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Zinc Application Methods Affect Agronomy Traits and Grain Micronutrients in Bread and Durum Wheat under Zinc-Deficient Calcareous Soil

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Abstract: Zn deficiency is a worldwide nutritional constraint in crop production particularly in cereals growing calcareous soils. In order to study the effect of different zinc-sulfate application methods on grain yield, agronomy traits and grain micronutrients of wheat, a pot experiment was carried out in a calcareous soil in factorial experiment at randomized complete block design (RCBD) with 30 treatments (6 Zn application methods, and 5 wheat genotypes) in four replications. Treatments were the first factor included six levels of applied Zn were (1) control (non Zn application), (2) soil application (5 mg Zn kg^{-1} soil), (3) seed application (3% (w/v) Zn for 1 kg seed), (4) foliar application at stem elongation and early grain filling stages (zinc sulfate was sprayed at a rate of 0.44 g Zn l^{-1}, (5) seed spray + foliar (combination of methods 3 and 4) and (6) soil + foliar (combination of methods 2 and 4), and also the second factor was five wheat genotypes including two spring bread wheat (‘Pishtaz’ and ‘Sivand’) and three spring durum wheat (‘Diyarbakır-81’, ‘Bisu-1’ and line ‘45558’). Both soil and foliar Zinc application methods could improve yield and grain Zn concentration; however, generally bread wheat had the better agronomic traits, grain yield as well as Zn, Fe, Cu and Mn concentrations in grain compared with durum wheat. The foliar Zn application was more effective in increasing Zn, Fe, Mn and ascorbic acid concentrations in grain. Different Zn treatments methods significantly increased Zn concentration and decreased phytate content of the wheat grain, as well as decreased grain phytate/Zn molar ratios. Thus, it seems that soil and foliar Zn application would improve the quantity and quality of the wheat yield in Zn-deficient soils. Therefore, fertilizer strategy (e.g., agronomic biofortification) appears as short-term solution to alleviate malnutrition problem.

Keywords: Zinc deficiency, Biofortification, Wheat, Grain quality, Soil Zn application

Çinko Uygulama Yöntemlerinin Çinko-Eksikliği Olan Kalkerli Topraklarda Ekmeklik ve Makarnalık Buğdayın Agronomik Özellikleri ve Tane Mikrobesin Maddeleri Üzerine Etkisi

Özet: Zn eksikliği, bitkisel üretimde ve özellikle kireçli topraklarda büyüyen tahıllarda dünya çapında bir besin kısıtlamasıdır. Farklı çinko sülfat uygulama yöntemlerinin buğday tahıl verimi, agronomik özellikleri ve tahıl mikrobesin maddeleri üzerine etkisini incelemek amacıyla, kireçli toprakta bir saksı denemesi, tesadüf blokları faktöryel deneme desenine göre dört tekrarlamalı, 30 uygulamalı (6 Zn uygulama yöntemi ve 5 buğday genotipi) olarak gerçekleştirilmiştir. Uygulamalarda birinci faktör olarak 6 adet Zn uygulama yöntemi [(1) kontrol (Zn uygulaması yok), (2) toprak uygulaması (5 mg Zn/kg toprak), (3) tohum uygulaması (%3 (w/v) Zn/1kg tohum, (4) kök uzatma ve erken tane dolum aşamasında yaprak gübrelemesi (çinko sülfat 0.44 g Zn/L oranında püskürtme), (5) tohum uygulaması + yaprak gübrelemesi (3. ve 4. uygulamaların kombinasyonu) ve (6) toprak uygulaması + yaprak gübrelemesi (2. ve 4. uygulamaların kombinasyonu)] ve ikinci faktör olarak 5 adet buğday genotipi [iki ilkbahar ekmeklik buğdayı ('Pishtaz' ve 'Sivand') ve üç ilkbahar makarnalık buğday ('Diyarbakır-81’, ‘Bisu-1’ ve ‘45558’ hattı] ele alınmıştır. Hem toprak hem de yaprak çinko uygulama yöntemleri verim ve tane Zn konsantrasyonu artırmıştır; bununla birlikte, genel olarak ekmeklik buğdaylar, makarnalık buğdaylara göre tane Zn, Fe, Cu ve Mn içeriklerinin yanı sıra agronomik özellikler ve verim bakımından daha iyi sonuçlar vermiştir. Yapraktan Zn uygulaması, tane Zn, Fe, Mn ve askorbik asit konsantrasyonlarının artırılmasında daha etkili olmuştur. Farklı Zn uygulama yöntemleri buğday tanelerinde belirgin bir şekilde Zn konsantrasyonu artırırken, fitik asit içeriğinin azalmasına; dolayısıyla da tanede fitik asit/Zn oranının azalmasına yol açmıştır. Sonuç olarak, toprak ve yapraktan Zn uygulaması, Zn eksikliği olan topraklarda buğday verim miktarını ve kalitesini artırabilmektedir. Bu nedenle, gübreleme stratejisi (tarımsal besin zenginleştirme) kötü beslenme sorunu hafifletmek için kısa vadeli bir çözüm olarak ortaya çıkmaktadır.

Anahtar kelimeler: Çinko eksikliği, Besin zenginleştirme, Buğday, Tahıl kalitesi, Toprak Zn uygulaması

Introduction

Zinc (Zn) plays multiple important roles in the various physiological and metabolic processes of plants (Marschner 1993). Also, Zn is an essential trace element for animal and human nutrition (Hambidge 2000). Zinc deficiency occurs worldwide in soils and plants, particularly in calcareous soils of arid and semi-arid regions. This is often related to low phyto-availability and high fixation of Zn due to high pH, free calcium carbonate, low organic matter, submerged soil conditions, imbalanced application of NPK fertilizers, and high bicarbonate content of irrigation water (Alloway 2009; Rehman et al. 2012). It is estimated that more than 40% of wheat crop is cultivated on severely low Zn soils (Alloway 2004; Malakouti 2007), which produces poor grain yield with low Zn content, leading to Zn deficiency in human.

Micronutrient malnutrition affects over three billion people around the world, especially in the developing countries, and the numbers are increasing (Welch and Graham 2004; Graham 2008; White and Broadley 2009). Zinc, Fe and iodine are the mineral micronutrients most frequently deficient in humans. Zinc malnutrition ranks the fifth in term of leading cause of disease in developing high-mortality countries (WHO 2002).

A major factor causing Fe and Zn deficiencies is their low bioavailability in cereals and legumes based diets (Hurrell 2001). These plant-based diets contain relatively high levels of anti-nutrient factors, such as phytic acid (PA), fibre, and tannins, which leads to a marked reduction in bioavailability of these nutrients (Raboy 2001). Thus, it is important to improve the micronutrient quality of staple foods by increasing the levels of Zn and Fe and/or decreasing the content of the anti-nutritional chemicals.

Currently, improving the grain Zn concentration of cereal crops is a high-priority research area. Biofortification of staple foods is the most promising strategy to alleviate micronutrient deficiency (Brinch-Pederson et al. 2007; Johns and Enzaguirre 2007). There are several approaches to biofortify crops, including agronomic biofortification (soil and foliar application) (Rengel et al. 1999; Cakmak 2008; Sadeghzadeh et al. 2009), genetic engineering techniques (Lucca et al. 2006; Brinch-Pederson et al. 2007), conventional and molecular breeding (Welch and Graham 2004; Mayer et al. 2008; Sadeghzadeh 2013), and molecular markers (Lonergan et al. 2009; Sadeghzadeh et al. 2010; Sadeghzadeh et al. 2015). Among these approaches, agronomic biofortification could be applied as a short time strategy to improve yield and alleviate nutritional problems.

