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REGULAR ARTICLE

Grain zinc, iron and protein concentrations and zinc-efficiency in wild emmer wheat under contrasting irrigation regimes

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Abstract Micronutrient malnutrition, and particularly deficiency in zinc (Zn) and iron (Fe), afflicts over three billion people worldwide, and nearly half of the world's cereal-growing area is affected by soil Zn deficiency. Wild emmer wheat [*Triticum turgidum* ssp. *dicoccoides* (Körn.) Thell.], the progenitor of domesticated durum wheat and bread wheat, offers a valuable source of economically important genetic diversity including grain mineral concentrations. Twenty two wild emmer wheat accessions, representing a wide range of drought resistance capacity, as well as two durum wheat cultivars were examined under two contrasting irrigation regimes (well-watered control and water-limited), for grain yield, total biomass production and grain Zn, Fe and protein concentrations. The wild emmer accessions exhibited high genetic diversity for yield and grain Zn, Fe and protein concentrations under both irrigation regimes, with a considerable potential for improvement of the cultivated wheat. Grain Zn, Fe and protein concentrations were positively correlated with one another. Although irrigation regime significantly affected ranking of genotypes, a few wild emmer accessions were identified for their advantage over durum wheat, having consistently higher grain Zn (e.g., 125 mg kg^{-1}), Fe (85 mg kg^{-1}) and protein (250 g kg^{-1}) concentrations and high yield capacity. Plants grown from seeds originated from both irrigation regimes were also examined for Zn efficiency (Zn deficiency tolerance) on a Zn-deficient calcareous soil. Zinc efficiency, expressed as the ratio of shoot dry matter production under Zn deficiency to Zn fertilization, showed large genetic variation among the genotypes tested. The source of seeds from maternal plants grown under both irrigation regimes had very little effect on Zn efficiency. Several wild emmer accessions revealed combination of high Zn efficiency and drought stress resistance. The results indicate high genetic potential of wild emmer wheat to improve grain Zn, Fe and protein concentrations, Zn deficiency tolerance and drought resistance in cultivated wheat.

Keywords *Triticum turgidum* ssp. *dicoccoides* · Zinc · Iron · Zinc-efficiency · Drought · Grain quality

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Introduction

Wheat (*Triticum* spp.) is the major staple food crop in many parts of the world in terms of cultivated area and food source, contributing 28% of the world edible dry matter (DM) and up to 60% of the daily calorie intake in several developing countries (FAO 2006). Therefore, the composition and nutritional quality of the wheat grain has a significant impact on human health and well-being, especially in the developing world. Micronutrient malnutrition, and particularly deficiency in Zn and Fe, afflicts over three billion people worldwide (Welch and Graham 2004; Bouis 2007), resulting in overall poor health, anemia, increased morbidity and mortality rates, and lower worker productivity (Cakmak et al. 2002; Demment et al. 2003; Hotz and Brown 2004). Producing micronutrient-enriched cereals (biofortification), either agronomically or genetically, and improving their bioavailability are considered promising and cost-effective approaches for diminishing malnutrition (Bouis 2003; Poletti et al. 2004; Welch and Graham 2004; Ghandilyan et al. 2006; Distelfeld et al. 2007). This solution, however, requires a comprehensive exploration of potential genetic resources and an in-depth understanding of their micronutrient accumulation mechanisms.

Micronutrient deficiencies in soils are also a critical problem for cereals productions causing severe reductions in yield and nutritional quality of the grains. Nearly half of the world's cereal-growing area is affected by soil Zn deficiency, particularly in calcareous soils of arid and semiarid regions (Graham and Welch 1996; Cakmak 2002), suffering also from water deficit. Various morphological and physiological parameters have been suggested to explain the genotypic differences in expression of high Zn deficiency tolerance (Zn efficiency, the ability of a genotype to grow better in a Zn-deficient soil) (Cakmak et al. 1998; Rengel 2001). Among cultivated wheats, durum wheat [*Triticum turgidum* ssp. *durum* (Desf.) MacKey] is the most sensitive to Zn deficiency (Graham et al. 1992; Kalayci et al. 1999). The sensitivity of plants to Zn deficiency is usually more pronounced under water-limited soil conditions (Ekiz et al. 1998; Bagci et al. 2007). Thus, developing new cultivars, combining improved tolerance to Zn deficiency in soils and increased Zn concentration in grain is a high-priority research topic, especially for the arid and semiarid regions.

