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REGULAR ARTICLE

Differences in grain zinc are not correlated with root uptake and grain translocation of zinc in wild emmer and durum wheat genotypes

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Abstract

Background and aims Cereal-based foods fall short of providing adequate dietary zinc (Zn) to human beings. Developing new genotypes with high genetic capacity for root uptake and grain deposition of Zn is an important challenge. There is a large genetic variation for grain Zn concentration among and between wheat species, especially within wild emmer wheat (*Triticum turgidum ssp. dicoccoides*) that can be exploited in order to understand the physiological mechanisms contributing to grain Zn accumulation.

Methods Eight different wild emmer genotypes and two durum wheat (*Triticum durum*) cultivars were used to investigate root uptake, root-to-shoot translocation and remobilization (i.e., retranslocation) from flag leaves into grains of $^{65}ZnSO_4$-treated plants. The initial seed Zn concentrations of wild emmer wheat and durum genotypes used in the experiments were different, ranging from 45 to 73 mg kg^{-1} and from 35 to 40 mg kg^{-1}, respectively. Plants were grown in nutrient solution for the experiments investigating root uptake and shoot transport of Zn by using ^{65}Zn labeled $ZnSO_4$ and in soil medium for the experiments studying shoot and grain Zn concentrations and ^{65}Zn translocation from flag leaves into grains. The treatment of flag leaves with ^{65}Zn was realized by immersion of flag leaves into $^{65}ZnSO_4$ solution for 15 seconds and for 5 times during the anthesis and early milk stages.

Results Wild emmer and durum wheat genotypes expressed highly significant differences in root uptake and root-to-shoot translocation of ^{65}Zn and translocation of ^{65}Zn from flag leaves into grains. However, none of these parameters showed a significant correlation either with the initial seed Zn concentrations at sowing or the grain Zn concentrations at harvest. The durum wheat cultivars with higher grain yield had lower concentration of Zn both in seeds at sowing or in grains at harvest, while wild emmer genotypes with lower grain yield capacity had higher concentration of Zn both in seeds at sowing or in grains at harvest. The concentration or content (total amount) of Zn in shoot during the early growth stage also did not correlate with the initial seed Zn concentrations.

Conclusions Differences in grain Zn concentration of wild emmer and cultivated wheats could not be explained by root Zn uptake and Zn translocation from flag leaf into grains during seedling and reproductive growth stages, respectively. It seems that there are

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additional key factors affecting the expression of genetic variation for grain Zn accumulation.

Keywords Zinc uptake · Zinc translocation · Grain zinc · Wild emmer wheat · Durum wheat

Introduction

Cereals are the most important food crops globally and provide more than half of the daily calorie intake of human populations (FAOSTAT 2011). In terms of protein supply, wheat is the major cereal crop providing 21 % of the daily protein consumption per capita compared to 13 % from rice and 4 % from maize on the global scale (FAOSTAT 2011). Grain yield of wheat has been continuously improved over the past several decades through breeding and management efforts (Edgerton 2009; Grassini et al. 2013). These increases were, however, associated with a significant decline in grain concentrations of protein and minerals, especially micronutrients such as zinc (Zn) and iron (Fe) (Fan et al. 2008; Morgounov et al. 2013). Since modern wheat cultivars are inherently low in grain Zn, increases in grain yield further reduced grain Zn through dilution. Moreover, up to 50 % of cereal-cultivated soils globally have low amounts of plant-available Zn (i.e., chemically soluble Zn) which additionally reduces grain Zn concentrations (Cakmak 2008). Therefore, Zn deficiency in human populations not surprisingly coincides with geographical distribution of soils with limited Zn availability as well as poverty. Dietary Zn deficiency causes diverse health problems in human beings, especially in children, such as impairments in brain function and development and vulnerability to deadly infectious diseases due to immune dysfunction.

Cultivated wheat usually has grain Zn concentrations ranging from 20 to 35 mg kg^{-1} (Rengel et al. 1999; Cakmak et al. 2004) or lower than 20 mg kg^{-1} when grown on potentially Zn deficient soils (Cakmak et al. 2010a). These values are too low to meet the daily required Zn intake. Wheat is categorized as a poor source of dietary Zn intake not only due to low levels of total Zn, but also high grain phytate which is known to reduce the bioavailability of Zn in the diet and thus absorption of Zn in the digestive tract (Welch and House WA 1982; Lönnerdal 2000).

"Biofortification" is a newly adopted term in the scientific literature defined as enrichment of edible parts of food crops with both total and bioavailable amount of micronutrients through agricultural approaches such as plant breeding and fertilizer strategy (White and Broadley 2005; Pfeiffer and McClafferty 2007). The biofortification approach is widely believed to be the most sustainable and cost-effective way for alleviation of micronutrient deficiency problems in humans compared to other solutions such as dietary diversification, supplementation and fortified food consumption (Bouis and Welch 2009). Biofortification of wheat with Zn involves genetic (breeding-based) and agronomic (fertilizer-based) strategies. Strength and limitations of these strategies have been reviewed extensively elsewhere and it has been highlighted that these strategies are not separate solutions to the problem; rather, they are synergistic (Cakmak 2008; Cakmak et al. 2010a; Velu et al. 2014).

