



Agro-Biofortification of Zinc and Iron in Wheat Grains

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Abstract

Zinc (Zn) and Iron (Fe) are essential micronutrients for both human and plants, but their deficiency is prevalent in the world, especially in developing countries including India and Pakistan. Therefore, zinc and iron biofortification of wheat and other cereal crops is being urgently addressed and highly prioritized as a research topic. Thus, a field study was planned in a silty-loam soil under conventional tillage, located at Peshawar, Khyber Pakhtunkhwa, Pakistan, in winter 2016–2017 to examine the influence of foliar zinc rates (0.1, 0.2, 0.3 and 0.4% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) and foliar iron rates (0.5, 1, 1.5 and 2% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) on physiology, phenology, yield indices and grain Zn and Fe contents of wheat. The results specified that leaf area per tiller, flag leaf area, productive tillers, yield components, biomass yield, grain Zn and Fe contents were significantly improved with various foliar Zn and Fe levels. The plants fertilized with 0.3% Zn and 1% Fe exhibited higher grain yield. Higher grain Zn content was observed in plants fertilized with foliar Zn at 0.4% and Fe at 0.5%. Higher grain Fe content was noted in the plants fertilized with 0.1% Zn and 2% Fe. It can be concluded that the plants fertilized with 0.3% foliar Zn and 1% Fe resulted in improved yield components and yield, whereas the plants fertilized with 0.1% Zn and 2% Fe showed an improved wheat grain quality (Zn and Fe content). Hence, foliar fertilization of 0.3% Zn and 1% Fe is recommended for higher wheat yields and better grain quality under the edaphoclimatic conditions studied.

Keywords Micronutrients · *Triticum aestivum* L. · Leaf fertilization · Nutrients · Flag leaf area

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Agronomische Biofortifikation von Zink und Eisen in Weizenkörnern

Zusammenfassung

Zink (Zn) und Eisen (Fe) sind Mikronährstoffe für Menschen und Pflanzen. Weltweit herrscht jedoch ein weit verbreiteter Fe- und Zn-Mangel, insbesondere in Entwicklungsländern wie Indien und Pakistan. Die Biofortifikation von Weizen und anderen Getreidepflanzen mit diesen Mikronährstoffen wird deshalb intensiv thematisiert und als Forschungsthema hoch priorisiert. Daher wurde im Winter 2016–2017 eine Feldstudie in einem schlammigen Lehmboden unter konventioneller Bodenbearbeitung in Peshawar, Khyber Pakhtunkhwa, Pakistan, geplant, um den Einfluss einer Blattdüngung mit Zink (0,1, 0,2, 0,3 und 0,4% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) und Eisen (0,5, 1, 1,5 und 2% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) auf die Physiologie, Phänologie, Ertragsindizes und Korn-Zn- und Fe-Gehalte von Weizen zu untersuchen. Die Ergebnisse zeigten, dass die Blattfläche pro Stock, die Fahnenblattfläche, die produktive Bestockung, die Ertragskomponenten, der Biomassertrag sowie die Korn-Zn- und -Fe-Gehalte durch verschiedene Blattbehandlungen mit Zn und Fe signifikant verbessert wurden. Die mit 0,3% Zn und 1% Fe gedüngten Pflanzen wiesen einen höheren Kornertrag auf. Ein höherer Zn-Gehalt der Körner wurde bei Pflanzen beobachtet, die mit 0,4% Zn und 0,5% Fe gedüngt wurden. Bei den mit 0,1% Zn und 2% Fe gedüngten Pflanzen wurde ein höherer Fe-Gehalt festgestellt. Daraus kann geschlossen werden, dass die mit 0,3% Zn und 1% Fe gedüngten Pflanzen zu verbesserten Ertragskomponenten und Erträgen führten, während die mit 0,1% Zn und 2% Fe gedüngten Pflanzen eine höhere Weizenkornqualität (Zn- und Fe-Gehalt) aufwiesen. Daher wird eine Blattdüngung von 0,3% Zn und 1% Fe für höhere Weizenträge und eine bessere Kornqualität unter den untersuchten edaphoklimatischen Bedingungen empfohlen.

