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Iron Biofortification of Wheat Grains with Various Iron Fertilization Techniques

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ABSTRACT

Most Pakistani soils are deficient in available iron (Fe), which limits crop productivity. Biofortification offers a sustainable solution by enhancing nutrient content in edible parts. This field experiment, conducted at the Nuclear Institute of Agriculture (NIA), Tandojam during the 2023–24 Rabi season, evaluated the effectiveness of iron application methods on wheat (variety NBW-232). The study used a randomized complete block design (RCBD) with four treatments and three replications on silty loam soil. Treatments included: T1 (control), T2 (12 kg Fe ha⁻¹ via broadcasting), T3 (12 kg Fe ha⁻¹ as Fe-coated urea), and T4 (seed priming with 2% FeSO₄·7H₂O). A uniform NPK dose (150-80-50 kg ha⁻¹) was applied. Results showed that Fe application significantly improved growth, yield, and Fe accumulation, with Fe-coated urea (T3) being the most effective. Iron coated urea (T3) succeeded to obtain the highest number of grains per spike (60.2), grain yield (5533.3 kg ha⁻¹), nitrogen (2.9%), and Fe content (119.3 mg kg⁻¹), while reducing phytic acid (1.807 mg g⁻¹) concentration ($p \leq 0.01$). In conclusion, Fe-coated urea markedly enhanced wheat productivity and its nutritional value.

KEYWORDS: malnutrition, biofortification, Fe-application, novel variety, calcareous soil

INTRODUCTION

Iron (Fe) is a vital micronutrient required by all living organisms, including plants, animals, and humans, for essential metabolic functions throughout their life cycles. In humans, iron is critical for oxygen transport, enzymatic activity, immune function, and cognitive development. However, iron deficiency remains one of the most widespread nutritional disorders globally, particularly in developing countries, where insufficient dietary intake of iron-rich foods contributes to anemia and related health problems (Kumar *et al.*, 2024). Despite its abundance in the Earth's crust as the fourth most common element, the biological availability of iron is limited due to the low solubility of iron compounds, particularly in the form of iron hydroxide, under natural environmental conditions (Baral *et al.*, 2024).

The application of Fe-coated urea not only enhances crop yield and grain nutritional quality but also offers economic advantages. Nitrogen losses via ammonia volatilization, leaching, and runoff can be controlled with coated urea application, thereby improving fertilizer use efficiency and lowering input costs for farmers. This dual benefit makes Fe-coated urea an attractive option for sustainable crop production. Furthermore, biofortification initiatives through micronutrient-enriched fertilizers are increasingly recognized as a cost-effective strategy to address hidden hunger, particularly iron deficiency, which remains a global public health concern.

Biofortification not only improves the nutritional status of staple crops but also contributes to long-term economic and social benefits by reducing malnutrition-related health problems (Bouis & Saltzman, 2017; Cakmak *et al.*, 2020).

Malnutrition in its various forms including under-nutrition, micronutrient deficiency, and over-nutrition affects more than 30% of the global population. Among these, iron deficiency is one of the most prevalent and significant nutritional challenges, especially in developing countries (Naresh *et al.*, 2019). It is estimated that approximately two billion people suffer from iron deficiency globally, with women, children, and populations relying heavily on cereal-based diets being most vulnerable (Naveed *et al.*, 2020). Staple crops such as wheat, rice, and maize, which form the basis of daily diets in many countries, typically contain low levels of iron and other essential micronutrients. Therefore, improving the micronutrient content of these crops is critical for addressing widespread deficiencies (Kiran *et al.*, 2022).

Wheat (*Triticum aestivum* L.) is one of the most important staple crops, supplying nearly 20% of the daily calories and protein intake in the human diet. It plays a central role in food security, serves as a primary source of essential nutrients (Singh *et al.*, 2023). However, wheat grains generally contain low levels of bioavailable iron, making them insufficient

to meet the daily dietary requirements (Gupta *et al.*, 2021).

One of the key factors limiting the iron content in wheat grains is soil-related constraints. While most soils contain adequate total iron, its availability to plants is often restricted, particularly in calcareous and alkaline soils that are high in calcium carbonate. These soils, common in arid and semi-arid regions including parts of Pakistan, tend to immobilize iron, making it unavailable for plant uptake and leading to iron-deficient crops (Sharma *et al.*, 2018). It is estimated that about 30% of the world's cultivated soils are iron-deficient, which in turn affects crop quality and human nutrition (Mahender *et al.*, 2019). In such conditions, added iron fertilizers may quickly convert into insoluble forms, further limiting their effectiveness.