Success of a breeding program aiming at improving grain Zn depends not only on the availability of Zn in the growth medium, but also the availability of large genetic variation for root uptake and grain accumulation of Zn. Extensive screening studies on cultivated modern wheat cultivars showed that genetic variation for grain Zn is very limited and not promising for exploitation in breeding programs (Rengel et al. 1999; Cakmak et al. 2010a; Zhao et al. 2009). In recent years, the wild progenitor of cultivated durum and bread wheat, *Triticum turgidum ssp. Dicoccoides* (wild emmer wheat) has drawn much attention due to its exceptionally high variation in grain Zn and also compatibility with the breeding-based biofortification efforts (Cakmak et al. 1999; Ortiz-Monasterio and Graham 2000; Peleg et al. 2008). For example, screening of over 800 genotypes of wild emmer wheat under greenhouse conditions showed that grain Zn concentrations vary from 14 to 190 mg kg^{-1} (Cakmak et al. 2004), indicating that wild emmer wheat is a highly promising genetic material. Probably, 6B is the candidate chromosome carrying the genes affecting grain Zn concentration. It was shown that *Gpc-B1* on chromosome 6B is a major locus affecting grain Zn concentration, probably by inducing early senescence and mobilization of Zn from vegetative tissue into grain (Uauy et al. 2006; Distelfeld et al. 2007).

Remobilization of Zn from vegetative issue is not the only driver in enhancement of grain Zn. An enhanced root Zn uptake and pool of vegetative Zn reserves, especially during the grain filling period, are further important factors contributing to grain Zn (Waters et al. 2009; Kutman et al. 2012; Hussain et al. 2016). Indeed, grain Zn accumulation is under influence of

various physiological steps starting from roots to shoots and from shoots into grains (Waters and Sankaran 2011; Olsen and Palmgren 2014). A high genetic capacity to absorb Zn effectively from growth medium may greatly contribute to grain Zn through i) increasing Zn pools in vegetative tissue for subsequent transportation of Zn into grain, and ii) direct delivery of Zn into seeds during the reproductive growth stage. According to Waters et al. (2009) and Kutman et al. (2012), Zn remobilization from vegetative tissues into grain is a major pathway for increasing grain Zn, when the growth medium is not supplied with adequate Zn.

To our knowledge there is no published report about the genetic variation in root uptake, shoot transport and grain translocation of Zn among different genotypes of wild emmer wheat differing markedly in grain Zn concentrations. By using eight different wild emmer genotypes along with two durum wheat (*Triticum durum*) cultivars with large variation in seed Zn, this study investigated root uptake, root-to-shoot translocation and retranslocation from flag leaves into grain and their relation to "seed" or "grain" Zn concentrations (i.e., "seed" is designated to define the initial seed material used in planting, whereas "grain" is designated for the fully-matured seeds harvested from the experimental plants). A radioactive isotope of Zn (^{65}Zn) was utilized to measure trace amounts of Zn during root uptake, root-to-shoot translocation and remobilization from flag leaves into grains.

Materials and methods

Seed material

Seeds of eight wild emmer wheat genotypes (*Triticum turgidum* L. ssp. *Dicoccoides*: TTD 172, TD 153, TD 531, TD 678, TTD 96, , TTD 27, TD 536, TD 510) and two durum wheat cultivars (*Triticum turgidum* L. ssp. *Durum* [Desf.] Saricanak-98 and Balcali-2000) were kindly provided by Dr. Hakan Ozkan, Cukurova University Field Crops Department and were selfed two times under field conditions at the research farm of Cukurova University prior to use in the experiments described below. A part of the seed material from each genotype was analyzed for total Zn by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Vista-Pro Axial; Varian Pty Ltd., Mulgrave, Australia) prior to sowing. All seeds were first surface-sterilized in 80 % ethanol (*v*/v), vernalized for 2 weeks at 4 °C and then used in germination. One nutrient solution and two soil culture experiments were conducted as described below.

