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Assessment Iron Application to Improve Agro-Morphological Traits and Bio-Fortification of Grain Wheat Grown in Different Nitrogen Conditions

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ABSTRACT

Bio-fortification of food crops is a cost-effective and sustainable agricultural strategy to decrease malnutrition arising from micronutrients deficiencies. Accordingly, the pot experiments have been conducted to investigate the effectiveness of various foliar-applied iron (Fe) fertilizers in increasing agro-morphological traits and grain micronutrients concentrations of bread wheat plants under different nitrogen (N) nutrition regimes via factorial experiment based on randomized complete block design with four replications at Maragheh University during 2014-15 growing seasons. The Fe fertilizers treatment included: non application Fe, $FeSO_4$, and Fe EDTA. The applied Fe foliar sprayed with the rate of 0.25% (w/v) at the stem elongation and early milk stages. The rates of soil-applied N consisted: low (100 mg N kg^{-1} soil), medium (200 mg N kg^{-1} soil) and high (400 mg N kg^{-1} soil) until full maturity under greenhouse conditions. Analysis of variance revealed that agro-morphological traits and grain micronutrient concentrations significantly differed among Fe treatments, and significant interactions existed between Fe and N treatment on harvest index and grain Zn, Fe, Cu and Mn concentrations. Application of various foliar Fe fertilizers had either a high positive effect on agro-morphological traits or remained effective on grain Fe concentration. Also, the effect of $FeSO_4$ on all characteristics studied was higher than that of FeEDTA. The results showed that the increasing soil application of N significantly enhanced grain Zn concentrations and decreased grain Cu concentrations of wheat. Generally, application of N and Fe fertilizers represents an important agronomic practice in increasing agronomic traits and grain micronutrients such as Fe and Zn. Therefore, the plant N status deserves special attention in bio-fortification of food crops with Fe.

Keywords*:* *Fe EDTA, Harvest index, Micro nutrient, Yield.*

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ABBREVIATION: Number of grain per spike (NGS), Number of fertile spikelet per spike (NFSS), Thousand grain weight (TGW), Grain yield (GY), Biological yield (BY), Harvest index (HI), Peduncle weight (PedW), Penultimate weight (PenW), Other inter node weight (OtherW), Spike length (SL), Peduncle length (PedL), Penultimate length (PenL), Other inter node length (OtherL), Nitrogen (N), Zinc (Zn), Iron (Fe), Copper (Cu), Manganese (Mn), Sulfur (S), Phosphorus (P), Field capacity (FC), Dry weight (DW).