Phattarakul et al. (2012) reported that Zn application increased grain yield and grain Zn concentrations. Gomaa et al. (2015) also reported increased grain yield with foliar application of micronutrient as compared to soil application. Nasiri et al. (2010) reported that foliar Zn application at both stem elongation and flowering stages had more beneficial effects on these characters as compared with spray at only one stage. Similar results were reported by Bharti et al. (2013), Mathpal et al. (2015) and Imran et al. (2015). Previously, many reports have estimated the wheat response to exogenous application of Zn (both soil and foliar applied), but a little is known regarding combined application of Zn. Therefore, the present study was conducted to determine the effect of different Zn application methods on (a) agronomy and morphology traits, (b) grain phytate, ascorbic acid, and Zn concentrations and (c) correlations of these components in durum and bread wheat grain.

Materials and Methods

The experiment was conducted on the Faculty of Agriculture, Maragheh University of Maragheh city, Iran (37º22′ N latitude, 46º16′ E longitude and altitudes of 1542 m) in 2014 year. The soil of the experimental site had a clay loam texture with pH (H_2O) 7.2, 20% $CaCO_3$ and 0.4% organic matter. The concentration of DTPA-extractable Zn was 0.4 mg kg^{-1} soil (Lindsay and Norvell 1978), which is lower than the widely accepted critical Zn concentration of 0.5 mg kg^{-1} (Sims and Johnson 1991). The mean annual precipitation and mean annual temperature were 297 mm and 14.1°C, respectively (Table 1).

Pot experiment was carried out in plastic pots (PVC) with 20 cm diameter and 30 cm depth, containing 3.5 kg soil. Before sowing, the soil was mixed homogenously with a basal treatment of 200 mg N kg^{-1} soil as $Ca(NO_3)_2.4H_2O$ and 100 mg P kg^{-1} soil as KH_2PO_4. The pot experiment was carried out in factorial design in randomized complete block design (RCBD) with 30 treatments (6 Zn application methods, and 5 wheat genotypes) in four replications. The first factor was six levels of applied Zn were (1) control (non Zn application), (2) soil application (5 mg Zn kg^{-1} soil), (3) seed application (3% (w/v) Zn for 1 kg seed), (4) foliar application at stem elongation and early grain filling stages (zinc sulfate was sprayed at a rate of 0.44 g Zn l^{-1}), (5) seed spray + foliar (combination of methods 3 and 4) and (6) soil + foliar (combination of methods 2 and 4). Foliar application of Zn was performed in very late afternoon to avoid possible leaf damage caused by salts on sunny day and at high day temperature. The second factor was five wheat genotypes including two spring bread wheat ('Pishtaz' and 'Sivand') and three spring durum wheat ('Diyarbakır-81', 'Bisu-1' and line '45558'). The seeds were provided by Dryland Agricultural Research Institute (DARI) of Iran (Table 2). Fourteen seeds were sown in each pot and daily watered by deionized water, and the seedlings were thinned to seven seedlings per pot at 3 to 4-leaf stage. Foliar Zn treatments (as $ZnSO_4.7H_2O$) were applied along with 0.01% (v/v) Tween as surfactant and nitrogen at the rate of 1% urea.

At maturity, five plants were randomly harvested from each pot to measure the plant height, number of fertile spikelet per spike (FSS), number of grain per spike (GN), thousand kernel weight (TKW), biomass, grain yield and harvest index (HI). Subsamples of grains from each pot were washed with distilled water and rapidly dried with tissue papers before oven drying at 65°C for 48 hours. Then, grain samples were finely ground in a mill, ashed at 550°C for 6 h, and dissolved in 2 M hydrochloric acid (HCl) (Chapman and Pratt 1961). Concentrations of Zn, Fe, Mn and Cu in the digest solutions were determined by Atomic Absorption Spectrophotometer (AAS-6300 Shimadzu).

For phytate measurement, 60 mg finely-ground grain samples were extracted with 10 mL of 0.2 N HCl at room temperature for 2 h under continuous shaking. Phytate in the extract was determined by indirect method that uses absorption of pink color developed by un-reacted Fe (III) with 2,2′-bi-pyridine (Haug and Lantzsch 1983) at 519 nm with a Elisa (BioTek, Powre Wave XS2, USA). Molar concentration of phytate and Zn in grain was used to calculate phytate/Zn ratio.

A modification of Okamura (1980) and Thimmiah (2009) method was used for ascorbic acid measurement. A sample of 0.5 g finely-ground grain sample was dissolved in 1.5 ml of 5% (w/v) metaphosphoric acid. After centrifugation at 18000 g for 15 min, 200 µl collected supernatant was mixed with 200 µl of 150 mM-NaH_2PO_4 buffer (pH 7.4), 200 µl of water, 100 µl of 10 mM-dithiothreitol; and 100 µl of 0.5% (w/v) N-ethylmaleimide and left at room temperature for 15 min. Then, the samples were vortex-mixed and incubated at room temperature for >30 s. To each sample was then added 400 µl of 10% (w/v) trichloroacetic acid, 400 µl of 44% (v/v) H_3PO_4, 400 µl of 4% (w/v) bipyridyl in 70% (v/v) ethanol and 200 µl of 3% (w/v) $FeCl_3$. After vortex-mixing, samples were incubated at 37 °C for 60 min and were recorded at 525 nm with a spectrophotometer (Shimadzu, UV-2100, Kyoto, Japan). The concentration of ascorbic acid (AsA) was calculated from a standard curve using a series of standard solutions (0-70 µmol) of ascorbate (Sigma Aldrich, Germany).

All data were subjected to one-way analysis of variance (ANOVA) using SAS software (Version 8.0) and Duncan's Multiple Range Test at P=0.05 was used in pairwise comparisons of means.

Table 1. Monthly of climatic parameters in the growing season

Months	Temperature (°C)			Precipitation	Relative Humidity (%)		
	Max	Min	Average	(mm)	Max	Min	Average
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. List of genotypes used in the experiment

Number	Code	Name and/or pedigree	Wheat type	TKW†
1	Pishtaz	Pishtaz	Bread	39
2	Sivand	Sivand	Bread	38
3	Diyarbakır-81	Diyarbakır-81	Durum	40
4	Bisu-1	Bisu-1//CHEN-1/TEZ/3/HUI//CIT71/Cll	Durum	29
5	45558	45558	Durum	36

Source: Dryland Agricultural Research Institute (DARI) of Iran.

† TKW: Thousand kernel weight.

Results and Discussion

Agronomy traits

Significant differences were observed among genotypes for grain yield, biomass, thousand kernel weight (TKW), number of grain per spike (GN) and number of fertile spikelet per spike (FSS) (Table 3). Bread wheat genotypes had the higher grain yield and biomass than durum wheats (Table 4).

Grain yield is an ultimate end product of many yield-contributing components, physiological and morphological processes taking place in plants during growth and development. Zn application significantly increased grain yield, biological yield and GN, HI and FSS of all genotypes as compared to control (Table 3, 4 and 5). Maximum increase in grain yield (83%) was observed in soil application of Zn where minimum increase (6.7%) was observed in seed spray Zn treatment (Table 4). In this case, Gomaa et al. (2015) also reported increased grain yield of wheat with foliar application of micronutrient as compared to soil application. Also, Bharti et al. (2013) showed that increased in grain yield (15.9% higher than control) and biological yield was recorded by the combined application of soil and foliar spray of zinc sulphate (20 kg Zn/ha) as compared to control in wheat genotypes differing in their Zn efficiency.

