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Study of genetic diversity in different wheat species with various genomes based on morphological characteristics and zinc use efficiency under two zinc-deficient growing conditions

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ABSTRACT

Screening of cash crops to tolerate and grow under low levels of micronutrients is important issue in the plant breeding programs. Thus, the study screened the tolerance of 50 wheat genotypes to zinc (Zn) deficiency in the calcareous soil. The Zn treatment was carried out with application of 5 mg kg^{-1} (+Zn) and without (-Zn) to the collected soils with initial Zn extractable of 0.5 mg Zn kg^{-1} soil. The results revealed that the supplementary application significantly increased shoot dry matter, shoot Zn concentration and shoot Zn content compared to the without Zn application (control), but Zn utilization decreased under Zn application. There was considerable genetic variation in Zn efficiency (55 - 118 %), shoot Zn concentration (11.8 - 27.0 and 14.3 - 39.6 mg kg^{-1} DM under deficient and sufficient Zn, respectively), shoot Zn content (0.56 - 2.02 and 0.90 - 2.83 µg plant^{-1}, under deficient and sufficient Zn, respectively) and Zn utilization efficiency (39 - 87.2 and 31.2 - 71.5 mg DM µg^{-1} Zn under deficient and sufficient Zn, respectively) within wheat genotypes. Cluster analysis based on Zn efficiency, and shoot dry matter at deficient and adequate Zn conditions classified the genotypes into four clusters. Over the two conditions, the most Zn-efficient and Zn-unefficient genotypes were 'Ankara-98' and 'Altintoprak-98' and 'Pg"S' and 'Zarin', respectively. Most durum genotypes had a greater Zn efficiency than modern bread wheat genotypes, therefore these genotypes could be effectively used to breed the new cultivars with high Zn efficiency for calcareous soils.

Key words: durum wheat; bread wheat; zinc concentration; zinc deficiency; zinc efficiency; biofortification

Abbreviations: Zn - Zinc, DAS - days after sowing, DM - dry matter, PVC - plastic pots, FC - field capacity, DARI - Dryland Agricultural Research Institute, AAS - atomic absorption spectrophotometer, ANOVA - analysis of variance, DMRT - Duncan's multiple range test, SE - standard error, SOD - superoxide dismutase, CA - carbonic anhydrase.

IZVLEČEK

PREUČEVANJE GENETSKE RAZNOLIKOSTI DVEH VRST PŠENICE Z RAZLIČNIMA GENOMOMA NA OSNOVI MORFOLOŠKIH LASTNOSTI IN UČINKOVITOSTI IZRABE CINKA V DVEH RAZMERAH NJEGOVE POMANKLJIVE OSKRBE

Preverjanje poljščin na rastno strpnost majhnim koncentracijam mikrohranil je pomemben izziv v rastlinskih žlahtniteljskih programih. V raziskavi je bila preverjena toleranca 50 genotipov pšenice na pomanjkanje cinka (Zn) na apnenčastih tleh. Obravnavanja s cinkom so obsegala uporabo (5 mg Zn kg^{-1} , +Zn) in neuporabo cinka (-Zn) v tleh z začetno vsebnostjo ekstraktibilnega Zn 0,5 mg Zn kg^{-1} tal. Izsledki so pokazali, da je dodajanje cinka značilno povečalo vsebnost suhe snovi poganjkov in vsebnost cinka v njih v primerjavi s kontrolo, a hkrati zmanjšalo učinkovitost njegove izrabe. Med genotipi je bila ugotovljena znatna genetska variabilnost v učinkovitosti izrabe cinka (55 - 118 %), v koncentraciji Zn v poganjkih (11,8 - 27,0 in 14,3 - 39,6 mg kg^{-1} DM v razmerah pomankljive in zadostne oskrbe s cinkom), v vsebnosti Zn (0,56 - 2,02 in 0,90 - 2,83 µg na rastlino, v razmerah pomankljive in zadostne oskrbe s cinkom) in v učinkovitosti izrabe cinka v ramerah pomankljive (39 - 87,2) in zadostne oskrbe s cinkom, (31,2 – 71,5 mg DM/µg Zn). Klasterska analiza, osnovana na učinkovitosti izrabe Zn in vsebnosti suhe snovi poganjkov v razmerah zadostne in pomankljive oskrbe s cinkom je genotipe razdelila v štiri skupine. V obeh rastnih razmerah sta Zn najučinkoviteje izrabljala genotipa 'Ankara-98' in 'Altintoprak-98' in najmanj učinkovito genotipa 'PgS' in 'Zarin'. Večina genotipov trde pšenice je imelo večjo učinkovitost izrabe cinka kot genotipi krušne pšenice, zato bi te lahko učinkovito uporabili pri žlahtnenju novih sort pšenice, ki bi dobro uspevale na apnenčastih tleh z veliko učinkovitostjo izrabe cinka.

Ključne besede: trda pšenica; krušna pšenica; vsebnost Zn v tleh; pomankanje Zn; učinkovitost izrabe Zn; biofortifikacija

Okrajšave: Zn – cink, DAS – dnevi po setvi, DM – suha snov, PVC – plastični lonci, FC – poljska kapaciteta, DARI – Dryland Agricultural Research Institute, AAS – atomski absorpcijski spektrofotometer, ANOVA – analiza variance, DMRT – Duncanov test, SE – standardna napaka, SOD – superoksid dismutaza, CA – karboanhidraza

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1 INTRODUCTION

Zinc deficiency is one of the common restricting factors in crops production, especially cereals, in world (Alloway, 2008). This scarcity is severed in calcareous soils of rainfed areas due to low availability caused by high levels of calcium carbonates, low total Zn contents, high pH and high phosphate in the soil (Alloway, 2009). Thirty percent of world's cultivated soils are estimated to be inadequate in zinc, chiefly in the Mediterranean region and Asia (Suzuki et al., 2006; Alloway, 2009). The investigations has been estimated that approximately up to 40 % of the soils under wheat production areas of Iran are encountered with a level of Zn-deficiency which has drastically influenced the crop performance (Broadley et al., 2007; Esfandiari et al., 2016; Esfandiari and Abdoli, 2016). Thus, in these areas loss of yield is the main concern of farmers. To deal with the problem, applications of different Zn-source of chemical fertilizers are proposed to enhance the plant growth and development, and finally increase crop yield (Sadeghzadeh et al., 2009; Bharti et al., 2013; Abdoli et al., 2014; Guo et al., 2016; Esfandiari et al., 2016).

Sensitivity to Zn deficiency in plants is species specific phenomena and among cereals, wheat is more sensitive than rye, triticale and barley (Cakmak et al., 1997, Cakmak et al., 1999; Blum, 2014). Also durum wheat has a more sensitivity to this deficit (Genc and McDonald, 2008). Studies have shown large variations in performance of bread and durum genotypes in Zn-deficient soils (Rengel and Graham, 1995; Cakmak et al., 1996, Cakmak et al., 1999; Kalayci et al., 1999; Torun et al., 2000; Moshiri et al., 2010; Velu et al., 2012; Abdoli et al., 2016; Yilmaz et al., 2017; Esfandiari et al., 2018). 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; Chatvaz et al., 2010). There is very promising progress in breeding of Zn biofortified cereal genotypes, particularly through the HarvestPlus program (Gomez-Coronado et al., 2016). Generally, the combination of plant breeding and agronomic biofortification is the most affordable and reasonable approach to attenuate Zn deficiency-related problems in humans, however also in crop production (Cakmak, 2008; Gomez-Coronado et al., 2016).

