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Full Length Research Paper

Foliar zinc fertilization improves the zinc nutritional value of wheat (*Triticum aestivum* L.) grain

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The objective of this research was to provide basic information for increasing the zinc (Zn) concentration and bioavailability in wheat grain. A field study was conducted on potential Zn-deficient soil (DTPA-Zn: 0.65 mgkg^{-1}) during 2007 to 2008 and 2008 to 2009 cropping seasons to determine the effect of foliar Zn application on grain Zn and phytic acid concentrations in wheat (*Triticum aestivum* L. cv. Xiaoyan 22) grown on a potential Zn-deficient soil with different N fertilization rates. The results show that foliar Zn application increased grain Zn concentrations in both cropping seasons, but had no significant effect on grain yield. Foliar Zn application decreased the phytic acid concentration and the phytic acid to Zn molar ratio in wheat grain. Wheat grain Zn concentrations were at a maximum and phytic acid to Zn molar ratios were at a minimum when foliar Zn was applied during the early grain filling stage. The increase in grain Zn concentration due to foliar Zn application was higher in the no N fertilizer treatment as compared to the N fertilized treatments. Foliar Zn application had no significant effect on wheat grain protein concentration. In summary, these results indicate that foliar Zn application at the early grain filling stage significantly increased grain Zn concentration and bioavailability in wheat grown on potential Zn-deficient calcareous soils, thus improving the grain's nutritional value to humans.

Key words: Bioavailability, application time, phytic acid, zinc deficiency, potential Zn-deficient soil.

INTRODUCTION

Nearly 50% of the world's cereal-growing areas have soils with low Zn availability (Cakmak, 2002). The problem is most serious in arid and semiarid regions, especially where soil pH and $CaCO_3$ content are high and soil organic matter content is low (Cakmak et al., 1999; Mirzapour and Khoshgoftar, 2006). Soil Zn deficiency (<0.5 mgkg^{-1}) and potential Zn deficiency (0.5 to 1.0 mgkg^{-1}) are common in calcareous soils of northern China. These soils are often used for winter wheat (*Triticum aestivum* L.) production (Wei et al., 2006). Wheat grain Zn concentrations are inherently low and wheat production on Zn-deficient or potential Zn-deficient soils can result in further reductions in grain Zn concentration.

Zinc deficiency affects one third of the world's population (Hotz and Brown, 2001). The problem is especially serious in developing countries, where as much as 50 to 70% of the daily calorie intake is derived from grain cereals (Branca and Ferrari, 2002; Welch and Graham, 1999, 2005; Cakmak, 2008). The effects of Zn deficiency on human health are significant, potentially leading to growth retardation, reduced immunity, learning deficits, mental retardation, DNA damage, coronary heart disease and even cancer (Welch and Graham, 1999; Hotz and Brown, 2001; Gibson, 2006; Prasad, 2007). Increasing the Zn concentration and bioavailability in wheat grain could significantly improve human health in areas with Zn-deficient or potential Zn-deficient soil.

The Consultative Group on International Agricultural Research has promoted research aimed at developing new cultivars with higher grain Zn concentration and bioavailability (Bouis, 2003). However, there are few reports on the genetic control and molecular mechanisms involved in determining grain Zn concentrations (Ghandilyan et al., 2006; Lucca et al., 2006; White and Broadley, 2005). The development of cultivars tolerant to

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Table 1. Selected physical and chemical properties of the soil (0 to 20 cm) at sowing in 2007.

Soil property	Value
pH	7.98
Organic matter ($g \cdot kg^{-1}$)	14.0
NO_3^--N ($mg \cdot kg^{-1}$)	63.6
NH_4^+-N ($mg \cdot kg^{-1}$)	6.53
Available P ($mg \cdot kg^{-1}$)	24.9
Available K ($mg \cdot kg^{-1}$)	166
$CaCO_3$ ($g \cdot kg^{-1}$)	75.2
DTPA-Zn ($mg \cdot kg^{-1}$)	0.65

soil Zn deficiency may take considerable time and resources. Furthermore, even cultivars with high Zn uptake efficiency are limited by soil Zn availability.