Plant domestication and breeding processes have led to increased crop productivity, but at the same time, it has narrowed the genetic basis of crop species (Ladizinsky 1998). Therefore, a major objective of modern breeding is to identify in the wild ancestors of crop plants valuable alleles that were "left behind" and re-introduce them into cultivated crops (Tanksley and McCouch 1997; Gur and Zamir 2004). Wild emmer wheat [*T. turgidum* ssp. *dicoccoides* (Körn.) Thell.] is the tetraploid (2n=4x=28; genome BBAA) progenitor of cultivated wheats (Feldman 2001). It is fully compatible with the tetraploid (BBAA) durum wheat and can be crossed with the hexaploid (2n=6x=42; BBAADD) bread wheat (*T. aestivum* L.) (Feldman and Sears 1981). Wild emmer offers a valuable source of allelic variation for various economically important traits (e.g. Nevo et al. 2002) including drought resistance (Peleg et al. 2005, 2007) and grain mineral concentrations (Cakmak et al. 2004).

The overall objective of this study was to explore the wild emmer wheat germplasm as a potential source for improving grain mineral concentrations and Zn-efficiency in cultivated wheat. In this paper we report on (i) the genetic variation in grain micronutrient and protein concentrations of wild emmer wheat accessions under two levels of water availability, (ii) genetic variation in Zn-efficiency of seeds originating from water-limited and well-watered conditions and (iii) the associations between grain mineral concentration, Zn efficiency and productivity.

Materials and methods

Plant material

Twenty two wild emmer wheat accessions (*T. turgidum* ssp. *dicoccoides*) as well as two durum wheat cultivars (*T. turgidum* ssp. *durum*) were tested for grain Zn, Fe and protein concentrations under well-watered and water-limited irrigation conditions and for the tolerance to Zn deficiency on a Zn-deficient calcareous soil. The wild emmer accessions tested in this study were collected along a naturally occurring gradient of water availability and represent a wide range of drought resistance capacity found in wild emmer wheat populations from Israel and surrounding regions (Peleg et al. 2005, 2007).

Characterization of wild emmer germplasm under Screenhouse conditions

The experiments were conducted during the winter at the experimental farm of The Hebrew University of

Jerusalem in Rehovot, Israel (34°47′ N, 31°54′ E; 54 m above sea level). Seeds were disinfected (3.6% Sodium Hypochloric acid, for 10 min), placed in moistened germination paper and vernalized (14 days, 4°C in dark). Seedlings were planted on natural sandy soil in an insect-proof screenhouse (0.27 by 0.78 mm pore size screen) with a polyethylene top to eliminate rainfall. The soil at this location is brown–red degrading sandy soil (Rhodoxeralf) composed of 76% sand, 8% silt and 16% clay. A split-plot factorial (accession × irrigation regime) block design with three replicates was employed; each block consisted of two main plots (for the two irrigation treatments), with various accessions in sub-plots. Each subplot consisted of four 80-cm long rows with plants spaced 5×25 cm, within and between rows, respectively. Two irrigation regimes were applied via drip system: well-watered control and water-limited (hereafter termed as “wet” and “dry”, respectively). The wet treatment was irrigated weekly with total amount of 750 mm, whereas the dry treatment was irrigated every other week with total amount of 250 mm. Water was applied during the winter months (December–March) to mimic the natural pattern of rainfall in the eastern Mediterranean region.

To minimize seed dispersal, plants were harvested when 50% of the plants in an individual plot reached maturity. All the above ground biomass was harvested and spikes were separated from the vegetative organs (stems and leaves). Spikes were oven-dried at 35°C (5 days) to preserved seed viability, while the vegetative parts were dried at 80°C (48 h) before dry weight was determined. Since wild emmer wheat is hardly threshable and spike DM is highly correlated with grain yield (r>0.93; unpublished data), we have used DM as an indicator of yield potential. Total DM was calculated as the sum of vegetative DM and spike DM.

A “drought-susceptibility index” (S) was calculated for plant productivity variable according to Fischer and Maurer (1978) as:

$$\mathbf{S} = \left(\mathbf{1} - \mathbf{Y_{dry}}/\mathbf{Y_{wet}}\right)/\left(\mathbf{1} - \mathbf{X_{dry}}/\mathbf{X_{wet}}\right)$$

where Y_{dry} and Y_{wet} are the mean performances of a certain genotype under the respective treatments and X_{dry} and X_{wet} are the mean performances of all genotypes under these treatments, respectively.