Root uptake and root-to-shoot translocation of ^{65}Zn in young seedlings grown in nutrient solution

Seeds were germinated for five days at 24 °C in perlite moistened with saturated $CaSO_4$. Seedlings were transplanted into pots containing 2.7 L of continuously aerated nutrient solution with the following composition (as μM): 2000 $Ca(NO_3)_2$, 1000 $MgSO_4$, 100 KCl, 200 KH_2PO_4, 700 K_2SO_4, 1 H_3BO_3, 0.5 $MnSO_4$, 0.2 $CuSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$, 100 FeEDTA and 1 $ZnSO_4$. Plants were grown in a computer controlled growth chamber for nine days (light intensity: 450 μmol m^{-2} s^{-1}, light-dark cycle: 16–8 h, temperature: 24-20 °C, humidity: 65–75 %) and the nutrient solutions were renewed every three days. Nine days after transplant (i.e. 9 DAT), half of the plants were supplied with 1 μM $ZnSO_4$ labeled with 77 KBq of ^{65}Zn from a source of $^{65}ZnCl_2$ at 37 MBq whereas the other half was reserved for total Zn analysis by ICP-OES. Following ^{65}Zn treatment, nutrient solution was sampled at 15 min intervals to determine the decrease in ^{65}Zn activity using a gamma counter (Perkin Emler 2480 WIZARD^2 Automatic Gamma Counter, USA). On the third sampling (i.e. at 45 min after ^{65}Zn treatment) ^{65}Zn activity in nutrient solution was reduced by approximately 50 % and the uptake experiment was terminated by transferring the roots to 1 mM $CaSO_4$ for 10 min and subsequently to 1 mM Na_2EDTA for 15 min to exchange and chelate apoplasmic root Zn (von Wiren et al. 1996). All solutions were then immediately changed with the non-radioactive versions. Plants were harvested as shoot and root samples 24 h after the ^{65}Zn treatment (i.e. 10 DAT) to calculate root-to-shoot Zn translocation rate over 24 h along with root Zn uptake rate over 45 min. The activity of ^{65}Zn in the root and shoot samples were analyzed by gamma counting. The data collected as counts per minute (CPM) was converted to Zn concentration using standards of known activity and concentration.

For the determination of total Zn concentration, shoot or root samples were dried at 70 °C, milled in a vibrating agate cup mill (Pulverisette 9, Fritsch GmbH, Idar-Oberstein, Germany) and subjected to microwave-assisted acid digestion (MarsExpress, CEM Co.,

Matthews, USA) in a 1:5 mixture of 30 % H_2O_2 (*w/v*) and 65 % HNO_3 (*w/v*). Zinc concentration in digested samples was analyzed by ICP-OES. Zinc analysis results were checked against the certified Zn concentrations of standard reference materials (SRM 1547 - Peach Leaves and SRM 1567a - Wheat Flour) obtained from the National Institute of Standards and Technology (Gaithersburg, USA).

Shoot Zn concentrations of plants grown in soil

The soil used in greenhouse experiments was from the Central Anatolia region of Turkey and had a clayey-loam texture with low organic matter (15 g kg^{-1}), high $CaCO_3$ (180 g kg^{-1}) and high pH (8.0 in 1:1 H_2O). Soil DTPA-extractable Zn concentration (Lindsay and Norvell 1978) was 0.1 mg kg^{-1}. The experiment was carried out in a greenhouse in plastic pots containing 3.0 kg of soil supplied with a basal treatment of 0.5 mg Zn kg^{-1} soil (as $ZnSO_4$), 200 mg N kg^{-1} soil (as $Ca[NO_3]_2$), 100 mg P kg^{-1} soil (as K_2HPO_4), 2.5 mg Fe kg^{-1} soil (as FeEDTA) and 25 mg S kg^{-1} soil (as K_2SO_4). All nutrients were mixed thoroughly with soil prior to sowing. Initially 12 seeds were sown in each pot and the seedlings were thinned to eight per pot following emergence. Shoots were harvested at 40 days after sowing (i.e. 40 DAS) for determination of dry matter production and total Zn concentration by ICP-OES as described above.

Grain yield, grain Zn concentrations and Zn translocation from flag leaves into seeds

Seeds were sown in plastic pots containing 3.0 kg of soil that received same amount of nutrients described above. At the stem elongation stage, plants were top-dressed with 200 mg N kg^{-1} soil (as $Ca[NO_3]_2$) and shoots were supported with nylon coated wiring attached to a wooden pole to prevent lodging of wild emmer genotypes. Part of the pots were used in the experiment investigating translocation of Zn from flag leaves into seeds.

To measure translocation of Zn from the flag leaf into the seeds, the flag leaf of each plant (approximately 10 cm from leaf tip) was treated with 0.1 % (*w/v*) $ZnSO_4$ solution labeled with 1480 KBq of ^{65}Zn and containing 0.01 % Tween®20. In each application, the flag leaf was incubated for five seconds in the application solution three times. Leaf applications were repeated five times at 2 or 3 days intervals between the Zadoks scale (Zadoks et al. 1974) stages 69 (anthesis completed) and 77 (late milk) for the genotypes tested. On the next day after the final application, the flag leaf of each plant was sequentially rinsed in dH_2O and 1 mM $CaCl_2$ to remove Zn that was not taken up by the leaf tissue, but adhered to the flag leaf surface. Fully matured plants were harvested, hand-threshed and weighed for above ground biomass production and seed yield. The cleaned seeds were further analyzed for ^{65}Zn activity and total Zn concentration as described above. Flag leaf and the remainder of shoot (straw) were also analyzed for ^{65}Zn activity to calculate the retranslocated portion of Zn in the seeds.