Zinc fertilization has been used widely in recent years to alleviate Zn deficiency in crops. Application methods include direct soil amendment, pre-sowing seed soaking and foliar spray. Zinc is easily immobilized in the soil solution; therefore, foliar Zn application is generally the most effective means for increasing grain Zn concentrations (Savithri et al., 1999).

Phytic acid, polyphenol and cellulose negatively affect grain Zn bioavailability to humans (Welch and Graham, 2004). Phytic acid possesses strong ion sequestering power, forming insoluble complexes with Zn^{2+} that are poorly adsorbed by humans. This is one reason why phytic acid can have a significant influence on human health (Welch and Graham, 2005). Phytic acid is the main storage form for P in grain (Liu et al., 2007). Several studies indicate that soil Zn application reduces P uptake by plants (Fagerial, 2001); therefore, we hypothesized that Zn fertilization could affect grain phytic acid concentration. Few studies have addressed the effect of Zn application on phytic acid concentrations in crops grown on potential Zn-deficient soils.

Nitrogen fertilizers are widely used in agricultural production; however, recent reports suggest the necessity of limiting N fertilizer use in an attempt to reduce environmental pollution (Cirilo et al., 2009). Zinc uptake by crops could be affected by changes in N fertilization practices; therefore, it is important to investigate the effect of foliar Zn application under different soil N conditions. The objective of this study was to investigate the effects of foliar Zn application and application time on grain Zn and phytic acid concen-trations in wheat grown on a potential Zn-deficient soil with different N fertilization rates.

MATERIALS AND METHODS

Experiment site

This study was conducted at the No. 1 Experiment Farm, Northwest A & F University, Yangling, Shaanxi, China during the 2007 to 2008 and 2008 to 2009 winter wheat growing seasons. The elevation at the site was 525 m. The climate is semi-humid with an average annual temperature of 13°C and an average annual rainfall of 600 mm. The soil was classified as an Earth-cumuli Orthic Anthrosol. Soil samples were taken in each plot and they were mixed. Selected physical and chemical properties of the soil are shown in Table 1. The wheat cultivar used in this study was 'Xiaoyan 22'.

Experimental design

The experiment utilized a split-split-plot design with five replications. The main plot factor was time of foliar Zn application: jointing stage (April 1 to 15), flowering stage (April 15 to 29), early grain filling stage (April 29 to May 13), and late grain filling stage (May 13 to 27). The split-plot factor was N fertilizer rate: no N application (N0), 120 kg $N \cdot ha^{-1}$ (N1) and 240 kg $N \cdot ha^{-1}$ (N2). The N fertilizer was applied to the soil as urea at sowing. The split-split-plot factor was foliar Zn application rate: foliar application with distilled water containing no Zn (Zn0) and foliar application with 0.3% $ZnSO_4 \cdot 7H_2O$ (Zn1). The foliar Zn treatments were applied in the late afternoon (16:00 to 18:00 h) every six days (three times) within the chosen growth stage. The total amount of Zn applied to wheat was 2.5 kg $Zn \cdot ha^{-1}$. Superphosphate fertilizer (100 kg P_2O_5 ha^{-1}) was applied to all treatments at sowing. The wheat was irrigated in December during both cropping seasons.

Sample collection and chemical analyses

At harvest, two 50 cm-long rows of wheat were collected from each treatment combination and threshed. The grain was rinsed with distilled water, oven-dried at 70°C and weighed to determine yield. Afterwards, the grain was ground with a stainless steel grinder and stored for analysis of Zn, phytic acid, and N. Grain Zn was determined by atomic absorption spectrometry (AA320CRT, Shanghai, China). Phytic acid concentration was determined by ion exchange resin chromatography (Liang et al., 2007). Grain N concentration was determined by Kjeldahl digestion and multiplied by 5.7 to obtain grain protein concentration (You et al., 2007).

Statistical methods

Statistical differences among groups were determined by analysis of variance (ANOVA) using SAS 8.1 software (SAS Institute Inc., Cary, NC, USA) followed by the least significant difference (LSD) test for multiple comparisons among groups. Differences were

Table 2. Effect of foliar Zn and soil N application on wheat yield (kg·ha^{-1}) in the 2007 to 2008 and 2008 to 2009 growing seasons.