INTRODUCTION

Foods derived from plants are major contributors to the micronutrients requirements for most people in the world, especially in developing countries and/or in regions with predominantly cereal-based diets. Nevertheless, staple food crops (such as cereals, especially maize, rice, and wheat) have not enough grain zinc and iron concentrations to meet human nutrition requirement. When plant crops are cultivated on micronutrient-deficient soils, they have low Zn and Fe content and also low consequently bioavailability (Cakmak *et al*., 2010a; Abdoli *et al*., 2014; Esfandiari and Abdoli, 2016; Esfandiari *et al*., 2016). So that, improvement and increasing Fe and Zn concentration in food crops is an important global challenge due to high incidence of micronutrients deficiency in human populations (White and Broadley, 2009; Bouis *et al*., 2011). Environmental factors (such as consumption of macro- and micro-fertilizers) and use of agronomic biofortification strategy can exert larger influences on grain yield (GY), agro-morphological traits and grain micronutrients concentration of food crops (Mishra *et al*., 2015; Esfandiari *et al*., 2016). Soil fertility is an important factor, which determines the growth of plant. Soil fertility is determined by the presence or absence of nutrients i.e. macro and micronutrients, which are required in minute quantities for plant growth (Zayed *et al*., 2011). Increasing price of fertilizers worldwide, necessary for production economy, ground water pollution, and soil structure degradation due to uncontrolled and unwise consumption of chemical fertilizers are the problems that have to be solved by proper methods. Spraying or foliar nutrition is a method for decreasing chemical fertilizers consumption and their environmental hazards, especially nowadays that toxin reduction policy and optimizing fertilizer consumption is under discussion. An important strategy to increase the concentration of trace elements in grains is fertilizing the plants with soil or spraying (Cakmack, 2002). In Iran calcareous soil conditions lead to imbalance use of chemical fertilizer (especially high use of phosphorus), lack of crop rotation, low use of manure fertilizer, unused of macronutrient in past, nowadays the lack of these elements in soil and human food is more evident and it makes the community suffer from deficiencies of these elements. The important combined effects of Fe and Zn lack is low blood Iranian (Persian anemia), which is also mentioned in foreign sources. However, the carried research in this field in Iran indicated the impact of micronutrients in increasing the yield quality and quantity in different crops (Ghazvineh and Yousefi, 2012). Calcareous soils, high soil and water pH, high concentration of HCO_3 (Bicarbonates), instability and misapplications of different fertilizers are the main reasons for Fe deficiency in soils and plants. Quantitative and qualitative yield of crops can be affected by the utilization of micronutrient fertilizer (Abadia *et al.,* 2011). Intensive and multiple cropping, cultivation of crop varieties with heavy nutrient requirements, and the unbalanced use of chemical fertilizers, especially nitrogen and phosphorus fertilizers, have caused a reduction in yields with symptoms of micronutrient deficiencies in crops. While balanced fertilization and micronutrient applications will increase yield and crop quality, it will also insure better peoples' health (Seilsepour, 2005). The purpose of the use of micronutrient elements in the process of crop production is to improve the quality and quantity of products and to enrich them in addition to increasing production. So far, much many studies have been done on the effect of each micronutrient on increasing the quality and quantity of wheat (Hung *et al.,* 2009). Nutritional disorders creating deficiency symptoms can be affected by other factors such as poor drainage, soil salinity and unbalanced fertilizer application. Availability of micronutrients such as Fe, Mn and Zn is much affected by pH and $CaCO_3$ content and soil texture usually micronutrient-deficiency problems are bound in calcareous soil of arid and semi-arid regions (Zeidan *et al*., 2010). Wheat grown under such deficient condition has low yield and micronutrients contents in grains (Amberger, 1991). Wheat grown under such deficient condition has low yield and micronutrients contents in grain. Each element of these micronutrients has its own function in plant growth for example. Potarzycki and Grzebisz (2009) reported that zinc exerts a great influence on basic plant life processes, such as (i) nitrogen metabolism-uptake of nitrogen and protein quality; (ii) photosynthesis-chlorophyll synthesis, carbon anhydrase activity; reported that Zn-deficient plants reduce the rate of protein synthesis and protein content drastically Mn is required for biological redo system, enzyme activation, oxygen carrier in nitrogen fixation (Romheld and Marachner, 1995). Micronutrient deficiency can greatly disturb plant yield and quality, and the health of domestic animals and humans (Welch, 2003). The role of microelements in maintaining balanced plant physiology is becoming clearer every day as a result of studies on their reactions and the disturbances caused by their deficiency. Micronutrients are essential elements for life (Malakouti, 2008). Micronutrients also play key roles in the release of carbon dioxide, and in optimizing the function of vitamin A and the immune system (Marschner, 1995). Aciksoz *et al*. (2011) reported it can be suggested that N fertilizer management and spraying Fe together with urea may represent important agronomic practices to contribute to increasing grain Fe (and Zn) concentrations in food crops. The plant N status deserves a special attention in efforts to bio-fortify food crops with Fe and Zn. Nitrogen is one of the most growth improving nutrients in plant production. In a recent study, Aciksoz *et al*. (2010) reported that at a given N supply, shoot and grain concentrations of Fe were not affected by increasing application of Fe fertilizers. But, it has recently been shown that N deficiency enhances Fe mobilization in old leaves and favors Fe re-translocation from source to sink in vegetative growing barley plants (Shi *et al*., 2012). Iron deficiency has developed in almost 30 percent of the soils under cultivation around the world. In addition, iron absorption decreases in the soils with law organic matters (Havlin *et al*., 2005). Numerous experiments have been conducted about the application of trace elements in the world, whose results have showed that their application not only increases quantitative and qualitative yield, but also increases their amounts in wheat grains (Malakouti and

Tehrani, 2005). Iron (Fe) deficiency is the most common cause of anemia worldwide. According to a recent report, WHO (2002) stated that anemia affects nearly two billion people, and pregnant women and pre-school children are under greatest risk of Fe deficiency anemia (Welch and Graham, 2004; McLeon *et al*., 2009). Iron (Fe) is one of the important micronutrients in plant productions, which deficiency of this element causes interveinal chlorosis on younger leaves and finally decreased the production and reduce grain quality of the plant crops (Kabir *et al*., 2016; Pal *et al*., 2016). Iron is an essential nutrient for plants as it is required in many functions including chlorophyll biosynthesis, respiration, and photosynthesis. Fe deficiency is the most common micronutrient deficiency in human populations affecting the health of people worldwide (Cakmak, *et al.,* 2010b). The role of essential microelements copper and zinc was proved in forming of more than 200 enzymes (Spiro, 1983). Among the micronutrients iron plays an important role in plant photosynthesis and respiration. Iron is considered as one of the micronutrients for plants. Although micronutrients are needed by plants in small amounts, they play an important role in plants growth and development such as their role in enzymes' activities, growth, cell differentiation, formation of flower, and crop quality improvement. The plants are found in many parts of the world who suffer from iron deficiency. There are some plants in many parts of the world that suffer from the shortage of iron (Cesco *et al*., 2010). Welch and Graham (2002) and Cakmak (2008) suggested that Fe deficiency in wheat grain can be alleviated by breeding and selection of cultivars that could absorb more Fe from the soil and accumulate it in the grain, whereas Yip (1997) proposed that Fe deficiency could be overcome by food fortification. Furthermore, it has been reported that N deficiency can significantly reduce grain Fe, Zn and other nutrients concentrations in wheat (Hou *et al*., 2011; Esfandiari *et al*., 2017). In addition, Beres *et al*. (2008) and Abedi *et al*. (2011) stated that N deficient decreased the GY due to its effect on the number of fertile spikelet per spike and number of grains per spike. While, N fertilization represents an important agronomic practice in increasing grain Fe (Aciksoz *et al*., 2011). There are various inorganic and chelated forms of Fe fertilizers that are used and tested for correction of Fe deficiency chlorosis in crop plants, such as $FeSO_4$, FeEDTA, FeDTPA, FeEDDHA, Fe-citrate and FeIDHA (iminodisuccinic acid) However, the effectiveness of those Fe compounds in overcoming Fe deficiency chlorosis is highly variable depending on their stability, penetration ability through the leaf cuticle and mobility/translocation following diffusion into leaf tissue (Rodriguez-Lucena *et al*., 2010a). Inclusion of urea in the spray solution of Fe compounds has been shown to stimulate penetration of Fe into the leaf tissue (Rodriguez- Lucena *et al*., 2010b). In addition, the effectiveness of inorganic and the chelated forms of Fe fertilizers ($FeSO_4$, Fe EDTA, Fe DTPA, Fe EDDHA, Fe-citrate) in overcoming Fe deficiency is highly variable depending on their solubility, stability, penetration ability through leaf cuticle, mobility and translocation following diffusion into the leaf tissues (Fernandez *et al*., 2009). Due to rapid conversion of Fe into unavailable forms when applied to calcareous soils and poor mobility of Fe in phloem, soil and/or foliar Fe fertilization appears to be less effective than Zn fertilization in enrichment of cereal grains (Cakmak, 2008).