Statistical analysis

A complete randomized design was employed in all experiments. Seed Zn concentration at sowing was determined with three replicates. Root Zn uptake experiments were conducted with five independent replications (pots). Soil culture experiments at 40 DAS and maturity were carried out with three and four independent replications (pots) respectively. Significant differences among mean values were determined by Fisher's least significant difference (LSD) test at 5 % probability level by JMP (SAS Institute, Cary, USA). Pearson correlation analysis was performed in Statistix (Analytical Software, Tallahassee, USA) and the statistical significance of the relationship was expressed in $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$ levels.

Results

Seed Zn concentrations of the wild emmer genotypes grown under the same conditions and used for sowing in the experiments of this study were significantly different (Table 1). The highest seed Zn concentrations were found in wild emmer genotypes ranging from 44.5 (TD 153) to 72.9 mg kg^{-1} (TD 678), whereas durum wheat cultivars Saricanak-98 and Balcali-2000 had the lowest seed Zn concentration at sowing (Table 1). On average, seed Zn concentration at sowing time was 53 % higher in the wild emmer genotypes compared to the durum wheat cultivars.

Both wild emmer and durum wheats exhibited significant differences in shoot dry matter production in nutrient solution at 10 DAT and in soil culture at 40 DAS (Table 1). In the nutrient solution experiment at 10 DAT,

Table 1 Seed Zn concentration at sowing, shoot dry matter production at 10 days after transplant (DAT) to nutrient solution, 40 days after sowing (DAS) in soil and at maturity (straw dry weight) and grain yield of wheat cultivars and wild emmer genotypes grown in nutrient solution (10 DAT) or soil culture (40 DAS and maturity)

	Seed Zn		Shoot dry matter production				Maturity			
Genotype	at sowing		10 DAT (nutrient sol.)		40 DAS (soil)		Straw dry wt.		Grain yield	
	(mg kg^{-1})		(mg plant^{-1})		(mg plant^{-1})		(g plant^{-1})		(g plant^{-1})	
Saricanak-98	39.7	i	175	f	531	a	8.4	d	2.70	a
Balcali-2000	35.2	j	132	g	492	abc	8.0	d	3.03	a
TTD 172	59.4	d	189	ef	393	e	9.3	cd	1.32	b
TD 153	44.5	h	234	bcd	421	de	13.1	a	0.71	cd
TD 531	47.2	g	257	b	515	ab	12.0	abc	1.40	b
TD 678	72.9	a	240	bc	464	abcd	12.5	a	1.15	bcd
TTD 96	71.0	b	208	cdef	430	cde	9.0	d	0.67	d
TTD 27	63.4	c	352	a	461	bcde	9.6	cd	1.17	bcd
TD 536	49.0	f	217	cde	528	ab	11.9	abc	1.28	bc
TD 510	51.3	e	327	a	472	abcd	9.8	bcd	0.60	d
$LSD_{0.05}$	1.6		40		69		2.7		0.58	

durum wheat genotypes had much less shoot dry matter production compared to the wild emmer genotypes. However, at 40 DAS, the differences in shoot dry weight between all genotypes were less marked with the exception of TTD 172 which had the lowest shoot dry weight both at 10 DAT and at 40 DAS among all wild emmer genotypes (Table 1). At maturity, the straw dry matter production in wild emmer wheats was about 53 % higher than that of durum wheats. There was a high variation in straw dry matter production among wild emmer genotypes ranging from 9.0 (TTD 96) to 13.1 g plant^{-1} (TD 153) whereas durum wheat cultivars produced about 8.2 g plant^{-1}. In case of wild emmer genotypes, there was a general trend of higher grain yield with higher biomass production, but some genotypes had very low (TD 153) or high (TTD 172) grain yield as compared to their straw weight (Table 1). Durum wheat genotypes produced the highest grain yield, but had the lowest straw weight, translating to a very high harvest index which was about 3.6 fold higher than that of wild emmer genotypes (data not shown).

Shoot Zn concentration at 10 DAT was significantly different among and within wild emmer and durum wheat genotypes (Table 2). TTD 172 with the lowest shoot dry weight at 10 DAT had the highest shoot Zn concentration. In contrast to plants harvested at 10 DAT, soil-grown plants harvested at 40 DAS did not show significant difference in shoot Zn concentration (Table 2). Similar to Zn concentration, shoot Zn content (i.e. total Zn uptake by shoots) was different among the genotypes at 10 DAT, but not at 40 DAS. At 10 DAT both durum wheat genotypes had the lowest Zn content, with values being significantly lower than five out of eight wild emmer genotypes.