Grains obtained under the two irrigation regimes were used for analysis of Zn, Fe and N concentrations. Nitrogen in the grain was determined by the indophenolblue procedure following Kjeldahl digestion. Grain nitrogen concentration was multiplied by 5.7 to obtain grain protein concentration (GPC). Grain concentrations of Zn and Fe were determined by inductively coupled plasma-optical emission spectroscopy (Varian-Vista-Pro, Australia). Measurements of minerals have been checked by using the certified values of the related minerals in the reference leaf and grain samples received from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

Greenhouse experiment for Zn efficiency

The seeds harvested from the field experiments in Rehovot, Israel, under two irrigation regimes were used to investigate Zn deficiency tolerance (Zn efficiency) of the genotypes under greenhouse conditions at Sabanci University, Istanbul by using a Zn-deficient soil from Central Anatolia, a region having an acute Zn deficiency in soils (Cakmak et al. 1996a). The soil used for Zn deficiency tolerance experiment had a clayey loam texture, pH 7.6, and 0.1 mg diethylenetriamine pentaacetic acid-extractable Zn per kg soil. About 10–12 seeds were sown in 1.8 kg soil in plastic pots with (+Zn=5 mg Zn kg^{-1} soil) and without (−Zn=0 mg Zn kg^{-1} soil) Zn applications in form of $ZnSO_4.7H_20$. Before potting, the soil was mixed homogenously with a basal treatment of 200 mg N kg^{-1} soil as $Ca(NO_3)_2$ and 100 mg P kg^{-1} soil as KH_2PO_4. The pots were thinned to 6 seedlings per pot after emergence and daily watered by using deionized water. After 35 days of growth under greenhouse conditions, shoots were harvested, washed with deionised water and dried at 70°C. Zinc efficiency ratio was calculated as the percentage of DM produced under Zn-deficiency relative to DM produced under Zn fertilization. The dried shoot samples were used for analysis of Zn as described above.

Statistical analysis

The JMP® 6.0 statistical package (SAS Institute 2005) was used for conducting the statistical analyses, unless indicated otherwise. A factorial model was employed for the analysis of variance, with accession and blocks considered as random effects and the irrigation regime as a fixed effect. Comparison between genotypes was based on Duncan's least significant difference at the 5% probability level. Principal component analysis (PCA) was used to determine the associations among the variables measured using SPSS ver.15 (SPSS 2006). PCA was based on the correlation matrix and was presented as biplot ordinations of populations (PC scores). Two components were extracted using Eigen values >1 to ensure meaningful implementation of the data by each factor.

Results

Analysis of variance carried out for the 22 wild emmer wheat accessions and two durum wheat cultivars grown in field with (wet) and without (dry) adequate irrigation revealed a significant effect of irrigation regime only on spike DM and total DM (Table 1). Significant effect of accession, as well as irrigation × accession interaction were noted for all variables. It is worth noting that the same results were obtained also for the 22 wild emmer wheat accessions when analysed without the two durum cultivars.

Limited water application (250 mm) reduced plant productivity (spike DM and total DM) of all genotypes by 33 and 37%, respectively compared to the well-watered conditions (Table 2). The S represents the loss of yield under drought compared to well-watered conditions (Fischer and Maurer 1978); genotypes with low S values are thought to be more drought resistant. There was a large variation in the S values among the 22 wild emmer accessions both for the spike DM and total DM (Table 2). The accessions MM 5/2 and MM 5/4 had very low S values for both yield parameters. The accession 18-39 had the lowest S value in the case of spike DM, but not for the total DM (Table 2). Both durum wheat cultivars showed an intermediate S values, in accordance with our previous results (Peleg et al. 2005)

Grain mineral analysis of the 22 wild emmer accessions showed a large genetic variation for Zn, Fe and protein concentrations under both irrigation regimes (Table 3). The wild emmer accessions exhibited higher grain Zn concentration under both treatments (e.g., range: 69–140 mg kg^{-1} and 71–134 mg kg^{-1} for the dry and wet treatments, respectively) as compared to the durum cultivars (e.g., range: 49–55 mg kg^{-1} and 53–56 mg kg^{-1}, respectively; Table 3). Similar to Zn, the wild accessions exhibited also higher grain Fe concentration (52–80 mg kg^{-1} and 48–88 mg kg^{-1} for the dry and wet treatments, respectively) as compared to the durum cultivars (30–33 mg kg^{-1} and 38–47 mg kg^{-1}, respectively; Table 3). The accessions 24–39, KH 5/1, MM 5/2 and MM 5/4 had both very high Zn (greater than 90 mg kg^{-1}) and high Fe (greater than 70 mg kg^{-1}) concentrations under both treatments. GPC showed also a significant variation among the wild emmer accessions under both irrigation regimes (e.g., 174–287 and 164–382 g kg^{-1} for the dry and wet treatments, respectively) and greater values as compared to the two durum cultivars (e.g., 149–165 and 165–184 g kg^{-1}, respectively; Table 3). The accession 15-T-6 contained very high GPC under both irrigation regimes. This accession together with the accession 9-72 had the highest grain Zn concentration (139 mg kg^{-1}) among all accessions under the dry treatment.