For example in wheat, foliar application of Zn fertilizers improved grain Zn concentration by up to 2- or 3-fold depending on the plant availability of Zn in soils (Cakmak *et al*., 2010a), whereas increases in grain iron concentration by foliar spray of $FeSO_4$ or Fe chelates did not exceed 36% (Zhang *et al*., 2010). Increasing soil application of nitrogen significantly enhanced ir concentrations of shoot and grains (Aciksoz *et al*., 2010). There are similar raiders reported by other investigators that show an increase in the grain micronutrients concentrations (especially zinc and iron) due to increased nitrogen application (Shi *et al*., 2010; Cakmak *et al*., 2010b). In recent studies it has been shown that the plant nitrogen status is an important factor in enrichment of cereal grains with iron. Increasing molecular evidence is available showing that remobilization from vegetative tissue and translocation into seed of nitrogen and iron (as well as zinc) is maintained by the similar genetic mechanisms (Waters *et al*., 2009), resulting in a positive correlation between grain iron and N concentrations (Distelfeld *et al*., 2007). Studies under both field and the greenhouse conditions demonstrated that increasing soil nitrogen application significantly improved shoot and grain iron concentrations (Shi *et al*., 2010). Similarly, foliar spray of urea enhanced grain iron (Kutman *et al*., 2010). However, in the above greenhouse and field experiments, plants were grown under different nitrogen treatments, but no treatments with either soil or foliar applications of iron fertilizers were tested. The present study was conducted to determine the effect of soil nitrogen supply and foliar application of iron fertilizers on (i) agronomic and morphologic traits, (ii) grain micro nutrients concentrations and (iii) correlations of between measured characteristics.

MATERIALS AND METHODS

Plant Materials and Treatments Information

Current research was conducted to investigate the effectiveness of various foliar-applied iron (Fe) fertilizers in increasing agro-morphological traits and grain micronutrients concentrations of bread wheat plants under different nitrogen (N) nutrition regimes via factorial experiment based on randomized complete block design with four replications. 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. 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-ethylen etriamine pentaacetic 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). Plants were grown under non-greenhouse conditions at the University of Maragheh (37° 22′ N; 46° 16′ E, altitude of 1542 m) during 2014-15 growing seasons. 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 $ZnSO_4.7H_2O$. Different amounts of N and Fe fertilizers were incorporated in the soil, depending on the experimental treatments described below. In current experiment, an effect of foliar applied Fe EDTA and $FeSO_4$ (and non-use of Fe) was studied on grain micronutrients concentration in plants grown at different soil N applications. Soil N applications treatment included 100 (low), 200 (medium) and 400 (high) mg N per kg soil added as $Ca(NO_3)_2$. The N treatments with 100 and 200 mg N kg^{-1} were applied before potting.

In the case of 400 mg N kg^{-1} soil application, first 200 mg N kg^{-1} was incorporated into soil before potting, and the remaining amount was applied in two equal portions at the stem elongation and flowering stages. Foliar treatment of FeEDTA was conducted at the rate of 0.25% (w/v). In the case of $FeSO_4$ application, foliar solution contained the same amount of Fe that was present in the 0.25% (w/v) FeEDTA. Tween has been used as a surfactant at 200 $mg.l^{-1}$. Foliar spraying with Fe fertilizers was continued until run-off by using a hand-sprayer. Spraying was conducted twice: at the stem elongation and early milk stages. Irrigation of plant in the pots (90% of FC) and weeds were controlled from pots close to physiological maturity of plants.

Measured Traits

Measurement of yield attributes and agro-morphological traits

At maturity, five plants were randomly harvested from each pot to measure the number of grain per spike (NGS), number of fertile spikelet per spike (NFSS), thousand grain weight (TGW), grain yield (GY), biological yield (BY), harvest index (HI), peduncle weight (Ped W), penultimate weight (Pen W), other inter node weight (Other W), spike length (SL), peduncle length (Ped L), penultimate length (Pen L), and other internode length (Other L). Harvest index was measured by dividing grain yield to biomass production. Samples of grains were analyzed for the quality traits.

