Zinc Fertilization Methods on Zinc Absorption and Translocation in Wheat

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Abstract

To increase grain Zn concentration of wheat grown on calcareous soil, field and hydroponic culture experiments were conducted to investigate the effects of various Zn fertilization methods on Zn absorption and translocation in wheat. A completely randomized block design was used in the field experiment comprising five Zn treatments (0, 7.5, 15, 30, and 45 kg Zn/ha as $ZnSO_4 \cdot 7H_2O$) and wheat cultivar (Zhengmai 9023). The hydroponic

experiment used a completely randomized block design with two factors (Zn supplementation to root and foliar spray of Zn). Results showed that in the field experiment, Zn fertilization significantly increased the amount of soil diethylene triamine penlaacetic acid-Zn (DTPA-Zn), whereas there was no significant effect on Zn concentration in grain. Furthermore, the utilization rate of Zn fertilizer was only 0.98%, 0.64%, 0.29%, and 0.14% with treatments of 7.5, 15, 30, and 45 mg Zn ha^{-1}, respectively. In contrast, the hydroponic experiment showed that both foliar spray and Zn supplied to roots significantly increased Zn concentration in grain, with the greatest concentration found in shoots. Results suggested that lower absorption and translocation were the inhibitory factors to increase grain Zn concentration in calcareous soil. Consequently, Zn fertilization to potentially Zn-deficient calcareous soils is not effective method to increase grain Zn concentration.

Keywords: Zn foliar spray, Zn absorption, Zn translocation, Calcareous soil, Wheat, Hydroponic

1. Introduction

Zinc is an important essential trace element for both plants and humans (Kaya et al., 1999; Asad and Rafique, 2000; Hao et al., 2007). However, Zn deficiency appears to be the most widespread and frequent micronutrient deficiency in crops worldwide, resulting in severe losses in yield and nutritional quality. Zn deficiency in humans is a critical nutritional and health problem in the world (WHO, 2002; Alloway, 2004; Gunes et al., 2007). It affects, on average, one-third of the world's population, ranging from 4 to 73% in different countries (Hotz and Brown, 2004). In China, Zn deficiency was also prevalent, with 60% of children suffering from Zn deficiency (Yang, 2007). In developing countries, a large proportion of dietary intake of Zn is derived from cereal grains. The concentration of Zn in cereal grains is generally low and the bioavailability is also reduced due to the existence of anti-nutrition factors such as phytic acid. Increasing Zn concentration of cereal grains has been identified as a way of addressing human Zn deficiency (Garnett and Graham, 2005; Muminjanov et al., 2007; Pahlavan-Rad and Pessarakli, 2009).

A number of attempts have been made to increase Zn concentration in grain crops. An important strategy for increasing micronutrient concentration in grain is breeding or cultivating more resistant genotypes (cultivars). However, the environment can have significant effects on grain Zn concentration and there is significant interaction between genotype and the environment (Cakmak et al., 2004). In addition, genotypic variation for grain Zn concentration among wheat cultivars is relatively narrow and limited. Use of Zn in soil amendments and as foliar sprays have gained acceptance in recent days. Studies have shown that soil amendment with Zn fertilizer increased Zn concentration in wheat grain in soil with extreme Zn deficiency (Yilmaz et al., 1997; Cakmak, 2002). Yilmaz et al. (1997) found that foliar Zn supplementation could increase Zn concentration in grain. A two-year field experiment showed that foliar application of Zn significantly increased Zn concentration in wheat grain (Pahlavan-Rad and Pessarakli, 2009).

However, increased Zn concentration in grain due to fertilization is mainly associated with extreme Zn deficient soils, such as in Turkey (Cakmak, 1996; 1999). In China, wheat is mainly grown on calcareous soils in which Zn is potentially deficient, with DTPA-Zn on average being 0.51 mg/kg. In calcareous soils there are many factors to affect Zn availability, such as high pH, high $CaCO_3$ and low organic matter (Graham and Rengel, 1993; Alloway, 2008). The lower bioavailability of Zn in soil directly affects grain Zn concentration and human health. However, Zn fertilization on calcareous soil showed no significant effects on grain Zn concentration (Hao et al., 2003). Little is known about mechanisms of Zn absorption in calcareous soils and Zn translocation within wheat plants. In this experiment, we tested for factors inhibiting Zn absorption in wheat cultivated on calcareous soil.