Table 1 Analysis of variance of the effect of genotype and irrigation regime on yield components: spike DM and total DM, and grain micronutrient (Zn and Fe) and protein concentrations (GPC) in 22 wild emmer wheat accessions and two durum wheat cultivars grown in Rehovot field experiment

Source of variance	d.f.	Sum of square				
		Spike DM	Total DM	GPC	Zn	Fe
Genotype (G)	23	87,456 ***	306,877 ***	40.2 ***	43,720 ***	17,996 ***
Irrigation (I)	1	38,621 ***	17,103 ***	0.03 n.s.	8.51 n.s.	0.84 n.s.
G × I	23	52,666 ***	145,341 *	16.2 *	13,773 ***	3,470 *
Experimental error	97	129,195	498,039	29.9	15,456	7,139

*,*** and n.s. indicate significance at $P \leq 0.05$, 0.001 or non-significant effect, respectively.

Table 2 Spike DM, total DM and drought susceptibility index (S) of 22 wild emmer wheat accessions and 2 durum wheat cultivars grown under well-watered (wet) and water-limited (dry) irrigation regimes under field conditions in Rehovot

Genotype	Spike DM			Total DM		
	Dry (g m^{-2})	Wet (g m^{-2})	S	Dry (g m^{-2})	Wet (g m^{-2})	S
Wild emmer wheat						
12-2	681	1,241[a]	1.77[a]	1,396[a]	2,630[a]	1.84
12-3	1,195[a]	1,406[a]	0.59	2,272[a]	2,716[a]	0.64[a]
12-4	1,131	1,301[a]	0.51[a]	1,742[a]	1,920[a]	0.36[a]
13-B-89	620	771	0.77	1,392[a]	1,928[a]	1.09
15-T-6	511	725	1.16	1,060	1,213	0.49[a]
16-34	586	1,017[a]	1.66[a]	1,509[a]	2,061[a]	1.05
16-40	920[a]	1,110[a]	0.67	1,320[a]	1,533	0.54[a]
18-27	675	796	0.60	1,181	1,854[a]	1.42
18-39	586	612	0.16	1,067	1,296	0.69
18-60	531	692	0.91	876	1,590	1.76
19-1	501	705	1.14	824	1,443	1.68
19-36	452	560	0.75	901	1,134	0.81
24-39	569	1,018[a]	1.73[a]	1,101	2,300[a]	2.04[a]
33-48	593	699	0.59	1,289	1,415	0.35[a]
33-58	594	697	0.58	1,108	1,753[a]	1.44
33-8	528	699	0.96	1,052	1,352	0.87
9-72	505	993[a]	1.93[a]	1,263	1,905[a]	1.32
KH 5/1	445	694	1.40[a]	1,216	1,767[a]	1.22
KH 5/3	613	761	0.76	1,076	1,403	0.91
MM 5/2	811[a]	863	0.24[a]	1,081	1,171	0.30[a]
MM 5/4	919[a]	1,005[a]	0.33[a]	1,735[a]	1,825[a]	0.19[a]
P 2/3	728	837[a]	0.51[a]	1,384[a]	1,562	0.45[a]
Mean	649	864	0.89	1,251	1,724	0.98
Durum wheat						
Inbar	435	541	0.77	880	1,194	1.03
Svevo	601	783	0.91	828	1,228	1.28

[a] Wild emmer wheat accessions significantly differing form mean value of the two durum wheat cultivars are marked

Seeds originated from all genotypes grown under both irrigation regimes were used in a greenhouse study to compare their shoot Zn concentration and tolerance to Zn deficiency. All variables (shoot DM, shoot Zn concentrations and Zn efficiency) were significantly influenced by genotypes, and in most cases also by the maternal environment and the genotype × maternal environment interaction (Table 4). On average, the source of seeds had little effect on the shoot DM production of the genotypes under Zn deficiency (Table 5). At adequate Zn application, plants derived from the seeds of the drought-stressed plants tended to have greater shoot DM production than the plants derived from the seeds of the well-watered plants (Table 5). Zinc deficiency reduced shoot growth of all genotypes, more clearly in the plants originated from the seeds of the drought-stressed plants (Table 5). Irrespective of the source of seeds, most of the genotypes behaved similarly in their tolerance to Zn deficiency (Zn efficiency). The accessions 12-3, 12-4, 15-T-6, 18-27 and 33-8 were affected from the source of seeds and showed differential reaction to Zn deficiency (Table 5). Generally, Zn deficiency tolerance of all genotypes was, on average, higher in the case of the plants derived from seeds of well-watered plants than the plants derived from the seeds obtained from drought conditions. Among both sources of the seeds, the accessions MM 5/4, 24-39, 18-60, MM 5/2 and KH 5/3 exhibited consistently higher Zn deficiency tolerance compared to other wild emmer accessions and also durum wheat genotypes. The accession 33-48 was particularly sensitive to Zn deficiency. There was no consistent relationship between high Zn efficiency and seed Zn concentrations. For example, one of the most Zn in-

