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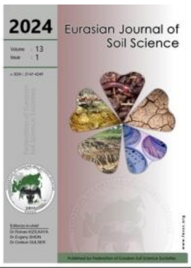

Enhancing iron concentration in bread wheat through Fe-EDTA fortification

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Abstract

Iron (Fe) malnutrition in humans is a global concern which can be revised by improved Fe density in staple crops. A field experiment was performed to evaluate the effect of chelated iron on growth, yield and iron concentration in bread wheat (cv. Moomal) at Tando Jam Pakistan. The treatments included, Control (No Fe-EDTA), Soil supplement of Fe-EDTA (@ 2 kg Fe ha^{-1}), Soil + foliar supplement of Fe-EDTA (@ 2 kg ha^{-1} and 0.2% Fe at booting, flowering, and milky stage), and Foliar supplement of Fe-EDTA (@ 0.2% Fe at booting, flowering and milky stages). The defined growth and yield traits of wheat were increased with Fe-EDTA applications over control treatment. Among different Fe-EDTA application methods, there was no significant difference for most of the growth and yield parameters (excluding spike length, number of spikelets $spike^{-1}$, and 1000 grain weight). The amount of Fe in wheat grains was significantly higher in all Fe-EDTA treatments over control, with maximum value (86.54 ± 5.57 mg kg^{-1}) in the treatment where Fe-EDTA was applied in soil + foliar. Similarly, a high Fe build up in surface soil was obtained with treatment of Fe-EDTA in soil + foliar. Overall, with various Fe-EDTA treatments, an increase of 21.2 to 29.1% in grain yield and 1.9 to 4.3 times in Fe concentration of wheat grains was achieved in current study. It is suggested that the Fe should be included in wheat production technology to attain better yield and Fe concentration in grains.

Keywords: Fe-EDTA, Fe-Malnutrition, Biofortification, Wheat production, Fe fertilization.

Introduction

Iron (Fe) is an important element essentially required by all living organisms (Taskin and Gunes, 2022). In humans, Fe insufficiency is a prevalent and significant nutritional deficit that poses a substantial threat to human health. According to an estimate, 34% of the population of the world suffers from Fe deficiency, out of which majority belongs to developing countries (Owaidah et al., 2020).

Among various human groups, children, adolescents, and young and pregnant women are more susceptible to iron deficiency (Abu-Ouf and Jan, 2015; Ramzani et al., 2016; Ali et al. 2020). The primary health consequences associated with iron deficiency comprehends fetal loss, impaired mental abilities, impaired immunity, and reduced work capability (Gombart et al., 2020; Grzeszczak et al., 2020; Midya et al., 2021).

In developing countries, the primary factor contributing to iron malnutrition can be attributed to the consumption of diets with low iron content and/or poor iron assimilation from the ingested food (Liberal et al., 2020). In these countries, the cereal crops are the major food crops which are inherently low in Fe and other micronutrients (Senguttuvel et al., 2023). Low Fe concentration in cereal crops may be associated to low Fe content in soils and its accessibility to plants (Zou et al., 2019). According to an estimate, 30% soils of the

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world are Fe deficient (Boamponsem et al., 2017; Mahender et al., 2019). The problem of Fe deficiency is most common in alkaline and calcareous soils (Rashid, 1996; Hsieh and Waters, 2016). Cultivation of crops in Fe deficient and/or alkaline-calcareous soils reduces the crops growth and productivity, and Fe concentration in grains (Hafeez et al., 2021; Turan et al., 2022).

Agronomic biofortification is a term used to describe the practice of augmenting the nutritional composition of food crops through fertilization techniques (Akram et al., 2020; Bughio et al., 2021). In case of Fe malnutrition in resource-poor populations, agronomic biofortification has emerged as a promising strategy to improve iron intake and combat iron deficiency (Bhardwaj et al., 2022; Kiran et al., 2022). Iron can be supplemented to crop plants using different sources including $FeSO_4$, Fe-EDTA, Fe-DTPA, Fe-EDDHA, Fe-citrate and Fe-IDHA (Chatterjee et al., 2018; Shaddox et al., 2019; Ay et al., 2022). However, the efficiency of Fe sources in promoting crop development and increasing Fe content is influenced by many factors. These factors encompass the solubility and stability of the Fe sources, their ability to permeate through the cuticle when applied foliar, their capacity to be taken up by root cells when applied to the soil, and their ability to be transported to the shoot (Fernández and Brown, 2013).

