AJA. VOL (1) ISSUE 1, 2014:11-16

Azarian Journal of Agriculture

www.azarianjournals.ir

Azarian Journals

Research article **ISSN:2383-4420**

Effects of foliar application of zinc sulfate at different phenological stages on yield formation and grain zinc content of bread wheat (cv. Kohdasht)

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Article Info

Accepted:
29 Sep. 2014

Keywords:
Zinc, Foliar spray, Grain filling, Grain Zn content, Wheat.

ABSTRACT

Zinc (Zn) is an essential mineral nutrient for plant and human growth, and dietary Zn deficiency is a worldwide nutritional problem. Two-year field experiments were conducted during the 2013 and 2014 to evaluate the effects of foliar application of zinc sulfate at the developmental stages on agronomy traits and grain Zn content. The following foliar applications of Zn (0.44 g Zn/lit) at the phenological stages were used: (1) no Zn fertilizer addition (control), (2) stem elongation stage and (3) stemming and grain filling stages. The results showed that foliar Zn application at the stemming and grain filling stages was much more effective than foliar Zn application at the stem elongation stage on grain yield and its components of wheat. Foliar application at stemming and grain filling stages had no significant effect on the harvest index whereas significantly increased plant height. Zn concentration was not changed when plants were sprayed at early stem elongation while significantly increased grain yield and its components. In general, the most effective treatments to ameliorate Zn deficiency were foliar application at stemming and grain filling stages.

INTRODUCTION

Zinc (Zn) is an essential trace element for the growth and development of humans (Salgueiro et al. 2000; Brown et al. 2001) animals and plants (Sommer 1928; Broadley et al. 2007). Zinc is an integral component of approximately 300 enzymes, including dehydrogenases, aldolases, isomerases, transphosphorylases, RNA and DNA polymerases, and various synthetases (Vallee and Falchuk 1993). Zn deficiency caused by inadequate dietary intake is a global nutritional problem in human populations (Cakmak 2008; Kutman et al. 2011). Recent estimates indicate that over two billion of the world population is affected by Zn deficiency (Cakmak et al. 2010a).

Wheat is one of the most widely grown cereals worldwide and plays a critical role in food security. In several developing countries, such as Iran, wheat is responsible for about half of the protein and daily calorie intake (Gallagher 1984). It is estimated that more than 40% of the wheat crop is cultivated on severely low Zn soils (Alloway 2008) which produces poor yields of grain with low Zn content. An excessive and monotonous consumption of wheat-based products rapidly results in Zn malnutrition because wheat is inherently low in Zn (Cakmak et al. 2010a). Increasing concentration of Zn in wheat grains and other staple foods is, therefore, an important challenge and a high-priority research task.

Many soils are calcareous and thus low in phytoavailable zinc (Zn) in central Iran (Afyuni et al. 2007). Soil application of Zn sulfate before sowing is the most common approach to correct Zn deficiency in crops (Alloway 2008). But foliar Zn applications of Zn are also used, usually at the mid tillering or at early anthesis stages of growth (Brennan 1991; Cakmak et al. 2010a). Foliar application of Zn seems to be an effective method of ameliorating Zn plant deficiency as well as a useful method for increasing Zn concentration of grain; although the effectiveness of this method of Zn application is not always high. For example, Thiruppathi et al (2001) announced that there are two ways to add Zn (foliar application and fertilization), in these ways the absorption of nitrogen, phosphorus and potassium and also increased harvest index, seed yield and its components in sesame. But, Wang et al (2012)

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reported that soil and foliar Zn application did not significantly affect the biomass and grain yield of maize and wheat. One reason for the controversy of the results may be the large differential responses of wheat cultivars to Zn application treatments (Wagner 1993). Foliar application of Zn fertilizers is an effective agronomical practice in crop production, with substantial influence on both yield and particularly grain quality (Khoshgoftarmanesh et al. 2010). Foliar Zn application resulted in a significant increase in starch content of maize (Leach and Hameleers 2001) and doubled the grain Zn concentration of maize (Peck et al. 2008). To have a measurable effect on human health, biofortification should increase the Zn concentration in wheat grain to about 40-45 mg/kg (Ortiz-Monasterio et al. 2007). The targeted levels of Zn in grain for better human nutrition can be easily reached by optimizing rate and timing of foliar Zn application (Cakmak et al. 2010b). Fertilizer strategy (e.g., agronomic biofortification) appears as a highly effective short-term solution to micronutrient malnutrition problem and should be implemented in the target countries, at least until currently on-going breeding programs develop promising wheat varieties with high grain Zn concentrations (Cakmak 2008; Zhao and McGrath 2009; Cakmak et al. 2010b). The present study was conducted to determine the effectiveness of foliar application Zn at the phenological stages on the following: (i) grain yield and dry matter yield of wheat and (ii) Zn concentrations (nutritional quality) of wheat grain.