Table 3 Grain Zn, Fe and protein concentration of 22 wild emmer wheat accessions and two durum wheat cultivars grown under well-watered (wet) and water-limited (dry) irrigation under field conditions in Rehovot

Genotype	Grain Zn		Grain Fe		Grain protein	
	Dry (mg kg^{-1})	Wet (mg kg^{-1})	Dry (mg kg^{-1})	Wet (mg kg^{-1})	Dry (g kg^{-1})	Wet (g kg^{-1})
Wild emmer wheat						
12-2	115[a]	99[a]	73[a]	60	247[a]	267[a]
12-3	124[a]	87[a]	71[a]	48	276[a]	213
12-4	108[a]	104[a]	69[a]	67[a]	231[a]	261
13-B-89	95[a]	71	65[a]	56	201	164
15-T-6	139[a]	109[a]	75[a]	63	287[a]	315[a]
16-34	101[a]	88[a]	71[a]	67[a]	229[a]	203
16-40	99[a]	86[a]	72[a]	66[a]	258[a]	256
18-27	82	92[a]	64[a]	54	231[a]	233
18-39	94[a]	133[a]	62[a]	81[a]	242[a]	258
18-60	91[a]	101[a]	60[a]	62	243[a]	167
19-1	69	104[a]	58[a]	60	223	246
19-36	90[a]	109[a]	63[a]	77[a]	266[a]	265
24-39	113[a]	127[a]	80[a]	88[a]	222	263
33-48	85[a]	101[a]	58[a]	60	212	262[a]
33-58	78	90[a]	52[a]	56	174	196
33-8	101[a]	90[a]	72[a]	57	214	186[a]
9-72	139[a]	99[a]	61[a]	48	210	209
KH 5/1	95[a]	118[a]	80[a]	81[a]	246[a]	231
KH 5/3	114[a]	94[a]	75[a]	64	275[a]	207
MM 5/2	126[a]	113[a]	76[a]	78[a]	257[a]	238
MM 5/4	121[a]	127[a]	71[a]	83[a]	255[a]	204
P 2/3	90[a]	108[a]	68[a]	84[a]	242[a]	382[a]
Mean	103	102	68	67	238	238
Durum wheat						
Svevo	49	53	33	47	165	165
Inbar	55	56	29	38	149	184

[a] Wild emmer wheat accessions significantly differing form mean value of the two durum wheat cultivars are marked

Table 4 Analysis of variance of the effect of genotype and maternal irrigation regime on 22 wild emmer wheat accessions and two durum wheat cultivars grown with and without zinc fertilization

Source of variance	d.f.	Sum of square				
		Shoot DM		Zn-efficiency	Shoot Zn concentration	
		−Zn	+Zn		−Zn	+Zn
Genotype (G)	23	1.36 ***	2.13 ***	14,683 ***	125 *	13,009 ***
Maternal Irrigation Regime (MI)	1	0.14 ***	0.41 ***	435 n.s.	4.18 n.s.	790 ***
G × MI	23	0.35 ***	0.49 ***	7,193 **	86 n.s.	3,002 **
Experimental error	97	2.11	3.74	15,762	462	1,462

Single, double, and triple asterisks and n.s. indicate significance at $P \leq 0.05$, 0.01, 0.001 or non-significant effect, respectively

Table 5 Shoot DM production, shoot concentration of Zn and Zn deficiency tolerance (Zn efficiency) of 22 wild emmer wheat accessions and 2 durum wheat cultivars grown for 35 days with (+Zn: 5 mg Zn kg^{-1} soil) and without (−Zn) Zn application on a Zn deficient calcareous soil under greenhouse conditions. Data are means of three replicates