A little information is present in literature, where the efficiency of Fe-EDTA has been tested with respect to improving Fe density in cereal grains. Many studies have been performed to evaluate the impacts of Fe application (as Fe-Sulphate) for growth and yield improvement (e.g., Abbas et al., 2009; Ali, 2012). Hence, this study is an effort to enhance Fe in wheat crop through agronomic approach (fertilizer application). Wheat (*Triticum aestivum* L.) is selected for this study because it is a chief source of diet in humans particularly in South Asian region, Central Asia and Middle Eastern countries where it provides approximately 50% of the everyday calorie intake (Cakmak et al., 2010; Sial et al., 2018). In Pakistan, wheat is a staple food crop and is cultivated generally on more than 9.0 million hectares area (PES, 2022). We hypothesize that this form of Fe will be more bioavailable to the plants with the ultimate profit of yield and Fe quantity in wheat grains. The present research was laid out with the objectives (i) to determine the effect of Fe application (as Fe-EDTA) on growth, yield and Fe quantity in wheat grains, and (ii) to propose the appropriate method of Fe application that enhances production, and Fe in wheat grains and soil.

Material and Methods

The experiment was performed at the field of Southern Wheat Section, Agriculture Research Institute (ARI) Tando Jam (25°25'00.2"N, 68°32'39.9"E) during Rabi season of 2017-18. A piece of land (10 m × 15 m = 150 m^2) was ploughed, levelled and divided into 16 equal experimental units of 6 m^2 (3 m × 2 m) for the experiment. The seeds of wheat variety "Moomal" were hand drilled at the seed rate of 50 kg per acre.

Experimental Design and Treatments

The research was organized in Randomized Complete Block Design (RCBD). The experiment included four treatments, each repeated four times; T1: Control (No Fe), T2: Soil supplement (2 kg Fe ha^{-1}), T3: Soil + Foliar supplement (2 kg Fe ha^{-1} + 0.2% Fe at booting, flowering and milky stage), and T4: Foliar supplement (0.2% Fe at booting, flowering and milky stage).

Fertilization scheme

The NPK fertilizers were supplemented to all plots @ 168 kg N, 84 kg P_2O_5 and 60 kg K_2O ha^{-1} as recommended by Khokhar (2015). The recommended amount of phosphorus, potassium and a fraction of N was supplemented at the time of sowing, while remaining nitrogen was given as Urea in 2 equivalent splits. The 1^{st} split of N was applied with second irrigation while the 2^{nd} split was given with third irrigation.

Agronomic observations

At harvest, five healthy plants were randomly selected from each replication plot for the selected agronomic observations. Plant height and spike length were recorded with measuring scale. Tillers and spikelets number were manually counted. Thousand grains were counted and weighed on digital balance. For yield purpose, the plants from every replication plot were reaped using 0.5 m^2 wooden frame. Afterwards, the plants were threshed manually and separated into grains and straw, which were weighed on a digital balance. Harvest index was computed by adopting the formula as given by Iqbal et al. (2017).

$$\text{Harvest index (\%)} = \text{grain yield (g)} / \text{biological yield (g)} \times 100$$

Plant analysis

The grains from each replication plot were grinded using grinder machine (Geepas model No. GCG289). The flour samples were digested and analyzed for iron concentration by following the procedure as outlined by Estefan et al. (2013). In brief, 01-gram wheat flour was added with di-acid mixture (HNO_3-$HClO_4$, 2:1), left for overnight, digested, cooled, filtered and raised to 50 ml with distilled water, and analyzed for the Fe concentration using Atomic Absorption Spectrometer (AAS, NOVAA 400, Germany).