Schlüsselwörter Mikronährstoffe · *Triticum aestivum* L. · Blattdüngung · Nährstoffe · Fahnenblattfläche

Introduction

Micronutrients hidden hunger in plants and human is an emerging issue and has become cry of the day (Singh 2009). Nearly 50% of the world cereals cultivated soils are zinc (Zn) deficient whereas iron (Fe) deficiency is mostly recorded in arid regions with high pH and calcareous soils (Kumar et al. 2016). These nutrients play an important role in plant physiological and metabolic functions and are required in trace amount for plant growth, development and yield (Tahir et al. 2013; Mousavi et al. 2013). Agronomic biofortification is one of the prominent agriculture techniques to address hidden hunger and improve grain micronutrients concentration (Ramzan et al. 2020). Biofortification of the crop plants with microelements is providing a sustainable solution for higher yield and suboptimal intake of micronutrients (Blancauert et al. 2017). It is a possible technique to overcome malnutrition in developing countries (Ngozi 2013) by applying micronutrients directly to the soil or plant leaves (De-Valença et al. 2017) which can potentially improve grain quality and productivity of wheat crop. Agronomic biofortification is one the best strategies to improve grain Zn and Fe contents (Cakmak et al. 2010).

Zinc is an imperative element for plant growth and plays an important role as functional, structural and regulatory cofactor of many enzymes. The foliar application of Zn increased the number of wheat grains per spike and the seed yield (Tabatabai et al. 2015). Moreover, Zn application is one of the important approaches for agronomic biofortification of valuable crops for human health. Cakmak (2010)

reported that foliar application of Zn significantly increased Zn grain concentration and was prominent when Zn was sprayed at late growth stage (milk and dough). Hussain et al. (2012) indicated that soil Zn application increased grain yield (29%), whole-grain Zn concentration (95%), and whole-grain estimated Zn bioavailability (74%).

Iron has several imperative roles in plant growth and development processes such as chlorophyll synthesis, respiration and photosynthesis. Fe is also an essential component of several plant enzymes contributing to oxidation and releasing energy as the result of the breakdown of different carbohydrates and nucleic acids (Havlin et al. 2014). Plants that are grown in Fe deficient soils are reported to have low Fe content in their edible portion which ultimately causes Fe deficiency in humans (White and Broadley 2009).

Therefore, the current study performed with foliar application of Zn and Fe to wheat crop in alkaline soil aimed to improve yield and nutrient availability in grains. Thus, the objectives of the study were to investigate the suitable levels of Zn and Fe, their main effects as well as their interactive effects on the performance of wheat for achieving high yield and grains biofortified with Zn and Fe.

Materials and Methods

Experimental Site and Location

The experiment was setup in silty-loam soil under conventional tillage system, located at Peshawar, Khyber

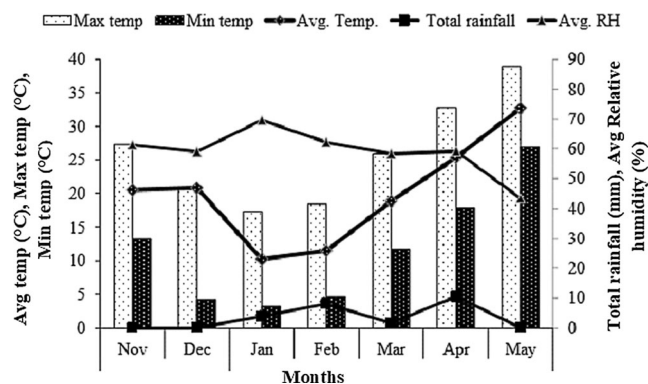


Fig. 1 Meteorological data for wheat growing season from November 2015 to May 2016 (Digital weather station of Agronomy Research Farm, The University of Agriculture Peshawar—Pakistan)

Pakhtunkhwa, Pakistan in winter 2015–2016. The experimental farm is located at 34.01° N latitude, 71.35° E longitude, at an altitude of 350m above sea level in Peshawar valley. Peshawar is located about 1600km north of the Indian Ocean and has semi-arid subtropical continental type of climate according to Koppen classification. The mean annual rainfall is very low (300 to 500mm), 60–70% rainfall occurs in summer, while the remaining 30–40% rainfall occurs in winter (digital weather station of agronomy research farm, University of Agriculture Peshawar). The soil of the experimental site was classified as Alfisol according to the Soil Survey Staff (2014). Rainfall, temperature and relative humidity data were collected and are summarized in Fig. 1.