Accessions	Shoot DM				Zn-efficiency		Shoot Zn Concentration			
	−Zn		+Zn				−Zn		+Zn	
	Dry[a] (g $plant^{-1}$)	Wet[a] (g $plant^{-1}$)	Dry (g $plant^{-1}$)	Wet (g $plant^{-1}$)	Dry (%)	Wet (%)	Dry (mg kg^{-1} DW)	Wet (mg kg^{-1} DW)	Dry (mg kg^{-1} DW)	Wet (mg kg^{-1} DW)
Wild emmer wheat										
12-2	0.38	0.39	0.51	0.53	74	72	10.2	10.2	94[b]	89
12-3	0.36	0.52	0.66	0.7	54	74	15.8[b]	10.3	94[b]	87
12-4	0.34[b]	0.38	0.48[b]	0.50	59	78	10.4	9.0	88[b]	99
13-B-89	0.44	0.34	0.56	0.42	77	80	10.1	7.9	83	104[b]
15-T-6	0.43	0.43	0.72[b]	0.49	59	89	8.9	8.6	78	88
16-34	0.37	0.44	0.54	0.69	69	65	9.1	8.6	84	84
16-40	0.37	0.32	0.64	0.54	58	60[b]	8.1	9.4	79	86
18-27	0.28[b]	0.24[b]	0.48[b]	0.30[b]	57	78	8.4	10.8	58	57[b]
18-39	0.30[b]	0.14[b]	0.47[b]	0.19[b]	65	76	9.5	13.2	62	79
18-60	0.39	0.30[b]	0.48[b]	0.30[b]	82	101	10.2	9.3	66	74
19-1	0.36[b]	0.26[b]	0.46[b]	0.34	77	78	9.4	10.2	84	85
19-36	0.18[b]	0.07[b]	0.27[b]	0.15[b]	67	47[b]	9.5	12.0	92[b]	88
24-39	0.47	0.37	0.57	0.41	82	89	9.4	10.2	71	69
33-48	0.21[b]	0.13[b]	0.46[b]	0.22[b]	45[b]	57[b]	8.8	9.0	92[b]	81
33-58	0.24[b]	0.39	0.40[b]	0.41	59	93	8.9	8.2	79	71
33-8	0.25[b]	0.07[b]	0.36[b]	0.16[b]	70	44[b]	9.9	8.8	89	87
9-72	0.41	0.32	0.53	0.49	78	67	8.9	10.5	79	79
KH 5/1	0.32[b]	0.12[b]	0.49	0.16[b]	66	72	9.2	11.3	87[b]	99[b]
KH 5/3	0.43	0.20[b]	0.54	0.23[b]	81	86	9.0	10.0	79	102[b]
MM 5/2	0.49	0.35	0.52	0.44	94	79	7.6	8.3	76	85
MM 5/4	0.4	0.39	0.47[b]	0.44	85	88	10.6	11.8	68	72
P 2/3	0.29[b]	0.11[b]	0.35[b]	0.14[b]	84	77	9.5	9.9	69	90
Mean	0.41	0.39	0.55	0.49	71	80	9.3	9.9	79	79
Durum wheat										
Svevo	0.46	0.39	0.6	0.49	76	79	9.1	9.8	68	73
Inbar	0.46	0.48	0.67	0.63	69	76	8.9	9.3	74	77

[a] Seeds were used in this experiment originated from maternal plants grown under well-watered (wet) and water-limited (dry) conditions

[b] Wild emmer wheat accessions significantly differing for the durum wheat cultivars are marked

efficient accession 33-48, had higher seed Zn concentrations than many other Zn-efficiency accessions (Tables 3 and 5). The source of seeds was less effective on the shoot concentration of Zn under both Zn treatments (Table 5). With exception of the accession 12-3, all genotypes were very similar in shoot Zn concentration under Zn deficient conditions. Shoot Zn concentration was affected by both Zn treatment and genotype.

PCA of the 22 wild emmer wheat accessions extracted two major principal components (Eigenvalues >1) that accounted collectively for 75.58% and 79.74% of the variance for the dry and wet treatments, respectively (Fig. 1). Under the dry treatment, principal component 1 (PC1, X-axis, Fig. 1a) explained 42.95% of the dataset variation, and was loaded positively with GPC, Zn, and Fe. PC2 (Y-axis, Fig. 1a) explained 32.63% of the dataset variation, and was positively loaded by spike DM and total DM. Under the wet treatment, principal component 1 (PC1, X-axis, Fig. 1b) explained 50.49% of the dataset variation, and was loaded positively with GPC, Zn and Fe and negatively

