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Evaluation Morphological Characteristics and Mineral Nutrients Content of Bread Wheat (*Triticum aestivum* L.) Affected Soil Application of Nitrogen and Iron Fertilizers

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ABSTRACT

This research was conducted to evaluate the effect of soil application of nitrogen (N) and iron (Fe) fertilizers on morphological traits and shoot mineral contents of bread wheat via factorial arrangement based on randomized complete block design with four replications. The factors included three level of nitrogen (100, 200 and 400 mg N kg^{-1} dry soil applied) as $Ca(NO_3)_2$ and three rate of Iron (No iron, Fe EDTA and $FeSO_4$, applied at the rate of 10 mg Fe kg^{-1} soil). The results of the present study show that shoot fresh matter production and plant height was significantly enhanced by increasing N supply at each Fe treatment. So that, application of 400 mg N kg^{-1} soil has the greatest effect on shoot fresh matter production and plant height. Compared with the control treatment, which did not receive extra N fertilizer, N fertilization at 200 and 400 mg kg^{-1} were decreased shoot Fe, copper (Cu) and manganese (Mn) concentration as well as shoot zinc (Zn) and Cu content in wheat. The mean shoot Fe concentrations were 246.9, 247.3 and 314.9 mg kg^{-1} in control, Fe EDTA and $FeSO_4$ treatments, respectively. Also, the effect of $FeSO_4$ on shoot zinc (Zn), Fe and Mn concentrations and also shoot Zn and Cu contents were higher than that of Fe EDTA. In generally, the higher shoot concentration and content of mineral nutrients were observed on the $FeSO_4$ treatment in 100 mg N kg^{-1} soil and the lower these traits were observed on the treatment of no Fe at all of soil N supply. Application of Fe fertilizer, in combination with optimum N fertilization or other agronomic practices that can increase mineral nutrients in shoot and seed of wheat (especially Zn and Fe), is required to produce improved seed quality for human health.

Key words: *Copper, Manganese, Plant height, Shoot dry weight.*

ABBREVIATION

Shoot fresh weight (SFW), Shoot dry weight (SDW), Plant height (PH), Nitrogen (N), Zinc (Zn), Iron (Fe), Copper (Cu), Manganese (Mn), Sulfur (S), Phosphorus (P), Phytosiderophore (PS), Yellow stripe 1 (YS1).