Experimental Treatments and Details

The experimental area was irrigated before sowing of wheat for weeds to germinate and then ploughed with cultivator for a fine seed bed preparation. Four treatments were applied as foliar sprays with a mini-directed jet sprayer in each treatment including zinc levels (0.1, 0.2, 0.3 and 0.4% $ZnSO_4 \cdot 7H_2O$) and iron levels (0.5, 1, 1.5 and 2% $FeSO_4 \cdot 7H_2O$). Foliar sprays were applied four times with a manual sprayer pump calibrated with five liters, one every 10 days in the flagging to grain filling stages. The volume of foliar spray was $0.08 L m^{-2}$. The control plants were sprayed with deionized water. All the treatment combinations in 4×4 factorial schemes and a control (16+1) were randomly allotted to the experimental units using randomized complete block design with four replications. The foliar fertilization of Zn and Fe was done in the late afternoon to avoid the possible harm caused by high temperature and salts accumulation during the sunny day.

Crop Husbandry

Wheat variety “Atta Habib-2010” was sown at the seed rate of $120 kg ha^{-1}$ on flat beds with 30cm apart rows. Plot size was $2.4 m \times 4 m$. Sowing was done on 17 November 2016 with the help of a drill machine. The seeds were treated with Amedacloprid and Topsin-M as systemic insecticide and fungicide, respectively. The recommended dose of nitrogen ($120 kg ha^{-1}$) was applied in the form of urea (46% N) in two equal splits. Phosphorus was applied in the form of diammonium phosphate (DAP, 46% P_2O_5) at the rate of $90 kg ha^{-1}$ where potassium (K_2O) at the rate of $50 kg ha^{-1}$ was applied from muriate of potash (MOP, 60% K_2O) at the time of sowing. The crop was irrigated with surface irrigation system by mean canal system, through Warsak Canal system of River Kabul. Harvesting was done manually after 184 days of sowing. For weeds control affinity™ 50WG (Isoproturon + Carfentrazone) weedicide at the rate of $2 kg ha^{-1}$ was sprayed in the morning time 60 days after sowing. Each plot was harvested and threshed separately.

Soil Analysis

Soil was randomly collected from different locations at the depth of 0.0–0.20m before the implantation of the experiment for physio-chemical analysis. The soil of the trail site was clay loam in texture, alkaline and calcareous, with a low organic matter, macro and micro-nutrients. Soil texture was determined by pipette method as described by Tagar and Bhatti (2001). Soil organic matter was determined by wet digestion method as described by Nelson and Sommers (1996). Electrical conductivity and soil pH were estimated according to methodology of McLean (1983) and available P, K, Zn and Fe were determined with proposed methodology of Soltanpour and Schwab (1977). Nitrogen was determined by Kjeldahl procedure according to the proposed methodology of Bremner and Mulvaney (1982). The

Table 1 Chemical properties of pre-harvest soil sample

Soil property	Unit	Values
Clay	%	2.8
Silt	%	50
Sand	%	47.2
Texture	–	Silty loam
pH (1: 5)	–	8.67
EC (1: 5)	$d S m^{-1}$	0.08
Organic matter	%	1.53
N content	%	0.1
P content	$Mg kg^{-1}$	34.52
K content	$Mg kg^{-1}$	70.2
Zn content	$Mg kg^{-1}$	1.18
Fe content	$Mg kg^{-1}$	0.65

physio-chemical analysis of the soil is summarized in Table 1.

Observations Recorded

Leaves of ten tillers in four central rows were randomly selected in each experimental plot at anthesis stage, the product of leaf length and width was multiplied with a correction factor (CF) and leaves number and then divided by the number of tillers. Leaf area per tiller was noted.

$$\text{Leaf area per tiller (cm}^2\text{)} = \frac{\text{Number of leaves} \times \text{avg.leaf length (cm)} \times \text{avg.leaf width (cm)} \times \text{CF}}{\text{Number of tillers}}$$

$$\text{CF} = 0.65.$$

Five plants were randomly selected from every experimental plot for the flag leaf area by calculating leaf length and width. The following formula was used for mean single leaf area.

$$\text{Leaf area} = \text{Leaf length} \times \text{Leaf width} \times \text{CF}(0.65)$$

For productive tillers one-meter long rows at three randomly selected positions in each experimental plot were calculated. The unproductive tillers were calculated by subtracting productive tillers per m² from the total tillers per m². Spikes of ten randomly selected plants were taken, threshed and then number of grains was calculated. After threshing, 1000 grains were calculated from each plot and weighed using electronic balance. After harvesting, threshing, cleaning and drying, the grain yield of wheat was estimated at 14% moisture content. Likewise, straw yield was recorded by subtracting grain yield from the total biomass yield.