Estimation of grain micronutrient concentrations

Grain samples from each replicate were washed with distilled water and dried at 70°C for 48 h, milled to pass through a 0.5 mm sieve, and stored for analysis. Powdered grain samples were dry-ashed for 6 h at 550°C. Ash sample of 0.5 g was weighed in a silicon evaporating dish, and wet-acid digested with nitric acid on a hot plate at 75°C for 45 min and solubilized with 25 ml of 0.2 N HCl (Chapman and Pratt, 1961). Zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) concentrations were analyzed by the atomic absorption spectrophotometer (AA-6300, Shimadzu, Japan). The values were expressed on dry weight basis (mg kg^{-1} dry weight).

Statistical analysis

Data analyzed via SAS software ver. 8.0 (SAS Institute Inc., Cary, NC, USA), and mean comparisons was performed by Duncan's multiple range test (DMRT) at 5% probability level (Duncan 1955). The means ± standard error (SE) was used to compare the data.

RESULTS AND DISCUSSION

Grain yield and its components

Based on the results of the analysis of variance, significant differences were observed among foliar Fe treatments for number of grain per spike (NGS), number of fertile spikelet per spike (NFSS), thousand grain weight (TGW), grain yield (GY), and biological yield (BY). But, N application had no significant effect on these traits (Table 1). The interaction between foliar Fe treatments and soil N supply significantly affected the harvest index (Table 1). Foliar application of Fe fertilizers generally increased NGS, NFSS, TGW, GY, and BY compared with control. Among the foliar Fe treatments, the highest NGS and NFSS were obtained in the $FeSO_4$ treatment (17.8 grains and 7.77 fertile spikelet per spike, respectively) and the lowest these traits were obtained in the no Fe treatment (9.05 grains and 5.60 fertile spikelet per spike, respectively) (Table 2). Also, GY was increased by 16.9% and 52.4% for the supply of

FeEDTA and $FeSO_4$, respectively on foliar sprayed at the stem elongation and early milk stages (Table 2). In addition, the mean BY was 0.713, 0.832 and 1.045 g $plant^{-1}$ in control (no Fe application), FeEDTA and $FeSO_4$ treatments, respectively (Table 2). Grain yield in wheat is the result of number of grains per spike and grain weight. In this research, the effects of the application of iron (Fe) fertilizer on BY were through increased GY, peduncle and penultimate dry weight, and grains number; which led to an increment in BY. There are similar results reported by other investigators that show an increase in the BY due to application of Fe (Safyan *et al*., 2012; Rawashdeh and Florin, 2015; Kabir *et al*., 2016). Singh *et al*. (2018) reported the rate of N application at sowing caused an increase in grain and straw yield up to the N rate of 120 kg N ha^{-1} and a decrease at higher rate of N. The increase in grain yield was primarily determined by the increase in the number of grains pot^{-1} or number of grains $spike^{-1}$. The split application of 160 kg N ha^{-1} increased the grain and straw yield more than split application of 120 kg N ha^{-1}.

Table 1. Analysis of variance of the effect of increasing soil N supply and foliar application of Fe fertilizers on grain yield and its components in bread wheat

S.O.V	df	NGS	NFSS	TGW	GY	BY	HI
Replication	3	8.34 ^{ns}	0.439 ^{ns}	4.87 ^{ns}	0.014 **	0.023 ^{ns}	44.2 *
Iron (Fe)	2	258.8 **	18.1 **	222.8 **	0.081 **	0.338 **	13.6 ^{ns}
Nitrogen (N)	2	2.80 ^{ns}	0.093 ^{ns}	4.41 ^{ns}	0.005 ^{ns}	0.001 ^{ns}	63.8 *
Fe × N	4	3.47 ^{ns}	0.097 ^{ns}	3.54 ^{ns}	0.005 ^{ns}	0.009 ^{ns}	47.0 *
Error	24	2.86	0.182	3.34	0.003	0.016	14.7
CV (%)	-	13.6	6.72	5.84	14.2	14.9	8.75

^{ns},* and**: non-significant and significant at 5 and 1% probability level, respectively.

NGS: Number of grain per spike, **NFSS**: Number of fertile spikelet per spike, **TGW**: Thousand grain weight, **GY**: Grain yield, **BY**: Biological yield, **HI**: Harvest index.

Table 2. Effect of foliar application of Fe fertilizers on grain yield and its components, and agro-morphological traits in bread wheat. Foliar Fe treatments include: no iron, 0.25% (w/v) Fe EDTA and 0.25% (w/v) $FeSO_4$. Foliar $FeSO_4$ fertilizer contained the same amount of Fe that was present in the Fe EDTA solution.

Foliar Fe treatments (0.25% [w/v])	NGS	NFSS	TGW (g)	GY (g $plant^{-1}$)
No Fe	9.05 ^{*c}	5.60 ^{b}	33.9 ^{a}	0.307 ^{c}
FeEDTA	10.6 ^{b}	5.68 ^{b}	33.8 ^{a}	0.359 ^{b}
$FeSO_4$	17.8 ^{a}	7.77 ^{a}	26.4 ^{b}	0.468 ^{a}
Foliar Fe treatments (0.25% [w/v])	**BY (g $plant^{-1}$)**	**Ped W (mg)**	**Pen W (mg)**	**SL (cm)**
No Fe	0.713 ^{c}	67.2 ^{b}	47.0 ^{b}	4.63 ^{b}
FeEDTA	0.832 ^{b}	74.0 ^{ab}	56.2 ^{ab}	4.89 ^{b}
$FeSO_4$	1.045 ^{a}	85.3 ^{a}	61.3 ^{a}	6.06 ^{a}

*Means, in each column, followed by similar letter are not significantly different at the 5% probability level via Duncan multiple range test.