Among the wild emmer genotypes, the highest seed Zn concentrations (above 50 mg kg^{-1}) were recorded in TTD 27 and TTD 96 whereas the durum wheat cultivars Saricanak-98 and Balcali-2000 had the lowest Zn concentrations. On average, wild emmer genotypes had about 81 % higher Zn concentration than the durum wheats. With exception of TTD 27, durum wheats grains had more total Zn content (i.e. Zn uptake per plant) than wild emmer genotypes (Table 2). On average, durum wheats accumulated about 59 % more Zn than wild emmer genotypes. Durum wheat cultivars had higher grain yield and lower grain Zn concentrations, while wild emmer genotypes had lower grain yield and higher grain Zn concentrations.

The shoot concentrations and contents of Zn did not show any significant relation to the Zn concentrations of the seeds used at sowing (Fig. 1). Both the Zn concentrations of seeds at sowing and the Zn concentrations of harvested grains showed a negative correlation with grain yield of the genotypes. However, there existed a positive and significant correlation between Zn concentrations of seeds and

Table 2 Shoot Zn concentration and content at 10 days after transplant (DAT) and 40 days after sowing (DAS) and grain Zn concentration and content at maturity of wheat cultivars and wild emmer genotypes grown in nutrient solution (10 DAT) or soil culture (40 DAS and maturity)

Genotype	Shoot Zn at 10 DAT (nutrient sol.)				Shoot Zn at 40 DAS (soil)		Grain Zn at maturity (soil)			
	Concentration		Content		Concentration	Content	Concentration		Content	
	(mg kg^{-1})		(μg plant^{-1})		(mg kg^{-1})	(μg plant^{-1})	(mg kg^{-1})		(μg plant^{-1})	
Saricanak-98	74.6	d	12.2	de	51.7	27.3	22.5	c	60.4	ab
Balcali-2000	92.4	bc	12.6	e	46.9	23.1	22.2	c	68.2	a
TTD 172	148.2	a	28.6	b	52.3	20.5	41.4	ab	52.0	abc
TD 153	82.7	cd	19.4	cd	45.7	19.2	30.1	bc	18.4	d
TD 531	74.6	d	19.5	c	50.2	25.8	27.9	bc	38.1	bcd
TD 678	82.6	cd	18.4	c	46.8	21.6	48.9	a	57.3	ab
TTD 96	75.5	d	15.5	cde	56.8	24.4	51.5	a	35.1	bcd
TTD 27	99.9	b	36.6	a	54.0	24.8	53.1	a	60.6	ab
TD 536	74.9	d	16.2	cde	52.8	28.1	27.6	bc	35.4	bcd
TD 510	78.0	cd	25.4	b	47.2	22.2	43.1	ab	26.0	cd
$LSD_{0.05}$	14.7		5.9		n.s.	n.s.	16.7		27.0	

grains ($r = 0.92$, $P < 0.001$) (Fig. 2). Seed and grain concentrations of other micronutrients (e.g. Fe, Mn and Cu) had no significant correlation (see Table S1, available online). The results of the root uptake and root-to-shoot translocation of ^{65}Zn at 10 DAT showed existence of significant differences among wild emmer and durum wheat genotypes (Table 3). On average, the durum wheat cultivars had about two-fold higher root ^{65}Zn uptake and root-to-shoot translocation rate. Of the wild emmer genotypes, TD 536 expressed the lowest ^{65}Zn root uptake and shoot translocation, whereas TD 678 expressed the highest ^{65}Zn uptake and translocation values (Table 3). There was a highly significant correlation between root uptake and shoot translocation of ^{65}Zn (data not shown).

The experiment with ^{65}Zn-mobilization (translocation) from flag leaves into grains showed that the durum wheat cultivars had much higher (almost 4-fold) ^{65}Zn activity in grains than the wild emmer genotypes (Table 3). The ^{65}Zn-mobilization ratios, calculated by dividing ^{65}Zn activity in grains by the total activity in the flag leaves and straw were also substantially different among the genotypes tested, especially within the genotypes of wild emmer wheat (Table 3). As was the case with ^{65}Zn activity, the durum wheat cultivars had also much greater (about 3.2-fold) ^{65}Zn-mobilization ratio compared to the wild emmer genotypes. Of the emmer wheat genotypes, TD 678 and TD 536 exhibited the lowest (4.6 %) and highest (16.4 %) ^{65}Zn mobilization rates, respectively (Table 3). There was a negative relationship ($p < 0.05$) between ^{65}Zn mobilization and seed or grain Zn concentrations (Fig. 3).