Among different Zn application methods, 5 mg Zn kg^{-1} of soil application was the most effective that increased biomass, TKW, GN and FSS compared to control (Table 4, 5). There was a significant difference in TKW, GN and FSS among the different methods Zn application for all the five genotypes (Table 3, 5). So that the highest TKW was observed in soil + foliar Zn application in 'Diyarbakır-81' genotype, whereas the greatest GN and FSS were obtained by soil Zn application in 'Phishtaz' genotype (Table 5). But under control (no Zn application) the bread wheat genotype 'Sivand', under combination of soil + foliar Zn application the durum wheat genotype '45558', and under foliar Zn application the durum wheat genotype 'Bisu-1' the lowest TKW, GN and FSS, respective (Table 5). The most probable reason of these results might be due to the role of Zn in chlorophyll (Chl) biosynthesis, maintaining Chl a/b ratio, maintenance of photosynthetic machinery and biosynthesis of auxin, which regulate the remobilization of carbohydrates to the grains (Rehman et al. 2012). Moreover, the positive effects of Zn on plant may be due to its effects as a metal component or regulatory in some enzymes (Vallee and Falchuk 1993), which have essential roles in plant metabolism, and maintenance of membrane structure and function (Marschner 1993; Abd El-Hady 2007; Rehman et al. 2012).

Table 3. Analysis of variance for the effect of different Zn application methods on grain yield and yield components, agronomy and morphology traits and grain quality of bread and durum wheat

Source of variance	d.f.	Mean squares					
		Grain yield	Biomass	HI†	TKW	GN	FSS
Replication	3	0.22 ns	0.89 ns	104 ns	62 ns	17.3 ns	1.6 ns
Genotypes (G)	4	1.1 **	10.3 **	59 ns	790 **	744 **	41.7 **
Zn application (Zn)	5	0.39 **	2.81 **	106*	28 ns	92 **	3.5 *
G × Zn	20	0.11 ns	0.95 ns	42 ns	46 *	31 *	2.7 *
Error	87	0.11	0.68	37	26	20	1.6
CV (%)		38	27	19	16	25	14.6

Continued Table 3.

Source of variance	d.f.	Mean squares				
		Length				Plant height
		Awn	Spike	Peduncle	Penultimate	
Replication	3	1.81 ns	0.07 ns	1.15 ns	0.83 ns	1.17 ns
Genotypes (G)	4	69.8 **	71.9 **	241.2 **	14.9 **	473 **
Zn application (Zn)	5	3.07 **	1.16 **	21.9 **	1.66 *	44.5 **
G × Zn	20	0.82 ns	0.75 **	4.68 ns	0.68 ns	12.1 *
Error	87	0.64	0.34	3.24	0.61	6.91
CV (%)	-	9.47	10.6	9.33	11.5	6.68

Continued Table 3.

Source of variance	d.f.	Mean squares						
		Ascorbic acid	Nutrient concentrations in grain				Phytic acid content	Phytate/Zn molar ratios
			Zn	Fe	Cu	Mn		
Replication	3	69713 **	0.001 ns	3.58 ns	7.73 **	884 **	8277 **	1934 **
Genotypes (G)	4	69352 **	1973 **	1108 **	18.7 **	1431 **	1690 **	2114 **
Zn application (Zn)	5	34771 **	753 **	14.5 *	5.50 **	131 **	965 **	2299 **
G × Zn	20	16351 **	89.9 **	44.6 **	6.09 **	42.3 **	187 ns	576 **
Error	87	5796	2.47	6.02	0.26	0.64	221	141
CV (%)	-	50.8	5.47	3.68	3.35	2.89	41.5	30.7

ns, * and **: Non significant, significant at 5% and 1% levels of probability, respectively.

† HI: Harvest index, TKW: Thousand kernel weight, GN: Number of grain per spike, FSS: Number of fertile spikelet per spike, Zn: Zinc, Fe: Iron, Cu: Copper, Mn: Manganese.

Table 4. The effect of different Zn application methods on grain yield, biological yield, harvest index, length of awn, peduncle and penultimate, and phytic acid content of bread and durum wheat

Treatments	Grain yield (g plant^{-1})	Biomass (g plant^{-1})	Harvest index (%)	Length (cm)			Phytic acid content (mg g^{-1})
				Awn	Peduncle	Penultimate	
Genotypes							
Pishtaz	0.850 a	2.412 a	33.8	7.2 cd	20.3 b	7.7 a	24.7 b
Sivand	0.741 a	2.458 a	29.7	6.9 d	16.0 c	6.8 b	29.6 b
Diyarbakır-81	0.527 b	1.607 b	33.1	11 a	23.0 a	6.9 b	44.9 a
Bisu-1	0.347 b	1.080 c	31.5	9.3 b	15.9 c	5.5 c	38.9 a
45558	0.396 b	1.198 bc	32.3	7.5 c	21.1 b	6.8 b	40.9 a
Zinc application methods							
Control	0.435 c	1.523 bc	28.0 b	7.9 c	18.3 cd	6.6 bc	46.5 a
Seed spray	0.464 bc	1.399 c	33.2 a	8.5 ab	19.2 bc	6.8 abc	41.8 ab
Soil	0.797 a	2.370 a	33.3 a	8.4 ab	20.6 a	7.2 a	35.4 bc
Foliar	0.679 ab	2.026 ab	34.4 a	8.9 a	20.1 ab	6.9 ab	31.6 c
Seed spray + Foliar	0.497 bc	1.499 c	32.7 a	8.7 a	19.7 ab	6.5 bc	29.9 c
Soil + Foliar	0.561 bc	1.690 bc	30.9 ab	8.0 bc	17.9 d	6.4 c	29.7 c

Means followed by the same letter within columns are not significantly different ($P < 0.05$) according to Duncan's test.

In terms of the length of awn, spike, peduncle and penultimate and plant height, significant differences were observed between genotypes and different Zn application methods (Table 3). Both soil and foliar Zn applications significantly increased length of awn, peduncle, penultimate, and plant height (Table 4, 5). Our results are in agreement with Movahhedy-Dehnavy et al. (2009) and Abdoli et al. (2014) results who observed significant differences in plant height in safflower and bread wheat treated with foliar applied Zn.

The interaction between wheat genotypes and different methods of Zn application significantly affected the length of spike and plant height (Table 3). According to the results obtained, highest length of spike was by soil Zn application of 'Pishtaz' and 'Sivand' genotypes (8.4 and 8.3 cm, respectively) and lowest by seed spray Zn of '45558' genotype (Table 5). Also under soil Zn application, durum wheat genotype 'Diyarbakır-81' had the highest (47.8 cm) and under seed spray Zn the durum wheat genotype 'Bisu-1' the lowest (31.9 cm) plant height (Table 5). In generally, maximum spike and plant length was seen in soil Zn application, whereas the minimum ones were observed in seed spray (Table 5). Our findings are in close conformity with Zeb and Arif (2008) who reported that zinc application methods (soil and foliar) significantly affect the plant height. In this case, Marschner (1993, 1995) reported that Zn was required for the synthesis of tryptophan, the precursor of the formation of indole acetic acid (IAA), which results in improved cell division and growth of plant (Abdoli et al. 2013)

Grain quality

Grain ascorbic acid (AsA) concentration ranged from 84.3 to 199.9 μM g^{-1} under various Zn treatments (Table 6). Increase in grain AsA concentration was 137% with combination of seed spray + foliar Zn application, 121% with foliar Zn application and 85.2% with combination of soil + foliar Zn applications (Table 6). Soil application of Zn greatly increased grain yield and agronomy and morphology traits, but remained less effective in increasing AsA concentration in grain (Table 4, 5 and 6). In agreement with our results, Bharti et al. (2013) reported that the AsA content increased gradually with increasing rates of Zn in both the crop seasons. The interaction of Zn application and genotypes significantly affected AsA concentration in grain (Table 3), and the mean comparison showed that under no Zn application (control), bread wheat 'Sivand' had the lowest (38.4 μM g^{-1}); whereas under foliar treatments durum wheat '45558' had the highest (348 μM g^{-1}) AsA concentration in grain (Table 6).