Soil sampling and Analysis

Composite soil samples were gathered at the depth of 0-15 cm before land preparation and after harvest of wheat crop using stainless steel auger. The samples (prior to wheat sowing) were thoroughly processed and subjected to the determination of various properties (pH, EC, organic matter content, lime content, texture and Fe) by adopting the standard procedures drafted by Estefan et al. (2013). For Fe analysis, 10 grams soil was added 20 mL AB-DTPA solution, which was shaken on a mechanical shaker, filtered and subsequently used for the determination of Fe concentration by AAS. We recognized that the experimental soil was clay in texture, moderately alkaline in reaction (pH 8.25 ± 0.2), non-saline (EC 0.29 ± 0.06 dS m^{-1}), marginal in organic matter content (1.20 ± 0.12%), moderately calcareous (7.73 ± 0.13%) and adequate in Fe concentration (5.65 ± 0.25 mg kg^{-1}). Soil samples, after harvest of crop, were randomly collected from each replication, processed, and analyzed for Fe concentration only.

Statistical analysis

The gathered plant and soil data were subjected to normality test, Anderson-Darling test, prior to conduct of Analysis of Variance (ANOVA) approach by Minitab 17 software. A one-way ANOVA technique was used to determine the significant difference among treatments' means, at a *P* value of 0.05. Any significant data was further subjected to Tukey's (HSD) test to compute the level of significant difference among treatments.

Results

Effect of Fe-EDTA application methods on growth and yield components of bread wheat

The supplementation of Fe-EDTA resulted in significant improvements in selected aspects of wheat growth and yield compared to the control treatment (Figure 1 and 2, $P<0.05$). The applications of Fe-EDTA resulted in an increase of plant height to 20.1%, tillers per plants to 15.3%, spike length to 20.9%, spikelets per spike to 24.5%, 1000-grain weight to 68.7%, straw yield to 18.3%, grain yield to 29.1%, and harvest index to 4.2% over the control treatment. Except for spike length, number of spikelets per spike, and 1000 grain weight, most of the growth and yield characteristics were found to be statistically identical among the various Fe-EDTA application methods. Notably, the combined application of Fe-EDTA (soil + foliar) resulted in substantially longer spikes, and a greater value for spikelets and grain weight.