Factor	2007 to 2008	2008 to 2009	Mean
Jointing stage	5162^{a}	3954^{a}	4558
Flowering stage	5021^{a}	4254^{a}	4638
Early grain filling stage	4953^{a}	4133^{a}	4543
Late grain filling stage	4816^{a}	4193^{a}	4505
N0	4474^{c}	3860^{b}	4167
N1	5064^{b}	4196^{b}	4630
N2	5426^{a}	4346^{a}	4886
Zn0	4959^{a}	4118^{a}	4539
Zn1	5017^{a}	4150^{a}	4584

Multiple comparisons were conducted among different levels of the same factor in one column ($P<0.05$, n = 5). N0, N1 and N2 represent soil N fertilization rates of 0, 120, 240 kg N·ha^{-1}; Zn0 and Zn1 indicate foliar Zn application rates of 0 and 2.5 kg $ZnSO_4 \cdot 7H_2O$ ha^{-1}, respectively.

Table 3. Effect of foliar Zn application at different growth stages and N fertilizer amount on wheat grain Zn concentration (mg·kg^{-1}).

Factor	2007 to 2008		Change (%)	2008 to 2009		Change (%)	Mean		Change (%)
	Zn0	Zn1		Zn0	Zn1		Zn0	Zn1	
Jointing stage	33.3^{b}	46.9^{b}	41	26.5^{ab}	43.6^{b}	65	29.9	45.3	51
Flowering stage	38.9^{a}	55.3^{a}	42	28.2^{ab}	45.5^{b}	61	33.5	50.4	50
Early grain filling stage	35.7^{ab}	59.7^{a}	67	26.3^{b}	54.6^{a}	108	31.0	57.2	84
Late grain filling stage	35.7^{ab}	55.8^{a}	56	29.6^{a}	48.2^{ab}	63	32.7	52.0	59
N0	33.6^{b}	54.2^{a}	61	26.3^{c}	46.6^{b}	77	30.0	50.4	68
N1	35.1^{ab}	52.6^{a}	50	26.9^{b}	46.0^{b}	71	31.0	49.3	59
N2	39.0^{a}	56.6^{a}	45	29.8^{a}	51.3^{a}	72	34.4	54.0	57

Multiple comparisons were conducted among different levels of the same factor in one column ($P>0.05$, n = 5). N0, N1 and N2 represent soil N fertilization rates of 0, 120 and 240 kg N.ha^{-1}; Zn0 and Zn1 indicate foliar Zn application rates of 0 and 2.5 kg $ZnSO_4 \cdot 7H_2O$ ha^{-1}, respectively.

considered statistically significant when $P < 0.05$ (n = 5).

RESULTS

Wheat grain yield

On potential Zn-deficient soils, foliar Zn application had no significant effect on wheat yield (Table 2). Soil N application, as expected, increased wheat yield significantly. Averaged across both cropping seasons, wheat yields in the N1 and N2 treatments were 1.11 and 1.17 times those in the N0 treatment.

Grain Zn concentrations

Grain Zn concentrations in the Zn0 treatments was averaged as 35.9 mg kg^{-1} in 2007 to 2008 and 27.7 mg kg^{-1} in 2008 to 2009 (Table 3). Foliar Zn application increased grain Zn concentrations regardless of application time or N fertilizer amount. Grain Zn concentrations in the Zn1 treatments were 1.41 to 2.08 times those in the Zn0 treatments. Grain Zn concentrations were highest when foliar Zn was applied at grain filling, especially the early grain filling stage. Soil N application increased grain Zn concentrations in both the Zn0 and Zn1 treatments, but the increase was not always significant in the Zn1 treatment.

Grain phytic acid concentrations

Grain phytic acid concentrations in the Zn0 treatments averaged as 8.41 g kg^{-1} in 2007 to 2008 and 7.85 g kg^{-1} in 2008 to 2009 (Table 4). Foliar Zn application reduced grain phytic acid concentrations regardless of application time or N fertilizer amount. Grain phytic acid concentrations in the Zn1 treatments were 0.80 to 0.92 times those in the Zn0 treatments. Time of foliar Zn application had a significant effect on grain phytic acid concentration in 2008 to 2009, but not in 2007 to 2008.

Table 4. Effect of foliar Zn application at different growth stages and N fertilizer amount on wheat grain phytic acid concentration ($g \cdot kg^{-1}$).