Table 5. Mean comparison of interactions between genotypes and different Zn application methods on thousand kernel weight, number of grain per spike, number of fertile spikelet per spike, length of spike and plant height of bread and durum wheat

Zinc application methods	Pishtaz	Sivand	Diyarbakır-81	Bisu-1	45558	Mean	Change by Zn application (%)
Thousand kernel weight (g)							
Control	27.7 $^{f-m}$	21.7 ^{m}	36.1 $^{a-f}$	32.8 $^{b-h}$	28.8 $^{e-m}$	29.4	-
Seed spray	23.0 $^{j-m}$	23.5 $^{i-m}$	37.0 $^{a-e}$	34.4 $^{a-g}$	31.1 $^{c-k}$	29.8	1.4
Soil	23.1 $^{i-m}$	28.8 $^{e-m}$	40.6 ^{ab}	38.1 $^{a-d}$	31.7 $^{c-i}$	32.5	10.5
Foliar	22.7 $^{k-m}$	22.1 ^{lm}	37.6 $^{a-d}$	39.4 $^{a-c}$	31.4 $^{c-j}$	30.6	4.1
Seed spray + Foliar	31.1 $^{c-k}$	26.7 $^{g-m}$	38.6 $^{a-d}$	25.7 $^{h-m}$	30.4 $^{d-l}$	30.5	3.7
Soil + Foliar	27.0 $^{g-m}$	27.7 $^{f-m}$	42.1 ^{a}	35.2 $^{a-g}$	27.2 $^{g-m}$	31.8	8.2
Mean	25.8 d	25.1 d	38.6 a	34.3 b	30.1 c		
Number of grain per spike							
Control	18.6 $^{d-i}$	19.1 $^{d-i}$	12.8 ^{ij}	13.1 ^{ij}	13.4 $^{h-j}$	15.4 b	-
Seed spray	25.8 $^{b-d}$	19.6 $^{d-i}$	15.1 $^{f-j}$	12.5 ^{ij}	14.0 $^{g-j}$	17.4 b	13.0
Soil	34.4 ^{a}	24.6 $^{b-d}$	17.1 $^{e-j}$	14.2 $^{g-j}$	13.0 ^{ij}	20.6 a	33.8
Foliar	28.4 ^{ab}	27.9 $^{a-c}$	14.7 $^{g-j}$	12.4 ^{ij}	19.5 $^{d-i}$	20.6 a	33.8
Seed spray + Foliar	21.5 $^{b-g}$	22.4 $^{b-f}$	15.9 $^{f-j}$	12.5 ^{ij}	15.1 $^{f-j}$	17.4 b	13.0
Soil + Foliar	24.2 $^{b-e}$	20.9 $^{c-h}$	14.6 $^{g-j}$	13.1 ^{ij}	10.3 ^{j}	16.6 b	7.8
Mean	25.5 a	22.4 b	15.0 c	12.9 c	14.2 c		
Number of fertile spikelet per spike							
Control	8.4 $^{e-j}$	9.8 $^{b-f}$	7.1 ^{ij}	7.8 $^{f-j}$	9.0 $^{c-i}$	8.4 ab	-
Seed spray	10.0 $^{a-e}$	8.9 $^{d-i}$	7.8 $^{f-j}$	6.9 ^{ij}	8.0 $^{e-j}$	8.3 b	-1.2
Soil	11.9 ^{a}	11.4 ^{ab}	7.9 $^{e-j}$	7.5 $^{h-j}$	7.5 $^{h-j}$	9.2 a	9.5
Foliar	10.5 $^{a-d}$	11.0 $^{a-c}$	6.9 ^{ij}	6.6 ^{j}	8.9 $^{d-i}$	8.8 ab	4.8
Seed spray + Foliar	8.4 $^{e-j}$	9.7 $^{b-g}$	7.3 ^{ij}	6.9 ^{ij}	8.1 $^{e-j}$	8.0 b	-4.8
Soil + Foliar	9.5 $^{b-h}$	9.5 $^{b-h}$	8.1 $^{e-j}$	7.3 ^{ij}	7.6 $^{g-j}$	8.4 ab	0.0
Mean	9.8 a	10 a	7.5 bc	7.1 c	8.2 b		
Length of spike (cm)							
Control	7.0 $^{b-e}$	7.1 $^{b-e}$	4.4 $^{f-h}$	4.9 ^{fg}	3.9 ^{hi}	5.4 bc	-
Seed spray	7.8 $^{a-c}$	6.7 ^{de}	4.6 $^{f-h}$	4.1 $^{g-i}$	3.4 ^{i}	5.3 bc	-1.9
Soil	8.4 ^{a}	8.3 ^{a}	4.6 $^{f-h}$	4.4 $^{f-h}$	3.7 ^{hi}	5.9 a	9.3
Foliar	7.9 ^{ab}	7.6 $^{a-d}$	4.6 $^{f-h}$	4.0 $^{g-i}$	4.1 $^{g-i}$	5.7 ab	5.6
Seed spray + Foliar	6.6 ^{e}	6.9 $^{c-e}$	4.5 $^{f-h}$	4.2 $^{g-i}$	3.7 ^{hi}	5.2 c	-3.7
Soil + Foliar	7.1 $^{b-e}$	6.9 $^{c-e}$	5.2 ^{f}	4.5 $^{g-h}$	3.7 ^{hi}	5.5 bc	1.9
Mean	7.4 a	7.3 a	4.6 b	4.3 b	3.7 c		
Plant height (cm)							
Control	41.6 $^{b-f}$	33.7 ^{ij}	42.1 $^{b-f}$	35.2 $^{h-j}$	40.3 $^{c-g}$	38.6 cd	-
Seed spray	44.1 $^{a-c}$	37.1 $^{g-i}$	43.2 $^{b-e}$	31.9 ^{j}	39.0 $^{e-h}$	39.0 bc	1.0
Soil	44.9 ^{ab}	41.9 $^{b-f}$	47.8 ^{a}	33.5 ^{ij}	38.2 $^{f-h}$	41.2 a	6.7
Foliar	44.9 ^{ab}	39.6 $^{d-g}$	44.7 $^{a-c}$	33.5 ^{ij}	40.7 $^{b-g}$	40.7 ab	5.4
Seed spray + Foliar	42.7 $^{b-e}$	37.0 $^{g-i}$	43.5 $^{b-d}$	32.5 ^{j}	40.7 $^{b-g}$	39.3 bc	1.8
Soil + Foliar	42.4 $^{b-f}$	33.9 ^{ij}	41.4 $^{b-f}$	32.7 ^{j}	35.2 $^{h-j}$	37.1 d	-3.9
Mean	43.4 a	37.2 c	43.8 a	33.2 d	39.0 b		

Means followed by the same letter within columns are not significantly different ($P < 0.05$) according to Duncan's test. Mark dashes between the letters (-), represents other letters between them and is sorted alphabetically.