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INTRODUCTION

Wheat is the most widely grown crop globally and a major source of the carbohydrates and proteins in human nutrition. Micro nutrient malnutrition, and particularly deficiencies of zinc (Zn) and iron (Fe), is a serious health problem worldwide, affecting more than 40% of the world's population (Welch and Graham, 2004; Maret and Sandstead, 2006; Cakmak, 2008; OMS-WHO, 2009; Joshi *et al*., 2010). In many areas of the world, Fe deficiency can occur in plants grown on alkaline soils that are rich in Ca and Mg and in soils with low organic matter content (Rashid and Ryan, 2004; De Santiago and Delgado, 2006). Also, the pH of soil directly affects the uptake of iron by plants. If the pH exceeds 6.5, iron is converted to an insoluble form that cannot be absorbed by plants (Franzen and Richardson, 2000). Deficiencies of Zn and Fe cause various serious health complications such as the stunting, increased susceptibility to infectious diseases, impaired brain function and the mental development, poor birth outcomes and anemia (Kennedy *et al*., 2003; Hotz and Brown, 2004; Fraga, 2005). Also, iron deficiency can result in significant yield losses when wheat cultivars are grown on calcareous soils (Berg *et al*., 1993). Increasing Zn and Fe concentrations of staple food crops such as wheat is therefore an important global challenge (Kutman *et al*., 2011). There are various inorganic and the chelated forms of Fe fertilizers that are used in the soils with zinc deficiency such as $FeSO_4$, Fe EDTA, Fe DTPA, Fe EDDHA, Fe-citrate and Fe IDHA (imino disuccinic acid) (Fernandez *et al*., 2006; Rodriguez-Lucena *et al*., 2010). In this case, Goos and Johnson (2000) reported that foliar sprays of Fe EDTA significantly re-duced iron-deficiency chlorosis, while increased seed yield in soybean. But, Erdal *et al*. (2004) stated that Fe fertilizer to form of $FeSO_4$, which is cheaper than other Fe sources, can be used in soil and foliar fertilization. Whereas, Ghasemi-Fasaei and Ronaghi (2008) stated that due to the nutritional disorder and imbalance, it appears that neither soil application of Fe-EDDHA nor foliar application of Fe-sulfate is appropriate in correcting Fe deficiency in wheat grown on calcareous soils. Hence, growing Fe efficient wheat cultivars should be considered as an appropriate practice for Fe chlorosis prone calcareous soils of the world. On the other hand, nitrogen (N) nutrition of plants appears to be critical component for an effective bio fortification of food crops with Zn and Fe due to the several physiological and the molecular mechanisms which are under influence of N nutritional status (Cakmak *et al*., 2010). High N can increase the seed Zn and Fe concentrations by enhancing the seed protein concentration and thereby the sink strength of the seed for Zn and Fe (Morgounov *et al*., 2007; Zhao *et al*., 2009). Also, Kutman *et al*. (2011) in the study of the effect of supply of nitrogen (N) by amount of 50 mg N (for low N plants) and 250 mg N (for high N plants) per kg dry soil in the form of $Ca(NO_3)_2.4H_2O$ on durum wheat, stated that the Zn and Fe uptake per plant was enhanced up to 4-fold by high N supply. It appears likely that the mechanisms contributing to root uptake and root to shoot transport of Fe and Zn are positively affected by improving plant N status (Aciksoz *et al*., 2011). In contrast, Gao and Grant (2011) reported that the four N sources did not differ in their effect on seed yield and seed Zn, indicating that at the rate of N applied there were no differential fertilizer source effects on Zn availability. In addition, based on the results of our previous researches soil application of Fe-chelate had no significant effect on dry matter yield of some soybean genotypes (Ghasemi-Fasaei *et al*., 2003) and chickpea (Ghasemi-Fasaei *et al*., 2005) grown on calcareous soil with Fe deficient. This study was conducted under pot conditions to investigate the role of soil applied Fe fertilizers to improve shoot Fe and other mineral elements concentrations of wheat crop grown under increasing soil N supply in a calcareous soil.

MATERIALS AND METHODS

Field and Treatments Information

This research was conducted to evaluate the effect of soil application of nitrogen (N) and iron (Fe) fertilizers on morphological traits and shoot mineral contents of bread Wheat via factorial arrangement based on the randomized complete block design with four replications. The factors included three level of nitrogen (100, 200 and 400 mg N kg^{-1} dry soil applied) as $Ca(NO_3)_2$ and three rate of Iron (no iron, Fe EDTA and $FeSO_4$, ap-plied at the rate of 10 mg Fe kg^{-1} soil). Ten seeds of bread wheat (*Triticum aestivum* L. cv. Kohdasht) were sown in each plastic pot containing 3.5 kg soil from a Zn deficient region in Iran. Plants were grown under non-greenhouse conditions at the Maragheh University (37° 22′ N; 46° 16′ E).

Farm Management

The soil used in the experiment had a clay-loam texture and low organic matter (0.4%), abundant $CaCO_3$ (20%) and high pH (7.82 in H_2O). The di ethylene tri amine penta acetic acid (DTPA) extractable Zn and Fe concentrations were 0.6 and 3.1 $mg.kg^{-1}$ soil, respectively, measured by using the method described by Lindsay and Norvell (1978). Before potting, soil in all experiments was supplied with the following nutrients: 100 mg P kg^{-1} soil as KH_2PO_4, 25 mg S kg^{-1} soil as K_2SO_4 and 2 mg Zn kg^{-1} soil as $ZnSO_4.7H_2O$. Soil application of 100 and 200 mg N kg^{-1} was done before potting. 400 mg N kg^{-1} supply was made up of two portions: first half was incorporated into soil be-fore potting and the second half during the stem elongation stage, after dissolving in deionized water.

