Evaluation of Turkish durum wheat (*Triticum turgidum* var. durum) genotypes based on quantitative traits and shoot zinc accumulation under zinc-deficient calcareous soil

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Abstract

This experiment was carried out in a Zn-deficient calcareous soil to study the effects of Zn deficiency on shoot dry weight, shoot content, and concentration of Zn, and also to identify new sources of Zn efficiency for further improvement of Zn efficiency with (+Zn = application of 5 mg Zn/kg soil) and without Zn supply (-Zn = non-application of zinc) on 50 durum wheat genotypes for 45 days. Variance analysis for shoot dry matter, shoot Zn concentration, shoot Zn content, and Zn utilization efficiency revealed that these traits were significantly ($P < 0.01$) affected by Zn application and durum wheat genotypes. Results revealed that dry weight of shoot and shoot Zn accumulation were considerably improved by Zn fertilizers. Furthermore, there was a considerable genetic variation in the expression of Zn deficiency symptoms (slight to severe), Zn efficiency (49-100%), shoot Zn concentration (7.1-20.1 and 10.4-33.1 mg Zn/kg dry weight under Zn deficient and sufficient, respectively), shoot Zn content (0.31-1.47 and 0.7-2.9 µg/plant under Zn deficient and sufficient, respectively), and Zn utilization efficiency (59.2-139.1 and 34.3-94.0 g dry weight/µg Zn under Zn deficient and sufficient, respectively) within durum wheat genotypes. In general, the presence of lines (AAZ, 4025, 45868, 45558 and Azarbayjan) with greater Zn efficiency than Zn efficient durum wheat cultivars (Ege-88, Aydin-93 and Akcakale-2000) indicates that the new lines can be used to improve current levels of Zn efficiency in durum wheat genotypes.

Keywords: breeding; durum wheat, genetic diversity, shoot dry matter, zinc efficiency

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Introduction

Durum wheat (*Triticum turgidum* var. durum) is cultivated on about 200-300 thousand hectares across arable lands in Iran. This plant also has special economic importance because of its genetic characterization of resistance to common bunt, leaf and stripe rusts (Al-Naimi et al., 2000).

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Table 1
Physico-chemical properties of the soil used in this experiment

pH	Calcium carbonate, $CaCO_3$ (%)	Organic matter (%)	EC_e (dS/m)†	Texture
7.2	20	0.5	2.3	Clay-loam
Extractable P (mg/kg)	Extractable Fe (mg/kg)	Extractable Cu (mg/kg)	Extractable Zn (mg/kg)	
6.1	3.1	0.7	0.6	

†Electrical conductivity of soil saturation extract

Thus, durum wheat can increase the sustainability of farming systems under disease prevailing conditions (Sadeghzadeh and Alizadeh, 2005).

Micronutrients deficiency is one of the common restricting factors in durum wheat production. This scarcity is severe in calcareous soils of arid and semi-arid areas due to low availability caused by high levels of calcium carbonates. It has been estimated that approximately up to 40% of the soils under wheat production areas of the world suffer from levels of Zn-deficiency which drastically influences the crop performance (Broadley et al., 2007; Esfandiari et al., 2016). Also, soil Zn deficiency is one of the major factors limiting wheat production and productivity in the north-west of Iran. Meanwhile, application of different Zn-sources of chemical fertilizers is proposed to enhance the plant growth and product development (Sadeghzadeh et al., 2009; Abdoli et al., 2014; Esfandiari et al., 2016).

Sensitivity to Zn deficiency is different in various plants. Wheat is more sensitive than rye, triticale, and barley (Cakmak et al., 1997, 1999; Blum, 2014). Also, durum wheat shows more sensitivity to this deficit compared to bread wheat (Genc and McDonald, 2008). Genc and McDonald (2004) reported that Zn consumption decreased under Zn sufficient conditions. Also, they stated that there is a significant genotypic variation in terms of zinc use among wheat genotypes. Furthermore, studies have been shown large variations in performance of bread and durum genotypes in Zn-deficient soils (Cakmak et al., 1996, 1999; Kalayci et al., 1999; Torun et al., 2000; Moshiri et al., 2010; Velu *et al*., 2012; Narwal et al., 2012; Abdoli et al., 2016). Olfati et al. (2015) evaluating genetic variation of 142 wheat genotypes for grain iron (Fe) and Zn content and their relationships with grain yield and its components under dryland conditions of Iran, reported that the range of grain Fe and Zn content was from 70 to 109 and 31 to 61 mg/kg, respectively. Cakmak et al. (1998) reported that the average decrease in shoot dry matter production due to Zn deficiency were 15% for rye, 25% for triticale, 34% for barley, 42% for bread wheat, 63% for oat, and 65% for durum wheat. Khoshgoftarmanesh et al. (2011) reported that Ghods and Falat genotypes were the most tolerant and sensitive genotypes to Zn deficiency among thirty spring wheat genotypes in Iran, respectively. Several reports have showed extensively significant variation in wheat germplasm with respect to concentration of micronutrients (Zhao et al., 2009; Chatzav et al., 2010; Heidari et al., 2016; Amiri et al., 2018). Abdoli and Esfandiari (2017) reported that there is a large genetic diversity among wheat genotypes, as well as, Zn-deficient stress causing 7.3, 20.8, 18.6, and 22.1% reduction in plant height, grains number per spike, and biological and grain yield, respectively. Therefore, the selection and breeding of tolerant genotypes to low Zn content in the soil are logical ways to overcome the Zn deficiency in wheat and other crops (Genc and McDonald, 2008).

Regarding the role of wheat as a staple food crop, the aim of this study was to screen fifty genotypes of durum wheat for their potential to use zinc at early growth stages and also to identify desirable genotypes to use for further breeding programs.