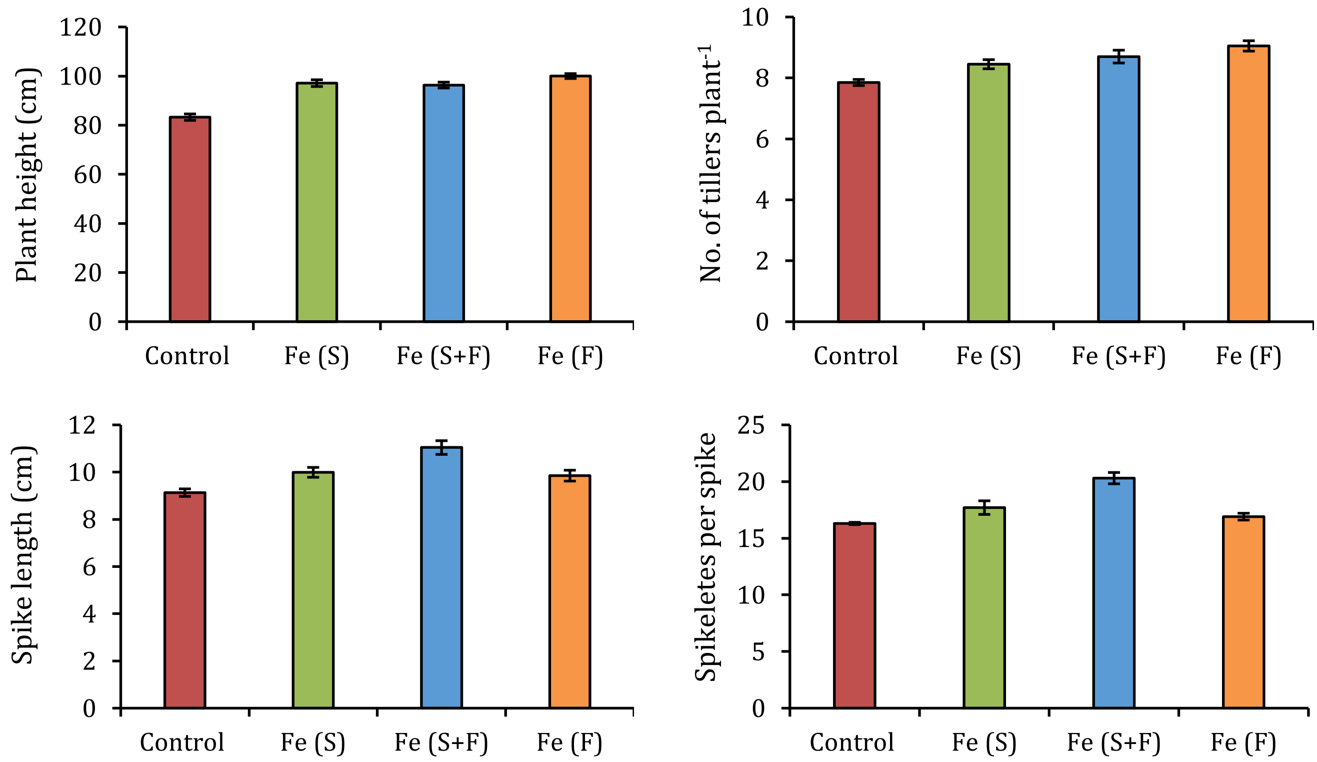

Figure 1. Impact of Fe-EDTA application on growth characteristics of bread wheat
S= (Soil Application), S+F= (Soil + Foliar), F= (Foliar Application)