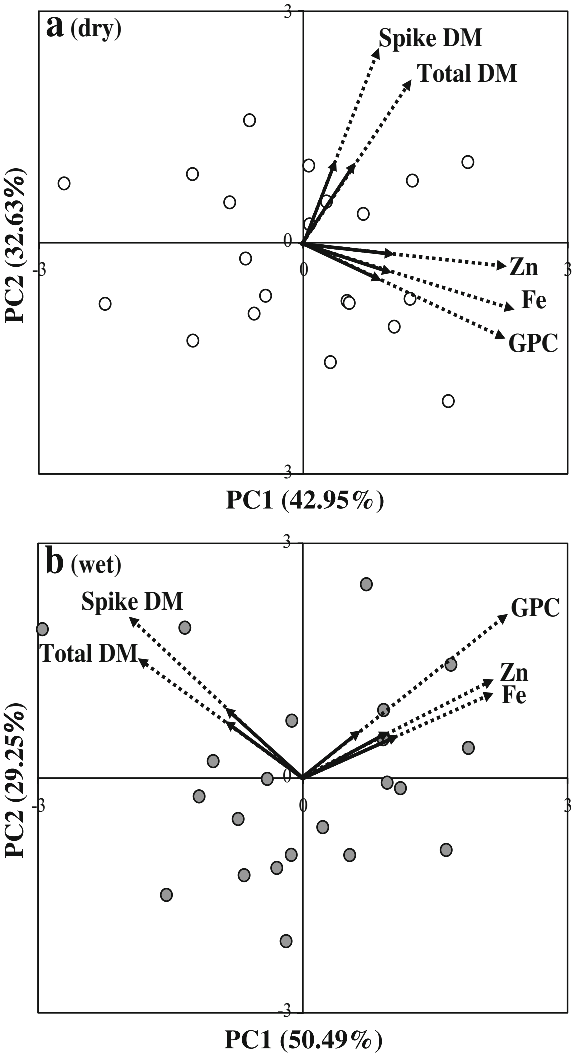

Fig. 1 Principal component analysis (based on correlation matrix) of continuous plant traits recorded on 22 wild emmer wheat accessions under water limited (white; **a**) and well watered (grey; **b**) field experiments. Biplot vectors are trait factor loadings for principal component 1 (*PC1*) and *PC2*

loaded by spike DM and total DM. PC2 (Y-axis, Fig. 1b) explained 29.25% of the dataset variation, and was positively loaded by spike DM and total DM. The PCA showed strong associations between the two components representing productivity (spike DM and total DM) (Fig. 1). This association was supported also by the high and positive correlations between yield components (r=0.67 $p\leq$0.001 and r=0.87 $p\leq$0.0001 for the dry and wet treatment, respectively). Grain mineral concentrations also showed strong association between them. Grain Zn concentration showed high and positive correlation with grain Fe concentration (r=0.57 $p\leq$0.006 and r=0.77 $p\leq$0.0001 for the dry and wet treatment, respectively). GPC was correlated positively with Zn (r=0.47 $p\leq$0.02) and Fe (r=0.6 $p\leq$ 0.006) only under the dry treatment.

Discussion

World cereal demand is growing at the present (for wheat, ca. 2% per year; Skovmand et al. 2001) in accordance with the global expansion of human populations. During the past several decades, the primary objective of plant breeding programs has been to increase yield, a quest that will remain a principal concern in providing the calorie intake required for the growing world population. However, equally important, but largely overlooked in breeding programs, is the nutrient composition and concentration, particularly the micronutrients, in the grains of staple food crops (Welch and Graham 1999; Cakmak 2002). Breeding programs directed towards increased yield have narrowed the genetic basis of modern crop plants. Therefore, it is essential and urgent to exploit genetic resources from relatives of wheat which harbour a richness of desirable genes.

Very high concentrations and substantial variation for Zn, Fe and protein in grain was found among the wild emmer wheat accessions (Table 3). Previous studies have showed the advantage of wild emmer wheat as compared with cultivated wheat for higher grain mineral concentrations (e.g. Cakmak et al. 2004). Our results demonstrate the huge potential of wild emmer wheat for improvement of grain mineral content. Under both irrigation regimes most of the wild emmer wheat accessions exhibited significantly higher concentration of Zn (up to 139 mg kg^{-1}), Fe (up to 88 mg kg^{-1}) and GPC (up to 380 g kg^{-1}) as compared with the two durum cultivars (Table 3). Increasing number of reports is available showing that wild and primitive wheat species generally contain more Zn than Fe in seeds, as reported by Monasterio and Graham (2000), Cakmak et al. (2004), Bonfil and Kafkafi (2000) and Distelfeld et al (2007). However, there is no explanation for this. It might be related to growth conditions or rather to the higher seed protein concentrations of wild wheat species (Nevo et al. 2002). Seed protein and seed Zn correlate very positively with each other and seed protein seems to be a sink for Zn (see Ozturk et al. 2006 and Cakmak et al. 2004 for further references and details).

Moreover, the results obtained in the current study show higher grain micronutrient concentrations as compared also with the results obtained in other studies on a wide range of cultivated wheat germplasm, showing lower grain Zn concentration (range 22–85 mg kg^{-1}) and grain Fe concentration values (range 25–73 mg kg^{-1}) (e.g. Welch 2001; Pomeranz and Dikeman 1983; Peterson et al. 1986; Morgounov et al. 2007).

The 22 wild emmer wheat accessions tested in the present study were previously characterized for their response to drought stress (Peleg et al. 2005). These wild emmer accessions represent a wide range of drought resistance, as measured by productivity under drought conditions, S and water-use efficiency. The study revealed significant genotype × environment interaction (G × E) for all grain mineral concentrations (Table 1). While certain accessions exhibited consistently high grain micronutrient concentrations across the two irrigation regimes (i.e. MM5/4, MM 5/2, KH 5/1 and 24-39), the ranking of other accessions was greatly affected by water availability. Furthermore, water availability had contrasting effect on different accessions resulting in either significantly increased Zn (e.g. 9-72, 15-T-6 and 12-3) or decreased Zn (19-1 and 18-39) in relation to water availability.