MATERIALS AND METHODS

Experimental site and soil properties

A two-year field experiment was conducted during the 2013 and 2014 growing seasons at Faculty of Agriculture, Maragheh University in Maragheh state of Iran (46º, 16′/E; 37º, 22′/ N, altitude = 1542 m). Weather characteristics of the study area through growing seasons for 2013 and 2014 were presented in Table 1. For analysis of basic soil properties, soil samples were collected at 0-30 cm depth from location before planting. The properties of the soils are shown in Table 2. Considering the critical deficiency level for the DTPA-extractable soil Zn (0.5 mg/kg) (Alloway 2008), soil in location was severely deficient in available Zn. For all treatments, about 100 kg N ha^{-1} as urea were incorporated into the 0-25 cm soil layer before planting. At the tillering stage, 100 kg N ha^{-1} in the form of urea was top-dressed. Irrigation was carried out every week during the plants growing period.

Plant material and experiment performance

Wheat cultivar used in this experiment was Kohdasht (*Triticum aestivum* L.) which was planted on the 11th April 2013 and 9th April 2014 to a depth of 4 cm. Plant density was 400 m^{-2}. Plot area was 1 × 1.2 m^2 containing six rows with 20 cm row spacing. The following three foliar application of Zn at the phenological stages: (1) no Zn fertilizer addition (control), (2) stem elongation stage (37 Zadoks scale) and (3) stemming and grain filling stages (37 and 71 Zadoks scale). In the foliar application, zinc sulfate was sprayed at a rate of 0.44 g Zn/lit (2 g for $ZnSO_4.7H_2O$). Foliar application of Zn was performed in the very late afternoon to avoid possible leaf damage caused by salts on sunny day and at high day temperature. The experimental design was laid out in a randomized complete block design with three replications.

Grain yield and morphology traits

Wheat was grown as following practices and harvested in the last two week of July in the following years. Ten plants were randomly selected from each plot for determining the data on yield attributes (number of grains per spike, number of fertile spikelet per spike, number of infertile spikelet per spike and thousand grain weight) and for determining plant height. The wheat crop was harvested from a net plot of 0.6 m^2 after leaving a 0.2 m border on each side. At harvest, grain and straw yields of wheat were recorded for each net plot and samples of grain were taken for the Zn analysis. Harvest index was measured by dividing grain yield to biomass production.

Zn concentration determination

The samples were washed with deionized water and dried at 70 ˚C for 48 h, ground, ashed at 550 ˚C for 8 h and the ash dissolved in 2 M hydrochloric acid (HCl) (Chapman and Pratt 1961). Concentrations of Zn in the digest solutions were determined by Atomic Absorption Spectrophotometer (model: AAS-63000SHIMADZU). The values were expressed on a dry weight basis (mg Zn/kg DM). Grain Zn content (ng Zn/g grain) was determined by multiplying grain dry matter with Zn concentration in the grain.

Table 1. Monthly weather parameters of the study area during growth season for 2013 and 2014

Months		Temperature (˚C)			Precipitation (mm)	Relative Humidity (%)		
		Max	Min	Average		Max	Min	Average
2013								
	Apr.	18.9	6.1	12.5	21.8	70	26	48
	May.	21.4	8.8	15.1	20.8	71	28	50
	Jun.	29.0	14.9	21.9	1.8	54	18	36
	Jul.	19.8	33.6	26.7	0.0	45	17	31
2014								
	Apr.	17.2	5.3	11.2	24.4	71	28	50
	May.	25.1	12.1	18.6	21.7	64	25	50
	Jun.	29.6	15.3	22.4	7.0	52	18	35
	Jul.	34.8	20.9	27.9	3.7	48	18	33

Source: Meteorological Office, Iran.

Table 2. Physical and chemical properties of soil (0-30 cm)

EC (ds/m)†	pH	Organic C (g/kg)	Zn (mg/kg)	Sand (%)	Loam (%)	Clay (%)	Soil Texture
0.49	6.81	1.49	0.41	47.6	35.2	17.2	Lom

†Electrical conductivity of soil saturation extract

Statistical analysis

All of data were subjected to one-way analysis of variance (ANOVA) using SAS 8.0 software (SAS Institute Inc, 1998). Means were compared using Duncan's multiple range tests at $P < 0.05$.