Factor	2007to 2008		Change (%)	2008 to 2009		Change (%)	Mean		Change (%)
	Zn0	Zn1		Zn0	Zn1		Zn0	Zn1	
Jointing stage	8.33^{a}	7.43^{a}	– 11	7.23^{b}	5.95^{c}	– 18	7.78	6.69	– 14
Flowering stage	8.43^{a}	7.63^{a}	– 10	8.75^{a}	7.19^{a}	– 18	8.59	7.41	– 14
Early grain filling stage	8.28^{a}	7.00^{a}	– 16	7.41^{b}	6.58^{b}	– 118	7.85	6.79	– 1
Late grain filling stage	8.61^{a}	7.86^{a}	– 9	8.02^{ab}	6.42^{bc}	– 20	8.32	7.14	– 14
N0	10.13^{a}	8.82^{a}	– 13	8.93^{a}	7.25^{a}	– 19	9.53	8.04	– 16
N1	7.81^{b}	7.16^{b}	– 8	7.73^{b}	6.56^{b}	– 15	7.77	6.86	– 12
N2	7.29^{c}	6.47^{c}	– 11	6.90^{c}	5.80^{c}	– 16	7.10	6.14	– 14

Multiple comparisons were conducted among different levels of the same factor in one column ($P<0.05$, n = 5). N0, N1 and N2 represent soil N fertilization rates of 0, 120 and 240 kg $N \cdot ha^{-1}$; Zn0 and Zn1 indicate foliar Zn application rates of 0 and 2.5 kg $ZnSO_4 \cdot 7H_2O$ ha^{-1}, respectively.

Table 5. Effect of foliar Zn application at different growth stages and various nitrogen rates on the phytic acid to Zn molar ratio of wheat grain.

Factor	2007 to 2008		Change (%)	2008 to 2009		Change (%)	Mean		Change (%)
	Zn0	Zn1		Zn0	Zn1		Zn0	Zn1	
Jointing stage	25.5^{a}	15.8^{a}	– 38	27.2^{a}	14.2^{ab}	– 48	26.4	15.0	– 43
Flowering stage	23.8^{a}	13.8^{ab}	– 42	31.9^{a}	15.7^{a}	– 51	27.8	14.7	– 47
Early grain filling stage	22.3^{a}	11.7^{b}	– 47	28.7^{a}	11.7^{c}	– 59	25.5	11.7	– 54
Late grain filling stage	24.6^{a}	14.1^{ab}	– 43	27.6^{a}	13.3^{bc}	– 52	26.1	13.7	– 48
N0	30.9^{a}	16.3^{a}	– 47	34.3^{a}	15.5^{a}	– 55	32.6	15.9	– 51
N1	22.5^{b}	13.8^{b}	– 39	28.7^{b}	14.5^{a}	– 49	25.6	14.2	– 45
N2	18.7^{c}	11.4^{c}	– 39	23.4^{c}	11.1^{b}	– 52	21.1	11.3	– 47

Multiple comparisons were conducted among different levels of the same factor in one column ($P<0.05$, n = 5). N0, N1 and N2 represent soil N fertilization rates of 0, 120 and 240 kg $N \cdot ha^{-1}$; Zn0 and Zn1 indicate foliar Zn application rates of 0 and 2.5 kg $ZnSO_4 \cdot 7H_2O$ ha^{-1}, respectively.

Soil N application significantly reduced grain phytic acid concentrations in both crop years. Grain phytic acid concentrations in the N1 and N2 treatments were 0.72 to 0.90 times those in the N0 treatment.

Phytic acid to Zn molar ratio in wheat grain

The phytic acid to Zn molar ratio in the Zn0 treatments averaged as 24.1 in 2007 to 2008 and 28.9 in 2008 to 2009 (Table 5). Foliar Zn application reduced the phytic acid to Zn molar ratio of wheat grain regardless of application time or N fertilizer amount. The phytic acid to Zn molar ratios in the Zn1 treatments were 0.41 to 0.62 times those in the Zn0 treatments. Foliar Zn application time had a significant effect on the phytic acid to Zn molar ratios, with the minimum value observed when foliar Zn was applied at early grain filling. Soil N application significantly reduced the phytic acid to Zn molar ratios. Grain phytic acid to Zn molar ratios in the N1 and N2 treatments were 0.70 to 0.94 times those in the N0 treatment.