Grain Zn concentration was found to be positively correlated with Fe under both irrigation regimes (Fig. 1), indicating co-segregating of genes affecting both Zn and Fe. Indeed many previous studies reported a positive correlation between grain Zn concentration and grain Fe concentration in cereals (Cakmak et al. 2004; Morgounov et al. 2007 and references therein). Furthermore, recently, Distelfeld et al. (2007) reported on multiple pleiotropic effects of a gene (*Gpc-B1*) derived from wild emmer wheat on grain protein, Zn and Fe concentrations, and this effect seems to be associated with higher leaf senescence. In the current study, GPC was positively correlated with either Fe or Zn under drought conditions but not in well watered conditions. The reason for such differential relationship between grain micronutrients and protein under limited and adequate irrigated needs further study.

When selecting for high grain mineral concentration, breeders should always take into consideration the impact of selection on productivity. A negative association between grain mineral concentrations and yield in cultivated wheat was previously reported (Ortiz-Monasterio et al. 1997; Feil 1997; Calderini and Ortiz-Monasterio 2003; Garvin et al. 2006). In the present study, it was interesting to notice that the wild emmer accessions tested exhibited neither negative nor positive association between yield and grain mineral concentrations. Thus, the combination of high productivity with high grain minerals under water-limited conditions may be a feasible breeding objective. The PCA presented in the current study revealed several wild emmer wheat accessions which show this potential combination (Fig. 1), being the most promising candidate for future breeding programs. In addition, Zn deficiency in soils is a common problem under semi-arid conditions, like in Central Anatolia and different parts of India, Australia, Pakistan and China resulting severe decreases in grain yield (Graham et al. 1992; Cakmak et al. 1996a; Alloway 2004). Increasing evidence is available showing that Zn deficiency stress in plants becomes more pronounced under water-limited conditions (Graham et al. 1992; Ekiz et al. 1998; Bagci et al. 2007). Therefore, development of genotypes with high tolerance to both drought and Zn deficiency stress is a high priority research area, and combination of these traits with high grain micronutrient concentration would be a most desirable breeding goal. Among the wild emmer accessions tested, the accession MM 5/2 and MM 5/4 had consistently lower drought stress susceptibility (Table 2), greater Zn and Fe concentrations in grain (Table 3) and higher Zn deficiency tolerance (Table 5). These accessions and others are being exploited in a recently initiated breeding program in Israel and Turkey to improve cultivated wheats for the mentioned traits.

Durum wheat has been often reported to be very sensitive to Zn deficiency (Graham et al. 1992; Rengel and Graham 1996; Cakmak et al. 1997). This high sensitivity of durum wheat cultivars to Zn deficiency has been ascribed to their low capacity to take up adequate Zn under Zn-deficiency conditions and low release rate of Zn-mobilising phytosiderophores from roots into rhizosphere (Cakmak et al. 1996b; Rengel and Graham 1996). Current results demonstrate high genetic potential of wild emmer wheat to improve Zn-efficiency in the cultivated wheat. In many instances, high Zn efficiency is affected by grain Zn concentration and, therefore, special attention should be paid to grain Zn concentration when genotypes are compared for their genetic capacity to tolerate Zn deficiency (Rengel and Graham 1995; Yilmaz et al. 1998; Genc et al. 2000). In the present study, it was found that high Zn deficiency tolerance (Zn efficiency) is unrelated to grain Zn concentrations. Besides benefits to human nutrition and health, increasing grain Zn concentration is also of great importance for the better growth and tolerance to biotic and abiotic stress factors during seed germination and early seedling growth (Rengel and Graham 1995; Yilmaz et al. 1998; Welch 1999).

Conclusions and prospects for wheat improvement During a long evolutionary history, the wild emmer wheat has accumulated high genetic diversity for various biotic and a-biotic stress adaptations. The notion of using the wild emmer gene resources in wheat improvement has been repeatedly advocated since the discovery of the wild progenitor of the cultivated wheats about a century ago (Aaronsohn 1910). A high genetic diversity was found between wild emmer wheat accessions in terms of drought resistance, grain nutrients concentrations and Zn deficiency tolerance, with a considerable potential to improve both traits in the cultivated wheat grown in zinc poor soils, suggesting that wild emmer is a potential source for improvement of cultivated wheats. However, since both traits exhibited genotype × environment interactions, wild accessions showing high stability over various environments should be carefully selected as donor parents for breeding programs.

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