Measured Traits

Plants were harvested at the early flowering stage (e.g., Zadoks stage 5.5), when they were 45 days old. At harvest, whole shoots (all above-ground plant parts) were harvested, washed with de ionized water and dried at 70°C for determination of shoot dry weight. Then, shoot samples were finely ground in a mill, ashed at 550°C for 6 h, and dissolved in 2 M hydrochloric acid (HCl) (Chapman and Pratt, 1961). Concentrations of zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) in the digest solutions were determined by Atomic Absorption Spectrophotometer (AAS-6300 Shimadzu) and the ex-pressed based on plant dry weights ($mg.kg^{-1}$ dry weight). Content of Zn, Fe, Cu and Mn in the shoot ($\mu g.plant^{-1}$) were measured by multiplying amount of seedling dry matter by amount of Zn, Fe, Cu and Mn concentration in the shoot (Genc *et al*., 2000).

Statistical Analysis

All data were analyzed with using SAS software version 8.0 (SAS Institute Inc., Cary, NC, USA) and MSTAT-C software version 2.10 for DOS (MSTATC, 1989). Mean comparison was conducted using Duncan's multiple range test (DMRT) at $P < 0.05$ (Duncan, 1955). All figures were drawn with using M.S Excel software version 10.0.

RESULT

Analysis of variance was carried out in order to determine the significance of the effects of soil N supply and different soil Fe treatments as well as their double interactions on 11 selected traits of bread wheat plants grown under pot conditions (Table 1). According to the results of the ANOVA, the double interaction had significant effects on all reported traits except the shoot dry weight, plant height, shoot Fe concentrations and shoot contents of Fe and Mn (Table 1). Moreover, the main effects of Fe supply on all reported traits were significant except shoot fresh weight (SFW), shoot dry weight (SDW), plant height (PH) and shoot Fe content (Table 1).

Table 1. Result of analysis of variance of Shoot fresh weight (SFW), shoot dry weight (SDW), plant height (PH), and shoot concentrations and contents of Zn, Fe, Cu and Mn traits affected of soil N supply and different soil Fe treatments

S.O.V	df	SFW	SDW	PH
Replication	3	0.006 ns	0.0008 ns	46.8 ns
Iron (Fe)	2	0.157 ns	0.022 ns	37.6 ns
Nitrogen (N)	2	0.416 **	0.031 ns	74.9 *
Fe × N	4	0.247 **	0.019 ns	13.6 ns
Error	24	0.068	0.015	18.7
CV (%)	-	16.6	21.6	13.3

S.O.V	df	Shoot concentration			
		Zn	**Fe**	**Cu**	**Mn**
Replication	3	37.3 ns	308.8ns	0.466ns	2.63ns
Iron (Fe)	2	2753.4 **	18405.8 **	31.7 **	224.8 **
Nitrogen (N)	2	217.4 ns	4891.2 **	7.71 **	14.3 **
Fe × N	4	518.3 *	1401.7 ns	4.98 **	25.4 **
Error	24	134.9	636.1	0.518	2.35
CV (%)	-	27.0	9.35	18.7	4.96

S.O.V	df	Shoot content			
		Zn	**Fe**	**Cu**	**Mn**
Replication	3	8.73 ns	224.4 ns	0.447 ns	0.620 ns
Iron (Fe)	2	667.7 **	1138.5 ns	6.91 **	16.8 ns
Nitrogen (N)	2	110.4 **	3916.5 ns	4.20 **	47.4 ns
Fe × N	4	179.9 **	2810.4 ns	2.66 **	23.6 ns
Error	24	35.8	1702.1	0.483	16.3
CV (%)	-	24.1	27.0	32.2	23.2

ns, * and ** are non-significant and significant at 5 and 1% probability levels, respectively.