The aims of this study were (i) to screen fifty genotypes of durum and bread wheat for their potential to use of Zn element at early growth stages, (ii) to identify the most Zn-efficient and Zn-inefficient wheat genotypes to be utilized in further genetic studies, and (iii) assess the impact of Zn application on shoot dry matter, Zn concentration and content, and Zn utilization efficiency in wheat.

2 MATERIALS AND METHODS

2.1 Plant materials

Wheat genotypes including eight winter bread wheat (*Triticum aestivum* L.) and forty-two winter durum wheat (*Triticum durum* L.) were obtained from Dryland Agricultural Research Institute (DARI), Maragheh of Iran. The details of wheat genotypes are shown in Table 1.

2.2 Soil preparation and crop management

The used soils were collected from severely Zn-deficient soils of Moghanlou, Bijar state in the Kourdistan city 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 (Esfandiari, unpublished; Abdoli, 2017). The soil details of the location are shown in the Table 2. Critical Zn concentration deficiency was considered when the concentration declined below to 0.5 - 0.6 mg kg^{-1} (Sims and Johnson, 1991). Plastic pots (PVC, 20 × 35 cm) were filled with 3.5 kg soil of the combined samples and for Zn treatment pots the concentration raised up to 5 mg Zn kg^{-1} soil form the $ZnSO_4.7H_2O$ source based on the soil Zn concentrations of the sample (+Zn) and without Zn fertilization (-Zn). Before sowing, the soils in pots were mixed homogenously with a basal treatment of 200 mg N ($Ca(NO_3)_2.4H_2O$) kg^{-1} and 100 mg P (KH_2PO_4) kg^{-1} fertilizers. Fourteen seeds from every genotype were sown into each pot, and the pots were thinned to seven seedlings per pot after emergence and daily watered by using deionized water. The field capacity (FC) was determined by the gravimetric method following the method suggested by Souza et al. (2000), and the irrigation treatment was carried out based on the distinction between the mass of the dry soil and wet soil after saturation. Plants were harvested after 45 days of treatment; Zn concentration and content in shoot, as well as shoot dry mass, were measured.

2.3 Determination of Fe and Zn concentration and contents

After the mentioned time, the seedling samples were oven dried at 75 ºC for 48 hours and weighted, then samples were ashed at 550 ºC for 8 hours and dissolved in 1 % (v/v) hydrochloric acid (Chapman and Pratt, 1961). Concentrations of Zn and Fe within the digested solutions were determined by Atomic Absorption

Spectrophotometer (model: AAS-6300 Shimadzu) and the expressed based on plant dry mass (mg kg^{-1} DM). Content of Zn in the shoot (µg plant^{-1}) were measured by multiplying amount of seedling dry matter by amount of Zn concentration in the shoot (Genc et al., 2000).

Table 1: Name, description and 1000 grain mass (g) of durum and bread wheat genotypes

No.	Genotype	Wheat type	1000 grain mass (g)	Description/Origin
1	Altintoprak-98	Durum	39	Turkish variety
2	Ankara-98	Durum	43	Turkish variety
3	Cheheldaneh	Durum	-	Local variety for cold
4	Mirzabey-2000	Durum	39	Turkish variety
5	Imren	Durum	36	Turkish variety
6	Berkmen-469	Durum	31	Turkish variety
7	Tunca-79	Durum	30	Turkish variety
8	G-1252	Durum	-	Turkish variety
9	Kunduru-414-44	Durum	33	Turkish variety
10	Durbel	Durum	35	Turkish variety
11	Gokgol-79	Durum	33	Turkish variety
12	Ammar-9	Durum	33	CIMMYT
13	Pinor-2001	Durum	36	Turkish variety
14	Gerdish	Durum	-	Local variety for cold
15	Sarayolla	Durum	36	Turkish variety
16	Chesit-1252	Durum	39	Turkish variety
17	Geromtel-1	Durum	36	CIMMYT
18	Fatasel-185	Durum	37	Turkish variety
19	Altin-40-98	Durum	36	Turkish variety
20	Turabi	Durum	37	Turkish variety
21	Cakmak-79	Durum	37	Turkish variety
22	Tyten-2002	Durum	38	Turkish variety
23	Zardak	Durum	-	Local variety
24	Kiziltan-91	Durum	41	Turkish variety
25	Meram-2002	Durum	39	Turkish variety
26	Haurani	Durum	-	ICARAD material
27	Za-14-105	Durum	40	-
28	Ter-1//Mrf1/Stj2	Durum	35	-
29	Kumbet-2000	Durum	39	Turkish variety
30	Haran-95	Durum	41	Turkish variety
31	61-130	Durum	-	ICARAD material
32	Kunduru-1149	Durum	38	Turkish variety
33	Bcr/Gro1//Mgnl1	Durum	31	-
34	Selcuklu-97	Durum	35	Turkish variety
35	Yelken-2000	Durum	40	Turkish variety
36	GAP	Durum	41	Turkish variety
37	Saji	Durum	-	Iranian released variety for moderate cold condition
38	SonQarak-98	Durum	37	Turkish variety
39	Eminbey	Durum	41	Turkish variety
40	Viya-2005	Durum	43	Turkish variety
41	Kunduru	Durum	-	Turkish variety
42	Pg"S	Durum	-	ICARAD material
43	Azar-2	Bread	42	Iranian released variety
44	Homa	Bread	42	Iranian released variety
45	Pishgam	Bread	43	Iranian released variety
46	Ohadi	Bread	43	Iranian released variety
47	Sardari	Bread	40	Local variety
48	Gascogen	Bread	-	Iranian released variety
49	Rasad	Bread	-	Iranian released variety
50	Zarin	Bread	39	Iranian released variety

Table 2: Physical-chemical properties of the soil used in the experiment

Physical properties	Amount	Chemical properties	Amount
Calcium carbonate, $CaCO_3$ (%)	20	Extractable Fe (mg kg^{-1})	3.1
Organic matter (%)	0.5	Extractable Zn (mg kg^{-1})	0.5
pH (H_2O)	7.2	Extractable Cu (mg kg^{-1})	0.7
Electrical Conductivity, EC_e (dS m^{-1})	2.3	Extractable P (mg kg^{-1})	6.1
Silt (%)	45	Available N (%)	0.092
Clay (%)	39	Available P (mg kg^{-1})	6.1
Sand (%)	16	Available K (mg kg^{-1})	360
Texture	Clay-loam		

2.4 Estimated of Zn efficiency and Zn utilization efficiency

Zinc efficiency ratio expressed as relative shoot growth and was calculated as the percentage of shoot dry matter produced under Zn-deficiency relative to shoot dry matter produced under Zn fertilization. Zn utilization efficiency was calculated by dividing amount of produced shoot dry matter by content of Zn in the shoot [mg DM μg^{-1} Zn] (Genc and McDonald, 2004; Genc et al., 2006).

2.5 Statistical analysis

The experiment was performed as a factorial based on completely randomized block design (RCBD) with three replications at out-glasshouse in 2013-14 at University of Maragheh, Maragheh, Iran. Analysis of variance (ANOVA) was performed using SAS software ver. 9.1 (SAS Institute, 2011) and also Duncan's Multiple Range Test (DMRT) was used to compare the means ($P \leq 0.05$) (Duncan, 1955). The data were analyzed using SPSS software ver. 16 (SPSS, 2007) for cluster analysis of genotypes based on Square Euclidean distance and Ward method. The figures were drawn using Excel software ver. 10 and the means ± standard error (SE) was used to compare the data.