2. Materials and Methods

2.1 Field Experiment

A field experiment was conducted at No. 1 Experiment Farm, Northwest A&F University (China) on a potentially Zn-deficient calcareous soil. The altitude of the area is 525 m and the climate is semi-humid prone to drought with an average annual temperature of 13ºC and average annual rainfall of 600 mm. Some characteristics of soils at the experimental site at 0-20 cm depth are in Table 1.

The experimental design was complete randomized block design with three replications comprising five Zn treatments (0, 7.5, 15, 30, and 45 kg Zn/ha as $ZnSO_4·7H_2O$) and wheat cultivar Zhengmai 9023. The following treatment codes will be used throughout the paper: Zn_0, $Zn_{7.5}$, Zn_{15}, Zn_{30}, and Zn_{45}. Plantings were done on 18 October 2008 at a seeding rate of 500 grains m^{-2}. The area of each plot was 3 m×4 m=12 m^2. There were 15 plots, with 30 cm between plots and 1 m between blocks. During planting 120 kg P_2O_5 ha^{-1} supplied as superphosphate and 100 kg N ha^{-1} in the form of urea were applied as the basic fertilizers with an experimental drill. Wheat was harvested at full maturity. At early and late growth stages, soil samples were collected once a week, and at the

wintering and jointing stages soil samples were collected once a month.

2.2 Hydroponic culture experiment

A nutrient solution culture experiment was conducted in the Hydroponic Culture Lab, College of Resources and Environment, Northwest A&F University from March to June, 2008. Zhengmai 9023, as the test cultivar, is not sensitive to Zn deficiency. Wheat seeds were soaked in 55ºC water for 15 min, 3% H_2O_2 for 10 min, and then distilled water for 3 h. The seeds were germinated for 5 days (d) on moist filter paper, and seedlings were transferred to 4ºC to vernalize for 20 d. Seedlings were then selected for uniformity and transplanted into 1 L opaque containers, each covered with a polystyrol-plate with five holes. One seedling was fixed in each hole and one hole was used as an inlet for an aeration tube. A modified Hoagland solution was used with the following composition in mg/L: $Ca(NO_3)_2 \cdot 4H_2O$ 950, KNO_3 610, $MgSO_4 \cdot 7H_2O$ 490, $NH_4H_2PO_4$ 120, Fe-citrate 0.0025 plus in mg/L: H_3BO_3 2.86, $MnCl_2 \cdot 4H_2O$ 1.81, $CuSO_4 \cdot 5H_2O$ 0.08, $H_2MoO_4 \cdot 4H_2O$ 0.09. All chemicals were Analytical reagent (AR). The plants were grown in 1/2 strength Hoagland solution for one week, the solution was then replaced with full strength Hoagland solution with $ZnSO_4 \cdot 7H_2O$. The nutrient solution was aerated continuously. The pots were arranged on a laboratory bench in a completely randomized block design. The containers were kept in a growth chamber with day/night temperatures of 25/15ºC and a 10 hour-photoperiod at 550 μmol m^{-2} s^{-1}. The full strength nutrient solution in the pots was replaced on the fourth day. Plants were harvested two months after transplanting the seedlings to the nutrient solutions.

The experimental design was completely randomized blocks with two factors consisting of two levels of Zn supplementation in hydroponic medium (0 μmol/L and 3 μmol/L) and Zn treatment of leaves (0.15% $ZnSO_4 \cdot 7H_2O$, 0.48 mL/plant) at the jointing, heading and flowering stages. The control treatment was sprayed with distilled water. The following treatment codes will be used throughout the paper: Zn_0, Zn_3 and S_{Water}, $S_{Jointing}$, $S_{Heading}$, $S_{Flowering}$. Each treatment was replicated four times.

2.3 Chemical and statistical analyses

The plant samples were incinerated in a muffle furnace at 525ºC for 6 h, dissolved 1:1 in 5 mL HNO_3, and then transferred to 50 mL volumetric flasks and brought to volume with distilled water. The solutions were analyzed with an atomic absorption spectrophotometer (AA320CRT, Shanghai Analytical Instrument Overall Factory, China) to determine Zn concentration.