Materials and Methods

Experimental site and soil characters

In order to identify Zn deficient tolerance in durum wheat, fifty genotypes were evaluated

Table 2
Name, pedigree, code, seed Zn concentration, and content of durum wheat genotypes used in this experiment

	Name/pedigree	Code	Type	Seed Zn concentration (mg/kg dry weight)	Seed Zn content (µg/seed)
1	Amonos-97	Amonos-97	C	39.4	1.61
2	Balcali-2000	Balcali-2000	C	27.8	1.22
3	Zenit	Zenit	C	40.1	1.47
4	DiyarBakir-81	DiyarBakir-81	C	24.4	1.18
5	Artuklu	Artuklu	C	25.4	1.11
6	Fuatbey-2000	Fuatbey-2000	C	39.5	1.56
7	Svevo	Svevo	C	28.9	1.07
8	Gediz-75	Gediz-75	C	34.1	1.47
9	Firat-93	Firat-93	C	47.3	2.21
10	Ceylan-95	Ceylan-95	C	40.1	1.63
11	Dena	Dena	C	25.0	0.90
12	Ozbek	Ozbek	C	26.8	1.23
13	Saji	Saji	C	21.6	0.79
14	Ege-88	Ege-88	C	18.3	0.79
15	Aydin-93	Aydin-93	C	22.9	0.82
16	Akcakale-2000	Akcakale-2000	C	18.0	0.80
17	AJAIA-12/F3LOCAL(SEL.ETHIO.135.85)//PLATA-13/3/SOMBRA-20/4/SNITAN/5/SOMAT-4/INTER-8	A-INTER-8	BL	34.3	1.34
18	45717	45717	BL	38.6	1.33
19	45415	45415	BL	31.5	1.25
20	RCOL/THKNEE-2/3/SORA/2*PLATA-12//SOMAT	RCOL/THK	BL	38.8	1.24
21	46046	46046	BL	24.2	1.04
22	45430	45430	BL	27.1	1.30
23	45632	45632	BL	45.8	1.33
24	HYDRANASSA30/SILVER-5/3/AUK/GUIL//GREEN/10/PLATA-10/6/MQUE/4/USDA573//QFN/AA-7/3/ALBA-D/5/AVO/HUI/7/PLATA-13/8/THKNEE-11/9/CHEN/ALTAR84/3/HUI/POC//BUB/RUFO/4/FNFOOT	HYDRANAS	BL	28.3	0.85
25	45667	45667	BL	42.1	1.46
26	GREEN-14//YAV-10/AUK	GREEN-14	BL	36.6	1.47
27	SHAG-14/ANADE-1//KITTI-1/4/ARMENT//SRN-3/NIGRIS-4/3/CANELO-9.1	SHAG-14	BL	31.2	0.86
28	Mrf1/Stj2//Bcrch1	Mrf1/Stj2	BL	18.9	0.77
29	4341	4341	BL	19.1	0.67
30	Mrb3/Mna-1	Mrb3/Mna-1	BL	26.4	1.12
31	45704	45704	BL	28.6	1.10
32	SORA/2*PLATA-12//SOMAT-3/3/STORLOM/4/BICHENA/AKAKI-7	SORA/2	BL	15.9	0.51
33	46020	46020	BL	31.2	1.62
34	Bisu-1//CHEN-1/TEZ/3/HUI//CIT71/CII	Bisu-1	BL	26.5	1.20
35	4017	4017	BL	34.9	0.96
36	MEXICALI 75	MEXICALI 75	BL	23.6	0.94
37	RASCON-37/2*TARRO-2/3/AJAIA-12/F3LOCAL(SEL.ETHIO.135.85)//PLATA-13/4/SORA/2*PLATA-12//SOMAT-3	RASCON-37	BL	14.9	0.61
38	4303	4303	BL	24.6	1.13
39	Geromtel-1	Geromtel-1	BL	26.6	1.07
40	KC-3426	KC-3426	BL	35.7	0.98
41	46202	46202	BL	31.3	0.90
42	VRKS-3/7/ENTE/MEXI-2//HUI/4/YAV-1/3/LD357E/2*TC60//JO69/5/BISU/6/RYPS26-2/10/PLATA-10/6/MQUE/4/USDA573//QFN/AA-7/3/ALBA-D/5/AVO/HUI/7/PLATA-13/8/THKNEE-11/9/CHEN/ALTAR84/3/HUI/POC//BUB/RUFO/4/FNFOOT	VRKS-3	BL	18.5	0.71
43	45620	45620	BL	34.8	1.24
44	Gdr2	Gdr2	BL	27.8	1.05
45	Aday-19	Aday-19	BL	23.2	0.95
46	AAZ//ALTAR84/ALD/3/AJAIA/4/AJAIA-12/F3LOCAL(SEL.ETHIO.135.85)//PLATA-13/5/SOOTY-9/RASCON-37/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV-1/6/ARDENTE/7/HUI/YAV79/8/POD-9	AAZ	BL	24.4	0.81
47	4025	4025	BL	39.8	1.39
48	45868	45868	BL	26.1	1.09
49	45558	45558	BL	36.8	1.09
50	Azarbayjan (LR)/Wadalmes IRDW2003-04-140-OMAR-OMAR-OMAR-4MAR-OMAR	Azarbayjan	BL	27.4	1.04

Adapted from Dryland Agricultural Research Institute (DARI) of Iran; C and BL indicate cultivars and breeding lines, respectively.The seed Zn concentration values were based on 25 seeds per genotype.

under Zn sufficient and deficient conditions during 2014-2015 growing seasons in University of Maragheh in Iran. The soil was collected from severely Zn-deficient soils of Moghanlou, Bijar city in Kurdistan province of Iran (47° 56' E, 36° 08' N; 1478 m elevation from sea level), where previous study proved the decline of wheat yield due to Zn deficiency (Abdoli, 2017). The soil details of the location are shown in the Table 1. Critical Zn concentration deficiency was considered when the concentration declined below to 0.5-0.6 mg/kg soil (Sims and Johnson, 1991). Pursuant to soil test and before sowing, the soil was mixed homogeneously with basal fertilizers of 200 mg N [Ca $(NO_3)_2 4H_2O$]/kg soil and 100 mg P [KH_2PO_4]/kg soil.