Grain samples of 1.0g were collected from each treatment, grains were dried at room temperature and put in oven to remove the moisture. After that, grains were ground with a seed grinder. 0.5g of the sample were taken and 10ml concentrated HNO₃ added in a 250ml conical flask, then put in fume hood for a night. 4ml of HClO₄ was added on the very next day. The conical flask was put on a hard plate until white fume appears inside the flask, then the conical flask was taken out with opener and left to cool. The conical flask was washed with a water bottle for 2–3 times and was poured in a volumetric flask to make it 100ml. The extract was read for Zn and Fe on AAS (Atomic Absorption Spectrophotometer).

Statistical Analysis

The data were statistically analyzed according to the Fisher's analysis of variance technique and least significant difference (LSD) test at 5% probability was used to compare the differences among treatment means (Steel

et al. 1997). Data were analyzed by statistical software (Statistix 8.1). The possible interactions were graphically made using Sigma Plot.

Results

Assessment of Zinc and Iron as Foliar Agents for Enhancing Allometric and Yield Traits of Wheat

Analysis of variance exhibited that different foliar Zn and Fe levels had significant effect on leaf area per tiller. The comparison of unfertilized plants vs. fertilized plants was also found significant, whereas the interactive effect had insignificant response toward foliar Zn and Fe (Table 2). Planned mean values of the data indicated that greater leaf area per tiller (124) was derived at 0.3% Zn, which was statistically analogous with 0.4 and 0.2% Zn.

The leaf area per tiller (109.8) was lower with 0.1% Zn. Among different Fe levels, greater leaf area per tiller (121.6) was recorded at 1% Fe, which was statistically analogous with 0.5 and 1.5% Fe treated plants. Lesser leaf area per tiller (107.1) was noted with 0.5% foliar Fe. The control plants in comparison with the plants fertilized with Fe and Zn produced lower leaf area per tiller (98.5).

Data illustrated that flag leaf area of wheat was significantly affected by different Zn and Fe levels. The comparison of control vs. fertilized plants was also found significant, whereas the interaction of Zn with Fe was noted insignificant (Table 2). Data regarding flag leaf area specified that plants fertilized with 0.2% Zn resulted in higher flag leaf area (33.70), which was statistically similar with the flag leaf area (33.53) produced in plants fertilized with 0.3% Zn. Least flag leaf area (32.59) was observed in plants fertilized with 0.1% Zn. Among different Fe levels, plants fertilized with 1% Fe resulted in determined flag leaf area (33.99), which was followed by flag leaf area (33.26) obtained with 1.5% Fe. Lesser flag leaf area (32.80) was produced with 2% Fe. The flag leaf area (31.88) of control plants in comparison with fertilized plants was lesser.

Analysis of variance revealed that the number of productive tillers was significantly affected by different foliar Zn and Fe levels. The comparison of control vs. rest was also found significant, while the interactive effect of Zn and Fe was found non-significant (Table 2). Mean values of the data presented that Zn at the rate of 0.3% resulted in the maximum number of productive tillers (299), which was statistically similar with the number of productive tillers (293) produced with 0.2% Zn. The productive tillers (281) were lower with 0.1% Zn. Among different Fe levels, Fe at the rate of 1% produced the maximum number of productive tillers (298), which was statistically at par with the number of productive tillers (296) attained with the 0.5%

Table 2 Leaf area per tiller (cm²), flag leaf area (cm²), productive tillers (m²) and unproductive tillers (m²) of wheat as affected by zinc and iron

Zinc (%)	Leaf area tiller ⁻¹ (cm ²)	Flag leaf area (cm ²)	Productive tillers (m ²)	Unproductive tillers (m ²)
0.1	109.8 b	32.59 c	281 c	30
0.2	114.1 ab	33.70 a	293 b	29
0.3	124.0 a	33.53 a	299 a	30
0.4	118.8 ab	33.08 b	291 b	31
LSD for zinc (Zn)	9.5	0.41	5.6	Ns
Iron (%)				
0.5	120.3 a	32.85 c	296 a	31
1	121.6 a	33.99 a	298 a	30
1.5	117.7 a	33.26 b	288 b	30
2	107.1 b	32.89 c	282 c	29
LSD for iron (Fe)	9.5	0.41	5.6	Ns
Planned mean comparison	*	**	**	Ns
Control	98.5	31.88	244	30
Rest	116.7	33.23	291	30
Interaction				
LSD for Zn × Fe	Ns	Ns	Ns	Ns