Also, soil nitrogen supply had also significant main effects on all traits except SDW, shoot Zn concentration and shoot contents of Fe and Mn (Table 1). Application of Fe had no significant effect on shoot dry weight and neither did soil application of N (Table 2). Results showed that shoot fresh matter production and plant height was significantly enhanced by increasing N supply at each Fe treatment (Table 2). Application of 400 mg N kg^{-1} soil has the greatest effect on shoot fresh matter production and plant height (Table 2).

Table 2. Mean comparison effect of soil N supply and different soil Fe treatments on shoot fresh weight (SFW), shoot dry weight (SDW), plant height (PH), and shoot concentrations and contents of Zn, Fe, Cu and Mn.

Soil treatments ($mg.kg^{-1}$) Fe	N	SFW ($mg.plant^{-1}$)	SDW ($mg.plant^{-1}$)	PH (cm)	
No Fe		1.66 *a	0.577 a	32.8 a	
Fe EDTA		1.61 a	0.601 a	34.2 a	
$FeSO_4$		1.44 a	0.518 a	30.7 a	
	100	1.47 b	0.589 a	30.2 b	
	200	1.46 b	0.507 a	32.5 ab	
	400	1.78 a	0.601 a	35.2 a	
Soil treatments ($mg.kg^{-1}$)		**Shoot concentration ($mg.kg^{-1}$)**			
Fe	**N**	**Zn**	**Fe**	**Cu**	**Mn**
No Fe		8.2 b	246.9 b	2.60 c	28.0 b
Fe EDTA		15.6 b	247.3 b	3.22 b	28.7 b
$FeSO_4$		37.3 a	314.9 a	5.68 a	35.8 a
	100	24.9 a	290.6 a	4.74 a	32.1 a
	200	16.5 a	268.3 b	3.20 b	30.0 b
	400	19.7 a	250.2 b	3.56 b	30.5 b
Soil treatments ($mg.kg^{-1}$)		**Shoot content ($\mu g.kg^{-1}$)**			
Fe	**N**	**Zn**	**Fe**	**Cu**	**Mn**
No Fe		4.7 b	144.7 a	1.52 b	16.3 a
Fe EDTA		9.16 b	149.5 a	1.95 b	17.2 a
$FeSO_4$		19.3 a	163.5 a	2.99 a	18.6 a
	100	13.9 a	171.8 a	2.76 a	18.9 a
	200	7.8 b	135.9 a	1.58 b	15.1 b
	400	11.4 ab	150.0 a	2.14 b	18.1 ab

*Similar letters in each column show non-significant difference at 5% level in Duncan's multiple rang test.

Averaged over all Fe treatments, increasing the N supply from 100 to the 400 mg N kg^{-1} soil decreased shoot concentrations of Fe and Cu by about the 13.9% and 24.9%, respectively, whereas the decrease in shoot Mn concentration about 5.0% (Table 2). Increases in soil N application had the distinctly negative effect on shoot contents of Zn and Cu (Table 2). As an average of all Fe treatments, increasing N supply from low to high decreased shoot Zn concentration from 13.9 to the 11.4 $\mu g.kg^{-1}$. Shoot Cu concentrations were also similarly downgraded by N application (Table 2). When compared to the control plants (no Fe treatment), soil treatment with Fe EDTA and $FeSO_4$ had increasing effect on the concentrations of Cu in shoot (Table 2). The results showed that shoot Cu concentration increased from 2.60 $mg.kg^{-1}$ (control) to 3.22 and 5.68 $mg.kg^{-1}$ with Fe EDTA and $FeSO_4$, respectively (Table