Grain protein concentrations

Grain protein concentrations were slightly lesser in the Zn1 treatment than the Zn0 treatment, but the difference was not significant (Figure 1). Grain protein concentrations increased as the amount of N fertilizer increased (Figure 2). Grain protein concentrations in the N1 and N2 treatments were 1.23 and 1.44 times those in the N0 treatment in 2007 to 2008 and 1.24 and 1.42 times those in the N0 treatment in 2008 to 2009.

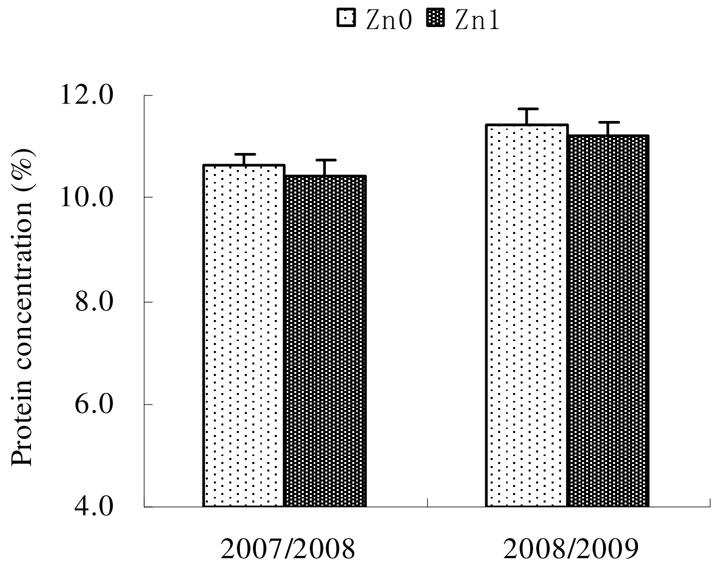


Figure 1. Effect of foliar Zn application on wheat grain protein concentrations during the 2007 to 2008 and 2008 to 2009 growing seasons. Zn0 and Zn1 indicate foliar Zn application rates of 0 and 2.5 kg $ZnSO_4 \cdot 7H_2O$ ha^{-1}, respectively.