Zn treatments generally increased grain Zn, Fe and Mn concentrations compared with control (Table 3, 6). Depending on various Zn treatments, grain Zn concentration ranged from 21.7 (at control) to 37.7 mg kg^{-1} dry weight (at foliar Zn application). It is notable that the effects of Zn application on grain Zn depended on methods and timing of application. Foliar application was more effective in improving Zn concentration in grain compared with soil application (Table 6). It is known that the nutrient uptake of roots depends on different mechanisms and these mechanisms are controlled by different factors (Mohr and Schopfer 1994). Adding Zn to soil is relatively inefficient because of the poor mobility of Zn in soil and because of rapid adsorption of Zn in calcareous and/or clayey soils with neutral or higher pH (Alloway 2004). A significant increase in grain Zn concentration with foliar sprays at stem elongation and early grain filling stages may be attributed to phloem mobility of Zn in wheat (Haslett et al. 2001). Recent evidence demonstrates that crease phloem is the key path for Zn delivery to the endosperm (Cakmak et al. 2010b). In agreement with the results of the present study, Zhang et al. (2010) reported a 68% increase in grain Zn concentration with foliar Zn application to wheat plants at grain-development stage. Foliar application of Zn at critical growth stages increased concentration and content of Zn in rice grains (Naik and Das 2008; Stalin et al. 2011), pea (Rafique et al. 2015) and bread wheat (Abdoli et al. 2014). But, Hussain et al. (2012) reported that the soil Zn application increased grain yield (29%), whole-grain Zn concentration (95%) and whole-grain estimated Zn bioavailability (74%). Bread wheats could accumulate more Zn, Fe, Cu and Mn concentrations in grain compared with durum wheats (Table 6). But, the highest phytic acid (PA) content and phytate/Zn molar ratio were observed for durum wheat genotypes and the lowest for bread wheat genotypes (Table 4 and 6).

In all genotypes, the concentration of Zn in grain dry matter was increased with Zn application, especially in 'Bisu-1' and '45558' genotypes (Table 6). According to the results obtained, highest Zn concentration in grain was by foliar Zn application of 'Sivand' genotype (48.4 mg kg^{-1} dry weight) and lowest by no Zn application of '45558' and 'Bisu-1' genotypes (6.3 and 6.9 mg kg^{-1} dry weight, respectively) (Table 6). Also, there was a significant difference in micronutrients concentration in grain among the different methods Zn application for all the five genotypes. So that under combination of seed spray + foliar Zn application, bread wheat genotype 'Sivand' had the highest and under seed spray Zn the durum wheat genotype '45558' the lowest concentration of Fe in grain (Table 6). Under foliar Zn application, bread wheat genotype 'Sivand' had the highest (42.1 mg kg^{-1} dry weight) and under seed spray as well as soil + foliar Zn application the durum wheat genotype '45558' the lowest (13.7 and 13.7 mg kg^{-1} dry weight, respectively) concentration of Mn in grain (Table 6). This variability may be related to differences in mechanisms involved in uptake, translocation and internal utilization of micronutrients (Fageria and Baligar 2003). Moreover, Mabesa et al. (2013) reported genotypic variation for increase in grain Zn concentration (1 to 10 mg Zn kg^{-1}) by foliar Zn application at heading stage of rice crop. Studies of natural variation revealed the existence of notable differences for Zn accumulation in wheat grains between different wheat genotypes in response to soil and foliar application of Zn (Cakmak et al. 1997; Khoshgoftarmanesh et al. 2013).

As shown in Figure 1, correlations between Zn concentration in grain and Mn concentration (r = 0.41*), and Fe concentration in grain (r = 0.30*) were significant for the five genotypes with Zn application. By contrast, Zn concentration in grain and phytate/Zn molar ratio (r = -0.40*) were negatively correlated. The relationships between Zn, Fe, Mn, AsA and PA were highly significant, but the correlation coefficients were very low. This might be partially due to the larger sample number calculated. Irrespective of the method, application of Zn significantly decreased Cu concentration in grain (Table 6). This might be due to competition between these two cations for the transport carriers in the phloem (Stephan and Scholz 1993). Zn application methods decreased phytate contents in grain and decreased phytate/Zn molar ratio compared with control (Table 4, 6). Minimum phytate/Zn molar ratio of 8.8 and 10 (76.8 and 73.7% less than control, respectively) in wheat grains was achieved with foliar Zn application and seed spray + foliar Zn application (Table 6). According to the results obtained, highest phytate/Zn molar ratio was by no Zn application of 'Bisu-1' and '45558' genotypes and lowest by foliar Zn application of 'Sivand' genotype (Table 6). Phytate/Zn molar ratio ranged from 38.0 (in the control) to 8.8 (foliar Zn application treatment). An increased Zn concentration in grain and decreased PA content in grain resulted in grain phytate/Zn molar ratio dropping to as low as 15 (Table 4, 6). Therefore, the molar ratio of grain PA to Zn can also be used as a bioavailability indicator (Simic et al. 2009). A Phytate complex with Zn and other minerals hinders their absorption into human body (Nolan et al. 1987). The phytate/Zn molar ratios < 20 is generally desirable for improving human nutrition (Turnlund et al. 1984; Weaver and Kannan 2002). This finding indicated that the effect of foliar Zn application on predicted Zn bioavailability was dose-dependent and that foliar Zn application is useful to increase Zn bioavailability not only in whole grain but also in wheat flour (Cakmak et al. 2010a; Kutman et al. 2011).

Table 6. Mean comparison of interactions between genotypes and different Zn application methods on ascorbic acid, Zn, Fe, Cu and Mn concentrations in grain and phytate/Zn molar ratio of bread and durum wheat.