3 RESULTS

3.1 Shoot dry matter and zinc efficiency

Shoot dry matter was influenced by genotype and Zn application (Table 3), and significant genetic differences were observed at both deficient and sufficient Zn supplies. Shoot dry matter varied from 33 ± 3 mg $plant^{-1}$ in ‘Zarin’ to 105 ± 5 mg $plant^{-1}$ in ‘Ankara-98’ at Zn deficient condition, and 41 ± 3 mg $plant^{-1}$ in ‘Durbel’ to 108 ± 12 mg $plant^{-1}$ in ‘Gascogen’ at Zn sufficient condition (Figure 1A). Zn application increased averages of shoot dry matter of genotypes from 54 mg $plant^{-1}$ to 68 mg $plant^{-1}$, which means 26 % rise in shoot dry matter, especially in durum wheats (Figure 1A). Shoot dry matter suppress due to Zn deficiency was different among the genotypes. At day 45, decreases in shoot growth and dry matter were more distinct in durum wheat genotypes (particularly in ‘Pg"S’, ‘Kunduru-414-44’ and ‘Viya-2005’). There was a positive relationship between shoot dry matter at deficient and sufficient Zn condition ($r = 0.591$, $P < 0.001$, $n = 50$, Figure 2).

Zn efficiency of genotypes was ranged from 55 to 118 % in ‘Pg"S’ and ‘Altintoprak-98’, respectively (Figure 1B). Mean Zn efficiency in bread wheats (83 %) was higher than durum wheats (73 %), but some durum wheats such as ‘G-1252’, ‘Tunca-79’, ‘Durbel’, ‘Ammar-9’, ‘Ankara-98’ and ‘Berkmen-469’ had greater Zn efficiency than the bread wheats.

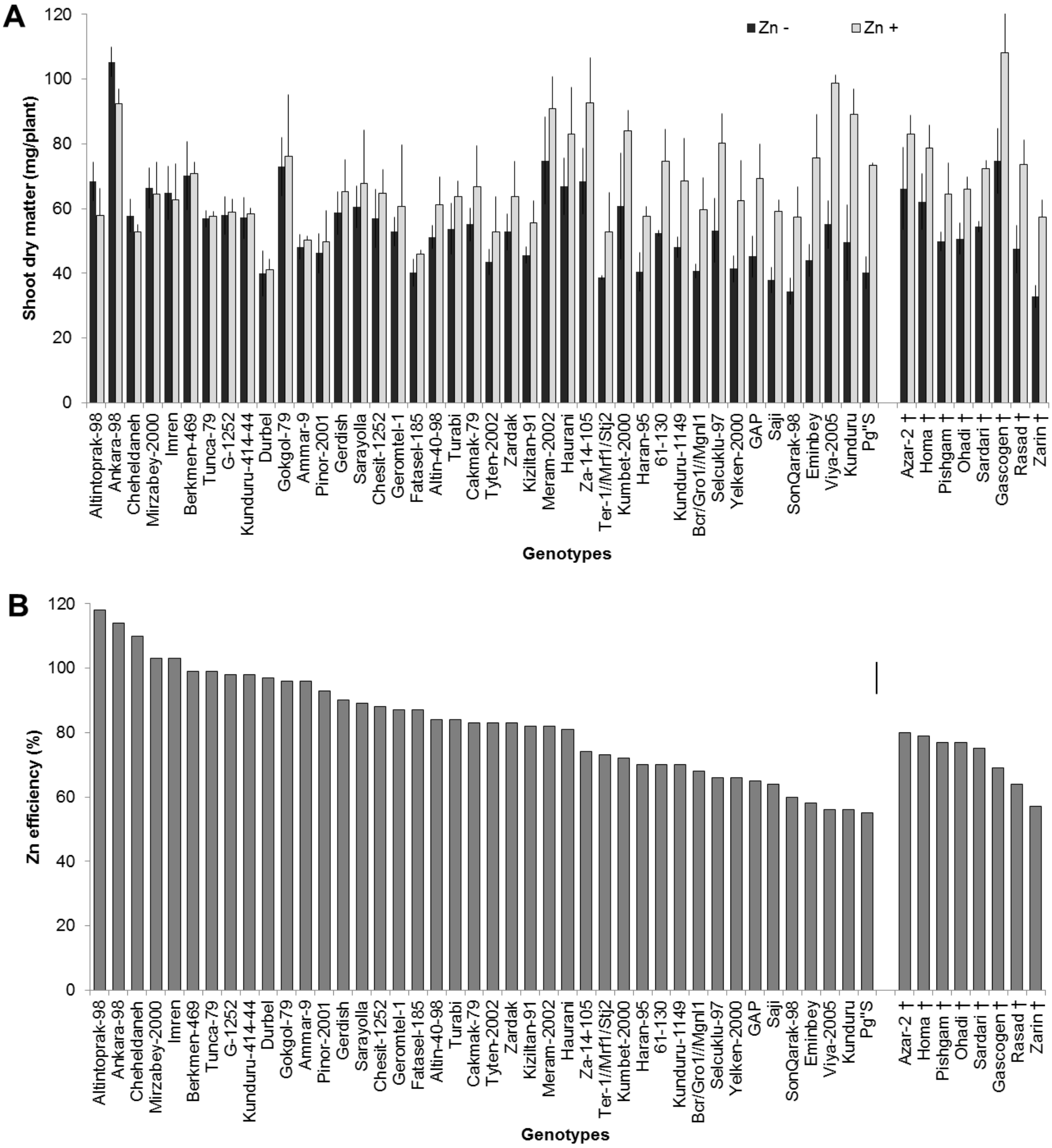

Figure 1: Effects of Zn fertilization (5 mg Zn kg^{-1} soil) on A: shoot dry matter (mg plant^{-1}) and B: Zn efficiency (%) in durum and bread wheat genotypes at 45 DAS. Vertical lines indicate standard error (SE) and vertical bar on the corners represent DMRT ($P < 0.05$) for the comparison between the genotypes. Zinc efficiency was calculated as [(shoot dry matter at -Zn/shoot dry matter at +Zn) × 100]. † Bread wheat.

Table 3: Analysis of variance (mean square) for the measured traits of in durum and bread wheat genotypes

Source of variance	df	Mean squares				
		Shoot dry matter	Shoot Zn concentration	Shoot Fe concentration	Shoot Zn content	Zn utilization efficiency
Replication	2	116 ns	1292 **	61041 **	3.87 **	5691 **
Zn fertilization (Zn)	1	13920 **	3184 **	13200 **	36.0 **	17144 **
Genotypes (G)	49	885 **	90.7 **	2984 **	0.938 **	395 **
Zn × G	49	228 ns	31.0 ns	1845 **	0.213 ns	122 ns
Error	198	206	33.9	505	0.245	123
CV (%)	-	23.4	25.6	12.9	34.8	22.7

ns, * and **: Non-significant and significant at the 5 % and 1 % levels of probability, respectively.
df: degrees of freedom, CV: coefficient of variance.