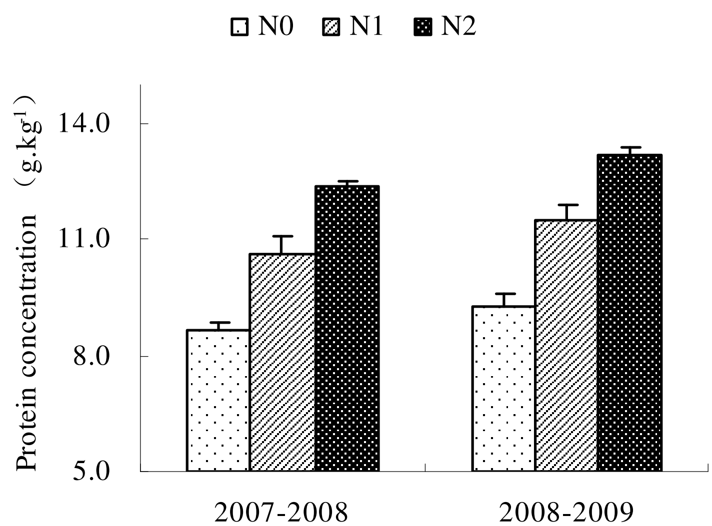


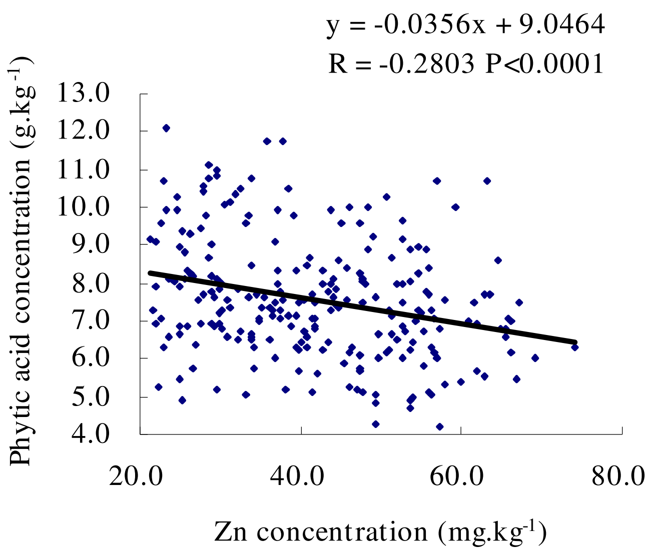
Figure 2. Effect of N fertilizer on wheat grain protein concentration in the 2007 to 2008 and 2008 to 2009 growing seasons. N0, N1 and N2 represent soil N fertilization rates of 0, 120 and 240 kg $N \cdot ha^{-1}$, respectively.

Correlations between Zn, phytic acid and protein concentration in wheat grain

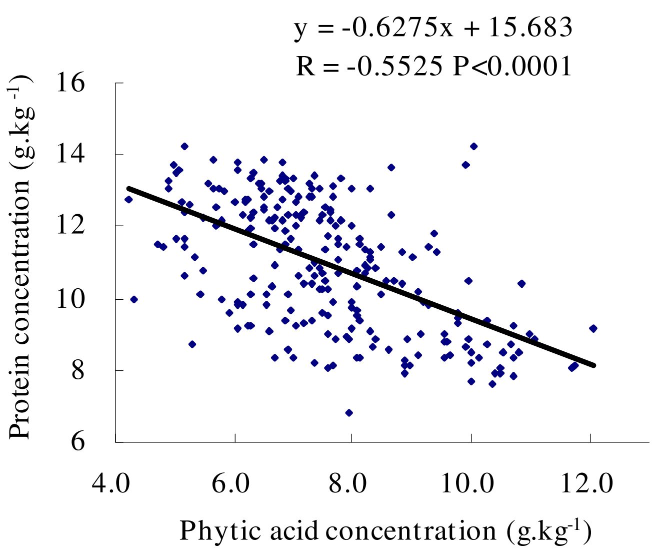
There was a significant negative correlation between grain Zn and phytic acid concentrations ($r = -0.2830$, $P < 0.0001$) and between grain phytic acid and protein concentrations ($r = -0.5525$, $P < 0.0001$) (Figure 3a, b and c). In contrast, there was no significant correlation between grain Zn and protein concentration (Figure 3c).

DISCUSSION

The goal of this research was to increase the Zn concentration and bioavailability in wheat grain in order to improve the health of humans who rely on wheat to meet the bulk of their nutritional needs. Preliminary experiments indicated that soil Zn application to potential Zn-deficient soil had no significant effect on grain Zn and phytic acid concentrations (data not shown). Therefore,

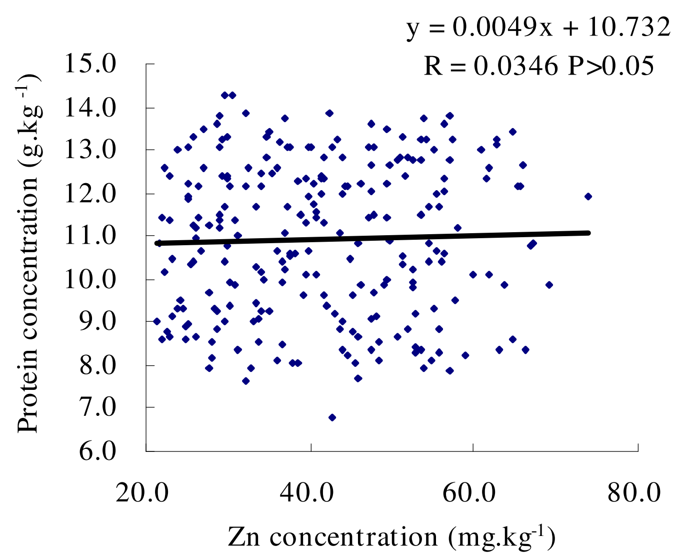


a



b

Figure 3. Correlations among grain Zn, phytic acid and protein concentrations during the 2007 to 2008 and 2008 to 2009 growing seasons.



c

Figure 3. Contd.