Zinc application methods	Pishtaz	Sivand	Diyarbakır-81	Bisu-1	45558	Mean	Change by Zn application (%)
Ascorbic acid (uM g^{-1})							
Control	57.8 ^{fg}	38.4 ^{g}	115 $^{d-g}$	69.7 $^{e-g}$	141 $^{d-g}$	84.3 d	-
Seed spray	122 $^{d-g}$	102 $^{d-g}$	119 $^{d-g}$	90.7 $^{e-g}$	284 $^{a-c}$	144 bc	70.1
Soil	63.2 ^{fg}	61.4 ^{fg}	126 $^{d-g}$	201 $^{b-e}$	189 $^{b-f}$	128 cd	51.8
Foliar	125 $^{d-g}$	101 $^{e-g}$	185 $^{b-f}$	175 $^{b-f}$	348 ^{a}	187 ab	121.1
Seed spray + Foliar	101 $^{e-g}$	105 $^{d-g}$	163 $^{c-g}$	338 ^{a}	294 ^{ab}	200 a	137.0
Soil + Foliar	87.0 $^{e-g}$	232 $^{a-d}$	183 $^{b-f}$	183 $^{b-f}$	96.6 $^{e-g}$	156 abc	85.2
Mean	92.5 d	106 cd	148 bc	176 b	225 a		
Zn concentrations in grain (mg kg^{-1} dry weight)							
Control	28.8 ^{ij}	37.9 ^{cd}	28.7 ^{ij}	6.9 ^{p}	6.3 ^{p}	21.7 f	-
Seed spray	34.0 ^{fg}	43.7 ^{b}	39.5 ^{c}	17.4 ^{o}	20.2 $^{l-n}$	31.0 c	42.9
Soil	20.4 ^{lm}	37.0 ^{de}	27.9 ^{j}	18.0 ^{no}	22.3 ^{kl}	25.1 d	15.7
Foliar	32.8 ^{gh}	48.4 ^{a}	42.0 ^{b}	30.8 ^{hi}	34.3 ^{fg}	37.7 a	73.7
Seed spray + Foliar	27.3 ^{j}	46.3 ^{a}	35.4 ^{ef}	21.8 ^{kl}	34.3 ^{fg}	33.0 b	52.1
Soil + Foliar	23.3 ^{k}	37.7 $^{c-e}$	23.7 ^{k}	17.0 ^{o}	18.2 $^{m-o}$	24.0 e	10.6
Mean	27.8 c	41.8 a	32.9 b	18.6 e	22.6 d		
Fe concentrations in grain (mg kg^{-1} dry weight)							
Control	76.9 $^{a-c}$	72.3 ^{de}	61.5 $^{i-k}$	67.3 ^{fg}	55.2 ^{lm}	66.7 ab	-
Seed spray	73.7 $^{a-d}$	73.1 $^{c-e}$	67.7 ^{fg}	64.0 $^{g-i}$	53.5 ^{m}	66.4 ab	-0.4
Soil	73.8 $^{a-d}$	69.4 ^{ef}	61.9 ^{ij}	60.8 $^{i-k}$	62.5 $^{h-j}$	65.7 b	-1.5
Foliar	77.2 ^{ab}	75.6 $^{a-d}$	66.6 ^{fg}	59.6 ^{jk}	60.3 $^{j-k}$	67.9 a	1.8
Seed spray + Foliar	68.2 ^{f}	77.5 ^{a}	66.2 $^{f-h}$	61.4 $^{i-k}$	62.4 $^{h-j}$	67.1 ab	0.6
Soil + Foliar	73.4 $^{b-d}$	72.9 ^{de}	61.7 ^{ij}	62.7 $^{h-j}$	57.7 ^{kl}	65.7 b	-1.5
Mean	73.9 a	73.5 a	64.3 b	62.6 c	58.6 d		
Cu concentrations in grain (mg kg^{-1} dry weight)							
Control	14.3 ^{mn}	19.7 ^{a}	14.6 $^{k-n}$	14.7 $^{j-n}$	16.0 $^{c-f}$	15.9 a	-
Seed spray	14.5 $^{l-n}$	17.1 ^{b}	15.7 $^{c-h}$	16.1 $^{c-e}$	14.8 $^{i-n}$	15.6 a	-1.9
Soil	13.4 ^{op}	16.3 ^{c}	15.0 $^{g-m}$	14.1 ^{no}	15.3 $^{e-l}$	14.8 b	-6.9
Foliar	15.7 $^{c-h}$	16.3 ^{c}	15.5 $^{c-j}$	11.3 ^{q}	15.4 $^{d-k}$	14.8 b	-6.9
Seed spray + Foliar	16.2 ^{cd}	16.0 $^{c-f}$	15.6 $^{c-i}$	15.4 $^{d-k}$	15.2 $^{f-l}$	15.7 a	-1.3
Soil + Foliar	14.8 $^{i-n}$	14.9 $^{h-n}$	15.8 $^{c-g}$	14.7 $^{j-n}$	13.3 ^{p}	14.7 b	-7.5
Mean	14.8 c	16.7 a	15.4 b	14.4 d	15.0 c		
Mn concentrations in grain (mg kg^{-1} dry weight)							
Control	33.9 ^{ef}	34.9 ^{de}	26.7 ^{lm}	21.2 ^{r}	16.7 ^{s}	26.7 d	-
Seed spray	36.0 ^{cd}	39.8 ^{b}	29.8 ^{j}	23.9 ^{p}	13.7 ^{u}	28.6 c	7.1
Soil	32.9 ^{fg}	39.2 ^{b}	28.4 ^{k}	24.8 ^{op}	22.6 ^{q}	29.6 b	10.9
Foliar	33.3 ^{f}	42.1 ^{a}	28.5 ^{k}	22.5 ^{q}	25.4 ^{no}	30.4 a	13.9
Seed spray + Foliar	30.2 ^{ij}	36.6 ^{c}	27.4 ^{kl}	14.7 ^{tu}	26.1 ^{mn}	27.0 d	1.1
Soil + Foliar	31.9 ^{gh}	31.1 ^{hi}	24.6 ^{op}	14.9 ^{t}	13.7 ^{u}	23.2 e	-13.1
Mean	33.0 b	37.3 a	27.6 c	20.3 d	19.7 e		
phytate/Zn molar ratio							
Control	12.3 $^{b-j}$	12.6 $^{b-j}$	17.1 $^{b-f}$	76.2 ^{a}	71.9 ^{a}	38.0 a	-
Seed spray	9.5 $^{d-j}$	9.5 $^{d-j}$	12.7 $^{b-j}$	22.3 ^{b}	21.7 $^{b-c}$	15.1 b	-60.3
Soil	15.3 $^{b-h}$	9.7 $^{d-j}$	13.3 $^{b-i}$	16.6 $^{b-g}$	19.2 $^{b-e}$	14.8 b	-61.1
Foliar	4.9 $^{h-j}$	2.0 ^{j}	11.3 $^{c-j}$	13.2 $^{b-i}$	12.7 $^{b-j}$	8.8 b	-76.8
Seed spray + Foliar	7.0 $^{f-j}$	4.2 ^{ij}	11.3 $^{c-j}$	18.3 $^{b-e}$	9.1 $^{e-j}$	10.0 b	-73.7
Soil + Foliar	6.1 $^{g-j}$	5.8 $^{g-j}$	18.7 $^{b-e}$	18.9 $^{b-e}$	20.2 $^{b-d}$	14.0 b	-63.2
Mean	9.2 b	7.3 b	14.1 b	27.6 a	25.8 a		

Means followed by the same letter within columns are not significantly different ($P < 0.05$) according to Duncan's test. Mark dashes between the letters (-), represents other letters between them and is sorted alphabetically.