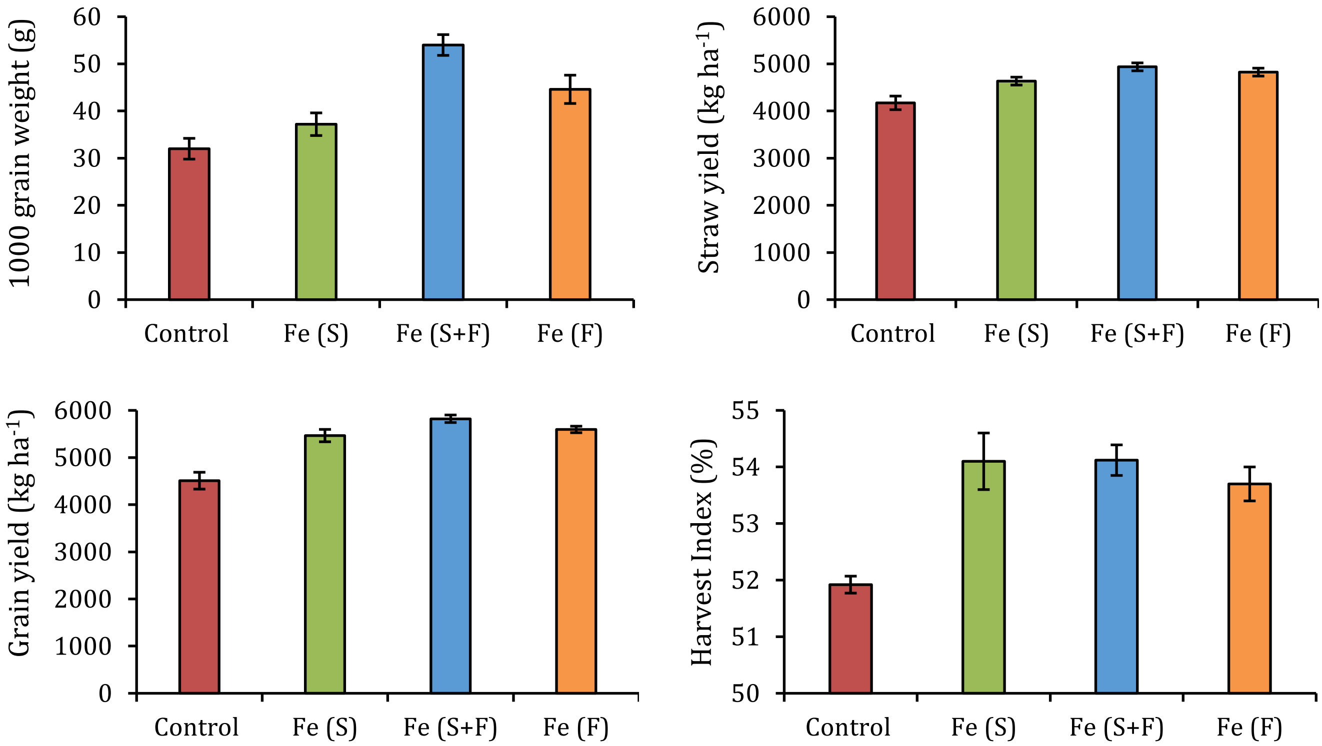

Figure 2. Impact of Fe-EDTA application on yield characteristics of bread wheat

Effect of Fe-EDTA application methods on Fe concentration in grains of bread wheat

The impact of Fe-EDTA applications on the Fe concentration in wheat grains was found statistically significant (P<0.05, Table 1). The wheat plants that received Fe-EDTA applications by defined methods exhibited a notable increase in Fe accumulation in their grains, ranging from 2 to 4 times higher compared to the plants of control group. Significant variations were also observed in Fe accumulation among the different Fe-EDTA application methods, whereby the plants treated with Fe-EDTA through a combination (soil + foliar) exhibited significantly highest Fe concentration (86.54 ± 5.57 mg kg^{-1}) than the other two methods (soil and/or foliar).

Table 1. Fe concentration in grains (mg kg^{-1}) of bread wheat as influenced by Fe-EDTA application

Fe application methods	Fe in grains (mg kg^{-1})	Times increase over control
Control	19.89 ± 2.90 ^{C}	---
Fe-EDTA in soil	38.63 ± 1.44 ^{B}	1.9
Fe-EDTA in soil + foliar	86.54 ± 5.57 ^{A}	4.3
Fe-EDTA foliar	50.60 ± 4.65 ^{B}	2.5

Each value is mean ± SE (*n* = 4); means followed by different letters show significant variation among each other (*P* < 0.05) as a function of Fe-EDTA application methods

Influence of Fe-EDTA application methods on Fe buildup in post-harvest soil

The outcome of Fe-EDTA applications on Fe concentration (mg kg^{-1}) in surface soil was highly significant (P < 0.05, Table 2). Wheat plants which were treated with Fe-EDTA through soil + foliar application significantly enhanced Fe concentration in soil (8.89 ± 1.07 mg kg^{-1}) than the control treatment (6.22 ± 0.25 mg kg^{-1}). With respect to control, the plots which were subjected to Fe-EDTA through various application methods increased the Fe concentration in soil from 3% to 42.9%. No significant difference was found among various Fe-EDTA application methods for Fe concentration in surface soil.

Table 2. Influence of Fe-EDTA application on Fe concentration (mg kg^{-1}) in postharvest soil

Fe application methods	Fe in soil (mg kg^{-1})	% increase over control
Control	6.22 ± 0.25 ^{B}	---
Fe-EDTA in soil	8.17 ± 0.36 ^{AB}	31.3
Fe-EDTA in soil + foliar	8.89 ± 1.07 ^{A}	42.9
Fe-EDTA foliar	6.41 ± 0.30 ^{AB}	3.0

Discussion

The application of Fe-EDTA in bread wheat resulted in substantial improvements in targeted growth and yield parameters, when compared to the control treatment. These findings highlight the importance of iron as a micronutrient for bread wheat and underscore the potential of Fe-EDTA application as an effective Fe source for promoting plant growth, optimizing yield, and improving overall productivity. Iron is very important to the growth of wheat because it is involved in important processes like photosynthesis, chlorophyll formation, enzyme activity, respiration, food uptake, and protein synthesis (Frossard et al., 2000; Wiedenhoeft, 2006). Similar positive impacts of iron on the upgrowth and productivity of wheat have been documented not only in Pakistan but also in other regions worldwide (Abbas et al., 2009; Habib, 2009; Armin et al., 2014; Bakhtiari et al., 2015).