the objective of this study was to investigate the effect of foliar Zn application and application time on Zn concentration and bioavailability in wheat grown on potential Zn-deficient soil. Foliar Zn application significantly increased grain Zn concentrations in wheat grown on potential Zn-deficient soil. Grain Zn concentrations in the Zn0 treatments averaged as 31.8 mg kg^{-1} across both cropping seasons. This was within the range of 20 and 35 mg kg^{-1} that has been reported for whole grain wheat flour in various countries (Rengel et al., 1999). Grain Zn concentrations in the Zn1 treatments of our study averaged as 51.2 mg kg^{-1}. With a whole grain flour intake of 400 g per day, grain from the Zn1 treatments should be able to supply the generally recommended dietary allowance of 15 mg Zn per day (National Research Council, 1989). Foliar application of 2.50 kg Zn ha^{-1} increased grain Zn content by 0.12 kg ha^{-1}. This was equivalent to a Zn uptake efficiency of 4.9%. The highest increases in grain Zn concentration occurred when foliar Zn was applied at early grain filling. This is similar to a previous report that grain Zn concentrations increased most when foliar Zn was applied during the later stages of wheat growth (milk and dough stages) (Cakmak et al., 2010). One expla-nation for these findings is that Zn accumulation rates in wheat grain are greatest during early grain filling (Ozturk et al., 2006).

Foliar Zn application reduced grain phytic acid concentrations and the phytic acid to Zn molar ratio. Grain phytic acid concentrations across both cropping seasons averaged as 8.13 g kg^{-1} in the Zn0 treatments and 7.01 g kg^{-1} in the Zn1 treatments. The phytic acid to Zn molar ratios averaged as 26.4 in the Zn0 treatments and 13.7 in the Zn1 treatments. Foliar Zn application at early grain filling resulted in the highest decrease in the phytic acid to Zn molar ratio. We found no reports in the literature regarding the effect of foliar Zn on grain phytic acid concentrations. One explanation for our result is that foliar Zn inhibited the conversion of inorganic P in grain to phytic acid. Additional research needs to be done to confirm this hypothesis. Soil Zn application to a Zn-deficient soil reduced grain phytic acid concentrations in 20 wheat cultivars from an average of 10.7 g kg^{-1} in unfertilized treatments to 9.1 g kg^{-1} in Zn fertilized treatments (Erdal et al., 2002). In the same study, the average phytic acid to Zn molar ratio decreased from 126 in the no foliar Zn (Zn0) treatments to 56 in the Zn fertilized (Zn1) treatments. These results indicate that soil Zn and foliar Zn application both decreased grain phytic acid concentrations.

Phytic acid is important because it can reduce Zn bioavailability by forming insoluble complexes with Zn. The phytic acid to Zn molar ratio is a common index for determining Zn bioavailability in food products (Wise, 1995; Grüner et al., 1996). The critical value for the phytic acid to Zn molar ratio is between 10 and 20. When the ratio is <10, phytic acid exerts little effect on the Zn absorption by humans. As the molar ratio exceeds 15, Zn absorption is inhibited, thus affecting the Zn nutritional value in humans (Gibson et al., 1997). Based on our results, we conclude that foliar Zn application can increase Zn bioavailability in grain grown on potential Zn-deficient soil.

There was a significant negative correlation between grain phytic acid and protein concentration in our study. In contrast, Raboy et al. (1991) reported a positive correlation between grain phytic acid and protein concentration. We were unable to explain the difference between the two studies. Grain protein concentrations are affected by many factors. Additional research needs to be done to determine the relationship, if any, between grain phytic acid and protein concentration. Overall, our results indicate that foliar Zn application did not cause a significant decline in grain protein concentration, thus the grain's nutritional quality was maintained.

Soil N application increased grain Zn concentrations. Shi et al. (2010) reported that long term N fertilization increased Zn concentration in wheat grain. In this study, grain Zn concentration and protein concentration increased, but grain phytic acid concentration decreased, as the amount of N fertilizer increased. In a future paper, we plan to examine interaction between foliar Zn and soil N application on wheat grain Zn concentration and bioavailability.

In summary, foliar Zn application at early grain filling can increase grain Zn concentrations and bioavailability in wheat grown on potential Zn-deficient soils. This could be a useful strategy for reducing human Zn deficiency and its related health problems.

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