Experimental design and treatments

A pot experiment was carried out as a factorial based on completely randomized block design (RCBD) with 100 treatments (2 Zn conditions and 50 durum wheat genotypes) in three replications. The first factor was two conditions of Zn (1) zinc deficient (without Zn fertilization; -Zn), and (2) normal Zn supply (soil application with 5 mg Zn/kg soil at planting form $ZnSO_4.7H_2O$ source; +Zn). The second factor was fifty durum wheat genotypes including 16 cultivars and 34 lines.

Plant material and growth conditions

Wheat genotypes of durum wheat (*Triticum turgidum* var. durum) were obtained from Dryland Agricultural Research Institute (DARI) of Iran. In the present experiment, seeds were harvested from the homogenous plants not treated with chemical fertilizers. The names and codes of durum wheat cultivars and lines used in this experiment are given in Table 2. Plastic pots (PVC, 20 × 35 cm diameter and depth, respectively) were filled with 3.5 kg soil. Fourteen seeds were sown in each pot at set distances and depths, and the pots were thinned to seven seedlings per pot after emergence and daily watered by using deionized water. The pots were kept in the greenhouse and mean temperature in the greenhouse was set at 24 ± 3° C. Irrigation of the plants in the pots (90 ± 5% of field capacity) and crop management practices such as weeds were controlled in pots close to sampling and harvest time of plants (coincided with 45 days after sowing).

Plant sampling, observations and measurements of ion contents

Several traits were measured at the end of the experiment. Visual symptoms (morphological traits) were recorded using a scale of 1 to 9 inclusive: 1 = healthy green plants, 2 = reduction in shoot growth, 3 = leaf symptoms (chlorosis areas) appearing on first leaves, 4 = chlorosis areas scattered across the first leaves, 5 = large chlorosis areas on the first leaves, 6 = leaves collapsing in the middle, 7 = chlorosis areas developing on second leaves, 8 = both first and second leaves turning pale yellow, and 9 = dead growing points (Genc and McDonald, 2004; 2008).

Forty-five days after sowing, the seedling samples were oven dried at 75° C for 48 hours, milled to pass through a 0.5 mm sieve, and stored for analysis. Powder samples were turned into ash at 550° C for 8 hours and dissolved in 1% (v/v) hydrochloric acid (Chapman and Pratt, 1961). Concentrations of Zn in the digested solutions were determined by Atomic Absorption Spectrophotometer (model: AAS-6300 Shimadzu) and the concentrations were expressed based on plant dry weight (mg/kg dry weight).

Zinc efficiency ratio was expressed as relative shoot growth and calculated as the percentage of shoot dry matter produced under Zn-deficiency relative to shoot dry matter produced under Zn fertilization. Shoot Zn content (µg/plant) was measured by multiplying amount of seedling dry matter by amount of Zn concentration in shoot (Genc et al., 2006). Zn utilization efficiency was calculated by dividing the amount of shoot dry matter produced by the content of Zn in shoot (mg dry weight/µg Zn) (Genc et al., 2006).

Statistical Analysis

The obtained data were subjected to analysis of variance (ANOVA) using SAS software version 8.0 (SAS Institute Inc., Cary, NC, USA). Duncan's Multiple Range Test (DMRT) at $P < 0.05$ was used for comparing means (Duncan, 1955). The data were analyzed using SPSS software version 16.0 (SAS Institute, 1987) for cluster analysis of durum wheat genotypes based on Square Euclidean distance and Ward method. Zn

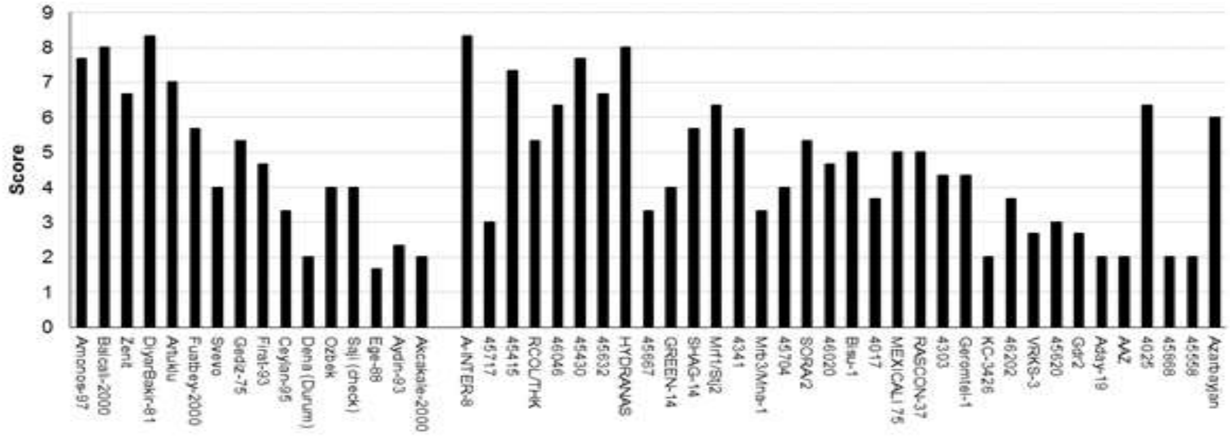

Fig. I. Zn deficiency scores in cultivars and lines of durum wheat at deficient and adequate Zn supply after 45 days (1 = no leaf symptoms, and 9 = severe symptoms); genotypes were ordered in increasing order of Zn efficiency. Cultivars and lines are located on the left and right side of figure, respectively.