Mean values of the different categories in each column with different letters discloses significant differences ($p \leq 0.05$) using LSD test

ns non-significant

** and * = significant at 1 and 5% level of probability, respectively

Fe. Lower number of productive tillers (282) was attained with 2% Fe. The control plants in comparison with the fertilized plants produced a lower number of productive tillers (244).

Analysis regarding unproductive tillers exhibited that number of nonproductive tillers was insignificantly affected by Zn and Fe levels. The comparison of control vs. rest and the interaction of Zn with Fe were found non-significant (Table 2). The rest plants in comparison with the control plants produced less number of nonproductive tillers. Analysis of variance revealed that different Zn and Fe levels significantly affected grains per spike of wheat. The comparison of control vs. rest was also found significant, while the interaction of Zn with Fe was found non-significant (Table 3). Mean values of the data showed that Zn at the rate of 0.3% produced more grains per spike (52), which was statistically similar with grains per spike (50) produced with 0.4% Zn. The grains per spike (48) was lower with 0.2% Zn.

Among different Fe levels, foliar spray of 1% Fe produced a higher number of grains per spike (54), which was followed by grains per spike (50) obtained with both 0.5 and 1.5% Fe. Lower grains per spike (45) were produced with 2% Fe. The control plants in comparison with the treated plants produced lower grains per spike (36).

Analysis of variance showed that different Zn and Fe levels significantly affected thousand grain weight of wheat. The comparison of control vs. rest was also found significant, while the interaction effect of Zn with Fe was found

non-significant (Table 3). Mean values of the data indicated that Zn at the rate of 0.3% attained a higher thousand grain weight (45.5), which was followed by thousand grain weight (44.4) produced with 0.4% Zn. Thousand grain weight (43.2) was less with 0.1% Zn. Among different Fe levels, Fe at the rate of 1% produced a higher thousand grain weight (45.4), which was followed by thousand grain weight (44.6) obtained with 0.5% Fe. Minimum thousand grain weight (42.9) was produced with 2% Fe. The control plants in comparison with the fertilized plants produced a less thousand grain weight (39.2).

Assessment of Zinc and Iron as Foliar Agents for Enhancing Grain and Biomass Yield Along With Zinc and Iron Content in Wheat

Grain yield was significantly affected by different Zn and Fe levels. The comparison of control plants vs. treated plants was also found significant, while the interaction had non-significant response for Zn and Fe (Table 3). Mean values of the data revealed that Zn at the rate of 0.3% ha⁻¹ produced higher grain yield (3775 kg ha⁻¹), which was statistically similar with the grain yield (3751 kg ha⁻¹) produced with 0.4% Zn. The grain yield (3496 kg ha⁻¹) was lower with 0.1% Zn. Among different Fe levels, Fe at the rate of 1% produced more grain yield (3802 kg ha⁻¹), which was statistically at par with the grain yield (3747 kg ha⁻¹) obtained with 0.5% Fe. Lower grain yield (3449 kg ha⁻¹) was produced with 2% Fe. The control plants in comparison with

Table 3 Grains per spike, thousand grain weight (g), grain yield (kg ha⁻¹) and biomass yield (kg ha⁻¹) of wheat as affected by zinc and iron

Zinc (%)	Grains spike ⁻¹	Thousand grain weight (g)	Grain yield (kg ha ⁻¹)	Biomass yield (kg ha ⁻¹)
0.1	49 b	43.2 c	3496 c	7863 c
0.2	48 ab	43.7 c	3592 b	8033 b
0.3	52 a	45.5 a	3775 a	8233 a
0.4	50 a	44.4 b	3751 a	8173 ab
LSD for zinc (Zn)	2.9	0.7	55	169.6
Iron (%)				
0.5	50 b	44.6 b	3747 a	8244 a
1	54 a	45.4 a	3802 a	8251 a
1.5	50 b	43.9 b	3616 b	8063 b
2	45 c	42.9 c	3449 c	7748 c
LSD for iron (Fe)	2.9	0.7	55	169.6
Planned mean comparison	**	**	**	**
Control	36	39.2	2919.1	6856
Rest	49	44.2	3653.5	8076
Interaction				
LSD for Zn × Fe	Ns	Ns	Ns	Ns

Mean values of the different categories in each column with different letters discloses significant differences ($p \leq 0.05$) using LSD test

ns non-significant

** = significant at 1% level of probability

the rest plants produced lower grain yield (2919 kg ha⁻¹). Higher grain yield was observed in the plants fertilized with 0.4% Zn over unfertilized plants.