2). In other words, soil application of Fe EDTA and $FeSO_4$ increased shoot Cu concentration by 23.8% and 118.5%, respectively. Our results indicated that when compared to the control plants (no Fe treatment), soil treatment with Fe EDTA and $FeSO_4$ had increasing effect on concentrations and contents of Zn, Fe and Mn in shoot (Table 2). It was seen that the effect of $FeSO_4$ on shoot Zn, Fe and Mn concentrations and also shoot Zn and Cu contents were higher than that of Fe EDTA (Table 2). Fe sulfate ($FeSO_4$) increased shoot Zn, Fe and Mn concentrations by 354.9, 27.5 and 27.9% as well as shoot Zn and Cu contents by 310.6 and 96.7%, respectively. But, there were non significant difference between the control (no Fe application) and Fe EDTA (Table 2). Due to increases in shoot fresh matter yield by increasing the N supply (Fig. 1), the total uptake of Zn and Cu per shoot (e.g., shoot content) was substantially increased by N supply at each Fe treatment (Fig. 2A, B).

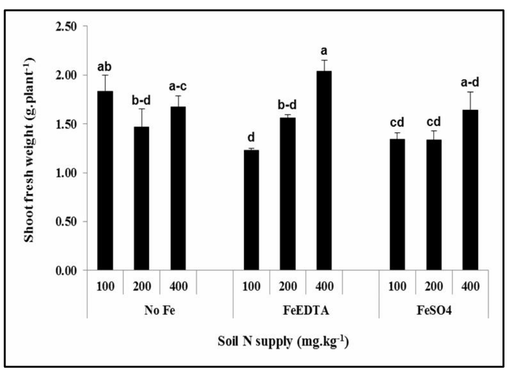

Fig 1. Effect of increasing the soil N supply on shoot fresh weight in bread wheat under different soil Fe treatments. Plants were grown in soils with low (100 mg N kg^{-1} soil), medium (200 mg N kg^{-1} soil) or high (400 mg N kg^{-1} soil) N supply for 45 days under pot conditions. Iron treatments were: no iron, Fe EDTA and $FeSO_4$, applied at the rate of 10 mg Fe kg^{-1} soil. Values are means of four independent replicates. Mean ± standard error (SE).

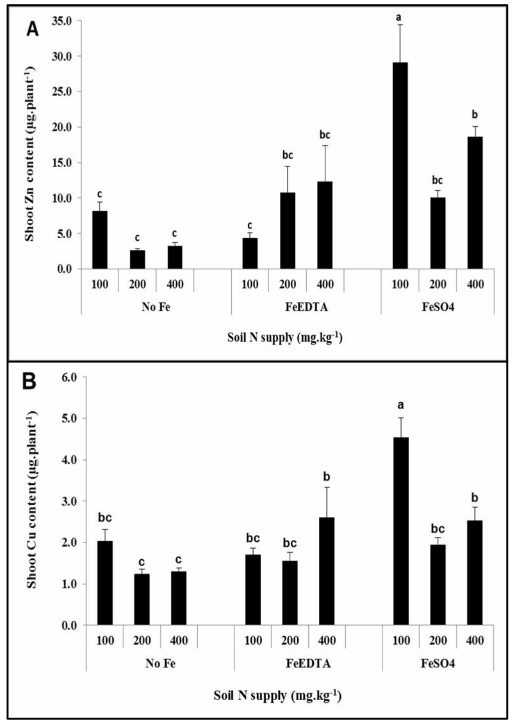

Fig. 2. Effect of increasing the soil N supply on shoot content (e.g., total accumulation) of Zn (A) and Fe (B) in bread wheat under different soil Fe treatments. Plants were grown in soils with low (100 mg N kg^{-1} soil), medium (200 mg N kg^{-1} soil) or high (400 mg N kg^{-1} soil) N supply for 45 days under pot conditions. Iron treatments were: no iron, Fe EDTA or $FeSO_4$, applied at the rate of 10 mg Fe kg^{-1} soil. Values are means of four independent replicates. Mean ± standard error (SE).