Table 3
Analysis of variance for studied traits of durum wheat genotypes

Source of variation	df	Mean squares (MS)			
		Shoot dry matter	Shoot Zn concentration	Shoot Zn content	Zn utilization efficiency
Replication	2	1653.1 **	606.2 **	2.01 **	2593.2 ns
Zn conditions (Zn)	1	34647.1 **	965.9 **	29.2 **	29690.8 **
Genotypes (G)	49	483.0 *	58.7 **	0.545 **	1958.9 ns
Zn × G	49	519.0 **	50.5 **	0.573 **	1780.8 ns
Error	198	315.1	30.8	0.266	1507.9
CV (%)	-	24.1	25.9	24.3	28.8

Ns, *, and ** indicate non-significant, significant at P < 0.05 and P < 0.01, respectively.

deficiency score, Zn efficiency, and shoot dry matter at deficient and adequate Zn supply were used for cluster analysis. Figures were drawn using Excel software version 10.0 and the means ± SE were used to compare the data.

Results

Symptoms of Zn deficiency appearing first as stunted shoot growth and followed by varied degree of chlorosis and necrosis of leaves depending on severity of Zn deficiency stress, became visible in durum wheat genotypes ‘45717’, ‘46046’, ‘45430’, ‘45632’, ‘A-INTER-8’, ‘HYDRANAS’, ‘Mrf1/Stj2’, and ‘Azarbayjan’ at Zn deficiency condition 45 days after sowing. At this stage, Zn efficient cultivars (such as ‘Dena’, ‘Ege-88’, ‘Aydin-93’, and ‘Akcakale-2000’) showed only reduction in growth, while Zn-inefficient genotypes (such as ‘Balkali-2000’ and ‘DiyarBekir-81’) turned pale yellow in both first and second young leaves (Fig. I).

Shoot dry matter and zinc efficiency

In this study, Zn fertilization significantly affected ($P \leq 0.01$) shoot dry matter of durum wheat genotypes (Table 3). So that, Zn fertilization (Zn sufficient condition) increased dry weight of shoots by 34.2% (Table 4). The analysis of variance revealed highly significant difference ($P \leq 0.05$) among genotypes for shoot dry matter. Also, interaction effect of Zn conditions × genotypes was significant on shoot dry matter at $P \leq 0.01$ (Table 3). Some of the genotypes that responded to Zn fertilization included ‘45717’, ‘45415’, ‘46046’, ‘45430’, ‘45632’, and ‘45667’ (Fig. II). The wheat cultivars such as ‘Ozbek’, ‘Saji’, ‘Ege-88’, ‘Aydin-93’, and ‘Akcakale-2000’ did not responded

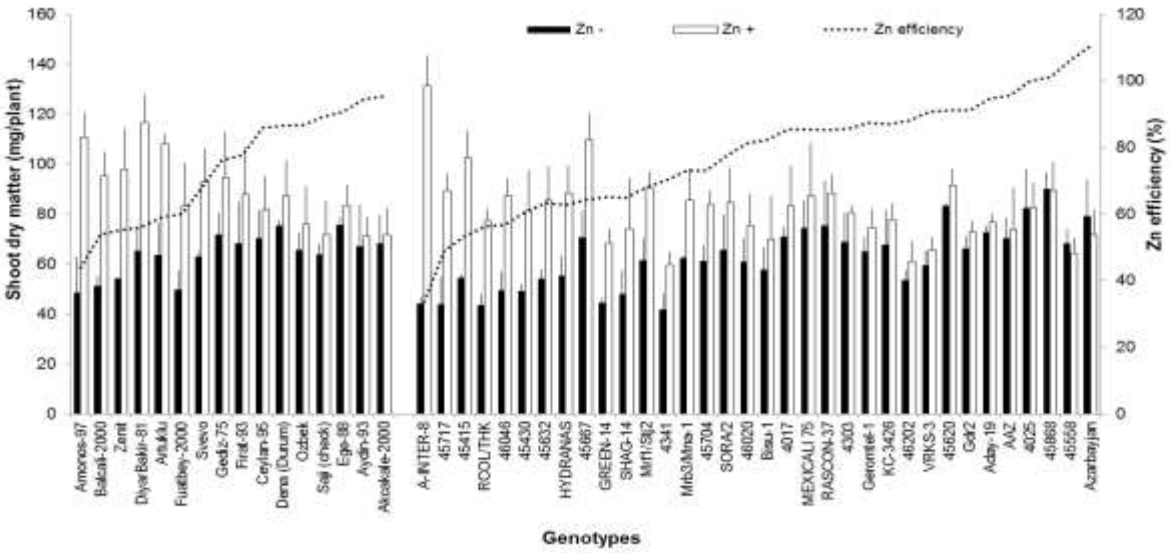

Fig. II. Effects of Zn fertilization (5 mg Zn/kg soil; +Zn) on shoot dry matter (mg/plant) and Zn efficiency (%) in cultivars and lines of durum wheat 45 days after sowing; The cultivars and lines are located on the left and right side of figure, respectively. Zinc efficiency was calculated as [(shoot dry matter at -Zn/shoot dry matter at +Zn) × 100]; Mean ± SE (n = 3).