Biomass yield was significantly affected by different Zn and Fe levels. The comparison of control vs. rest was also found significant while the interactive effect of Zn and Fe was found non-significant (Table 3). Mean values of the data shown that Zn at the rate of 0.3% produced higher biomass yield (8237 kg ha⁻¹) which was statistically similar with the biomass yield (8173 kg ha⁻¹) produced with 0.4% Zn. The biomass yield (7863 kg ha⁻¹) was lower with 0.1% Zn. Among different Fe levels, the plants fertilized with 1% Fe resulted in highest biomass yield (8251 kg ha⁻¹), which was statistically at par with the biomass yield (8244 kg ha⁻¹) attained with 0.5% Fe. Lower biological yield (7748 kg ha⁻¹) was produced with 2% Fe. The control plants in comparison with the fertilized plants produced lower biological yield (6856 kg ha⁻¹).

Analysis of variance showed that different Zn and Fe levels significantly affected grain Zn content of wheat. The comparison of control vs. rest was also found significant, while the interaction of Zn and Fe was found non-significant (Table 4). Mean values of the data revealed that increasing Zn level consistently increased the grain Zn content. Zn at the rate of 0.4% produced grains with higher Zn contents (39.1), which was statistically similar with the grain Zn content (38.7) obtained with 0.3% Zn. The grain Zn content (33.9) was lower with the application of 0.1% Zn. Among different iron levels, grain Zn content (38.3) was higher

Table 4 Grain Zn content (mg kg⁻¹) and grain Fe content (mg kg⁻¹) of wheat as affected by zinc and iron

Zinc (%)	Grain Zn content (mg kg ⁻¹)	Grain Fe content (mg kg ⁻¹)
0.1	33.9 b	47.82 a
0.2	34.1 b	46.76 b
0.3	38.7 a	44.26 c
0.4	39.1 a	40.87d
LSD for zinc (Zn)	1.5	1.1
Iron (%)		
0.5	38.3 a	42.10 c
1	36.5 b	41.96 c
1.5	35.7 b	46.54 b
2	35.2 b	49.11 a
LSD for iron (Fe)	1.5	1.1
Planned mean comparison	**	**
Control	30.3	41.7
Rest	36.4	44.9
Interaction		
LSD for Zn × Fe	Ns	**

Mean values of the different categories in each column with different letters discloses significant differences ($p \leq 0.05$) using LSD test

ns non-significant

** = significant at 1% level of probability

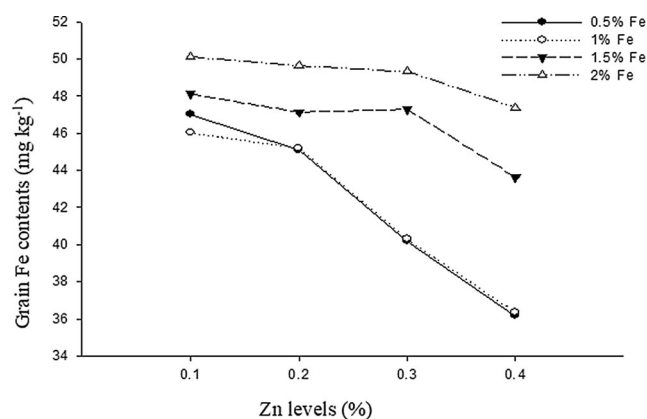


Fig. 2 Interaction of zinc and iron foliar fertilization regarding the grain Fe content of wheat

with Fe at the rate of 0.5%, which was followed by the Zn content of grain (36.5) recorded with the 1% Fe. Minimum grain Zn content was recorded with the 2% Fe. The control plants in comparison with the fertilized plants have lower Zn content (30.3).