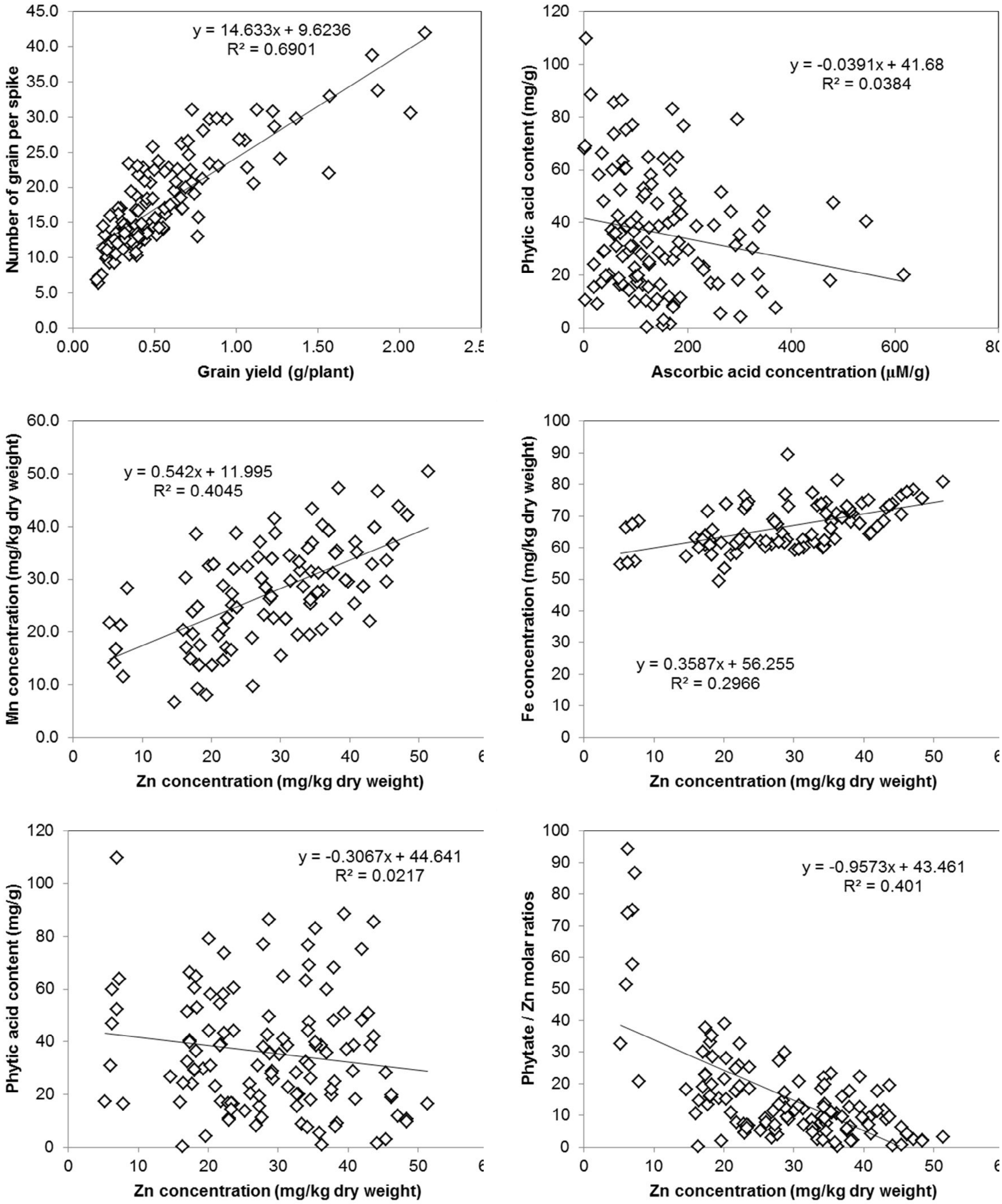

Figure 1. The relationship between grain yield with number of grain per spike, relationship between ascorbic acid concentration with phytic acid content, as well as relationship between Zn concentration with Mn and Fe concentration in grain, phytic acid content and phytate/Zn molar ratio of wheat

Conclusions

The results of this study showed that both soil and foliar applications of Zn were effective methods if a high grain yield and high morphology traits are desired. Also, foliar Zn application was much superior to soil application for increasing Zn, Fe and Mn concentrations in grain, even though much less Zn is applied in the foliar than the soil. The phytate/Zn molar ratio, however, was substantially decreased with the increase of Zn concentration in grain. Therefore, fertilizer strategy (e.g., agronomic biofortification) appears as short-term solution to alleviate malnutrition problem and foliar Zn application represents an effective approach to provide more dietary Zn from wheat-derived products to humans.

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References

Abd El-Hady BA. (2007). Effect of zinc application on growth and nutrient uptake of barley plant irrigated with saline water. Journal of Applied Sciences Research. 3(6), 431-436.

Abdoli M, Esfandiari E, Mousavi SB, Sadeghzadeh B. (2014). Effects of foliar application of zinc sulfate at different phenological stages on yield formation and grain zinc content of bread wheat (cv. Kohdasht). Azarian Journal of Agriculture. 1(1), 11-17.

Abdoli M, Saeidi M, Azhand M, Jalali-Honarmand S, Esfandiari E, Shekari F (2013). The effects of different levels of salinity and indole-3-acetic acid (IAA) on early growth and germination of wheat seedling. Journal of Stress Physiology and Biochemistry. 9(4), 329-338.

Alloway BJ (2004). Zinc in soils and crop nutrition. IZA Publications, Brussels.

Alloway BJ (2009). Soil factors associated with zinc deficiency in crops and humans. Environmental Geochemistry and Health. 31, 537-548.

Bharti K, Pandey N, Shankhdhar D, Srivastava PC, Shankhdhar SC (2013). Improving nutritional quality of wheat through soil and foliar zinc application. Plant, Soil and Environment. 59, 348-352.

Brinch-Pederson H, Borg S, Tauris B, Holm PB (2007). Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. Journal of Cereal Science. 46, 308-326.

Cakmak I (2008). Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant and Soil. 302, 1-17.

Cakmak I, Ekiz H, Yilmaz A, Torun B, Köleli N, Gültekin I, Alkan A, Eker S (1997). Differential response of rye, triticale, bread and durum wheats to zinc deficiency in calcareous soils. Plant and Soil. 188, 1-10.

Cakmak I, Kalayci M, Kaya Y, Torun AA, Aydin N, Wang Y, Arisoy Z, Erdem H, Yazici A, Gokmen O, Ozturk L, Horst WJ (2010b). Biofortification and localization of zinc in wheat grain. Journal of Agricultural and Food Chemistry. 58, 9092-9102.

Cakmak I, Pfeiffer WH, McClafferty B (2010a). Biofortification of durum wheat with zinc and iron. Cereal Chemistry Journal. 87, 10-20.

Chapman HD, Pratt PF (1961). Methods of Analysis for Soil, Plant and Water. Riverside, CA: Division of Agriculture Science, University of California.

Fageria NK, Baligar VC (2003). Methodology for evaluation of lowland rice genotypes for nitrogen use efficiency. Journal of Plant Nutrition. 26, 1315-1333.

Gomaa MA, Radwan FI, Kandil EE, El-Zweek SMA (2015). Effect of some macro and micronutrients application methods on productivity and quality of wheat (*Triticum aestivum* L.). Middle East Journal of Agriculture Research. 4, 1-11.

Graham RD (2008). Micronutrient deficiencies in crops and their global significance. In: Alloway, B.J. (Ed.), Micronutrient deficiencies in global crop production. Springer, Dordrecht. pp: 41-61.

Hambidge M (2000). Human zinc deficiency. Journal of Nutrition. 130, 1344s-1349s.

Haslett BS, Reid RJ. Rengel Z (2001). Zinc mobility in wheat: uptake and distribution of zinc applied to leaves or roots. Annals of Botany. 87, 379-386.

Haug W, Lantzsch H (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. Journal of the Science of Food and Agriculture. 34, 1423-1424.

Hurrell RF (2001). Modifying the composition of plant foods for better human health. In: Nösberger, J., Geiger, H.H., Struik, P.C. (Eds.), Crop Science: Progress and Prospects. CABI publishing, Bristol, pp. 53-64.

Hussain S, Aamer Maqsood M, Rengel Z, Aziz T (2012). Biofortification and estimated human bioavailability of zinc in wheat grains as influenced by methods of zinc application. Plant and Soil. 361, 279-290.

Imran M, Kanwal S, Hussain S, Aziz T, Maqsood MA (2015). Efficacy of zinc application methods for concentration and estimated bioavailability of zinc in grains of rice grown on a calcareous soil. Pakistan Journal of Agricultural Sciences. 52(1), 169-175.

Johns T, Enzaguirre PB (2007). Biofortification, biodiversity and diet: a search for complementary applications against poverty and malnutrition. Food Policy Journal. 32, 1-24.

Khoshgoftarmanesh AH, SanaeiOstovar A, Sadrarhami A, Chaney R (2013). Effect of tire rubber ash and zinc sulfate on yield and grain zinc and cadmium concentrations of different zinc-deficiency tolerance wheat cultivars under field conditions. European Journal of Agronomy. 49, 42-49.