Discussion

The eight wild emmer genotypes used in the present study had a large range in their seed Zn concentrations (i.e., 44.5–72.9 mg kg^{-1}), while the two durum wheat genotypes had the lowest seed Zn concentrations (i.e., 35.2 and 39.7 mg kg^{-1}). Some of the emmer genotypes had very high Zn concentrations in seeds (over 70 mg kg^{-1}) (Table 1). Such high seed Zn concentrations were also found in other studies involving screening of various wild emmer wheat germplasms (Cakmak et al. 2004; Gomez-Becerra et al. 2010). Differing seed Zn concentrations of the genotypes used in the experiment did not affect the shoot dry matter production of plants during the early growth stage (Table 1), probably because the experimental plants were grown under adequate Zn conditions. It is known that seeds with higher Zn concentrations contribute positively to growth of plants when grown under low Zn supply (Harris et al. 2007; Cakmak 2008).

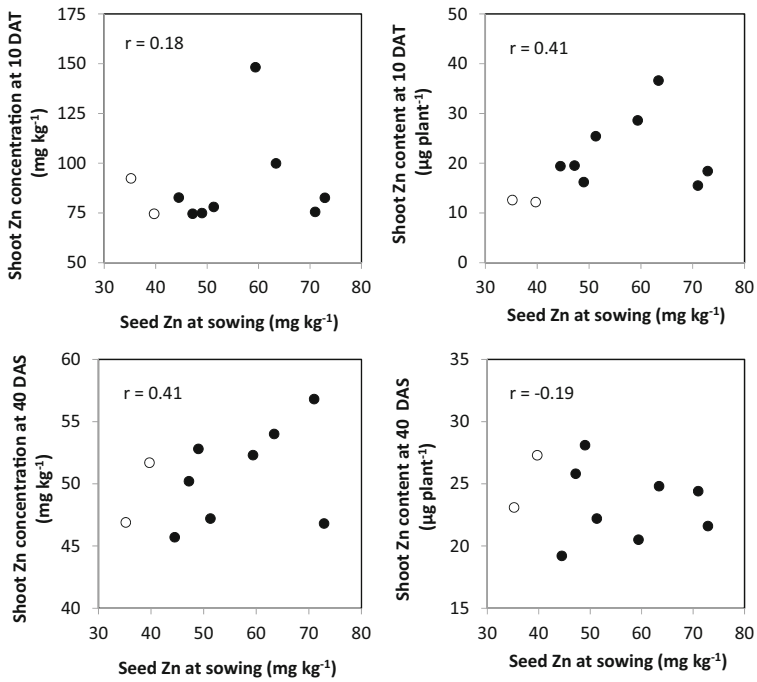

Fig. 1 Relationships between Zn concentrations of seeds used at sowing and shoot Zn concentrations and content of plants grown in nutrient solution (10 DAT) or in soil culture (40 DAS). Open circles are durum wheat cultivars whereas filled circles are wild emmer genotypes

Lower grain yield capacity of wild wheats has been often discussed as one of the major reasons for higher grain Zn concentrations compared to modern wheat cultivars (Cakmak 2008; Murphy et al. 2008; Zhao et al. 2009). Also in this study, wild emmer genotypes with much lower grain yield capacity had higher grain Zn concentrations (Table 1). However, for some of the wild emmer genotypes, grain Zn concentrations were not directly related to grain yield capacity. For example TTD 27 and TD 536 had more or less similar grain yield, but their grain Zn concentrations differed up to 2-fold. There are several reports showing that differences in grain Zn among genotypes of a given species are not always related to grain size or grain yield (Cakmak et al. 2004; McDonald et al. 2008; Velu et al. 2014). These observations suggest that besides environmental factors, there are specific physiological and genetic factors contributing to grain Zn. Root uptake, root-to-shoot transport and translocation (or remobilization) from vegetative tissue into grains are major determinants affecting accumulation of Zn in grains (Cakmak et al. 2010a; Waters and Sankaran 2011; Olsen and Palmgren 2014). Absorption of Zn by roots is the first step in the transportation of Zn from soil into developing grains. Published evidence shows that overexpression of certain Zn transporter proteins at the plasma membranes of root cells significantly contributes to grain Zn (Bashir et al. 2013; Olsen and Palmgren 2014).

The genotypes tested in the present study exhibit significant differences in shoot Zn concentrations and total amount of Zn per shoot (i.e., content) when grown in nutrient solution (Table 2). However, no relationship was found between the Zn concentrations of seeds used at sowing (Table 1) and the capacity of plants for Zn uptake by roots and for Zn translocation from roots to shoots (Table 2). Similarly, shoot Zn concentration and content during the early growth stage of plants grown in nutrient solution or soil culture (Table 2) did not show any relation to the concentration of Zn in seeds used in the experiment. Interestingly, all genotypes used in this study had more or less similar shoot Zn concentration and Zn accumulation (i.e., content) during the stem elongation stage, although these genotypes differed substantially in their seed Zn concentrations at sowing and grain Zn concentrations at harvest. Wild emmer genotypes with higher seed Zn concentrations were not superior to the durum wheat cultivars with lower seed Zn concentrations in terms of root Zn uptake and root-to shoot translocation capacity (Table 3). These results clearly show that root Zn uptake capacity of genotypes and shoot accumulation of Zn during early growth stage has no relation to the initial seed concentrations of