Analysis of variance showed that different Zn and Fe levels significantly affected grain Fe content of wheat (Fig. 2). The comparison of control vs. rest was also found significant, while the interactive effect of Zn with Fe was found significant (Table 4). Mean values of the data revealed that increasing Zn level consistently decreased the grain Fe content. Zn at the rate of 0.1% produced grains with higher Fe contents (47.82), which was statistically similar with the grain Fe content (46.76) obtained with 0.2% Zn. The grain Fe content (40.87) was lower with 0.4% Zn. Among different Fe levels, grain Fe content (49.11) was higher with Fe at the rate of 2%, which was followed by the Fe content of grain (46.54) recorded with 1.5% Fe. Minimum grain Fe content (41.96) was recorded with 1% Fe. The control plants in comparison with the fertilized plants have lower grain Fe content (41.7).

Discussion

Role of Zinc and Iron in Wheat Allometric and Yield Traits

Leaves are arranged on the stem to permit most of sunlight absorption and serve as the top most food producing organ. The increase in leaf area might be due to Zn involvement in the synthesis of auxin hormones, amino acid, indole acetic acid, division and expansion of cells which are improving leaf area expansion (Nadergoli et al. 2011). Prominent leaf area recorded at 1% Fe may be due to Fe is being obligatory for synthesis of chlorophyll, structure of chloroplast, component of ferredoxin in the process of photosynthesis

and fix nitrogen which lead to increase leaf area and crop production (Zayed et al. 2011).

Flag leaf has a pivotal function in production and accumulation of assimilates toward grain during grain filling. The enlargement of flag leaf area might be due to adequate availability of the nutrients, needed as nutritional supplements for influencing size and efficiency of the leaf canopy. The application of Zn and Fe prominently raise the concentration and total uptake of the nutrients in grain, flag leaves and grain protein contents (Ziaeiian and Malakouti 2001). The foliar application of Fe had significantly amplified the chlorophyll content and flag leaf area of the plants in comparison to no Fe fertilized plants (Rawashdeh and Sala 2014).

The improvement in the productive tillers might be due the fact that zinc application plays a key role in metabolic, physiological and biochemical processes which hasten assimilate partitioning to the flowers. Islam et al. (1999) demonstrated that zinc application enhanced spike length and fertile tillers per plant in the wheat crop. Khan et al. (2008) investigated that Zn application had positive impact on plant growth which lead to increase plant height, number of fertile tillers m⁻², spike length, grains per spike, biological yields and 1000-grain weight culminating in increase of grain yield. Maximum productive tillers were noted with 1% Fe, might be due to the fact that Fe has a marked role in cellular events and mitochondrial homeostasis which in turn effect the plant growth and development (Bashir et al. 2013). Application of Fe at different levels hastened the number of tillers per square meter, the 1000-grain weight and grain yield of the wheat (Abbas et al. 2012). Results indicated that the plant height, numbers of leaves, fertile tillers per m² were prominently augmented by the application of Fe in comparison with untreated treatment (Rawashdeh and Sala 2015). Yield and physiology of wheat crop was prominently improved with Zn and Fe application (Jalal et al. 2020).

In the current study we did not observe any significant change in unproductive tillers after application of zinc and iron (Table 1). However, it is reported that deficiency of Zn causes stunted growth, infertility and spikelet sterility (Hafeez et al. 2013). Arora and Singh (2004) proposed that tillers in wheat and barley are covered up genetically and micronutrients nutrition has a slight outcome in this feature.

Grains per spike is a directly yield contributing attribute in getting higher yield. The higher the grains per spike, the higher will be the yield. The prominence in grains per spike might be due to the reason that microelements had efficiently enhanced the enzymatic processes, photosynthesis and assimilates translocation to seeds. Wang et al. (2003) investigated that Zn fertilizer enhanced grains per spike, spike length and grain weight which subsequently improved the production of wheat. In a long-term experiment, Hao et al.

(2003) concluded that fertilization of Zn improved the nutritional value, grain number per spike and thousand grain weight per unit area. The grains per spike increase with 1% Fe might be due to the better starch utilization in seed and translocation of assimilate to developing grains, which increase the grain size and grains per spike. A field experiment showed that the application of micronutrients like Fe, Zn and Mn prominently increase the plant heights, spikes plant⁻¹, grains spikes⁻¹, 1000-grain weight, grains yield, biological yields and harvests index (Bameri et al. 2012). Fathi and Gholizadeh (2010a) demonstrated that increased level of Zn and Fe as soil fertilizer increases 1000-grain weight, number of spike m⁻², grains per spike and grain yield in barley plant.