The available Zn in the initial and post harvest soil samples was extracted with DTPA solution and the quantity was estimated with AAS (AA320CRT).

The results were analyzed statistically by analysis of variance and multiple comparisons of means according to standard procedures using Excel 2003 and DPS 3.01 statistical software (Ruifeng Information Technology Ltd. Co, Hangzhou, China). The level of significance (α) was 0.05 ($P \leq 0.05$).

Zn accumulation=Zn concentration × biomass yield

Zn utilization rate=(aboveground part Zn accumulation in - the Zn treatment)/Zn fertilizer level

Root%=root Zn accumulation/plant Zn accumulation

Shoot%=shoot Zn accumulation/plant Zn accumulation

Grain%=grain Zn accumulation/plant Zn accumulation

3. Results

3.1 Field Experiment

3.1.1 Changes of the amount of soil DTPA-Zn after Zn fertilization during the wheat life cycle.

The DTPA extractable Zn can estimate the probability of whether soil can provide sufficient Zn to the roots to meet plant demand (Robson, 1993). The amount of soil DTPA-Zn increased on average by 2.3, 3.5, 4.7, and 5.3 fold during the life cycle of wheat in the $Zn_{7.5}$, Zn_{15}, Zn_{30}, and Zn_{45} treatments, respectively, compared to the control (Zn_0 treatment)(Fig 1).

The change of soil DTPA-Zn was time- and Zn-level dependent. The amount of soil DTPA-Zn significantly increased one month after $Zn_{7.5}$ and Zn_{15} fertilization and then declined during 30 d to 50 d. The amount of soil DTPA-Zn slightly fluctuated in the Zn_{30} treatment during this period. From 50 d to 120 d, soil DTPA-Zn declined in the $Zn_{7.5}$ treatment and then stabilized. The trend of soil DTPA-Zn in the Zn_{30} treatment contrasted to that observed in the $Zn_{7.5}$ treatment. The amount of soil DTPA-Zn was sharply reduced in the Zn_{15} treatment at 200 d and then increased. The amount of soil DTPA-Zn showed no significant changes in the Zn_{45} treatment during the wheat life cycle ($P \leq 0.05$).

3.1.2 Effect of Zn fertilization on Zn concentration and accumulation in wheat tissues

Soil Zn fertilization had no significant effects on Zn concentration and accumulation in stems, leaves, and grains. In addition, there was no significant correlation between Zn concentrations and Zn levels and between Zn accumulations and Zn levels. However, Zn concentration and accumulation in glumes significantly increased with increasing supply of Zn in soil compared to the control (Zn_0 treatment).

3.2 Hydroponic Culture Experiment

3.2.1 Effects of foliar and root Zn supply on wheat biomass

Zinc applied to roots or Zn foliar spray showed inhibitory effects on root biomass compared to controls. In contrast to roots, Zn supplementation significantly increased grain biomass compared to controls, irrespective of the supply method. Zinc foliar spray significantly increased shoot biomass on the condition that Zn was absent in the nutrient solution. However, a combination of Zn applied to root treatment with foliar Zn treatment significantly reduced shoot biomass compared to controls.

3.2.2 Effects of foliar Zn and Zn added to root on Zn concentration and distribution in wheat tissues.

Zinc supplementation to roots increased root, shoot and grain Zn concentration by 6.74, 2.89, and 1.84 fold, respectively compared to the control (Zn_0 treatment). Foliar Zn significantly increased shoot and grain Zn concentration compared to controls. However, with Zn absent in the culture solution, Zn foliar spray slightly increased root Zn concentration compared to the control (Zn_0 treatment). In contrast to this, root Zn concentration showed significant reduction compared to controls in the Zn supplied to root combined with Zn foliar spray treatment.