NGS: Number of grain per spike, **NFSS**: Number of fertile spikelet per spike, **TGW**: Thousand grain weight, **GY**: Grain yield, **BY**: Biological yield, **PedW**: Peduncle weight, **PenW**: Penultimate weight, **SL**: Spike length.

The growth media application of Fe and Zn interacted with N to increase protein, Zn and Fe concentration in wheat grain. The foliar sprayed Zn and Fe at booting stage of wheat significantly increased the whole grain protein, total uptake and concentration of Fe and Zn in grain. Armin *et al*. (2014) reported the application of Nano-Fe concentration with 4% dose at tillering + stem elongation was the best treatment for obtaining the maximum economic yield in wheat. Svecnjak *et al*. (2013) reported that the high-N fertilization produced higher grain yields as well as grain N and trace element concentrations in wheat. According to the research of Akbari Moghaddam (2011), Sabbr *et al*. (2011), Maadi *et al*. (2012) and Mosanaei *et al*. (2017), the effect of N fertilizer was significant on the GY and its components of wheat. In general, the effects of $FeSO_4$ on NGS, NFSS, GY, and BY were higher than that of FeEDTA, except TGW (Table 2). It has been suggested that improvement number of grains in wheat plants might be due to the positive effect of Fe fertilization on chlorophyll formation, photosynthesis process, and improvement of photosynthetic material production; because of Fe plays a significant role in various physiological and biochemical pathways in plants (Marsh *et al*., 1963; Rout and Sahoo, 2015). Seilsepour (2005) during his investigation of Fe application in Iran, indicated that foliar application of Fe and Zn increased seed yield, protein content, and Zn concentration in wheat. Increase of grain yield, grain weight, grains per spike, spike length due to Fe fertilization was reported by Hemantaranjan and Garg (1988). Haghyghatneya and Rajai (2005) reported that the foliar application of Fe had more of an effect on economic and biologic yields than any other application method. Thus, ultimately these factors causing reduction of infertile spikelet's and increased fertile spikelet's and grain number per spike. Similar to earlier studies (Esfandiari *et al*., 2016), current study agreed that when Fe was applied at optimum rate at different growth stages, it increased the GY by increasing the number of fertile spikelet per spike and the number of grains per spike. Rawashdeh and Florin (2015) reported that foliar Fe application (either single or along with other micronutrients) can help achieve favorable results on quantitative and qualitative yield of wheat. Chaudry *et al*. (2007), Safyan *et al*. (2012), Zain *et al*. (2015) and Pal *et al*. (2016) reported that the application foliar of micronutrients (such as Fe and Zn) increased grain and straw yields of wheat and gerbera.

Agro-Morphological traits

The changes in agro-morphological traits were studied in wheat plants treated by increasing soil N fertilization and foliar spray of $FeSO_4$ and Fe EDTA. According to the results of the ANOVA, significant differences were observed among foliar Fe treatments for peduncle weight (Ped W) and penultimate weight (Pen W) at 5% level, and spike length (SL) at 1% level (Table 3). But, N fertilizer had no significant effect on agronomic and morphological traits (Table 3). In the present study, foliar application of Fe fertilizers generally increased peduncle weight, penultimate weight, and spike length compared with control (Table 2). So that the mean peduncle dry weight was 67, 74 and 85 mg in control (no Fe application), FeEDTA and $FeSO_4$ treatments, respectively (Table 2), as well as the mean penultimate dry weight was 47, 56 and 61 mg in control (no Fe application), FeEDTA and $FeSO_4$ treatments, respectively (Table 2). Furthermore, the effect of $FeSO_4$ on spike length was higher than that of FeEDTA (Table 2). The possible reason of increased weight of stem internodes (especially internodes of peduncle and penultimate) with adequate Fe application may be the result of delayed leaf senescence and sustained leaf photosynthesis during growth period. Some researchers have reported that the increase of stem internodes weight is resulted from the increase of micronutrients supply (Abdoli *et al.*, 2016). Zain *et al.* (2015) showed that the application of micronutrients substantially improved plant height and spike length of wheat. Kahrariyan *et al.* (2013) reported foliar application Fe in the early stages of growth (tillering stage) had a better effect on the morphological and physiological traits and it was due to the positive impact of iron in increasing of the leaf area index and consequently increasing of photosynthesis.