Higher shoot fresh weight was observed on the Fe EDTA treatment in 400 mg N kg^{-1} soil (2.04 $g.plant^{-1}$). But, lower shoot fresh weight was observed on the treatment of Fe EDTA at soil N supply by 400 mg N kg^{-1} soil (1.23 $g.plant^{-1}$) (Fig. 1). Higher shoot concentrations of Zn was observed on the $FeSO_4$ treatment in 100 mg N kg^{-1} soil with 53.2 $mg.kg^{-1}$ dry matter. But, lower shoot concentrations of Zn were observed on the treatment of no Fe at all of soil N supply with range 5.1 to 13.8 $mg.kg^{-1}$ dry matter (Table 3). Further more, higher shoot concentrations of Cu was observed on the $FeSO_4$ treatment in 100 mg N kg^{-1} soil with 8.0 $mg.kg^{-1}$ dry matter. But, lower shoot concentrations of Cu were observed on the treatment of no Fe at 200 and 400 mg N kg^{-1} soil with 2.39 and 2.31 $mg.kg^{-1}$ dry matter, respectively (Table 3). The highest and lowest shoot concentrations of Mn showed in the $FeSO_4$ treatment at 400 mg N kg^{-1} soil with 38.2 $mg.kg^{-1}$ dry matter and no Fe application at 200 and 400 mg N kg^{-1} soil with 26.1 to 26.3 $mg.kg^{-1}$ (Table 3). The higher shoot content of Zn was observed on the $FeSO_4$ treatment in 100 mg N kg^{-1} soil and the lower shoot content of Zn were observed on the treatment of no Fe at all of soil N supply and Fe EDTA treatment in 100 mg N kg^{-1} soil, respectively (Fig. 2A). Furthermore, higher shoot content of Cu was observed on the $FeSO_4$ treatment in 100 mg N kg^{-1} soil and lower shoot content of Cu were observed on the treatment of no Fe at 200 and 400 mg N kg^{-1} soil, respectively (Fig. 2B). In this experiment, shoot fresh weight showed positive correlation with SDW (R^2 = 0.81, $P < 0.01$) and PH (R^2 = 0.64, $P < 0.01$) (Table 4). Shoot Fe concentration showed positive correlation with shoot Zn (R^2 = 0.72, $P < 0.05$), Cu (R^2 = 0.78, $P < 0.05$) and Mn (R^2 = 0.85, $P < 0.01$) concentration and had positive correlation with shoot Zn (R^2 = 0.68, $P < 0.05$), Cu (R^2 = 0.65, $P < 0.05$) and Mn (R^2 = 0.67, $P < 0.01$) content of wheat (Table 4).

Table 3. Effect of increasing soil N supply on shoot concentrations of Zn, Cu and Mn in beard wheat under different soil Fe treatments.

Soil treatments ($mg.kg^{-1}$)		Shoot concentration ($mg.kg^{-1}$)		
Fe	**N**	**Zn**	**Cu**	**Mn**
No Fe	**100**	13.8 ± 3.8 ^{c}	3.11 ± 0.18 ^{de}	31.6 ± 0.5 ^{c}
	200	5.1 ± 0.2 ^{c}	2.39 ± 0.05 ^{e}	26.1 ± 0.3 ^{d}
	400	5.8 ± 0.6 ^{c}	2.31 ± 0.05 ^{e}	26.3 ± 0.3 ^{d}
Fe EDTA	**100**	7.8 ± 1.1 ^{c}	3.10 ± 0.21 ^{de}	29.9 ± 1.0 ^{c}
	200	22.0 ± 9.4 ^{bc}	2.96 ± 0.18 ^{de}	29.3 ± 0.4 ^{c}
	400	16.8 ± 6.8 ^{c}	3.61 ± 0.95 ^{cd}	27.0 ± 0.9 ^{d}
$FeSO_4$	**100**	53.2 ± 10.6 ^{a}	8.00 ± 0.25 ^{a}	34.7 ± 1.0 ^{b}
	200	22.3 ± 1.9 ^{bc}	4.27 ± 0.28 ^{bc}	34.6 ± 0.7 ^{b}
	400	36.5 ± 3.6 ^{ab}	4.76 ± 0.05 ^{b}	38.2 ± 1.2 ^{a}

Values are means of four independent replicates. Mean ± standard error (SE).