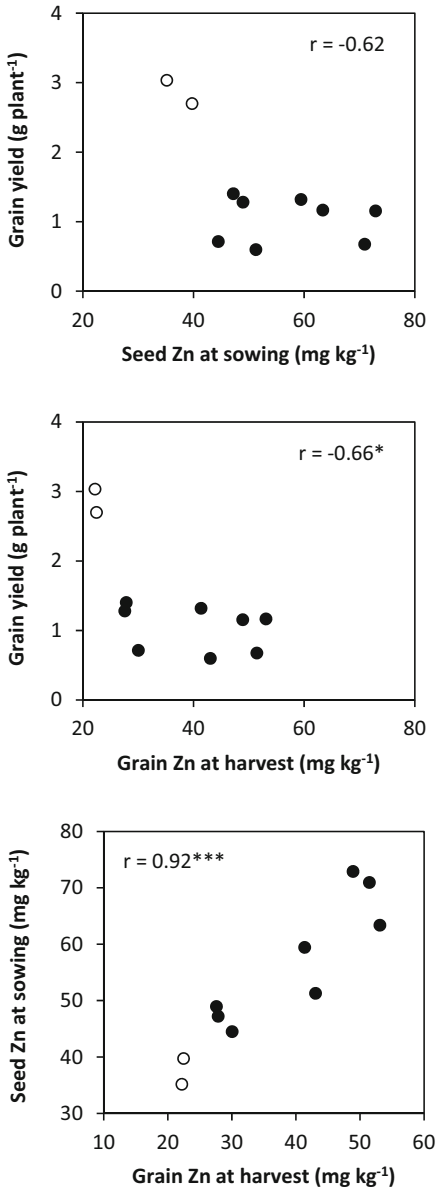

Fig. 2 Relationships between seed Zn at sowing with grain yield (top), grain Zn at harvest with grain yield (middle) and grain Zn at harvest with seed Zn at sowing (bottom). Open circles are durum wheat cultivars whereas filled circles are wild emmer genotypes. * and *** indicates significant relationships at $p \leq 0.05$, and $p \leq 0.001$ respectively

plants. Previously, similar observations were also made among different cereal species stating that grain Zn concentrations do not correlate to shoot Zn concentration or root Zn uptake capacity of plants. For example, rye has exceptionally high tolerance to Zn deficiency in severely Zn-deficient calcareous soils and exhibits much greater root Zn uptake capacity and root-to-shoot Zn translocation when compared to durum or bread wheat cultivars, but grain Zn concentration of rye is lower than the durum and bread wheat cultivars (Cakmak et al. 1997, 1998). Similarly in rice, enhancement in root Zn uptake is not necessarily associated with increases in grain Zn (Jiang et al. 2008), indicating that there are mechanisms other than root Zn uptake determining grain Zn accumulation.

Remobilization of Zn from vegetative tissue into seed represents an important source of seed Zn (Stomph et al. 2009; Waters et al. 2009; Sperotto 2013; Pottier et al. 2014). Thus, the available pool of Zn in vegetative tissue during the reproductive growth stage is of critical relevance for Zn-remobilization into grains. Foliar Zn application is, therefore, highly effective in improving grain Zn concentrations when foliar Zn fertilizers are applied during the reproductive growth stage (Cakmak et al. 2010b; Zhang et al. 2011; Mabesa et al. 2013). Zinc remobilization from leaf tissue into grain differs between genotypes of a given species as shown in rice and wheat (Phattarakul et al. 2012; Mabesa et al. 2013; Xu et al. 2012). Similarly also in the present study, it has been shown that the emmer wheat and durum wheat genotypes differed markedly in their ability to translocate Zn from flag leaves into grains. However, there was a distinct inverse relationship ($p < 0.05$) between the mobilization rate of ^{65}Zn from flag leaves and the Zn concentrations of grains harvested or seeds used at sowing (Fig. 3). In fact, the wild emmer genotype with highest Zn translocation capacity (i.e., TD 536) had the lowest seed Zn concentration among all wild emmer genotypes. A similar observation was made by Sperotto et al. (2013) in rice plants. Removal of flag leaves in rice plants grown under field conditions did not affect grain Zn (and also grain Fe) concentrations. It is obvious that besides the Zn pools in flag leaf tissue there are other important sources and mechanisms which contribute to grain Zn. For example, stem reserves of Zn play a critical role in improving grain Zn. Previous studies in wheat clearly showed that when Zn is sufficient in growth medium, Zn accumulates at large amounts in stem during vegetative growth stage, and then is depleted significantly during the grain development stage with corresponding increases in grain Zn (Pearson and Rengel 1994; Kutman et al. 2012). Very recently, in a doubled-haploid mapping population of barley, Hussain et al. (2016) showed a close relationship between grain Zn and remobilization of Zn from