Different Zn supply methods showed different effects on Zn distribution in wheat tissues. Zinc supplementation to roots significantly reduced Zn distribution in shoots and grains. However, root Zn distribution significantly increased with increasing Zn concentration in the nutrient solution. This suggests that with more Zn in the medium, more Zn will be rent in roots. In contrast to Zn supplied to roots, Zn foliar spray significantly reduced root Zn distribution; however, Zn foliar spray increased shoot and grain Zn distribution compared to the control (spray distill water treatment), irrespectively of Zn levels in the nutrient solution. In this hydroponic experiment, Zn foliar spray or Zn supplied to roots increased accumulation in shoots appropriately 70%-90% Zn compared to controls.

4. Discussion

4.1 Effect of Zn fertilization methods on Zn absorption in calcareous soil

The amount of soil DTPA-Zn is dynamic during the wheat life cycle. Fig. 1 shows that the amount of soil DTPA-Zn was reduced in wheat seedling, jointing and filling stages. This suggests that the demand of Zn for wheat was mainly during these stages and wheat absorbed Zn from soil solution primarily during these stages. This finding is consistent with Shao et al. (2005) who found that wheat absorbed Zn mainly at seedling and grain filling stages.

In this field experiment, Zn concentration in wheat tissues were not significantly increased with increasing Zn fertilizer levels in the soil with the exception of glumes. Furthermore, the utilization rate of Zn fertilizer was only 0.98%, 0.64%, 0.29%, and 0.14% with treatments of 7.5, 15, 30, and 45 kg Zn ha^{-1}, respectively. A question arises as to whether there exist factors that inhibited Zn absorption by wheat, such as lower Zn bioavailability in soil. In this field experiment, the amount of DTPA-Zn was sufficient for wheat. Therefore, there must be some factors that lowered Zn absorption by wheat. Based on this hypothesis, a hydroponic experiment were handy to offer an explanation for the observation made. In contrast to the field experiment, in the hydroponic experiment Zn supplied to roots significantly increased Zn concentration in wheat tissues. Therefore Zhengmai 9023 showed good ability to absorb Zn from solution. These two experiments demonstrated that even though Zn levels were high in calcareous soil, wheat did not absorb additional Zn from the treated soil solution. A conclusion can be made that the lower absorption of Zhengmai 9023 was controlled by inhibitory factors of the soil, not the physiology of the wheat cultivar. Previous research has shown that low Zn concentration in wheat tissues was due to the low Zn bioavailability in soil with extreme Zn deficiency (Yilmaz et al., 1997; Cakmak, 2002). However, from our study, it seems that low Zn bioavailability is not the dominant inhibitory factor for low grain Zn concentration in calcareous soil in potentially Zn deficiency soils.

Zinc content of plant uptake is controlled by many factors, such as the amount of soil DTPA-Zn, transport of Zn to root surfaces and the interactions between Zn and other nutrients in the soil or within the plants (Robson, 1993). Zinc absorption by plants involves a number of steps (Lasat et al., 1998). First, adequate Zn

bioavailability was necessary in the rhizoshpere. There are two pathways for Zn to move from the soil solution to the rhizosphere, mass flow and diffusion. In calcareous soils, diffusion is the dominant pathway for Zn to reach root zones. However, diffusion of Zn in calcareous soils is low due to low soil moisture, low organic matter and high pH (Nambiar, 1976; Holloway, 1996; Jiang et al., 2006; Alloway, 2008). Consequently, there is not sufficiently available Zn reaching the rhizosphere. Furthermore, some divalent ions (such as Cu^{2+}, Ca^{2+}, Fe^{2+}) can inhibit Zn absorption by sharing the same carrier site in roots or by competing for transportation proteins such as zinc-regulated iron regulated protein (ZIPs) (Bowen, 1969; Nambiar, 1976; Yang et al., 2004). Therefore, in calcareous soils, it is reasonable to increase Zn diffusion efficiency by increasing organic matter content and irrigation management. However, additional research is needed to determine the relationships between Zn diffusion efficiency and Zn concentration in wheat grain.