Table 4.
The average values of the study traits under zinc deficient (-Zn) and zinc sufficient (+Zn) conditions, and the percentage change of each traits after the application of Zn fertilizer in durum wheat genotypes

Traits	Conditions		Percentage change (%)
	Zinc deficient (-Zn)	Zinc sufficient (+Zn)	
Shoot dry matter (mg/plant)	62.8 b	84.3 a	+34.2
Shoot Zn concentration (mg Zn/kg dry weight)	13.6 b	17.2 a	+26.5
Shoot Zn content (µg/plant)	0.85 b	1.48 a	+74.1
Zn use efficiency (mg dry weight/µg Zn)	88.1 a	68.2 b	-22.6

Conditions for each trait with the same letters are not significantly different from each other at P≤ 0.05.

to application of Zn fertilization (Zn efficient cultivars), but other cultivars responded to Zn fertilization (Fig. II). Amongst the durum wheat genotypes, there were also significant genetic differences in shoot dry matter under both Zn deficiency and sufficiency. Under Zn deficiency, there was a 2-fold difference between the genotypes with highest ('45868') and lowest dry matter ('4341'). Unlike Zn deficiency, 'A-INTER-8' and '4341' genotypes represented the highest and lowest dry matter accumulators under Zn sufficiency, respectively (Fig. II). Zn efficiency (relative to shoot dry matter) differed markedly from 33.7 to 110% in between of durum wheat lines and cultivars. Our findings showed that genotypes of '45558', '45868', '4025', 'AAZ', and 'Aday-19' had significantly higher Zn efficiency than Zn efficient cultivars 'Saji', 'Ozbak', and 'Dena' (Fig. II). Within the durum wheat, Zn efficient cultivars achieved greater Zn efficiency than Zn-inefficient cultivars. Overall, there was a great range in Zn efficiency within the genotypes such as durum wheat cultivars.

Zn concentration and content in shoot

Zn fertilization significantly affected (P≤0.01) shoot Zn concentration and content, with significant differences among genotypes (Table 3). Results showed that application of Zn fertilization (Zn sufficient condition) increased shoot Zn concentration and content by 26.5 and 74.1%,

Table 5
Effects of Zn fertilization (5 mg Zn/kg soil; +Zn) on shoot Zn concentration (mg Zn/kg dry weight), shoot Zn content (μg/plant), and Zn utilization efficiency (mg dry weight/μg Zn) in cultivars and lines of durum wheat at 45 days after sowing