Table 3 Root Zn uptake and shoot Zn translocation, and grain ^{65}Zn activity and ^{65}Zn mobilization from flag leaf in wheat cultivars and wild emmer genotypes grown in nutrient solution for 10 days after transplant (DAT) or soil culture (maturity)

Genotype	10 DAT (nutrient solution)				Maturity (soil)			
	Root Zn uptake		Shoot Zn translocation		^{65}Zn activity		^{65}Zn mobilization	
	(nmol g^{-1} root dw. 45 min^{-1})				(CPM)		(%)	
Saricanak-98	205	b	87	a	4652	a	31.9	a
Balcali-2000	229	a	81	ab	4907	a	23.9	ab
TTD 172	105	e	55	c	1314	bc	11.8	cd
TD 153	120	d	46	d	474	c	6.3	d
TD 531	78	g	28	fg	1070	bc	5.7	d
TD 678	147	c	77	b	1622	bc	4.6	d
TTD 96	101	e	37	de	694	c	5.3	d
TTD 27	96	ef	46	d	2423	b	7.3	cd
TD 536	56	h	19	g	1025	c	16.4	bc
TD 510	81	g	33	ef	1078	bc	12.8	cd
$LSD_{0.05}$	12		9		1375		9.8	

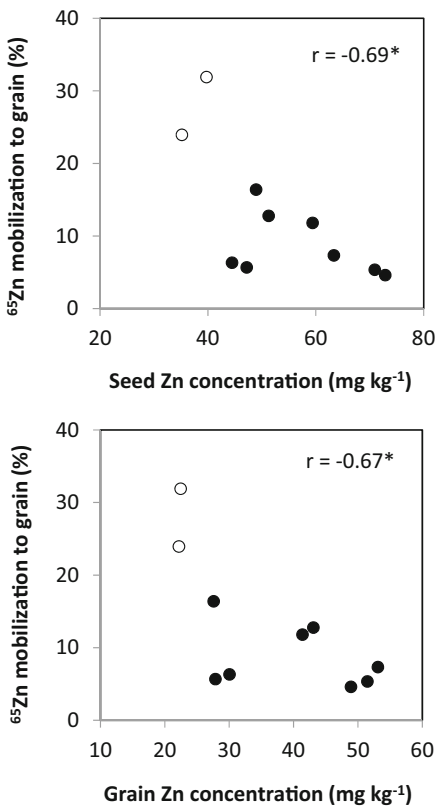

Fig. 3 The relationships between ^{65}Zn mobilization ratio to grain and seed Zn concentration at sowing (top) or grain Zn concentration at harvest (bottom). Open circles are durum wheat cultivars whereas filled circles are wild emmer genotypes. * indicates a significant relationship at $p \leq 0.05$

stem and leaves. It seems that grain Zn accumulation is a highly complex trait and most likely controlled by multiple genes with small or moderate effects on grain Zn. Therefore it is not surprising that a number of QTLs were identified associated with accumulation of Zn in grains (Distelfeld et al. 2007; Peleg et al. 2009; Srinivasa et al. 2014; Xu et al. 2012; Tiwari et al. 2016).

It is worth noting the highly significant ($p < 0.001$) and positive relationship between the Zn concentrations of the harvested grains and seeds used at sowing (Fig. 2). This correlation was still highly significant even when the low seed-Zn wheat cultivars were not considered ($p < 0.01$), suggesting that although quite variable the Zn accumulation capabilities of the wild emmer wheats were highly stable (i.e., in field and in greenhouse with different soils and management). Despite the high variation in grain Zn of wild emmer genotypes, their grain yield were only a fraction of the durum wheat cultivars. When durum wheats were omitted from the correlation, grain yield had no relation to grain or seed Zn in wild emmer genotypes, suggesting that selection for both traits (i.e. yield and grain Zn) is possible without compromising from each other. As found in the previous studies (Cakmak et al. 2004; Peleg et al. 2008), there was a close positive relationship between grain Zn and Fe concentrations, and this

correlation seems to be specific, because there was not a positive correlation between grain Zn and Mn (Table S1).

In conclusion, the variation in grain Zn concentration of wild emmer and cultivated wheats could not be explained by the differences in root Zn uptake and Zn translocation from flag leaf into grains during seedling and reproductive stages, respectively. Apparently, there are additional key factors affecting the expression of genetic variation for grain Zn in wild emmer wheat. The results also indicate that emmer wheat genotypes with higher grain yield and Zn concentration should be considered for breeding high-Zn cultivars, although the major physiology behind the variation in grain Zn accumulation remains elusive.

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