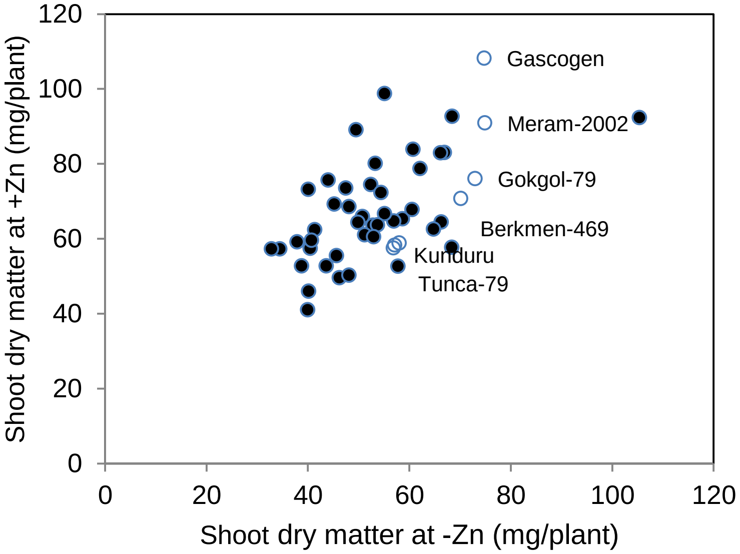

Figure 2: The relationship between shoot dry matter at deficient (-Zn) and sufficient Zn (+Zn) condition in durum and bread wheat genotypes at 45 DAS (r = 0.591, P < 0.001, n = 50). The 'Gascogen' and 'Meram-2002' genotypes which are Zn efficient and also responsive to Zn fertilizer, and also 'Gokgol-79', 'Berkmen-469', 'Kunduru' and 'Tunca-79' which are Zn efficient but not responsive to Zn fertilizer (empty circles). Closed circles represent reminder of genotypes studied.

3.2 Zn concentration and content in the shoot

Zn fertilization significantly affected (*P* < 0.001) shoot Zn concentration and content, with significant differences (*P* < 0.001) among genotypes (Table 3). Large genotypic diversity in shoot Zn concentration were observed under both no Zn application condition (11.8 mg Zn kg^{-1} DM in 'Ammar-9' to 27.0 mg Zn kg^{-1} DM in 'Saji') and with Zn application (14.3 mg Zn kg^{-1} DM in 'Pishgam' to 39.6 mg Zn kg^{-1} DM in 'Sarayollah') (Table 4). Although, shoot Zn concentration was higher in plants supplied with Zn (Table 4). Zn fertilization resulted in 28 % increase in Zn concentration. According to Figure 4 there was no significant correlation between shoot Zn concentration and dry matter production. Zinc content ranged from 0.56 µg plant^{-1} in 'Ter-1//Mrf1/Stj2' to 2.02 µg plant^{-1} in 'Ankara-98', and 0.90 µg plant^{-1} in 'Pishgam' to 2.83 µg plant^{-1} in 'Ankara-98' at deficient and sufficient Zn conditions, respectively (Table 5). Moreover, shoot Zn content was significantly correlated with shoot dry matter (r = 0.70, *P* < 0.001) and shoot Zn concentrations (r = 0.51, *P* < 0.001) (Figure 4).

Table 4: Effects of Zn fertilization (5 mg Zn kg^{-1} soil) on shoot Zn and Fe concentration (mg kg^{-1} DM) in durum and bread wheat genotypes at 45 DAS

No.	Genotype	Shoot Zn concentration (mg kg^{-1} DM)			Shoot Fe concentration (mg kg^{-1} DM)		
		-Zn	+Zn	Mean	-Zn	+Zn	Mean
1	Altintoprak-98	19.2 ± 2.8	27.9 ± 2.6	23.5 a-i	143 ± 19	184 ± 38	163 f-m
2	Ankara-98	19.5 ± 4.0	30.8 ± 2.8	25.1 a-h	177 ± 39	154 ± 20	166 e-l
3	Cheheldaneh	20.6 ± 5.4	25.1 ± 1.7	22.9 a-i	180 ± 25	182 ± 7	181 d-i
4	Mirzabey-2000	24.8 ± 5.7	31.2 ± 3.0	28.0 ab	179 ± 29	182 ± 25	181 d-i
5	Imren	23.7 ± 4.3	29.2 ± 0.0	26.4 a-f	181 ± 25	203 ± 5	192 c-f
6	Berkmen-469	21.2 ± 3.8	30.4 ± 3.6	25.8 a-g	185 ± 19	207 ± 25	196 b-e
7	Tunca-79	24.3 ± 4.4	31.4 ± 7.3	27.9 a-c	180 ± 26	132 ± 12	156 h-m
8	G-1252	22.4 ± 5.7	31.4 ± 7.3	26.9 a-d	158 ± 25	165 ± 5	162 f-m
9	Kunduru-414-44	25.5 ± 6.8	34.4 ± 8.1	30.0 a	219 ± 25	212 ± 19	215 a-c
10	Durbel	14.5 ± 3.2	23.9 ± 0.5	19.2 d-j	215 ± 16	154 ± 9	184 d-h
11	Gokgol-79	19.0 ± 2.8	28.1 ± 2.6	23.6 a-i	149 ± 29	158 ± 22	154 h-m