RESULTS AND DISCUSSION

Grain yield and its components

The results of both experiments (2013 and 2014) showed that foliar application of Zn at the phenological stages had significant effect on yield and its components (Table 3 and 4). Zn treatments significantly increased grain yield and biological yield of wheat in comparison to control (Table 3). The foliar spray of Zn at the stemming and grain filling stages was the most effective treatment on grain yield (83%). The present results are supported by Arif et al (2006) who reported that foliar application of micronutrients at tillering, jointing and booting stages help in improving yield of wheat. Zeidan et al (2006) reported that yield and its components in lentil are improved by foliar application of micronutrients. In this connection, Potarzycki and Grzebisz (2009) reported that zinc exerts a great influence on basic plant life processes, such as (i) nitrogen metabolism - uptake of nitrogen and protein quality; (ii) photosynthesis - chlorophyll synthesis and carbon anhydrase activity. Also, Hemantaranjan and Grag (1988) believe that more production of chlorophyll and IAA can cause delay in plant oldness and prolong the period of photosynthesis. This incident improves the production of carbohydrates and their transportation to the growing seeds. In the first year of the experiment, with foliar Zn application, average biological yield was increased by 84.7% at the stem elongation stage and by 183.5% at the stemming and grain filling stages. However, in the second year, foliar Zn application was less effective on biological yield and increased by 21.7% at the stem elongation stage and by 48.8% at the stemming and grain filling stages (Table 3). Kaya et al (2000) and Cakmak (2008) reported that zinc plays an important role in the biomass production. But, Wang et al (2012) reported that soil and foliar Zn application did not significantly affect the biomass and grain yield of maize and wheat. However, foliar Zn application significantly improved the grain Zn concentration of maize by 27% and 37% and of wheat by 28% and 89% during the first and second growing seasons, respectively. Romheld and Marschner (1991) and McCauley et al (2009) daresay the increase in the grain yield and biomass is attributable to the improved physiology of plants with the added Zn consequently correcting the efficiency of different enzymes (e.g., dehydrogenases, aldolases, isomerases, transphosphorylases and etc.), chlorophyll content, plant growth regulators (e.g., IAA and GA3), DNA transcription, tryptophan biosynthesis and improvement in nitrate conversion to ammonia in plant (Hemantaranjan and Grag 1988; Oosterhuis et al. 1991; Alloway 2008).

In both years, foliar spray of Zn at the phenological stages had no effect on the harvest index of wheat (Table 3). The highest thousand grain weight was obtained by foliar application of Zn at the stemming and grain filling stages (33.3 g), followed by application at the stem elongation stage (31.3 g) and the lowest thousand grain weight was obtained from control (Table 3). Yilmaz et al (1997) reported that following Zn fertilization, thousand grain weight showed an increase of 26% in wheat plants. During both study years, the application of Zn at the phenological stages significantly increased number of grains per spike and number of fertile spikelet per spike and also decreased number of infertile spikelet per spike (Table 4). The highest number of grains per spike (42 grain) was recorded from the treated plants with application of Zn at the stemming and grain filling stages and the lowest resulted from the treated plants with no Zn addition, respectively. There was no significant difference with no Zn

Table 3. The effect of foliar application of Zn at the phenological stages on grain yield, biological yield, harvest index and thousand grain weight of wheat (cv. Kohdasht)

Foliar Zn application stages	Years		Mean	Percentage change from control (%)
	2013	2014		
	Grain yield (g/m^2)			
No Zn (control)	118 ± 20	178 ± 20	148 c	
Stem elongation	215 ± 32	201 ± 11	208 b	40.5
Stemming and grain filling	304 ± 33	238 ± 28	271 a	83.1
CV (%)	21.9			
	Biological yield (g/m^2)			
No Zn (control)	321 ± 34	461 ± 6	391 c	
Stem elongation	593 ± 89	561 ± 7	577 b	47.6
Stemming and grain filling	910 ± 114	686 ± 61	798 a	104
CV (%)	19.7			
	Harvest index (%)			
No Zn (control)	37.0 ± 5.6	38.5 ± 4.0	37.7 a	
Stem elongation	36.3 ± 0.2	35.9 ± 1.1	36.1 a	-4.2
Stemming and grain filling	33.6 ± 0.7	34.5 ± 1.7	34.0 a	-9.8
CV (%)	12.3			
	Thousand grain weight (g)			
No Zn (control)	26.2 ± 0.5	35.6 ± 0.7	30.9 b	
Stem elongation	26.2 ± 0.2	36.4 ± 0.3	31.3 b	1.3
Stemming and grain filling	30.9 ± 0.7	35.8 ± 2.0	33.3 a	7.8
CV (%)	4.77			

Means in the same column followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's test. Data are means ± standard error (n = 3).

addition and the application of Zn at stem elongation stage (Table 4). The effect of Zn treatments on number of fertile spikelet per spike was almost similar to the trend obtained with number of grains per spike (Table 4). In this connection, Banks (2004) reported that the foliar application of Zn affected yield and its components of soybean and increased number of pods per plant. Also, Seifi Nadergoli et al (2010) reported that the highest number of seeds per pods, number of pods per plant and yield of common bean were obtained by foliar application at shooting, flowering and podding stages, respectively.

