Fe than of flour Zn to grain Zn.

Table 3. Iron and Zinc Concentrations and Contents in Grain of Winter Wheat As Affected by the Foliar Application of Fertilizers[a]

			concentration (mg kg^{-1})		content (g ha^{-1})	
location	cultivar	treatment[b]	Fe	Zn	Fe	Zn
Dongbeiwang	Jing411	T_1	25.6	25.4	120	120
		T_2	29.9	27.2	148	134
		T_3	34.5	25.8	159	119
		T_4	30.5	42.6	142	199
		T_5	29.3	36.3	148	183
		$LSD_{0.05}$	3.9	3.5	18	19
	Jingdong8	T_1	32.3	31.0	146	140
		T_2	36.3	32.3	167	149
		T_3	39.0	31.1	180	143
		T_4	35.6	47.4	161	214
		T_5	34.4	43.1	168	210
		$LSD_{0.05}$	4.0	5.2	18	18
Shangzhuang	Nongda211	T_1	30.7	30.9	136	137
		T_2	38.4	32.5	177	151
		T_3	39.9	26.3	177	118
		T_4	41.8	47.1	182	191
		T_5	36.3	39.5	176	191
		$LSD_{0.05}$	3.4	5.4	26	35

[a] Values are means of four replicate plots. [b] T_1, control; T_2, foliar application of ferric citrate; T_3, foliar application of $FeSO_4 \cdot 7H_2O$; T_4, foliar application of ferric citrate plus $ZnSO_4 \cdot 7H_2O$; T_5, foliar application of complex micronutrients.

Table 4. Iron and Zinc Distribution in Wheat Fractions of Jingdong8 and Nongda211 Cultivars As Affected by Foliar Application of Fertilizers[a]

			Fe (mg kg^{-1})			Zn (mg kg^{-1})		
location	cultivar	treatment[b]	flour	shorts	bran	flour	shorts	bran
Dongbeiwang	Jingdong8	T_1	10.2	91.3	128.3	11.2	84.1	101.4
		T_2	11.0	103.6	143.3	11.1	77.5	90.7
		T_3	13.2	123.6	183.0	10.9	82.9	105.2
		T_4	12.0	119.1	158.6	16.4	133.6	159.8
		T_5	11.6	110.7	157.4	15.0	126.5	146.7
		$LSD_{0.05}$	1.7	15.6	19.2	0.8	17.8	18.0
Shangzhuang	Nongda211	T_1	10.6	98.4	121.8	12.4	75.0	91.2
		T_2	12.5	113.1	143.8	12.6	79.8	88.8
		T_3	13.4	125.2	163.0	11.4	67.7	77.9
		T_4	12.8	114.3	161.2	18.4	114.6	138.8
		T_5	11.2	107.8	144.7	15.6	101.3	112.6
		$LSD_{0.05}$	1.7	16.3	14.8	2.2	11.3	12.2
	grand mean		11.9	110.7	150.5	13.5	94.3	111.3

[a] Values are means of four replicate plots (except for the grand means in the bottom row). [b] T_1, control; T_2, foliar application of ferric citrate; T_3, foliar application of $FeSO_4 \cdot 7H_2O$; T_4, foliar application of ferric citrate plus $ZnSO_4 \cdot 7H_2O$; T_5, foliar application of complex micronutrients.

Foliar application of micronutrients significantly affected the distribution of Fe and Zn in milling fractions (**Table 4**). Compared with control, foliar application of Fe alone (T_2 and T_3) or with Zn (T_4) increased Fe concentration in all three fractions but especially in bran. Application of a complex of micronutrients (T_5) increased Fe concentration in shorts and bran but not in flour. Foliar application of fertilizers containing Zn (T_4 and T_5) significantly increased Zn concentration in all three fractions, with increases ranging from 26 to 48% in flour, from 35 to 59% in shorts, and from 24 to 58% in bran. Moreover, the mean concentrations of Fe and Zn in the flour of the two cultivars were increased significantly from 10.4 to 12.4 mg kg^{-1} for Fe and from 11.8 to 17.4 mg kg^{-1} for Zn by application of ferric citrate plus $ZnSO_4$ (T_4); relative to the control, these represented increases of 19 and 47%, respectively.