Table 3. Analysis of variance of the effect of increasing soil N supply and foliar application of Fe fertilizers on agro-morphological traits in bread wheat

S.O.V	df	Ped W	Pen W	Other W	SL
Replication	3	140.6 ^{ns}	16.7 ^{ns}	146.9 ^{ns}	0.346 ^{ns}
Iron (Fe)	2	1010.3 *	632.3 *	720.8 ^{ns}	0.916 **
Nitrogen (N)	2	91.0 ^{ns}	66.3 ^{ns}	201.4 ^{ns}	0.308 ^{ns}
Fe × N	4	456.8 ^{ns}	164.2 ^{ns}	187.9 ^{ns}	0.261 ^{ns}
Error	24	278.3	196.8	388.0	0.224
CV (%)	-	22.1	25.6	31.9	9.10
S.O.V	**df**	**Ped L**	**Pen L**	**Other L**	
Replication	3	0.919 ^{ns}	0.383 ^{ns}	3.68 *	
Iron (Fe)	2	7.24 ^{ns}	0.575 ^{ns}	1.34 ^{ns}	
Nitrogen (N)	2	4.88 ^{ns}	0.430 ^{ns}	0.231 ^{ns}	
Fe × N	4	7.12 ^{ns}	0.164 ^{ns}	1.16 ^{ns}	
Error	24	3.29	0.404	1.08	
CV (%)	-	9.23	8.18	13.5	

^{ns},* and**: non-significant and significant at 5 and 1% probability level, respectively.

PedW: Peduncle weight, **PenW**: Penultimate weight, **OtherW**: Other internode weight, **SL**: Spike length, **PedL**: Peduncle length, **PenL**: Penultimate length, **OtherL**: Other internode length.

Grain quality, grain micronutrient concentrations and HI

The results of this study showed significant main effects of different foliar Fe treatments and soil N supply on concentrations of Zn, Fe, Cu and Mn in grain (Table 4). Also, the interaction between foliar Fe treatments and soil N supply significantly affected the grain concentrations of Zn, Fe, Cu and Mn (Table 4). Overall, Fe concentration in grain was raised by 2.8% and 37.0% for the supply of Fe EDTA and $FeSO_4$, respectively on foliage at the stem elongation and early milk stages. Similarly, the grain concentration of Zn was increased by 4.0% and 6.5% for foliar applied Fe EDTA and $FeSO_4$, as well as increasing amount of N used showed a significant increase in grain Zn content (Table 5). According to the results obtained, highest grain Zn concentrations was by foliar $FeSO_4$ application of 400 mg N kg^{-1} soil (47.7 mg kg^{-1} DW) and lowest by no Fe application of 100 mg N kg^{-1} soil (30.6 mg kg^{-1} DW) (Table 5). Also under soil N application with 100 mg kg^{-1} soil, foliar $FeSO_4$ application had the highest (116.2 mg kg^{-1} DW) and under soil N application with 100 and 200 mg kg^{-1} soil at no Fe application the lowest (79.8 and 79.6 mg kg^{-1} DW, respectively) grain Fe concentrations (Table 5). According to the results of this research, it is stated that when N application rate was increased either at sowing, average total uptake and accumulation of Fe and Zn in grain increased, ensuring the positive role of N rates (Cakmak, 2008; Shi *et al*., 2010). On the other hand, Zhang *et al*. (2010) stated that the grain Fe concentration was increased significantly from 29.5 mg kg^{-1} dry weight in the control to 37.8, 35.9 and 34.9 mg kg^{-1} dry weight by application of $FeSO_4$, ferric citrate plus $ZnSO_4$ and ferric citrate, respectively. Similar to the results of this study, Kabir *et al*. (2016) reported that $FeSO_4$ was the most efficient in biofortifying Fe in grain under Fe deficiency in pea plant. Pahlavan-Rad and Pessarakli (2009) announced that foliar application of Fe resulted in a 21% increase of grain Fe concentration and a 13% increase of grain Zn concentration. In pea plant (*Pisum sativum*), foliar Fe spray is particularly effective in enriching the grain with Fe if it is applied at during grain filling (Kabir *et al*., 2016). Khan *et al*. (2006) and Esfandiari *et al*. (2017) reported that Cu, Fe, Mn and Zn contents of leaf, straw and grain of wheat genotypes increased with the application of mineral fertilizers. The highest grain Cu concentrations was observed in foliar $FeSO_4$ application in 400 mg N kg^{-1} soil, whereas the greatest grain Mn concentrations was obtained by no Fe application in 400 mg N kg^{-1} soil (Table 5). But under foliar FeEDTA application at 200 mg N kg^{-1} soil the lowest grain Cu concentrations and grain Mn concentrations with 4.58 mg Cu kg^{-1} DW and 29.6 mg Mn kg^{-1} DW, respectively (Table 5).

Table 4. Analysis of variance of the effect of increasing soil N supply and foliar application of Fe fertilizers on grain concentrations of Zn, Fe, Cu and Mn in bread wheat

S.O.V	df	Zn	Fe	Cu	Mn
Replication	3	25.8 ^{ns}	29.5 *	0.121 ^{ns}	0.357 ^{ns}
Iron (Fe)	2	16.2 ^{ns}	3423.5 **	15.2 **	4.36 **
Nitrogen (N)	2	184.4 **	19.3 *	0.399 **	3.86 *
Fe × N	4	108.5 **	49.0 **	0.768 **	4.77 **
Error	24	21.0	7.02	0.066	0.767
CV (%)	-	12.6	2.86	4.41	2.79

^{ns},* and**: non-significant and significant at 5 and 1% probability level, respectively.
Zn: Zinc, **Fe**: Iron, **Cu**: Copper, **Mn**: Manganese.