NO.	Genotypes code	Zn concentration (mg Zn/kg dry weight)		Zn content (μg/plant)		Zn use efficiency (mg dry weight/μg Zn)	
		-Zn	+Zn	-Zn	+Zn	-Zn	+Zn
1	Amonos-97	20.1 (7.4)	24.7 (0.5)	1.15 (0.69)	2.18 (0.19)	62.0 (16.6)	40.6 (0.9)
2	Balcali-2000	9.9 (0.7)	20.2 (0.3)	0.50 (0.02)	1.92 (0.02)	101.8 (7.5)	49.5 (0.8)
3	Zenit	15.5 (4.4)	29.0 (6.7)	0.84 (0.22)	2.98 (1.11)	73.4 (16.1)	37.9 (7.2)
4	DiyarBakir-81	16.9 (0.8)	21.8 (4.1)	1.09 (0.12)	2.53 (0.52)	59.5 (2.8)	50.4 (11)
5	Artuklu	15.8 (4.7)	14.6 (2.1)	0.91 (0.12)	1.59 (0.28)	73.2 (16.8)	71.9 (12)
6	Fuatbey-2000	16.0 (5.8)	17.9 (0.3)	0.73 (0.17)	1.49 (0.31)	77.2 (20.5)	55.8 (1.0)
7	Svevo	12.7 (1.9)	21.7 (4.3)	0.81 (0.15)	2.11 (0.72)	82.2 (10.9)	49.9 (9.7)
8	Gediz-75	12.7 (1.7)	18.9 (3.6)	0.92 (0.21)	1.90 (0.60)	81.4 (9.8)	57.6 (12)
9	Firat-93	14.5 (4.0)	17.1 (1.3)	1.09 (0.53)	1.50 (0.31)	78.4 (16.9)	59.2 (4.1)
10	Ceylan-95	14.7 (3.9)	17.0 (0.7)	0.94 (0.07)	1.40 (0.27)	76.9 (16.2)	59.0 (2.6)
11	Dena	12.4 (4.5)	15.6 (0.3)	0.92 (0.31)	1.36 (0.20)	99.7 (26.6)	64.0 (1.2)
12	Ozbek	10.5 (0.2)	19.4 (1.8)	0.69 (0.08)	1.44 (0.29)	95.4 (1.5)	52.7 (5.5)
13	Saji	15.8 (3.8)	17.2 (2.4)	0.99 (0.18)	1.27 (0.35)	69.5 (13.3)	60.7 (9.6)
14	Ege-88	15.5 (4.8)	16.8 (0.5)	1.17 (0.35)	1.40 (0.18)	75.5 (17.8)	59.8 (1.7)
15	Aydin-93	19.5 (9.0)	17.4 (0.9)	1.15 (0.38)	1.23 (0.15)	72.4 (22.9)	57.8 (3.2)
16	Akcakale-2000	12.6 (0.0)	17.0 (3.1)	0.86 (0.14)	1.25 (0.35)	79.5 (0.0)	64.0 (14)
17	A-INTER-8	7.1 (0.2)	13.7 (1.6)	0.32 (0.02)	1.79 (0.24)	139.1 (3.1)	75.0 (9.5)
18	45717	12.6 (0.2)	29.2 (0.5)	0.56 (0.15)	2.59 (0.16)	79.1 (1.2)	34.3 (0.6)
19	45415	8.5 (0.2)	14.0 (0.3)	0.47 (0.01)	1.44 (0.14)	117.3 (2.3)	71.3 (1.7)
20	RCOL/THK	7.1 (0.2)	16.0 (2.4)	0.31 (0.04)	1.25 (0.24)	140.8 (3.4)	65.9 (11)
21	46046	14.6 (4.6)	25.5 (0.4)	0.72 (0.23)	2.22 (0.17)	80.7 (19.5)	39.3 (0.6)
22	45430	17.2 (0.3)	11.6 (1.9)	0.87 (0.02)	0.80 (0.12)	96.0 (3.0)	71.1 (10)
23	45632	13.7 (3.6)	21.6 (0.6)	0.76 (0.25)	1.86 (0.34)	82.6 (17.4)	46.4 (1.2)
24	HYDRANAS	16.2 (8.7)	12.2 (1.0)	0.79 (0.32)	1.06 (0.09)	98.9 (14.6)	83.7 (7.8)
25	45667	10.3 (4.6)	21.8 (0.4)	0.67 (0.24)	2.38 (0.20)	135.1 (21)	46.0 (0.9)
26	GREEN-14	15.0 (1.9)	15.2 (0.4)	0.65 (0.06)	1.04 (0.11)	90.3 (12.9)	65.7 (1.8)
27	SHAG-14	13.9 (0.5)	11.2 (0.3)	0.66 (0.12)	0.83 (0.22)	85.5 (3.4)	89.2 (2.7)
28	Mrf1/Stj2	12.0 (0.7)	10.5 (1.0)	0.81 (0.13)	0.92 (0.04)	115.9 (8.9)	89.4 (8.5)
29	4341	18.7 (8.8)	19.0 (0.3)	0.75 (0.32)	1.13 (0.12)	76.6 (24.6)	52.7 (0.9)
30	Mrb3/Mna-1	8.6 (0.2)	15.0 (2.2)	0.54 (0.01)	1.33 (0.35)	115.8 (2.3)	70.4 (12)
31	45704	16.0 (6.6)	11.8 (2.3)	1.05 (0.53)	0.99 (0.22)	82.3 (24.0)	94.0 (23)
32	SORA/2	13.1 (1.3)	14.4 (0.4)	0.86 (0.20)	1.23 (0.22)	77.7 (6.8)	69.6 (2.1)
33	46020	17.0 (6.4)	14.8 (0.3)	1.04 (0.41)	1.12 (0.21)	74.6 (20.5)	67.8 (1.5)
34	Bisu-1	18.3 (0.7)	13.6 (0.6)	1.06 (0.10)	0.97 (0.27)	91.8 (5.6)	73.7 (3.3)
35	4017	15.7 (6.7)	23.4 (0.3)	1.13 (0.51)	1.94 (0.36)	85.9 (25.8)	42.7 (0.6)
36	MEXICALI 75	10.2 (2.9)	12.5 (2.8)	0.78 (0.26)	1.19 (0.45)	112.2 (24)	92.0 (26)
37	RASCON-37	13.0 (0.7)	12.7 (0.1)	1.00 (0.29)	1.12 (0.08)	77.7 (3.9)	78.6 (0.6)
38	4303	9.3 (0.2)	33.1 (1.7)	0.64 (0.11)	2.65 (0.03)	108.0 (2.0)	30.4 (1.5)
39	Geromtel-1	12.4 (3.6)	11.9 (1.2)	0.83 (0.28)	0.90 (0.17)	92.8 (20.9)	86.0 (9.4)
40	KC-3426	19.2 (1.7)	17.6 (0.3)	1.47 (0.28)	1.38 (0.14)	82.1 (9.6)	56.8 (1.0)
41	46202	12.5 (0.2)	22.3 (0.6)	0.67 (0.05)	1.35 (0.15)	79.8 (1.1)	44.9 (1.2)
42	VRKS-3	11.5 (2.5)	11.1 (1.2)	0.72 (0.24)	0.74 (0.11)	90.4 (16.2)	91.7 (9.1)
43	45620	9.1 (0.2)	13.0 (2.0)	0.76 (0.01)	1.19 (0.22)	109.7 (2.2)	81.6 (15)
44	Gdr2	11.4 (3.9)	12.0 (1.5)	0.76 (0.28)	0.87 (0.11)	106.2 (27)	86.9 (12)
45	Aday-19	15.4 (4.4)	17.3 (0.3)	1.14 (0.37)	1.33 (0.08)	74.2 (16.5)	57.9 (1.2)
46	AAZ	11.8 (3.2)	11.9 (2.3)	0.81 (0.18)	0.90 (0.32)	96.2 (20.7)	93.0 (22)
47	4025	14.5 (4.6)	19.4 (1.9)	1.10 (0.20)	1.56 (0.05)	81.2 (19.5)	82.6 (5.7)
48	45868	15.4 (3.2)	19.3 (0.3)	1.35 (0.19)	1.72 (0.22)	69.9 (12.0)	52.0 (0.9)
49	45558	14.4 (0.4)	10.4 (0.1)	0.96 (0.05)	0.70 (0.09)	97.2 (3.9)	72.9 (0.7)
50	Azarbayjan	11.1 (3.1)	16.6 (1.2)	0.88 (0.19)	1.20 (0.21)	96.8 (20.2)	61.2 (4.8)

Numbers in parentheses indicate the standard error, SE (n = 3).

respectively (Table 4). Large genotypic diversity in shoot Zn concentration were observed under both non-Zn application condition (7.1 to 19.5 mg Zn/kg dry weight in 'RCOL/THK' and 'Aydin-93', respectively) and with Zn application (10.4 to 33.1 mg Zn/kg dry weight in '45558' and '4303', respectively) (Table 5). Shoot Zn concentration was higher in durum wheat plants supplied with

Table 6
Correlation of shoot dry matter with Zn efficiency, shoot Zn concentration, shoot Zn content and Zn utilization efficiency in durum wheat genotypes

Traits	Shoot dry matter	Zn efficiency	Shoot Zn concentration	Shoot Zn content	Zn utilization efficiency
Shoot dry matter	1				
Zn efficiency	-0.03 ns	1			
Shoot Zn concentration	-0.01 ns	-0.17 ns	1		
Shoot Zn content	0.49 **	-0.24 ns	0.84 **	1	
Zn utilization efficiency	0.01 ns	-0.01 ns	-0.89 **	-0.73 **	1

Ns, *, and ** indicate non-significant, significant at P ≤0.05, and P≤0.01, respectively.