12	Ammar-9	11.8 ± 1.4	20.4 ± 0.7	16.1 ij	205 ± 8	144 ± 2	174 d-k
13	Pinor-2001	19.0 ± 2.8	19.5 ± 4.1	19.2 d-j	159 ± 14	176 ± 14	168 e-l
14	Gerdish	23.4 ± 4.3	29.8 ± 2.3	26.6 a-f	244 ± 16	224 ± 14	234 a
15	Sarayolla	19.8 ± 3.4	39.6 ± 17	29.7 a	173 ± 34	157 ± 4	165 e-l
16	Chesit-1252	15.9 ± 0.5	30.4 ± 4.1	23.1 a-i	165 ± 15	172 ± 20	169 e-k
17	Geromtel-1	15.3 ± 2.2	24.9 ± 6.7	20.1 b-j	178 ± 16	167 ± 8	172 d-k
18	Fatasel-185	16.0 ± 2.9	25.0 ± 2.0	20.5 b-j	242 ± 15	188 ± 6	215 a-c
19	Altin-40-98	23.8 ± 3.4	32.0 ± 1.3	27.9 a-c	181 ± 16	176 ± 3	178 d-j
20	Turabi	19.1 ± 2.9	20.3 ± 0.0	19.7 b-j	141 ± 19	159 ± 2	150 i-m
21	Cakmak-79	18.0 ± 3.3	29.5 ± 2.4	23.8 a-i	229 ± 19	200 ± 20	215 a-c
22	Tyten-2002	16.6 ± 2.2	24.5 ± 1.7	20.6 b-j	183 ± 23	153 ± 7	168 e-k
23	Zardak	22.5 ± 5.5	26.1 ± 1.6	24.3 a-i	223 ± 28	157 ± 1	190 c-g
24	Kiziltan-91	22.2 ± 3.1	22.4 ± 0.2	22.3 a-j	190 ± 16	191 ± 10	191 c-f
25	Meram-2002	20.1 ± 2.4	27.8 ± 2.4	24.0 a-i	151 ± 25	176 ± 21	163 f-m
26	Haurani	25.1 ± 9.4	26.1 ± 3.0	25.6 a-g	178 ± 24	171 ± 16	174 d-k
27	Za-14-105	21.7 ± 3.1	25.4 ± 2.1	23.5 a-i	177 ± 17	183 ± 23	180 d-i
28	Ter-1//Mrf1/Stj2	14.5 ± 1.5	24.0 ± 3.1	19.2 d-j	190 ± 20	164 ± 2	177 d-j
29	Kumbet-2000	14.3 ± 0.7	31.7 ± 3.8	23.0 a-i	243 ± 18	149 ± 11	196 b-e
30	Haran-95	16.9 ± 2.6	21.6 ± 1.7	19.2 d-j	157 ± 22	159 ± 6	158 g-m
31	61-130	16.3 ± 0.2	24.0 ± 4.6	20.2 b-j	162 ± 13	110 ± 9	136 lm
32	Kunduru-1149	16.9 ± 1.4	31.5 ± 2.7	24.2 a-i	160 ± 13	165 ± 19	163 f-m
33	Bcr/Gro1//Mgnl1	16.9 ± 3.3	18.2 ± 0.4	17.5 g-j	161 ± 25	146 ± 10	153 h-m
34	Selcuklu-97	21.0 ± 4.9	32.5 ± 2.8	26.8 a-d	172 ± 15	170 ± 20	171 e-k
35	Yelken-2000	24.5 ± 3.3	30.2 ± 3.7	27.4 a-d	229 ± 27	215 ± 9	222 ab
36	GAP	19.6 ± 3.0	24.1 ± 0.9	21.8 a-j	167 ± 34	167 ± 9	167 e-l
37	Saji	27.0 ± 5.2	32.2 ± 8.1	29.6 a	179 ± 22	163 ± 15	171 e-k
38	SonQarak-98	24.0 ± 6.2	28.9 ± 6.2	26.5 a-f	132 ± 22	162 ± 2	147 j-m
39	Eminbey	16.6 ± 3.1	20.8 ± 0.4	18.7 e-j	138 ± 23	160 ± 2	149 i-m
40	Viya-2005	26.2 ± 2.7	26.8 ± 1.9	26.5 a-f	177 ± 17	168 ± 19	172 d-k
41	Kunduru	18.1 ± 1.7	28.8 ± 3.5	23.5 a-i	270 ± 28	136 ± 11	203 b-d
42	Pg"S	17.8 ± 0.9	24.1 ± 3.7	20.9 b-j	210 ± 16	157 ± 13	183 d-h
43	Azar-2 †	18.2 ± 3.3	23.5 ± 2.9	20.8 b-j	175 ± 27	133 ± 18	154 h-m
44	Homa †	19.2 ± 3.4	19.8 ± 1.9	19.5 c-j	163 ± 24	126 ± 14	144 k-m
45	Pishgam †	13.8 ± 2.1	14.3 ± 1.4	14.0 j	152 ± 27	141 ± 9	147 j-m
46	Ohadi †	17.6 ± 2.6	18.9 ± 0.2	18.3 f-j	145 ± 21	176 ± 6	161 f-m
47	Sardari †	16.3 ± 2.5	18.7 ± 0.0	17.5 g-j	170 ± 29	137 ± 9	153 h-m
48	Gascogen †	20.7 ± 3.1	22.9 ± 2.4	21.8 a-j	173 ± 25	208 ± 23	190 c-g
49	Rasad †	16.4 ± 2.1	17.8 ± 0.6	17.1 h-j	149 ± 27	116 ± 8	133 m
50	Zarin †	17.1 ± 3.1	18.5 ± 2.6	17.9 g-j	138 ± 31	179 ± 12	158 g-m
	Mean	19.5 b	26.0 a		180 a	167 b	