DISCUSSION

Mineral nutrients such as Zn and Fe availability are low in calcareous soils of Iran. The Fe chelate and Fe sulfate have been used as an effective source of Fe in correcting Fe deficiency in calcareous soils. In some cases, however, its application might cause nutritional disorder due to the antagonistic effect of Fe with other cationic micronutrients (in particular with Zn and Mn). The application of different forms of Fe had no effect on shoot fresh weight (SFW), shoot dry weight (SDW) and plant height (PH) (Table 2). Nonetheless, higher shoot fresh weight was observed on the Fe EDTA treatment in 400 mg N kg^{-1} soil and lower this trait was observed on the treatment of Fe EDTA at soil N supply by 400 mg N kg^{-1} soil (Fig. 1). Expressed that iron (Fe) is one of the essential elements for the plant and plays an important role in the many plant processes such as photosynthesis, respiration, nitrogen uptake and construction, and also in construction and development of chloroplasts in plants (Rashid and Ryan, 2004; Huber and Thompson, 2007; Ghafari and Razmjoo, 2015). According to the results of this research, Ghasemi-Fasaei and Ronaghi (2008) reported that neither soil application of Fe-EDDHA [chelate Fe ethylen diamine di (o hydroxy phenyl acetic acid)] nor foliar application of Fe sulfate had a significant effect on wheat dry matter yield. Also, Ryegrass grown in an alkaline soil did not respond to application of various Fe-chelates and $FeSO_4$ regarding growth and shoot concentrations of Fe (Ylivainio *et al*., 2004).

Table 4. Correlation coefficients between measured traits

Traits	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
(1)	1										
(2)	0.81**	1									
(3)	0.64*	0.50	1								
(4)	-0.17	-0.10	-0.38	1							
(5)	-0.35	-0.25	-0.68*	0.72*	1						
(6)	-0.28	-0.07	-0.41	0.94**	0.78*	1					
(7)	-0.28	-0.34	-0.67	0.76*	0.85**	0.68*	1				
(8)	-0.06	0.06	-0.28	0.99**	0.68*	0.95**	0.68*	1			
(9)	0.33	0.57	-0.25	0.54	0.65*	0.60	0.46	0.62	1		
(10)	-0.05	0.22	-0.24	0.89**	0.67**	0.96**	0.55	0.94**	0.73*	1	
(11)	0.42	0.54	-0.22	0.58	0.53	0.54	0.60	0.65	0.89**	0.66	1

1: Shoot fresh weight (SFW), **2**: Shoot dry weight (SDW), **3**: Plant height (PH), **4**: Zn concentration, **5**: Fe concentration, **6**: Cu concentration, **7**: Mn concentration, **8**: Zn content, **9**: Fe content, **10**: Cu content, **11**: Mn content.
ns, * and ** are non-significant and significant at 5 and 1% probability levels, respectively.