Plant height

Results showed that, plant height was significantly affected by foliar spray of zinc at the phenological stages (Table 4). The highest plant height was obtained by foliar application of Zn at the stemming and grain filling stages (61.8 cm) and the lowest plant height was obtained from control (50.2 cm) (Table 4). Comparing with control, plant height increased 4.8 and 23.1% by utilization of Zn at the stem elongation and also at the stemming and grain filling stages, respectively. Kaya et al (2000) reported that added Zn significantly increased plant height via increasing internodes distances.

Grain Zn concentration

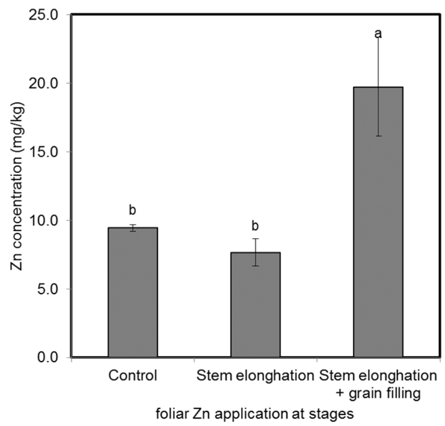
In this study, foliar spray of zinc sulfate at early stem elongation in general had no significant effect on the Zn concentration and Zn content while significantly increased grain yield of wheat cultivar (Figure 1). On average, the foliar Zn treatment at the stemming and grain filling stages increased the mean grain Zn concentration from 9.4 mg/kg in the control treatment to 19.7 mg/kg and also increased the mean grain Zn content from 248 ng/grain in the control treatment to 657 ng/grain (Figure 1 a,b). In well agreement with these observations, Cakmak et al (2010b) showed in field tests that increasing pool of Zn in the vegetative tissue during the reproductive growth stages (for example by spraying foliar Zn fertilizers) represents an important field practice in maximizing accumulation of Zn in grain.

The accumulation of trace elements in plants is affected by genetic and agronomic factors. For example, Zhang et al (2012) reported that foliar Zn application significantly increased the Zn concentration and the predicted bioavailability in both whole grain and flour of wheat as well as Cakmak (2008) announced that foliar Zn application alone or in combination with soil Zn application significantly increased the Zn concentration in wheat grain. Also, Khoshgoftarmanesh et al (2013) reported that soil application of 250 kg rubber tire ash/ha and foliar spray of 0.66 kg Zn/ha at tillering stage were the most effective treatments to ameliorate Zn deficiency and to increase Zn and decrease Cd concentration in grains of most wheat cultivars.

CONCLUSION

The results suggest that grain yield and its components in wheat plant were mostly affected by foliar Zn application at the stemming and grain filling stages rather than at the stem elongation stage. However, foliar Zn application at the stemming and grain filling stages significantly increased the Zn concentration and the predicted bioavailability in grain of wheat. Foliar Zn application showed a reliable adaptability in biofortification of wheat with Zn without causing any yield penalty. Foliar Zn application, therefore, represents a preferential agronomic practice to deliver more Zn from wheat-derived products to people in Iran and other countries.

(a) Zn concentration



(b) Zn content

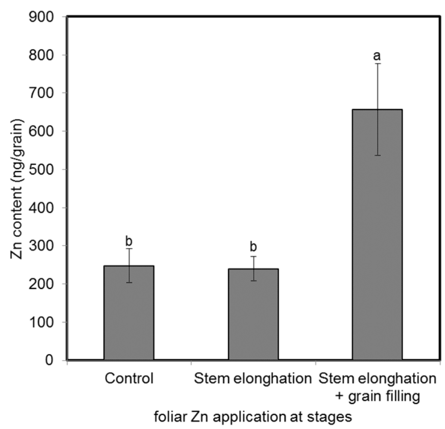


Figure 1. The effect of foliar application of Zn at the phenological stages on Zinc concentration (a) and content (b) in whole grains of wheat (cv. Kohdasht). Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's test. Error bars indicate ± standard error (n = 3).

ACKNOWLEDGMENT

The work reported here is a part of the Ph.D. dissertation of first author. The authors would like to thank their colleagues in Agricultural, university of Maragheh, Maragheh, Iran.

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