4.2 Effect of Zn fertilizer methods on Zn translocation within wheat plants

In the hydroponic experiment, Zn foliar spray significantly increased Zn concentration in grain. However, approximately 80% of Zn was distributed in shoots, only 10% in grain. Zinc supplied to roots combined with Zn foliar spray treatments reduced root Zn concentrations, but increased shoot and grain Zn distribution compared to Zn_3S_{water} treatment. This raises the question as to whether Zn in grain was derived from nutrient solution or from other wheat organs such as roots and shoots. It is generally accepted that Zn accumulation in grain is controlled by homeostatic mechanisms in the plant that regulate Zn absorption, translocation, and loading and unloading rates of phloem sap (Hao et al., 2007). Palmgren et al. (2008) reported that grain Zn originates from two sources: first, directly from the soil and second, from the remobilization of stored Zn in leaves. In wheat, re-translocation from leaves is important for Zn allocation to the grain (Palmgren et al., 2008). The redistribution of Zn may depend on age of the plant as well as on Zn content of the source organs (Page and Feller, 2005). For example, senescence or limited Zn supply may limit redistribution, especially during grain filling (Erenoglu et al., 2002; Jiang et al., 2007; Palmgren et al., 2008). This was consistent with Erenoglu et al. (2002) who reported that in wheat, remobilization of Zn from old leaves into generative organs is much greater under low supplies than adequate supplies of Zn. In contrast, Robson (1993) reported that remobilization of Zn from older into younger tissues was greater with adequate compared to deficient Zn supply. Although the source of grain Zn was uncertain in the hydroponic experiment, we determined that if wheat could absorb enough Zn from the solution, then Zn re-translocation to the grain from other organs was lower. The translocation of Zn in wheat plants was also low. It has been determined that over-expression of a Zn transporter can provide a new strategy for increasing Zn content of wheat (Ramesh et al., 2004). Palmgren et al. (2008) had determined that increased expression of genes encoding Zn transporters can increase Zn uptake in plants. Furthermore, with respect to root-to-shoot allocation of Zn, the Zn pump HMA4 seems to be a major player in dicots. However, it remains to be tested whether HMA4 might be used for transgenic biofortification approaches in cereals (Pahlavan-Rad et al., 2009).

5. Conclusion

We can conclude that in calcareous soils, Zn fertilization to potentially Zn-deficient calcareous soils was not an effective approach to increase grain Zn concentration by lower absorption. If wheat can absorb sufficient Zn from soil solution, Zn foliar spray will possibly have little effect on increasing Zn concentration in grain. This would depend on sufficient Zn being absorbed by roots. However, the low translocation of Zn in wheat plants was also an inhibitory factor to increase grain Zn concentration. In calcareous soils, methods in which to increase Zn absorption by wheat should be of high priority.

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Table 1. Characteristics of tested soil

Taxonomic classification	pH	Organic matter (g/kg)	Olsen-P (mg/kg)	Available-K (mg/kg)	$CaCO_3$ (g/kg)	DTPA-Zn (mg/kg)	Total-Zn (mg/kg)
Eum-Otrthic Anthrosols	7.98	13.79	17.2	166.2	65.1	0.67	69.78

Table 2. Effect of Zn fertilization on Zn concentration and accumulation in wheat tissues. [a,b,c]Values in the same column followed by the same letter are not significantly different at the $P = 0.05$ level

Treatment	Zn concentration (mg/kg)				Zn accumulation (g/ha)			
	Stem	Leaf	Grain	Glume	Stem	Leaf	Grain	Glume
Zn_0	22.50a	18.12a	31.32a	16.18b	69.37a	17.12a	164.38a	32.95b
$Zn_{7.5}$	17.68a	17.76a	33.14a	21.22ab	64.61a	20.15a	168.56a	41.20ab
Zn_{15}	15.82a	22.89a	37.10a	22.12ab	66.18a	28.29a	183.73a	56.33a
Zn_{30}	15.93a	22.78a	41.42a	21.22ab	59.16a	33.31a	214.21a	55.15a
Zn_{45}	26.50a	20.45a	34.77a	25.67a	102.49a	29.00a	169.85a	61.26a

Table 3. Effects of Zn supplied to roots and Zn foliar spray on wheat biomass (g). [a,b,c]Values in the same column followed by the same letter are not significantly different at the P = 0.05 level