In general, Cu concentration in grain was reduced by 4.2% for the supply of FeEDTA and increased by 34.7% for the supply of $FeSO_4$ (Table 5). Ghasemi-Fasaei and Ronaghi (2008) stated that Fe application increased Fe uptake but decreased that of Mn, Zn, and Cu in bread wheat. Irmak *et al*. (2012) reported that the increasing Fe application decreased the Cu content of leaf and grain. On the other hand, soil N is a particularly important issue as it plays a key role in achieving quantitatively and qualitatively high yields (Maadi *et al*., 2012). In this case, Svecnjak *et al*. (2013) reported that the trace element increments with high-N rate averaged 14.0% for Fe, 9.2% for Zn, 19.7% for Mn, 13.2% for Cu in grain of wheat. Gao *et al*. (2012) reported that the compared to the non-fertilized control, N fertilization increased protein concentration in grain and flour of bread wheat. However, N fertilization did not consistently affect grain Fe and Zn, or other quality traits.

According to the results obtained, highest harvest index (HI) was by foliar $FeSO_4$ application of 200 mg N kg^{-1} soil with 51.9% and lowest by foliar $FeSO_4$ application of 400 mg N kg^{-1} soil with 40.5% (Table 5). Zain *et al.* (2015) stated that the application of micronutrients substantially improved harvest index in wheat. Increasing N application rate from 100 to 200 mg N kg^{-1} soil resulted in increased HI (Table 5). Increasing rate of N fertilizer application was shown to increase the HI of canola and bread wheat in other studies (Faramarzi *et al.*, 2009; Kazemeini *et al.*, 2010; Maadi *et al.*, 2012).

Table 5. Effect of increasing soil N supply and foliar application of Fe fertilizers on HI and grain concentrations of Zn, Fe, Cu and Mn in bread. Plants were grown on soils with low (100 mg N kg^{-1} soil), medium (200 mg N kg^{-1} soil) and high (400 mg N kg^{-1} soil) N supply until full maturity under greenhouse conditions. Foliar Fe treatments were: no iron, 0.25% (w/v) Fe EDTA and 0.25% (w/v) $FeSO_4$. Foliar $FeSO_4$ fertilizer contained the same amount of Fe that was present in the Fe EDTA solution.

Soli N treatments (mg kg^{-1} soil)	**Foliar Fe treatments (0.25% [w/v])**			
	No Fe	**Fe EDTA**	**$FeSO_4$**	**Mean**
		Harvest index (%)		
100	43.3 ± 2.9	42.4 ± 2.8	42.4 ± 3.1	42.7 ^{b}
200	42.8 ± 2.5	44.5 ± 0.9	51.9 ± 1.7	46.4 ^{a}
400	43.9 ± 1.3	42.0 ± 0.8	40.5 ± 1.5	42.1 ^{b}
Mean	43.3 ^{*a}	43.0 ^{a}	45.0 ^{a}	
		Grain Zn concentrations (mg kg^{-1} DW)		
100	30.6 ± 0.3	37.4 ± 0.4	33.5 ± 0.6	33.8 ^{b}
200	38.0 ± 0.4	34.2 ± 1.5	31.2 ± 0.3	34.5 ^{b}
400	36.9 ± 0.5	38.1 ± 0.3	47.7 ± 6.7	40.9 ^{a}
Mean	35.2 ^{a}	36.6 ^{a}	37.5 ^{a}	
		Grain Fe concentrations (mg kg^{-1} DW)		
100	79.8 ± 1.0	85.8 ± 1.5	116.2 ± 0.8	93.9 ^{a}
200	79.6 ± 1.0	83.1 ± 2.1	111.6 ± 0.6	91.4 ^{b}
400	86.2 ± 1.1	83.9 ± 2.0	109.0 ± 2.6	93.0 ^{ab}
Mean	81.9 ^{c}	84.2 ^{b}	112.2 ^{a}	
		Grain Cu concentrations (mg kg^{-1} DW)		
100	5.59 ± 0.12	5.56 ± 0.15	6.87 ± 0.07	6.00 ^{a}
200	5.38 ± 0.04	4.58 ± 0.11	6.96 ± 0.05	5.64 ^{b}
400	4.89 ± 0.06	5.03 ± 0.07	7.50 ± 0.31	5.81 ^{b}
Mean	5.28 ^{b}	5.06 ^{c}	7.11 ^{a}	
		Grain Mn concentrations (mg kg^{-1} DW)		
100	31.3 ± 0.5	32.6 ± 0.4	31.1 ± 0.5	31.7 ^{a}
200	31.7 ± 0.2	29.6 ± 0.6	31.0 ± 0.1	30.7 ^{b}
400	33.2 ± 0.4	31.6 ± 0.5	30.6 ± 0.3	31.8 ^{a}
Mean	32.1 ^{a}	31.3 ^{b}	30.9 ^{b}	

*Means, in each column, followed by similar letter are not significantly different at the 5% probability level via Duncan multiple range test.

Values are means of four independent replicates ± standard error.