Zn (Table 5). According to the data analyzed, there was no correlation between shoot Zn concentration and dry matter production (Table 6). Zinc content ranged from 0.31 µg/plant in 'RCOL/THK' to 1.47 µg/plant in 'KC-3426' at Zn deficient conditions and also from 0.70 µg/plant in '45558' to 2.98 µg/plant in 'Zenit' at Zn sufficient conditions, respectively (Table 5). Moreover, shoot Zn content significantly correlated with shoot dry matter (R^2 = 0.49, P≤0.01) and shoot Zn concentrations (R^2 = 0.84, P≤ 0.01) (Table 6).

Zn utilization efficiency

Zn utilization efficiency (shoot dry matter produced per unit of Zn) also varied among the genotypes and was affected by Zn fertilization (Table 3). Unlike shoot Zn concentration and content, Zn utilization efficiency diminished in all wheat genotypes by Zn fertilization (Table 4), so that the highest and lowest decreases in Zn utilization efficiency were recorded in '4303' and '45704' genotypes, respectively (Table 5). Under Zn deficiency, Zn utilization efficiency varied from 59.5 to 140.8 in 'DiyarBakir-81' and 'RCOL/THK', respectively. Also, under Zn application, this attribute varied from 30.4 to 94.0 in '4303' and '45704', respectively (Table 5). Results of this experiment showed that Zn utilization efficiency negatively correlated with shoot Zn content (R^2 = -0.73, P≤0.01) (Table 6).

Cluster analysis

Genotypes were divided into six groups based on cluster analysis (Fig. III). The first group included twenty-five genotypes. The second group included genotypes numbers 43, 47, 48, 49, and 50. It should be noted that the genotypes recognized as Zn utilization efficient in this study in terms of seedling dry weight ('4025', '45868', '45558', and 'Azerbaijan') were also included in this group. Therefore, it can be concluded that tolerance to Zn deficiency (Zn efficient) above these genotypes is more than other genotypes in the study because of their ability to absorb better on Zn and to produce more dry matter of seedlings. The third group consisted of three genotypes. The fourth group included genotypes numbers 1, 2, 3, 6, 18, 19, 20, 21, 22, 23, 24, 26, and 27, which were sensitive to Zn deficiency so that they had the lowest Zn efficiency among other genotypes and their seedling dry weight increased by application of Zn (Zn sufficient conditions). The fifth group consisted of genotype number 29, which had the lowest seedling dry weight in both Zn sufficiency and deficiency conditions. Finally, the sixth group included genotype number 17, which was highly susceptible to Zn deficiency conditions (Fig. III).

Discussion

Zinc is an essential component of several enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients, and plays an important role in the production of dry matter and yield (Cakmak, 2008; Whiting et al., 2009; Eide, 2011; Esfandiari et al., 2016; Abdoli and Esfandiari, 2017). This study demonstrates that there were great differences in the expression of visual symptoms and Zn efficiency between cultivars and lines of durum wheat studied. The differences observed in Zn