The Fe concentration in wheat grains was significantly higher (two to four times) when the Fe-EDTA was applied in soil, foliar and/or soil + foliar than the control treatment. A rise in iron concentration in grains may be related to Fe application to wheat crop and its better mobility from soil to grains and/or leaf to grains. A good remobilization of Fe from shoots (77% of the whole shoot Fe) to grains of wheat has also been documented previously (Garnett and Graham, 2005). Many studies have shown a rise in iron accumulation in wheat grains with the submission of various Fe treatments (Habib, 2009; Pahlavan-Rad and Pessarakli 2009; Zhang et al., 2010; Aciksoz et al., 2011). However, in current study the increment in iron quantity was many orders of magnitude higher than the cited studies. A high Fe density in wheat grains might be associated to coordination of Fe and EDTA. The EDTA has been documented to escalate the concentration of coordinated ions in plants (Vassil et al., 1998). It is speculated that synthetic chelates (including EDTA) increase the mobility of ions by two mechanisms, (i) by destroying the physiological root barriers that are normally involved in controlling the uptake and mobility of ions, and (ii) destabilizing root surrounding plasma membrane (a barrier forming agent) by removal of its stabilizing ions (e.g., Zn^{2+} and Ca^{2+}) (Vassil et al., 1998). In addition, Lindsay (1995) suggested that chelating agents are useful because they increase the total Fe concentration in soil solution, enhance diffusion gradients and reduce Fe depletion zones in rhizosphere.

Among three Fe-EDTA treatments, there was significantly higher Fe concentration in wheat grains when the wheat crop was subjected to soil + foliar application of Fe-EDTA. Such an increase in wheat grains may be associated to exposure, uptake and absorption of Fe by both pathways (roots and leaves). The combined approach of soil and foliar application provides iron through both root and leaf pathways, increasing the chances of iron absorption by the plants. It facilitates efficient translocation of iron within the plant, aiding its transport to the developing grains. The synergy between soil and foliar applications may further enhance iron uptake and accumulation. These scientific reasons explain the observed increase in iron concentration in wheat grains.

There was a significantly higher Fe concentration in surface soil where the Fe-EDTA was applied in soil + foliar than the plots where no Fe-EDTA application was made. Relatively high Fe concentration in soil + foliar treatment is possibly because of exposure of soil with Fe by both means (soil + droppings during foliar spray). The retention of Fe in soil may be associated to the process of adsorption, whereby inorganic colloids (clays, metal(s) of oxides, hydroxides, carbonates and phosphates) and organic colloids (organic matter, and certain algae and bacteria) retain metal ions (Bradl, 2004). In current study, the texture of the soil was Clay (with > 46% clay fraction) and the organic matter was moderate (> 1%), hence a high adsorption of Fe was expected. A greater retention and lower solubility and bioavailability of Fe is also correlated to high pH and soil calcareousness (Bradl, 2004; Ramzani et al., 2016). In current study, the soil was moderately to slightly alkaline in reaction (pH > 8.0) and moderately calcareous in nature (> 7%), hence high retention of Fe was anticipated. The pH also affects to stability of chelates to bind Fe; it has been documented that EDTA loses significant Fe when the pH increases above 6.0 (Lindsay, 1995).

Despite yielding an obvious outcome in our study that documents the benefits of Fe usage in wheat husbandry, mainly by soil+foliar fertilization, the study also possesses certain limitations. The first limitation pertains to the extent of the experiment. Since the experiment spanned only one growing season, it is crucial to replicate it at multiple locations having varied environmental conditions, soil types and wheat varieties to reach to more robust ending. A second potential limitation relates tothe price of Fe-EDTA, which is relatively higher than the commonly used source of Fe ($FeSO_4$). Future studies should be devised where these two sources of Fe may be compared for their efficiency, economic returns and health benefits.

Conclusion

The applications of Fe-EDTA significantly enhanced the defined growth and yield components, and Fe concentration in wheat grains and post-harvest soil than control treatment. Among the different tested Fe application methods, the outcome of combined soil + foliar supplement of Fe-EDTA was relatively better for most of the parameters. We suggest that the combined soil + foliar application of Fe-EDTA (@ 2 kg Fe ha^{-1} + 0.2% Fe at booting, flowering and milky stage) should be adopted for selected wheat cultivar (Moomal) to attain maximum crop growth and yield, and Fe concentration in grains.

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