Means followed by the same letters in each column and each factor are not significantly different at 5 % level, according to Duncan's Multiple Range Test. Mean ± SE (n = 3). † Bread wheat.

Table 5: Effects of Zn fertilization (5 mg Zn kg^{-1} soil) on shoot Zn content (µg plant^{-1}) and Zn utilization efficiency (mg DM µg^{-1} Zn) in durum and bread wheat genotypes at 45 DAS

No.	Genotype	Shoot Zn content (µg plant^{-1})			Zn utilization efficiency (mg DM µg^{-1} Zn)		
		-Zn	+Zn	Mean	-Zn	+Zn	Mean
1	Altintoprak-98	1.34 ± 0.30	1.57 ± 0.09	1.45 b-l	54.4 ± 7.7	36.5 ± 3.3	45.5 c-i
2	Ankara-98	2.02 ± 0.32	2.83 ± 0.18	2.42 a	55.3 ± 10	33.0 ± 2.7	44.2 c-i
3	Cheheldaneh	1.16 ± 0.25	1.32 ± 0.09	1.24 c-l	54.6 ± 12	40.2 ± 2.8	47.4 c-i
4	Mirzabey-2000	1.60 ± 0.33	2.01 ± 0.32	1.80 a-d	46.7 ± 14	32.6 ± 2.9	39.7 g-i
5	Imren	1.59 ± 0.46	1.83 ± 0.33	1.71 b-f	45.4 ± 9.1	34.3 ± 0.0	39.9 g-i
6	Berkmen-469	1.47 ± 0.30	2.14 ± 0.23	1.80 a-d	50.6 ± 9.9	33.8 ± 3.6	42.2 e-i
7	Tunca-79	1.40 ± 0.31	1.82 ± 0.46	1.61 b-j	44.3 ± 8.9	34.9 ± 6.7	39.6 g-i
8	G-1252	1.36 ± 0.46	1.89 ± 0.52	1.62 b-i	49.9 ± 11	35.0 ± 6.9	42.5 e-i
9	Kunduru-414-44	1.51 ± 0.48	1.98 ± 0.39	1.74 b-f	45.0 ± 12	32.0 ± 6.1	38.5 hi
10	Durbel	0.60 ± 0.22	0.98 ± 0.08	0.79 l	75.5 ± 16	41.9 ± 0.8	58.7 b-d
11	Gokgol-79	1.40 ± 0.32	2.05 ± 0.40	1.73 b-f	54.7 ± 7.5	36.2 ± 3.1	45.5 c-i
12	Ammar-9	0.57 ± 0.09	1.03 ± 0.06	0.80 l	87.2 ± 11	49.1 ± 1.7	68.2 ab
13	Pinor-2001	0.91 ± 0.25	1.05 ± 0.39	0.98 h-l	55.1 ± 8.2	56.1 ± 11	55.6 b-g
14	Gerdish	1.38 ± 0.30	1.99 ± 0.42	1.69 b-h	46.4 ± 10	34.0 ± 2.7	40.2 f-i
15	Sarayolla	1.16 ± 0.10	2.27 ± 0.52	1.71 b-f	54.3 ± 11	34.1 ± 10	44.2 c-i
16	Chesit-1252	0.91 ± 0.17	1.99 ± 0.39	1.45 b-l	63.2 ± 2.1	33.9 ± 4.1	48.6 c-i
17	Geromtel-1	0.83 ± 0.18	1.76 ± 0.99	1.29 c-l	68.4 ± 9.9	45.3 ± 9.6	56.9 b-d
18	Fatasel-185	0.65 ± 0.16	1.15 ± 0.12	0.90 j-l	66.3 ± 11	40.5 ± 3.1	53.4 b-i
19	Altin-40-98	1.20 ± 0.09	1.94 ± 0.24	1.57 b-k	43.8 ± 6.4	31.4 ± 1.3	37.6 i
20	Turabi	0.98 ± 0.07	1.29 ± 0.10	1.14 e-l	54.8 ± 7.9	49.3 ± 0.1	52.1 c-i
21	Cakmak-79	1.02 ± 0.28	1.91 ± 0.23	1.47 b-l	59.7 ± 11	34.3 ± 2.7	47.0 c-i
22	Tyten-2002	0.73 ± 0.14	1.26 ± 0.22	1.00 g-l	62.3 ± 8.3	41.3 ± 2.9	51.8 c-i
23	Zardak	1.16 ± 0.23	1.66 ± 0.30	1.41 b-l	49.7 ± 11	38.6 ± 2.4	44.2 c-i
24	Kiziltan-91	1.01 ± 0.12	1.24 ± 0.16	1.13 e-l	46.9 ± 6.7	44.6 ± 0.3	45.8 c-i
25	Meram-2002	1.48 ± 0.30	2.56 ± 0.47	2.02 ab	51.4 ± 6.9	36.5 ± 3.2	44.0 c-i
26	Haurani	1.84 ± 0.89	2.25 ± 0.66	2.04 ab	50.9 ± 15	39.3 ± 4.1	45.1 c-i
27	Za-14-105	1.42 ± 0.09	2.40 ± 0.54	1.91 a-c	48.5 ± 8.1	39.9 ± 3.1	44.2 c-i
28	Ter-1//Mrf1/Stj2	0.56 ± 0.07	1.34 ± 0.48	0.95 i-l	70.8 ± 7.5	43.1 ± 5.4	57.0 b-d
29	Kumbet-2000	0.84 ± 0.18	2.66 ± 0.34	1.75 b-f	70.4 ± 3.7	32.4 ± 3.9	51.4 c-i
30	Haran-95	0.66 ± 0.07	1.25 ± 0.14	0.96 i-l	61.8 ± 8.7	47.0 ± 3.6	54.4 b-g
31	61-130	0.85 ± 0.01	1.70 ± 0.08	1.28 c-l	61.3 ± 0.6	44.5 ± 7.4	52.9 b-i
32	Kunduru-1149	0.82 ± 0.12	2.10 ± 0.28	1.46 b-l	59.9 ± 4.8	32.1 ± 2.5	46.0 c-i
33	Bcr/Gro1//Mgnl1	0.68 ± 0.11	1.09 ± 0.21	0.89 kl	64.5 ± 14	55.0 ± 1.3	59.8 bc
34	Selcuklu-97	1.18 ± 0.47	2.58 ± 0.25	1.88 a-d	54.0 ± 14	31.2 ± 2.5	42.6 e-i
35	Yelken-2000	1.04 ± 0.21	1.80 ± 0.18	1.42 b-l	42.5 ± 6.5	34.0 ± 3.8	38.3 hi
36	GAP	0.85 ± 0.05	1.68 ± 0.30	1.27 c-l	54.0 ± 9.8	41.7 ± 1.6	47.9 c-i
37	Saji	1.06 ± 0.28	1.85 ± 0.35	1.46 b-l	40.6 ± 9.4	34.7 ± 7.2	37.7 i
38	SonQarak-98	0.78 ± 0.11	1.66 ± 0.39	1.22 c-l	48.0 ± 13	37.4 ± 6.8	42.7 d-i
39	Eminbey	0.76 ± 0.20	1.59 ± 0.31	1.17 d-l	65.8 ± 15	48.0 ± 1.0	56.9 b-e
40	Viya-2005	1.47 ± 0.34	2.65 ± 0.24	2.06 ab	39.0 ± 3.9	37.7 ± 2.5	38.4 hi
41	Kunduru	0.86 ± 0.12	2.52 ± 0.12	1.69 b-h	56.2 ± 5.3	35.7 ± 3.9	46.0 c-i
42	Pg"S	0.71 ± 0.07	1.77 ± 0.29	1.24 c-l	56.6 ± 2.8	43.3 ± 6.1	50.0 c-i
43	Azar-2 †	1.26 ± 0.45	1.93 ± 0.21	1.59 b-k	59.1 ± 11	43.8 ± 5.0	51.5 c-i
44	Homa †	1.25 ± 0.37	1.55 ± 0.16	1.40 b-l	56.3 ± 12	51.5 ± 4.7	53.9 b-g
45	Pishgam †	0.68 ± 0.07	0.90 ± 0.10	0.79 l	76.6 ± 13	71.5 ± 7.1	74.1 a
46	Ohadi †	0.88 ± 0.11	1.25 ± 0.07	1.07 f-l	59.2 ± 8.1	52.9 ± 0.7	56.1 b-f
47	Sardari †	0.90 ± 0.16	1.36 ± 0.05	1.13 e-l	64.9 ± 12	53.4 ± 0.0	59.2 bc
48	Gascogen †	1.49 ± 0.02	2.52 ± 0.50	2.00 ab	50.3 ± 7.1	44.5 ± 4.2	47.5 c-i
49	Rasad †	0.78 ± 0.18	1.31 ± 0.13	1.05 f-l	63.2 ± 8.2	56.2 ± 1.8	59.8 bc
50	Zarin †	0.57 ± 0.15	1.06 ± 0.18	0.80 l	62.1 ± 11	56.0 ± 7.1	59.1 bc
	Mean	1.07 b	1.77 a		56.4 a	41.3 b	

Means followed by the same letters in each column and each factor are not significantly different at 5 % level, according to Duncan's Multiple Range Test. Mean ± SE (n = 3). † Bread wheat.

3.3 Fe concentration in the shoot

Shoot Fe concentration was influenced by genotype and Zn fertilization, and significant genetic differences were evident at both deficient and adequate Zn supply ($P < 0.001$) (Tables 3, 4). The amount of Fe in the shoots varied among genotypes and ranged from about 133 to 234 mg Fe kg^{-1} DM. Results showed that the shoot Fe concentration ranged from 132 ± 22 mg Fe kg^{-1} DM in 'SonQarak-98' to 270 ± 28 mg Fe kg^{-1} DM in 'Kunduru' at deficient Zn supply, and 110 ± 9 mg Fe kg^{-1} DM in '61-130' to 224 ± 14 mg Fe kg^{-1} DM in 'Gerdish' at adequate Zn supply (Table 4).

3.4 Zn utilization efficiency

Zn fertilization significantly affected ($P < 0.001$) Zn utilization efficiency, with significant variations ($P < 0.001$) among genotypes (Table 3). Zn utilization efficiency (shoot dry matter produced per unit of Zn) also varied among the genotypes and was affected by Zn fertilization. Unlike to shoot Zn concentration and content, Zn utilization efficiency decreased in all wheat genotypes by Zn fertilization ('Ammar-9' and 'Viya-2005', the highest and lowest decrease, respectively). Under Zn deficiency, Zn utilization efficiency varied from 39.0 ± 3.9 to 87.2 ± 11 in 'Viya-2005' and 'Ammar-9', respectively. At Zn application, it varied from 31.2 ± 2.5 to 71.5 ± 7.1 in 'Selcuklu' and 'Pishgam', respectively (Table 5).