Roomizadeh and Karimian (1996) observed that application of Fe-EDDHA had no significant effect on dry matter yield of soybean or even decreased it. The results indicate that the effect of $FeSO_4$ on shoot Zn, Fe and Mn concentrations and also shoot Zn and Cu contents were higher than that of Fe EDTA (Table 3, Fig. 2A, B), but Erdal *et al*. (2004) reported that plants fertilized with $FeSO_4$ contained less Fe when compared to Fe EDTA at harvest. This may be due to the faster utilization and transfer of Fe from $FeSO_4$ to the fruit. The results also showed that the plant used more Fe from Fe EDTA at early stages, but with progressing stages, Fe usage from $FeSO_4$ increased. Erdal *et al*. (2004) stated that Fe fertilizer to form of $FeSO_4$, which is cheaper than other Fe sources, can be used in soil and foliar fertilization. Shoot Fe concentration showed positive correlation with shoot Zn, Cu and Mn concentration and content of wheat (Table 4). The increase in shoot Zn concentration with Fe application may be due to increasing plant growth and metabolism. These results are in agreement with the findings of Sanchez-Raya *et al*. (1974) who reported that the synergistic effect of high levels of Fe with Mn absorption in tomato plants. The higher amounts of Mn, Zn, and Cu in control wheat in present study are probably due to the higher amounts of phyto siderophore (PS) under Fe deficient condition, in comparison with Fe sufficient condition. Be-cause, concentration of PS was considerably higher than that of Fe which might be responsible for the increase of Mn, Zn, and Cu in the xylem sap of Fe-deficient plants (Alam *et al*., 2001; Ghasemi-Fasaei and Ronaghi, 2008). The increase in seed quality of wheat in our experiment by application of Fe fertilizers may be due to the role of Fe in enhancing accumulation of assimilate in the shoot (Zeidan *et al*., 2010; Ghafari and Razmjoo, 2015). However, contrary to the results of this research; it is stated that concentration of zinc (Zn), copper (Cu) and manganese (Mn) decreased under Fe sufficient compared with Fe deficient condition (Alam *et al*., 2001; Lidon, 2002). Also, Karaman *et al*. (1997) reported that the application of different Fe sources increased Fe concentration but decreased P, Zn, Cu and Mn concentrations of bean (*Phaseolus vulgaris* L.). Ghasemi-Fasaei and Ronaghi (2008) stated that Fe application increased Fe uptake but decreased that of Manganese (Mn), zinc (Zn), and copper (Cu). Nitrogen (N) is one of the most yield-limiting nutrients for crop production in the world (Huber and Thompson, 2007). The results of this experiment showed that soil application of 200 and 400 mg N kg^{-1} increased mean plant height by 7.6 and 16.6%, respectively (Table 2). In this case, Khan *et al*. (2009) revealed that application of urea (as one of the forms of nitrogen) significantly increased plant height, spike length, biological yield and N uptake by the crop. Similar results have been reported by Arif *et al*. (2006), Aciksoz *et al*. (2011) and Kutman *et al*. (2011) for wheat. Nitrogen plays a vital role in increasing the dry matter, yield and seed quality of a crop (Liben *et al*., 2005). The results of our study indicate that the application of 400 mg N kg^{-1} soil has the greatest effect on shoot fresh matter production of wheat. So that the use of 400 mg N kg^{-1} soil compared to control (100 mg N kg^{-1} soil) increased the shoot fresh weight by 21.1% (Table 2). The results showed that increasing the N supply from 100 to 400 mg N kg^{-1} soil decreased shoot concentrations of Fe, Cu and Mn (Table 2). Besides that, in the present study, the negative impact of high N supply on content of cationic micronutrients in the shoot tissue of wheat (Table 2). But, studies under both field and greenhouse conditions demonstrated that increasing soil N application significantly improved shoot and seed Fe concentrations (Kutman *et al*., 2010; Shi *et al*., 2010). According to the results of this research, probably, high N nutrition decreased activity and abundance of Fe transporter proteins such as yellow stripe 1 (YS1) in root cell membranes.

CONCLUSION

In conclusion, the results of this study showed that the two Fe sources (Fe EDTA and $FeSO_4$) did not differ in their effect on fresh and dry weight of shoot and shoot Mn con-tent, indicating that at the form of Fe applied there were no differential fertilizer source effects on Mn availability. Also, compared with the control treatment, which did not receive extra N fertilizer, N fertilization at 200 and 400 mg kg^{-1} were decreased shoot Fe, Cu and Mn concentration as well as shoot Zn and Cu content in wheat. Overall, application with Fe EDTA treatment in 400 mg N kg^{-1} soil increased shoot fresh weight in wheat plants grown on the Fe deficient calcareous. However, $FeSO_4$ treatment in 100 mg N kg^{-1} soil significantly increased the concentration and total amount of Cu, Mn, and Zn in the aerial parts of plant. Application of Fe fertilizer, in combination with optimum N fertilization or other agronomic practices that can increase mineral nutrients in shoot and seed of wheat (especially Zn and Fe), is required to produce improved seed quality for human health.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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