Kutman UB, Yildiz B, Cakmak I (2011). Improved nitrogen status enhances zinc and iron concentrations both in the whole grain and the endosperm fraction of wheat. Journal of Cereal Science. 53, 118-125.

Lindsay WL, Norvell WA (1978). Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Science Society of America Journal. 42, 421-428.

Lonergan PF, Pallotta MA, Lorimer M, Paull JG, Barker SJ, Graham RD (2009). Multiple genetic loci for zinc uptake and distribution in barley (*Hordeum vulgare*). New Phytologist. 184, 168-179.

Lucca P, Poletti S, Sautter C (2006). Genetic engineering approaches to enrich rice with iron and vitamin A. Physiologia Plantarum. 126, 291-303.

Mabesa RL, Impa SM, Grewal D, Johnson-Beebout SE (2013). Contrasting grain-Zn response of biofortification rice (*Oryza sativa* L.) breeding lines to foliar Zn application. Field Crops Research. 149, 223-233.

Malakouti MJ (2007). Zinc is a neglected element in the life cycle of plants. Middle Eastern and Russian Journal of Plant Science and Biotechnology. 1(1), 1-12.

Marschner H (1993). Zinc uptake from soils. In: Robinson AD (Ed.), Zinc in soils and plants. Dordrecht: Kluwer Academic Publishers, pp: 59-77.

Marschner H (1995). Mineral nutrition of higher plants. 2nd Addition Academic Press Inc. pp. 890.

Mathpal B, Srivastava PC, Shankhdhar D, Shankhdhar SC (2015). Zinc enrichment in wheat genotypes under various methods of zinc application. Plant, Soil and Environment. 61(4), 171–175.

Mayer JE, Pfeiffer WH, Beyer P (2008). Biofortified crops to alleviate micronutrient malnutrition. Current Opinion in Plant Biology. 11, 166-170.

Mohr H, Schopfer P (1994). Plant Physiology. Springer-Verlag, Berlin, Heidelberg, New York.

Movahhedy-Dehnavy M, Modarres-Sanavy SAM, Mokhtassi-Bidgoli A (2009). Foliar application of zinc and manganese improves seed yield and quality of safflower (*Carthamus tinctorius* L.) grown under water deficit stress. Industrial Crops and Products. 30, 82-92.

Naik SK, Das DK (2008). Relative performance of chelated zinc and zinc sulphate for lowland rice (*Oryza sativa* L.). Nutrient Cycling in Agroecosystems. 81, 219-227.

Nasiri Y, Zehtab-Salmasi, S, Nasrullahzadeh S, Najafi N, Ghassemi-Golezani K (2010). Effects of foliar application of micronutrients (Fe and Zn) on flower yield and essential oil of chamomile (*Matricaria chamomilla* L.). Journal of Medicinal Plants Research. 4(17), 1733-1737.

Nolan KB, Duffin PA, McWeeny DJ (1987). Effects of phytate on mineral bioavailability. In vitro studies on Mg^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+} and Zn^{2+} (also Cd^{2+}) solubilities in the presence of phytate. Journal of the Science of Food and Agriculture. 40, 79-85.

Okamura M (1980). An improved method for determination of L-ascorbic acid and L-dehydroascorbic acid in blood plasma. Clinica Chimica Acta. 103(3), 259-268.

Phattarakul N, Rerkasem B, Li LJ, Wu LH, Zou CQ, Ram H, Sohu VS, Kang BS, Surek H, Kalayci M, Yazici A, Zhang FS, Cakmak I (2012). Biofortification of rice grain with zinc through zinc fertilization in different countries. Plant and Soil. 361, 131-141.

Raboy V (2001). Seeds for a better future: 'Low phytate' grains help to overcome malnutrition and reduce pollution. Trends in Plant Science. 6, 458-462.

Rafique E, Yousra M, Mahmood-Ul-Hassan M, Sarwar S, Tabassam T, Choudhary TK (2015). Zinc application affects tissue zinc concentration and seed yield of pea (*Pisum sativum* L.). Pedosphere. 25(2), 275-281.

Rehman H, Aziz T, Farooq M, Wakeel A, Rengel Z (2012). Zinc nutrition in rice production systems: A review. Plant and Soil. 361, 203-226.

Rengel Z, Batten GD, Crowley DE (1999). Agronomic approaches for improving the micronutrient density in edible portions of field crops. Field Crops Research. 60, 27-40.

Sadeghzadeh B (2013). A review of zinc nutrition and plant breeding. Journal of Soil Science and Plant Nutrition. 13, 905-927.

Sadeghzadeh B, Rengel Z, Li C (2009). Differential zinc efficiency of barley genotypes grown in soil and chelator-buffered nutrient solution. Journal of Plant Nutrition. 32, 1744-1767.

Sadeghzadeh B, Rengel Z, Li C (2015). Quantitative trait loci (QTL) of seed Zn accumulation in barley population Clipper x Sahara. Journal of Plant Nutrition. 38(11), 1672-1684.

Sadeghzadeh B, Rengel Z, Li C, Yang H (2010). Molecular marker linked to a chromosome region regulating seed Zn accumulation in barley. Molecular Breeding. 25, 167-177.

Simic D, Sudar R, Ledencan T, Jambrovic A, Zdunic Z, Brkic I, Kovacevic V (2009). Genetic variation of bioavailable iron and zinc in grain of a maize population. Journal of Cereal Science. 50, 392-397.

Sims JT, Johnson GV (1991). Micronutrients soil tests. In: Mordcvedt, J.J., Cox, F.R., Shuman, L.M., Welch, R.M. (Eds.), Micronutrients in Agriculture. SSSA Book Series No. 4, Madison, WI. pp: 427-476.

Stalin P, Das T, Muthumanickam D, Chitdeshwari T, Velu V (2011). Foliar nutrition of rice to enhance micronutrient concentration in grains. International Rice Research Notes (0117-4185), 1-6.

Stephan UW, Scholz G (1993). Nicotianamine: mediator of transport of iron and heavy metals in the phloem? Physiologia Plantarum. 88, 522-529.

Thimmiah SR (2009). Standard Methods of Biochemical Analysis. Kalyani Publishers, New Delhi, pp. 545.

Turnlund JR, King JC, Keyes WR, Gong B, Michel MC (1984). A stable isotope study of zinc absorption in young men: effects of phytate and α-cellulose. The American Journal of Clinical Nutrition. 40, 1071-1077.

Vallee BL, Falchuk KH (1993). The biochemical basis of zinc physiology. Physiological Reviews. 73, 79-118.

Weaver CM, Kannan S (2002). Phytate and mineral bioavailability. In: Reddy, N.R., Sathe, S.K. (Eds.), Food phytate. CRC Press, Boca Raton, pp: 211-223.

Welch RM, Graham, RD (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. Journal of Experimental Botany. 55, 353-364.

White PJ, Broadley MR (2009). Biofortification of crops with seven mineral elements often lacking in human diets-iron, zinc, copper, calcium, magnesium, selenium, and iodine. New Phytologist. 182(1), 49-84.

World Health Organization (2002). World Health Report 2002: Reducing Risks, Promoting Healthy Life. http://www.who.int/whr/2002/en/whr02_en.pdf (retrieved 13.07.2010).

Zeb T, Arif M (2008). Effect of zinc application methods on yield and yield components of maize. Department of Agronomy, NWFP Agricultural. University, Peshawar, Pakistan.

Zhang Y, Shi R, Rezaul KMD, Zhang F, Zou C (2010). Iron and zinc concentrations in grain and flour of winter wheat as affected by foliar application. Journal of Agricultural and Food Chemistry. 58, 12268-12274.