Zeidan *et al*. (2010) applied foliar Fe fertilizer (1.0% $FeSO_4$) and reported that Fe application increased protein and Fe contents of wheat grain. Narimani *et al*. (2010) showed that foliar spray of iron enhanced protein and yield of durum wheat. Several approaches were taken to cope with Fe deficiency in the wheat grain. Abbas *et al*. (2009) applied 0, 4, 8, and 12 kg ha^{-1} in the form of iron sulfate to the soil and showed that iron fertilization increased Fe and protein contents of the wheat grain. With application of 150 g ha^{-1} iron in the form of Fe_2O_3, Habib (2009) reported that iron and protein contents of the wheat grain were enhanced. Recent evidence in the literature indicates that the nitrogen nutritional status of plants has a positive influence on grain accumulation of Fe (Kutman *et al*. 2010), possibly by contributing to release of Fe mobilizing compounds from leaves and roots enhancing root uptake and transport of Fe via increasing pool of transporter proteins facilitating translocation and phloem transport of Fe via chelation with nitrogenous compounds and improving seed deposition of Fe by increasing amount of proteins in seeds (Cakmak *et al*., 2010b).

Correlation between measured traits

Correlation coefficient, which is used as a standard of measuring linear relationship between two variables, only has one mathematical interpretation, and does not refer to cause and effect relationships (Ozer *et al*., 1999). Some statistical methods, such as correlation analysis, indicate partial role of each component of yield in the amount of yield; also, they provide necessary information for choosing indirect traits in superior genotypes to have yield breeding (Zimmermann *et al*., 1994). Results showed that a positive significant correlation was found between GY with NGS (R^2 = 0.96, P < 0.01), NFSS (R^2 = 0.88, P < 0.01), BY (R^2 = 0.90, P < 0.01) and SL (R^2 = 0.76, P < 0.05), but a negative significant correlation was found between GY with TGW (R^2 = -0.79, P < 0.05) (Table 6). Also in this experiment, TGW showed negative correlation with NGS (R^2 = -0.93, P < 0.01) and NFSS (R^2 = -0.96, P < 0.01) (Table 6). In this experiment, grain Fe concentrations showed positive correlation with grain Cu concentrations (R^2 = 0.88, P < 0.01) (Table 6).

CONCLUSIONS

In general, this study increases the understanding of agronomic biofortification. The present research has shown that grain yield and its components of wheat differed substantially in response to iron (Fe) and nitrogen (N) supply, as well as application of various foliar Fe fertilizers had either a high positive effect on agro-morphological traits or remained effective on grain Fe concentration. Also, the effect of $FeSO_4$ on all characteristics studied was higher than that of Fe EDTA. It appears that foliar application of Fe-sulfate ($FeSO_4$) is appropriate in correcting Fe deficiency in wheat grown on calcareous soils. In addition, the results indicate an important role of N nutrition in enrichment of wheat grain with Zn. This N effect should be considered in breeding and fertilization programs focusing on enrichment of staple food crops with Fe and Zn.

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Table 6. Correlation coefficients between grain yield, agro-morphological traits and grain micronutrient concentrations under soil N supply and foliar application of Fe fertilizers in bread wheat

Traits	NGS	NFSS	TGW	GY	BY	HI	PedW	Pen W	Other W	SL	Ped L	Pen L	Other L	Zn	Fe	Cu	Mn
NGS	1																
NFSS	0.96**	1															
TGW	-0.93**	-0.96**	1														
GY	0.96**	0.88**	-0.79*	1													
BY	0.94**	0.93**	-0.88**	0.90**	1												
HI	0.41	0.25	-0.14	0.57	0.16	1											
PedW	0.65	0.65	-0.57	0.66	0.77*	0.02	1										
PenW	0.62	0.65	-0.55	0.63	0.82**	-0.11	0.80**	1									
OtherW	0.39	0.44	-0.40	0.36	0.66	-0.43	0.61	0.90**	1								
SL	0.88**	0.95**	-0.94**	0.76*	0.93**	-0.04	0.69*	0.74*	0.64	1							
PedL	-0.10	-0.12	0.25	0.08	-0.01	0.10	0.30	0.28	0.29	-0.05	1						
PenL	0.29	0.29	-0.20	0.34	0.38	0.03	0.01	0.53	0.56	0.33	0.27	1					
OtherL	0.01	0.08	-0.14	-0.05	0.25	-0.61	0.19	0.59	0.83**	0.31	0.21	0.59	1				
Zn	0.00	0.20	-0.18	-0.09	0.19	-0.60	0.28	0.43	0.62	0.43	0.44	0.26	0.60	1			
Fe	0.97**	0.97**	-0.98**	0.86**	0.90**	0.27	0.57	0.55	0.38	0.92**	-0.21	0.22	0.07	0.11	1		
Cu	0.86**	0.92**	-0.91**	0.75*	0.82**	0.13	0.74*	0.55	0.34	0.90**	-0.05	0.02	-0.01	0.27	0.88**	1	
Mn	-0.42	-0.39	0.28	-0.48	-0.53	-0.13	-0.52	-0.77*	-0.56	-0.35	-0.07	-0.52	-0.42	0.03	-0.28	-0.24	1

* and**: significant at 5 and 1% probability level, respectively.

NGS: Number of grain per spike, **NFSS**: Number of fertile spikelet per spike, **TGW**: Thousand grain weight, **GY**: Grain yield, **BY**: Biological yield, **HI**: Harvest index, **Ped W**: Peduncle weight, **Pen W**: Penultimate weight, **Other W**: Other inter node weight, **SL**: Spike length, **PedL**: Peduncle length, **Pen L**: Penultimate length, **Other L**: Other internode length, **Zn**: Grain Zn concentrations, **Fe**: Grain Fe concentrations, **Cu**: Grain Cu concentrations, **Mn**: Grain Mn concentrations.

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