Thousand grain weight is one of the imperative yield interposing factors which have a pivotal role in limiting yield potential of crops. The increase in yield attributing characters like spike length, grains per spike, effective tillers and 1000-grain weight with Zn application has been reported by Bameri et al. (2012). Application of Zn improved the nutritional value, grains per spike and thousand grain weight (Hao et al. 2003). Maximum grain weight is determined with 1% Fe. This perhaps due to the fact that application of Zn, Mn and Fe have key importance in crop growth, involving in photosynthesis, respiration, biochemical and physiological processes and thus attributed to higher yields (Zeidan et al. 2010).

Role of Zinc and Iron in Wheat Yield, Biomass and Zinc and Iron Content

Final grain yield is a product of aggregate effects of various growth and yield contributing parameters which are influenced by various agronomic practices under a specific set of ecological conditions. The enhancement in grain yield may due to the role of Zn as a catalyst in different growth processes like photosynthesis, respiration, hormone production, protein synthesis and other physiological activities (Ramkala et al. 2008). Zn assists the conversion of nitrate to ammonia in the plants and enhanced the wheat grain yield by 4–9% (Alloway 2008). The plots treated with 1% Fe resulted in higher grain yield might be due to the inclination of assimilates to the source (Chaudry et al. 2007). Fe has a vital role in plant respiration, photosynthetic reaction and chlorophyll synthesis that is why it increases the grain yield of wheat whereas a higher rate of Fe had insignificant influence on crop yield (Abbas et al. 2009b).

Biological yield is the mixture of seed and straw yield. It is the function of genetic characters of the crop, nutrient situation of the soil and the ecological pattern present in the surrounding of crop. The elevation in the biomass may be due to the Zn nutrition which in result expedites cell division, photosynthesis, respirations, nitrogen metabolism,

biochemical and physiological processes (Zeidan et al. 2010). Shoot yield contributes to overall yield of the crop, dry matter increasing with ZnSO₄ application (El-Nasharty et al. 2013). Significant biomass yield was noticed with fertilization of 1% Fe because it has a critical role in chlorophyll synthesis and photosynthesis, enzyme activities and plant respiration. Our results are also in agreement with Abbas et al. (2012) who demonstrated that different Fe doses enhance straw yield, spike length, 1000-grain weight and grain yield of wheat. Knany et al. (2009) exhibited that foliar spray with Zn, Mn and Fe in combination or alone had a prominent effect on the growth and yield characters of wheat. When Zn, Fe and Mn are applied separately they will have far better impact than applied in combination.

Enrichment of grain Zn contents might be due to Zn nutrition which empowers the plants to uptake better amount of Zn in leaves, it is then remobilized and allocated in grains (Jiang et al. 2008). Zn fertilization not only enriches nutritional quality but also involves progressively in production of grain in Zn-deficit soils (Cakmak 2008). Notable grain Zn content is recorded in the plants treated with 0.5% Fe. This is because Fe and Zn have an antagonistic relationship after the certain parallel concentration and Fe transitional phloem mobility (Abbas et al. 2009b).

Increasing Zn levels decreased grain Fe content at all Fe levels except 0.5% Fe, however, the decrease was more prominent at 1.5 and 2% Fe (Fig. 2). This might be due to the fact that Zn helps in accumulation of assimilate in the grains. Application of Zn predicted that Fe uptake enhances with augmentation of Zn to the certain level whereas, elevated level of Zn results in reduction of grain Fe uptake in wheat and this may be due the Fe transitional phloem mobility (Abbas et al. 2009b).

Conclusions

Foliar application of Zn and Fe alone or combination had a positive effect on yield and yield components of wheat. In calcareous soil, Fe and Zn are little available for plants, in this times, foliar application is a useful method to nourish plants. This study clearly demonstrated that foliar zinc fertilization at the rate of 0.3% in conjunction with iron at the rate of 1% improved wheat yield and yield components at maximum with the application in flagging to grain filling stages. Foliar zinc fertilization at the rate of 0.4% and iron at the rate of 2% were most interesting for an enriched wheat grain quality with maximum increment of Zn and Fe content.

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Conflict of interest A. Jalal, Shahenshah, M. Carvalho Minhoto Teixeira Filho, A. Khan, T. Shah, M. Ilyas and P.A. Leonel Rosa declare that they have no competing interests.

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