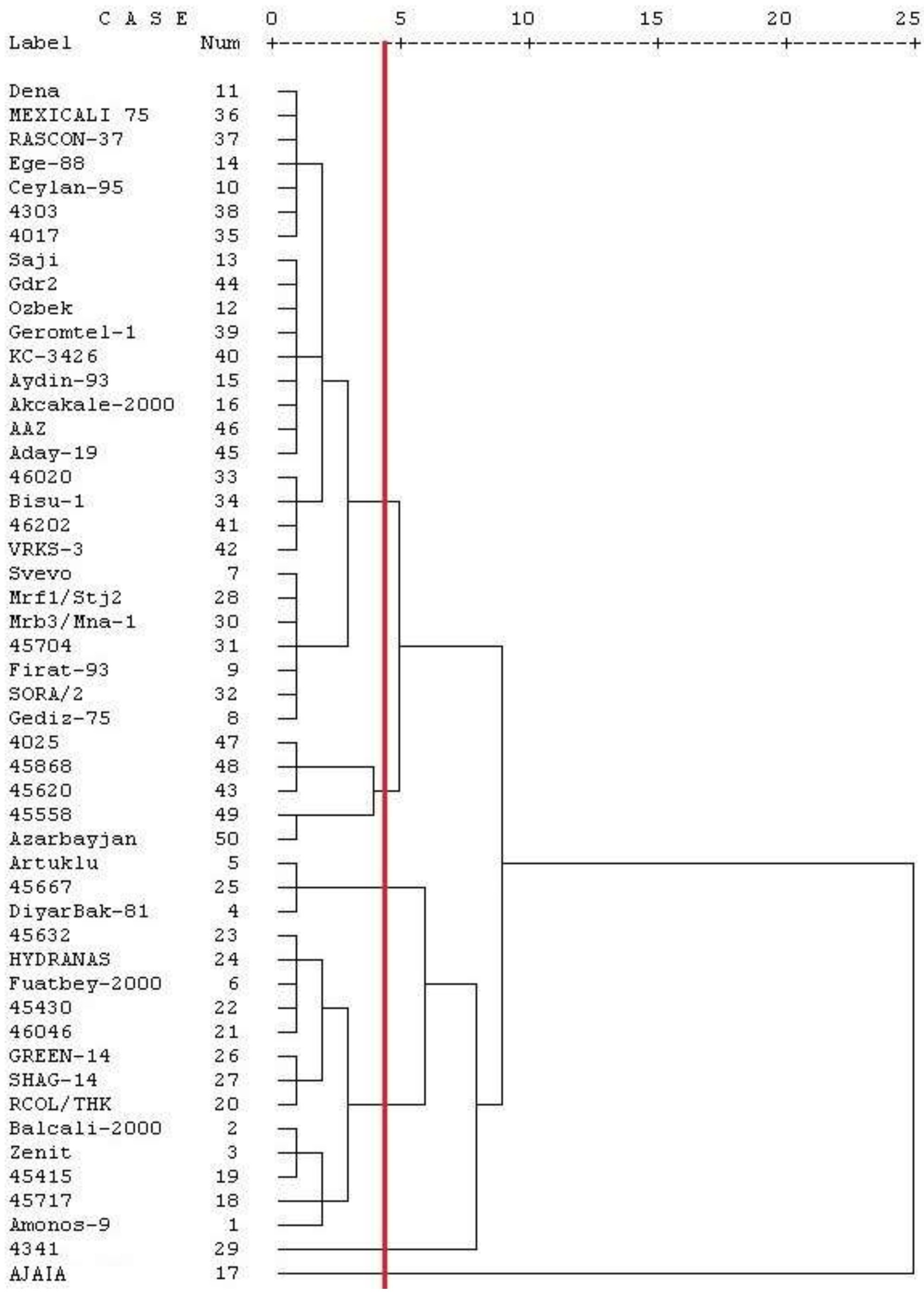

Fig. III. Dendrogram of fifty durum wheat genotypes resulted from Ward method cluster analysis based on mean Zn deficiency score, Zn efficiency (%), and shoot dry matter (mg/plant) under deficient and adequate Zn supply; numbers inside the figure are number of durum wheat genotypes.

efficiency seemingly is due to genetic make-up dissimilarities. McDonald et al. (2008) reported the same differences on the Zn content and concentration under the controlled growing conditions with diverse durum genotypes. Genc and McDonald (2008) in their research on the variation of Zn content and concentration in seeds noted that due to the weak correlation between Zn efficiency and Zn content or Zn concentration of seed, the related difference observed was mainly due to the genetic differences as well. Results of this study showed that there were significant differences among the wheat genotypes for all the traits. As results suggest, some durum wheat genotypes (9 genotypes) had higher or equal Zn efficiency with Zn efficient durum wheat cultivars ('Akcakale-2000', 'Aydine-93', 'Ege-88', and 'Saji') and there were no durum wheat genotypes with lower Zn efficiency than Zn-inefficient cultivars of durum wheat except 'A-INTER-8' (Fig. II).

It should be noted that some genotypes had low Zn efficiency (e.g., high reduction in shoot dry matter), but did not show severe symptoms ('45717', '45667', and 'GREEN-14') and vice versa ('4525' and 'Azarbayjan') (Figs. I and II). Kalayci et al. (1999) and Genc et al. (2000) reported that Zn efficiency based on shoot growth and severity of leaf symptoms does not always correlate, indicating that these two phenomena may be affected by Zn deficiency to a different degree, and perhaps controlled by different genes (Lonergan et al., 2001; Narwal et al., 2012). It is clear that under Zn deficiency, reduction in shoot growth is a result of inhibited synthesis or enhanced degradation of indole acetic acid (Cakmak et al., 1998) while chlorosis or necrosis of leaves are associated with oxidative damage caused by free oxygen radicals (Marschner and Cakmak ,1989; Cakmak, 2000; Song et al., 2009; Eide, 2011; Marreiro et al., 2017). Thus, it is possible that the effects of Zn deficiency on these processes may vary with genotypes.

In the present study, a significant but not a very strong correlation was observed between visual symptoms and Zn efficiency (R^2 = 0.41, $P \leq 0.05$) (data not shown). This has implications for evaluation protocols reliant on leaf symptoms only. It would be almost impossible to differentiate between those showing severe symptoms and reduction in shoot growth and those showing no leaf symptoms and severe reduction of shoot growth. Obviously, in evaluating Zn efficiency, genotypes showing no reduction in shoot growth and no leaf symptoms would be preferred. Thus, it is recommended that the evaluation be carried out at least at two levels, deficient and sufficient of Zn, by which both visual symptoms and reduction in shoot growth are considered jointly in the assessment of genotypes for Zn efficiency.

A non-significant correlation (R^2 = 0.047) between relative shoot Zn content and relative shoot dry matter (Zn efficiency) indicates that higher Zn content does not necessarily indicate higher Zn efficiency. The proportion of total Zn content that is physiologically available but not the total Zn content may be more important in terms of Zn efficiency (Cakmak et al., 1997). Several other researchers also reported that biochemical Zn utilization including the ability to maintain the activity of Zn requiring enzymes under Zn deficiency may play a role in Zn efficiency (Rengel, 1995; Hacisalihoglu and Kochian, 2003; Whiting et al., 2009). The correlation between Zn utilization efficiency and Zn efficiency at the plant level was no significant, too (Table 6). This is not surprising since Zn utilization efficiency is a function of Zn content which is based on Zn concentration estimated by chemical analysis. Zn-efficient genotypes did not always have a higher Zn utilization efficiency than Zn-inefficient genotypes (Table 5). For instance, despite similar Zn utilization efficiency, 'KC-3426' and '45558' showed greater Zn efficiency than 'Fuatbey-2000' and '45430' did. These results suggest that Zn utilization efficiency based on chemical analysis may not always indicate Zn efficiency, and there is a need to develop robust screening methods to predict physiologically active Zn (Genc and McDonald, 2008).

Cluster analysis is a method for allocating genotypes into qualitatively homogeneous stability subsets (Lin et al., 1986). Based on cluster analysis, the sample studied was clustered into six main groups. So that, the genotypes numbers 43 ('45620'), 47 ('4025'), 48 ('45868'), 49 ('45558'), and 50 ('Azarbayjan') at second group had high Zn efficient and shoot dry matter in both environments by, showed less reduction in shoot dry matter under Zn-deficient stress condition. It seems that durum wheat genotypes studied are suitable for cultivation in marginal lands that are constantly exposed to Zn deficiency during the growing seasons.

Conclusions

Results of this study showed that dry weight of shoots and shoots zinc accumulation considerably improved by Zn fertilizers. Also, the findings suggested the existence of genotypic variation for tolerance to Zn deficiency among durum wheat genotypes, which offers potential for the improvement of Zn efficiency in wheat breeding programs. Moreover, it is necessary to test more genotypes of durum wheat in future to reveal greater Zn efficiency values than those recognized here.

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