3.5 Genetic variation revealed by Zn efficiency and shoot dry matter

The result of cluster analysis for studied genotypes is presented in Figure 3. In the present study, cluster analysis separated 50 wheat genotypes into four main groups (Figure 3). Twenty-five wheat genotypes were placed in the first group (G-I), which these genotypes included 'Altintoprak-98', 'Cheheldaneh', 'Mirzabey-2000', 'Imren', 'Berkmen-469', 'Tunca-79', 'G-1252', 'Kunduru-414-44', 'Durbel', 'Gokgol-79', 'Ammar-9', 'Pinor-2001', 'Gerdish', 'Sarayolla', 'Chesit-1252', 'Geromtel-1', 'Fatasel-185', 'Altin-40-98', 'Turabi', 'Cakmak-79', 'Tyten-2002', 'Zardak', 'Kiziltan-91', 'Pishgam' and 'Ohadi'. These wheat genotypes had high Zn efficiency, and shoot dry matter values, thus they were considered the most desirable genotypes for both growth conditions. The second group (G-II) consists of twelve durum wheat genotypes and three bread wheat genotypes ('Ter-1//Mrf1/Stj2', 'Haran-95', '61-130', 'Kunduru-1149', 'Bcr/Gro1//Mgnl1', 'Selcuklu-97', 'Yelken-2000', 'GAP', 'Saji', 'SonQarak-98', 'Eminbey', 'Pg"S', 'Sardari', 'Rasad' and 'Zarin'). In this group, all genotypes had low Zn efficiency, thus they were susceptible to Zn deficiency and only suitable for non-Zn deficiency (adequate Zn) conditions. Six durum wheat genotypes as well as three bread wheat genotypes ('Meram-2002', 'Haurani', 'Za-14-105', 'Kumbet-2000', 'Viya-2005', 'Kunduru', 'Azar-2', 'Homa' and 'Gascogen') were clustered in the third group (G-III). Finally, the fourth group (G-IV) consists of one ('Ankara-98') genotype and this genotype have high shoot dry matter in both deficient and adequate Zn conditions (Figure 3).

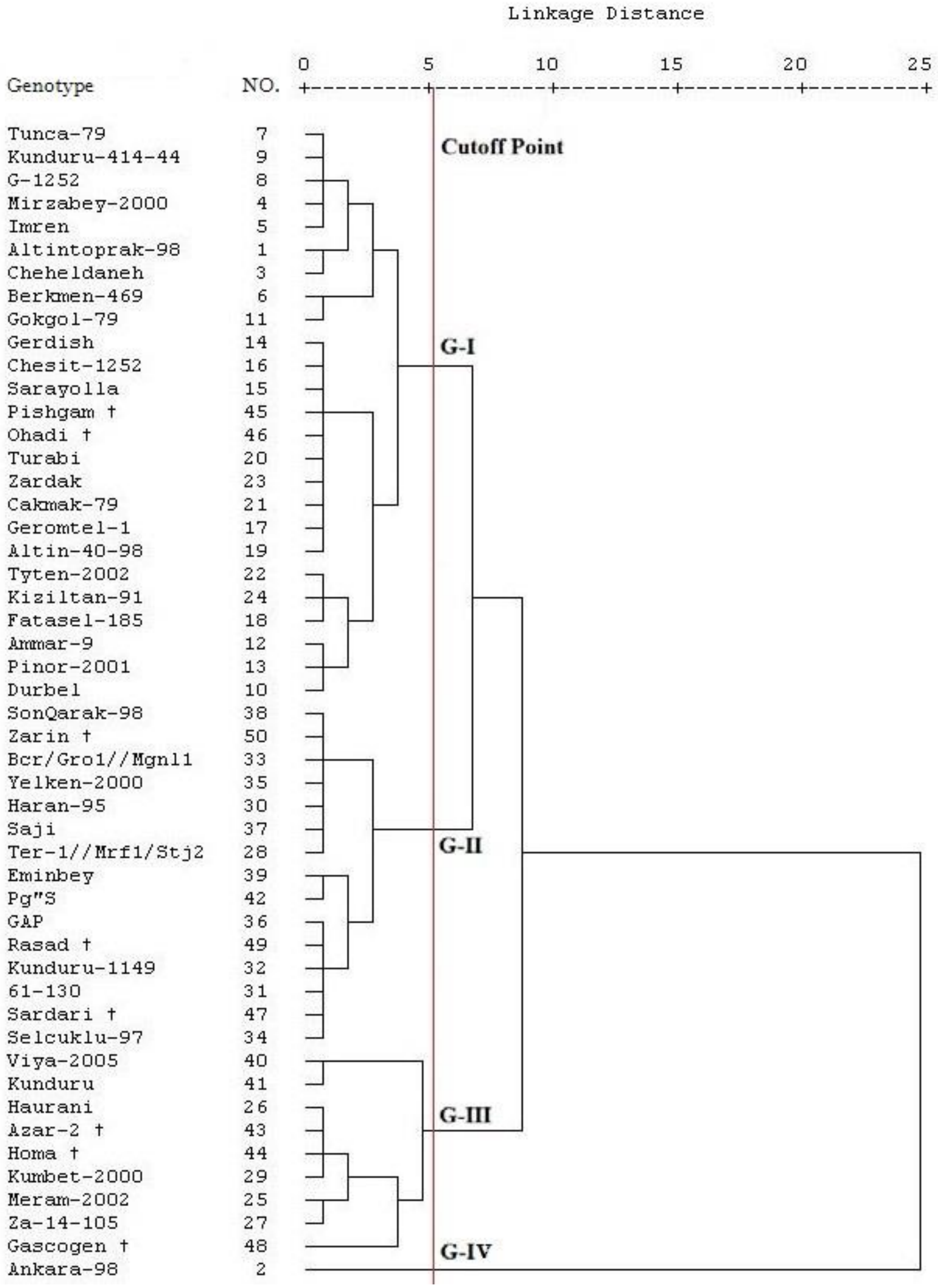

Figure 3: Dendrogram of 50 durum and bread wheat genotypes resulted from UPGMA cluster analysis based on mean Zn efficiency (%), and shoot dry matter (mg plant^{-1}) at deficient and adequate Zn supply. † Bread wheat.