Treatment		Root (g)	Shoot (g)	Grain (g)
Zn added to roots	Spraying time			
Zn_0	Control	$0.34\pm0.01a^{(note1)}$	$2.25\pm0.07e$	$0.30\pm0.02d$
	Jointing	$0.24\pm0.03e$	$2.30\pm0.09e$	$0.72\pm0.05b$
	Heading	$0.35\pm0.05a$	$2.83\pm0.11a$	$0.88\pm0.04a$
	Flowering	$0.31\pm0.04b$	$2.57\pm0.08b$	$0.84\pm0.03a$
Zn_3	Control	$0.28\pm0.05c$	$2.44\pm0.07c$	$0.66\pm0.02c$
	Jointing	$0.26\pm0.03de$	$2.30\pm0.06de$	$0.76\pm0.03b$
	Heading	$0.27\pm0.03cd$	$2.42\pm0.05cd$	$0.89\pm0.07a$
	Flowering	$0.25\pm0.06de$	$2.29\pm0.08e$	$0.87\pm0.06a$
p values of the significance of the LSD				
Zn		P<0.001	P<0.001	P<0.001
S		P<0.001	P<0.001	P<0.001
Zn×S		P<0.001	P<0.001	P<0.001

Table 4. Effects of Zn supplied to root and Zn foliar spray on Zn concentration and distribution. [a,b,c]Values in the same column followed by the same letter are not significantly different at the P = 0.05 level.

| Treatment		Root (mg/kg)	Shoot (mg/kg)	Grain (mg/kg)	Root %	Shoot %	Grain %
Zn to root	Foliar Zn						
Zn_0	Control	49.98d	25.38f	18.68d	22.81b	77.19c	7.15d
	Jointing	57.58d	38.84e	19.95cd	13.58c	86.42b	12.20a
	Heading	58.94d	47.65d	20.76c	13.41c	86.59b	10.53b
	Flowering	54.72d	56.42c	19.32d	10.37d	89.63a	9.11c
Zn_3	Control	404.71a	106.49b	33.74b	30.28a	69.72e	5.61e
	Jointing	340.89c	125.94a	36.88a	23.28b	76.72cd	6.93d
	Heading	368.18b	126.37a	37.77a	24.49b	75.51d	7.72d
	Flowering	376.63b	126.98a	36.59a	24.36b	75.64cd	7.62d
p values of the significance of the LSD							
Zn		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
S		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Zn×S		P<0.001	P<0.001	P=0.068	P<0.001	P<0.001	P<0.001
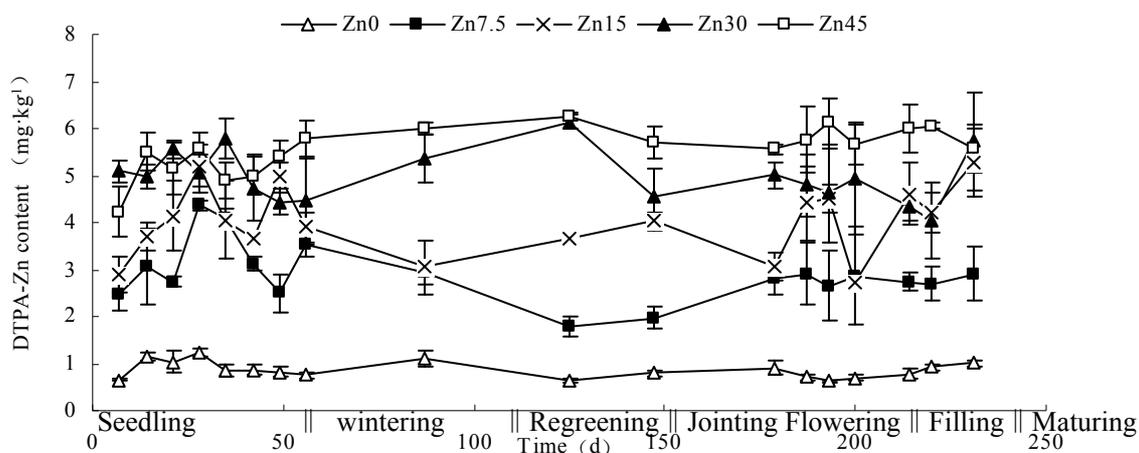
Figure 1. Changes in the amount of soil DTPA-Zn after Zn fertilization during the wheat life cycle