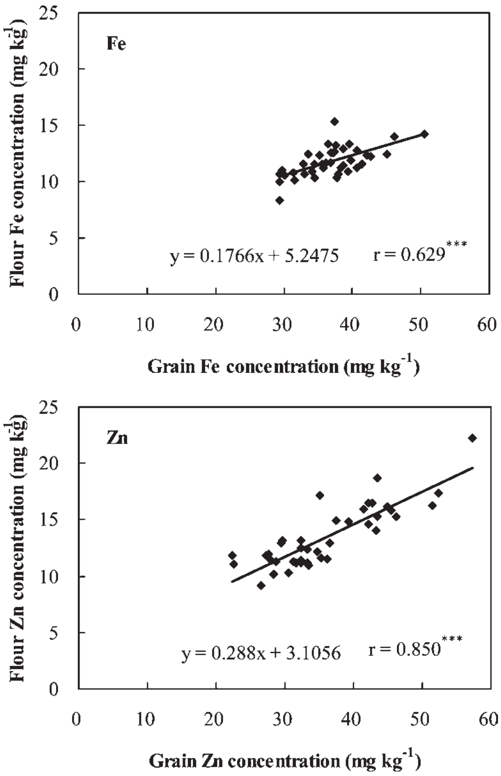

Figure 1. Pearson correlation of Fe and Zn concentrations between wheat grain and flour derived from Jingdong8 and Nongda211 cultivars (n = 40).

Molar Ratio of Phosphorus to Iron (P/Fe) and Zinc (P/Zn). Foliar application of Fe alone (T_2 and T_3) or with Zn (T_4) decreased P/Fe in grain and flour of Nongda211 but not in grain or flour of Jingdong8 (**Table 5**). Foliar application of a complex of micronutrients (T_5) did not affect P/Fe in grain or in flour of either cultivar. However, foliar application of fertilizer containing $ZnSO_4$ (T_4 and T_5) decreased P/Zn by 15−30% in grain and by 18−29% in flour. Foliar application of ferric citrate plus $ZnSO_4$ reduced P/Fe and P/Zn in grain and flour of Nongda211 but not in grain or flour of Jingdong8 (**Table 5**).

DISCUSSION

In this study, foliar application of micronutrients did not increase significantly the yield of three wheat cultivars, a finding that is consistent with some results (*33*, *34*) but is inconsistent with other results obtained from nutrient deficient soils (*23*−*25*, *32*). The soils in the current study, however, were not obviously deficient in Fe and Zn. As shown in **Table 1**, the available levels of Fe and Zn (DTPA-Fe, DTPA-Zn) in the two fields were higher than the critical levels in China (5 mg kg^{-1} for Fe and 0.5 mg kg^{-1} for Zn).

A significant but low increase of grain Fe concentrations was shown among the three cultivars by foliar Fe application (**Table 3**). The current findings agreed with results conducted in West Asia (*24*, *32*, *33*) and are consistent with previous results indicating that Fe can move from leaf to grain (*28*, *31*, *44*) but are inconsistent with results found in Canada (*34*). It is notable that the effects of foliar Fe application on grain Fe concentration depended on climate, soil condition, and timing of application.

The increase of grain Fe was less efficient than of grain Zn in all three cultivars by foliar micronutrients (**Table 3**), which was consistent with previous results (*24*, *32*, *33*). The failure of foliar

Table 5. Molar Ratio of Phosphorus to Iron (P/Fe) and Phosphorus to Zinc (P/Zn) in Wheat Grain and Flour As Affected by Foliar Application of Fertilizers[a]

			grain		flour	
location	cultivar	treatment[b]	P/Fe	P/Zn	P/Fe	P/Zn
Dongbeiwang	Jingdong8	T_1	188	229	170	189
		T_2	182	238	160	209
		T_3	162	236	152	212
		T_4	183	160	169	152
		T_5	188	174	179	154
		$LSD_{0.05}$	18	24	NS[c]	27
Shangzhuang	Nongda211	T_1	184	213	154	153
		T_2	151	208	138	159
		T_3	145	260	132	179
		T_4	140	154	136	109
		T_5	169	181	160	111
		$LSD_{0.05}$	16	30	20	18

[a] Values are means of four replicate plots. [b] T_1, control; T_2, foliar application of ferric citrate; T_3, foliar application of $FeSO_4 \cdot 7H_2O$; T_4, foliar application of ferric citrate plus $ZnSO_4 \cdot 7H_2O$; T_5, foliar application of complex micronutrients. [c] NS, no significant difference.

applications of Fe to cause large increases in grain Fe might be explained in three ways. First, the foliar-applied Fe could have increased the plant-available Fe concentration in the plant cytoplast (*30*), whereas most of the Fe was compartmentalized and stored as precipitated Fe in the apoplast or buffered with ferritin to avoid toxicity (*38*, *45*, *46*). Then only the small portion of the Fe combined with ligands such as nicotianamine (NA) proteins was physiologically active and could be retranslocated to grain during senescence by the phloem pathway (*28*, *31*, *44*, *47*). A second possible explanation for the lack of large increase in Fe concentration in grain is that the synthesis of chelates (e.g., NA) or the storage capacity of grain (e.g., phytate, ferritin, N-containing compounds) might not have increased synchronously with the enhanced Fe concentration in leaves and thereby limited the retranslocation of Fe into grain (*27*, *44*, *48*). Finally, the unloading process of phloem transport from maternal to filial tissues could have been restricted because of xylem discontinuity (*29*, *49*).