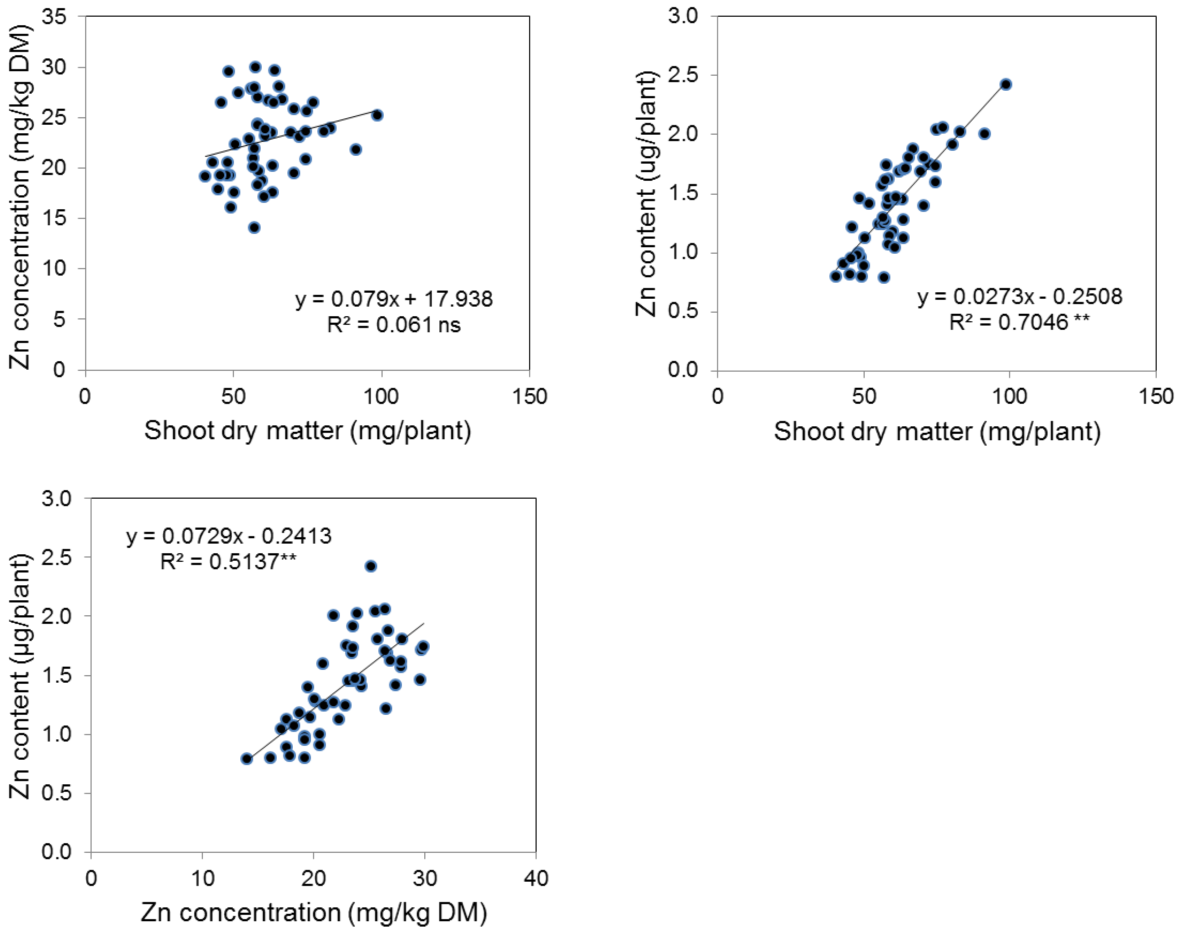

Figure 4: Relationship between shoot dry matter with shoot Zn concentration and content, also shoot Zn content with shoot Zn concentration in eight bread wheat and forty two durum wheat genotypes grown for 45 DAS. ns, * and **: Non-significant and significant at the 5 % and 1 % levels of probability, respectively

4 DISCUSSION

Wheat genotypes exhibited a variation in their performance, which has been exploited in this study, and there was great difference in Zn efficiency between durum and bread wheat genotypes (Figures 1A, B). At the current experiment, we did not measure the Zn content and concentration at seeds, however, since, the seeds were harvested from the homogenous plants not treated with chemical fertilizers, so, the differences observed in Zn efficiency seemingly is due to genetic make-up dissimilarities. McDonald et al. (2008) reported the same differences on the Zn content and concentration at 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 concentration of seed, the related difference observed was main part due to the genetical differences as well. Most of durum wheats (26 genotypes) had higher Zn efficiency than Zn efficient bread wheats and there were no durum wheats with lower Zn efficiency than Zn-inefficient bread wheat except ‘Eminbey’, ‘Viya-2005’, ‘Kunduru’ and ‘Pg"S’ (Figure 1B). Cakmak et al. (1999) presented that durum wheat had the least Zn-efficiency among cereals, and this was partly attributed to the lack of D genome. However, Cakmak et al. (1999) reported in *Aegilops tauschii* Coss. (DD) demonstrated genetic variation in Zn efficiency within this species as well. In the present study, the existence of Zn-inefficient bread wheat genotype (‘Zarin’) despite the presence of the D genome, and equivalent or greater Zn efficiency in some durum wheats compared to bread wheat show that the D genome might not necessarily be the source of Zn efficiency.

The higher Zn efficiency of durum and bread wheat genotypes can also use to produce new cultivars of wheat through plant breeding program. However, this targeted breeding approach requires screening of a large number of genotypes or cultivars of both species for identification of Zn efficiency sources. In such screening studies, it is important to remember that donors should be selected based on their performance under contrasting Zn availability. It is obvious that high yielding genotypes below Zn deficiency and responsive to Zn fertilizer (‘Gascogen’ and ‘Meram-2002’ bread and durum wheat genotypes, respectively) are extremely desirable for cropping on Zn-deficient soils (Figure 1A), whereas those with high Zn efficiency simply due to low yield potential under Zn sufficiency are not (‘Kunduru-414-44’ and ‘Tunca-79’). Moreover, genotypes with high yield under Zn deficiency, and also responsive to Zn fertilizers can be identified simultaneously by two level testing where the second level aims to identify Zn-efficient and responsive genotypes (Figures 1A, B). Therefore, identification and cultivation of Zn-efficient genotypes that could use Zn efficiently is a realistic alternative to Zn fertilizer application in some edaphic environments (Hacisalihoglu et al., 2004; Gomez-Coronado et al., 2016).

Our results revealed significant variation among durum and bread wheat genotypes for dry matter and other measured traits (Figure 1; Tables 5, 4). One of the helpful test in breeding programs is seedling test, it could be possible to screened and predict yield response in short time. According to some previous work, there were significant correlations between seedling responses and yield in bread wheat (Kalayci et al., 1999). Genc et al. (2000) reported that Zn efficiency at the seedling stage were higher than maturity or vice versa in some genotypes. On the other hand, it seems that some efficient genotypes are identified and enter the crossing program or the next generation (Graham, 1984). In previous studies Rengel (1999), Gao et al. (2005), Genc et al. (2006) and Genc and McDonald (2008) evaluated differences in Zn efficiency in Zn-efficient and Zn-inefficient wheat by a number of Zn efficiency mechanisms such as Zn uptake by the roots, translocation to the shoots and physiological efficiency (utilization). In this research we did not study on Zn uptakes and transportation in roots and shoot. Thus, the evaluation of relative importance of these individual components was impossible. However, Zn uptake was the main factor in determination of Zn efficiency in barley and bread wheat, respectively (Gao et al., 2005; Sadeghzadeh et al., 2009). But, Hacisalihoglu et al. (2001) showed that there is no correlation between Zn efficiency and Zn compartmentation or xylem translocation in wheat. Furthermore, it was reported that superoxide dismutase (SOD) and carbonic anhydrase (CA) were two importance enzymes to improve Zn efficiency (Hacisalihoglu et al., 2001), therefore it seems that Zn-efficient genotype with more efficient biochemical utilization of cytoplasmic Zn could be response to Zn deficiency, and this may be an important contributor in wheat phenotypic characteristics.

Soil Zn application at 5 mg kg^{-1} significantly decreased Fe concentration in the shoots of wheat genotypes (Table 4). Decrease in Fe concentration in plant was observed and this may be attributed to its increased uptake with the application of Zn showing synergistic effect with Zn. Our findings are contradictory to Rathore et al. (1974), who showed that increasing either element (Zn, Fe and/or Mn) decreased the toxic effect of others and implied a mutual antagonistic effect on Zn uptake. 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.

Cluster analysis based on Zn efficiency, and shoot dry matter at deficient and adequate Zn conditions classified the genotypes into four clusters (Figure 3). Cluster analysis has been generally used for description of variation between genotypes and grouping based on Zn efficiency, shoot dry matter and stress tolerance indices (Genc and McDonald, 2004).

5 CONCLUSIONS

The present study showed the existence of genotypic variation for tolerance to Zn deficiency among bread and durum wheat genotypes, which offers potential for the improvement of Zn efficiency in wheat breeding programs. In addition, Zn fertilization improved shoot dry matter and shoot Zn content and concentration of bread wheat genotypes compared to durum wheat genotypes under calcareous soil. Screening Zn tolerant genotypes using cluster analysis discriminated ‘Ankara-98’ and ‘Altintoprak-98’ genotypes as the most Zn-efficient and ‘Pg"S’ genotype among durum wheat and ‘Zarin’ genotype among bread wheat as the most Zn-inefficient. Moreover, it is necessary to test of more cultivars or genotypes of both wheat species in future to reveal greater Zn efficiency values than those recognized here. Also, seedling responses measured in the present study need to be affirmed at maturity in future studies.

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