The status of grain Zn differs from that of grain Fe. It is well-known that the concentration of Zn in wheat grain can be improved by foliar Zn applications, especially on Zn-deficient soils (*13*, *21*, *24*, *25*, *32*, *33*). The current results indicate that grain Zn concentration could also be improved on a marginally Zn-deficient soil (the Shangzhuang location) or on a Zn-sufficient soil (the Dongbeiwang location) (**Table 3**). A reasonable explanation is that additional Zn supplied by foliar application was readily retranslocated from vegetative organs to developing reproductive organs such as developing grain by phloem (*19*−*21*). However, the extent of increased Zn concentration by foliar application in the current study was less than that in previous studies (*6*, *13*, *21*, *23*−*25*, *33*). One possible explanation is that Zn in the soil of the current study was sufficient to produce a relatively high concentration of Zn in grain. In addition, the current finding also demonstrated that Fe and Zn concentration in wheat grain could be enhanced simultaneously by foliar complex micronutrients, which is consistent with previous results (*24*, *32*, *33*).

The uneven distribution of Fe and Zn within wheat grain increases the nutritional problems associated with heavy reliance on wheat-derived food: the lowest concentrations of these micronutrients occur in the flour, which is the fraction most used for making food products. Regardless of treatment in the current study, the concentrations of Fe and Zn were always lower in flour than in bran or shorts. This finding agrees with previous results indicating that milling greatly decreases Fe or Zn concentration in flour (*8*, *40*, *41*). Grain Fe and Zn were mostly concentrated in the bran and shorts in the current study, which agrees with the recent results (*21*, *36*, *40*, *41*). The current results also showed that flour Fe and Zn concentrations were positively correlated with grain Fe and Zn concentrations (**Figure 1**), indicating that flour Fe and Zn concentrations might be improved by increasing grain Fe and Zn concentrations. This finding quite agreed with recent results (*37*) and further clarified the question of whether foliar Fe can increase wheat endosperm (flour) Fe concentration (*12*, *21*). Moreover, the findings confirmed that Fe and Zn concentrations in wheat flour were enhanced simultaneously by foliar application of ferric citrate plus $ZnSO_4$ (**Table 4**), which is similar to the results gained by optimal nitrogen input (*41*). However, it seemed easier to increase Zn than Fe in flour (**Table 4**; **Figure 1**). A working model proposed recently for the translocation of Fe and Zn from phloem to the storage sites in the developing barley and wheat grain indicated that a similar transport system for Fe and Zn exists within grain tissues and that NA plays a key role in transporting Fe and also Zn as NA-Fe and NA-Zn from the nucellar projection to the endosperm (*29*, *50*). The current results suggest that the affinities of transporters to NA-Fe or NA-Zn may be different in transporting Fe or Zn into endosperm. The Fe localization was largely limited to bran and shorts, whereas Zn localization seemed more even among fractions (**Table 4**), which was the same with results in spelt wheat (*36*). An explanation would be that the deposition of Fe by protein storage vacuole (PSV) rich in aleurone and embryo may work to keep iron homeostasis for avoiding oxidative damage; consequently, Fe transport into endosperm was strictly limited (*29*, *38*, *39*, *46*). Therefore, the low affinity of Fe to NA, the peripheral localization of iron, and the strong deposition of Fe by PSV may contribute to lower efficiency in the increase of flour Fe by foliar Fe application.

Determining the bioavailability to humans of micronutrients in plant foods is difficult but important (*4*, *42*). The P/Zn in both grain and flour, mostly reflecting Zn concentration, was decreased significantly by foliar application of $ZnSO_4$, indicating enhanced Zn bioavailability (**Table 5**). This result was consistent with the previous results (*43*, *51*). The decrease in P/Zn caused by the foliar application of Zn, however, was greater than the decrease in P/Fe caused by foliar application of Fe (**Table 5**). It is estimated that about 5% of the Fe and 25% of the Zn in cereal grains is bioavailable (*52*). Therefore, researchers had suggested that, to have a measurable biological effect on human health, grain Fe and Zn concentrations should be increased by at least 25 and 10 mg kg^{-1}, respectively (*53*). In the present study, increase of Zn by foliar application matched this goal, but the increase of Fe by foliar application did not (**Table 3**). Regardless, the increase of Fe and Zn in flour by foliar application (**Table 4**) may be remarkable because of the much lower phytate concentration and existence of highly bioavailable ferritin in the endosperm (*29*, *41*).

In conclusion, foliar application of Fe and Zn did increase the concentrations of these micronutrients and potential bioavailability of Zn in both wheat grain and flour, but increases were greater for Zn than for Fe. Significant differences existed between Fe and Zn in localization within wheat grain fractions. The inherently peripheral localization of Fe within wheat grain and strictly limited transport of Fe to endosperm will make it more difficult to increase Fe concentration in flour products and thereby improve human nutrition and health. The situation of Zn is promising because Zn is more readily transported into endosperm with higher bioavailability than Fe.

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