Given these challenges, agronomic biofortification has emerged as a promising and sustainable approach to enhance the micronutrient content of food crops. Unlike conventional food fortification, which involves adding nutrients during food processing, biofortification aims to increase the concentration of essential micronutrients like iron and zinc in crops during their growth cycle, using either plant breeding or nutrient management techniques (Ramzan *et al.*, 2020). Among the agronomic techniques, iron fertilization through soil application, seed priming, and foliar sprays has shown varying levels of success in improving grain iron content. The nitrogen status of a plant can influence iron translocation and accumulation in grains, suggesting that combining nitrogen and iron fertilization could be an effective strategy for improving both yield and nutritional value (Riaz *et al.*, 2017; Wang *et al.*, 2023).

Increasing the concentration and availability of iron in wheat grains can significantly improve dietary intake, particularly in regions with high consumption of wheat-based foods. Furthermore, the use iron-coated urea, offers a practical solution for enhancing nutrient use efficiency and addressing soil nutrient

imbalances. These methods not only improve crop yield but also contribute to human health by enriching the N and Fe content of food (Szerement *et al.*, 2022).

In light of the widespread iron deficiency in both soils and human and nitrogen losses, this study was designed to evaluate the effect of various iron fertilization techniques on wheat performance, nutrient uptake, and grain quality under the specific soil and climatic conditions of Tandojam, Sindh, Pakistan. The core aim of the research was to assess the impact of different Fe application methods including soil broadcasting, seed priming, and Fe-coated urea on the growth, nitrogen and iron content, and yield of wheat. Specifically, this study sought to determine the most efficient mode of iron fertilization that enhances both agronomic performance and nutritional value of wheat grains.

MATERIAL & METHODS

Experimental details: A field experiment was carried out to evaluate the N and Fe content of wheat plants towards Fe applications with various fertilizing techniques during the year 2023-24. A study was conducted at the experimental field of Nuclear Institute of Agriculture (NIA) Tandojam, Sindh. A three replication Randomized Complete Block Design (RCBD) was used to layout the experiment in a plot size of 4.5m × 3.6m = (16.2 m²). Total piece of land was 194.4 m² the land was prepared as per recommended practices before commencement of planting season. Wheat variety (Advance line) "NBW-232" was used in this experiment, the treatments were four, and the application of Fe was applied in three different ways. The source of iron was ferrous sulphate heptahydrate FeSO₄ · 7H₂O. Further, all treatments received the recommended basal dose of phosphorous (80 kg P₂O₅ ha⁻¹) in the form of Di-ammonium phosphate (DAP) 46% P₂O₅ and potassium (50 kg K₂O ha⁻¹) as sulphate of potash (SOP) 50% K₂O. Details regarding treatments are shown in (Table 1).

Table 1. Details of the applied treatments

	Treatments	Fertilizer dose.
T1	Straight Fertilizers (control)	Recommended NPK (150 kg N, 80 kg P ₂ O ₅ , 50 kg k ₂ O ha ⁻¹)
T ₂	Fe application with broadcasting	Recommended NPK+12 kg Fe ha ⁻¹
T ₃	Fe application with coated urea	Recommended PK +12 kg Fe ha ⁻¹
T ₄	Fe application with Seed priming	Recommended NPK+2% of Fe

Note: All treatments received the recommended basal dose of N, P₂O₅, and K₂O, with the Fe treatments applied in different forms as specified excluded coated urea.

Cultivating practices: The wheat crop was harvested at physiological maturity after the wheat were sown at the recommended seed rate of 50–60 kg ha⁻¹ and all suggested cultural and management procedures were followed in the early stages (approximately after 120 days). The canal water was used for irrigation. The first water was applied after 25 days of cultivation and a total of three irrigations were applied, each at 25-30 days intervals, depending on the conditions of the soil.

Nitrogen as urea (containing 46% N) was applied at the rate of 150 kg per hectare, uncoated urea was applied in three splits and coated urea was applied once a time at the seedling stage. Coating of iron+urea was done manually in the laboratory of the department of Soil Science, Sindh Agriculture University, Tandojam. Iron + urea were coated with natural polymers in the form of vegetable oil (Babar et al., 2025). The oil is readily accessibility available

in local markets. The ratio for coating was maintained at 2:1, according to Babar et al., (2024). Seed priming (2% Ferrous Sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)) was prepared by taking 20g of Ferrous sulphate and dissolved in 1000mL of distilled water and soaked the seed in solution for 4-6 hours.

Soil observations: Before planting the crop, soil samples were taken from the experimental field to

analyze the soil's chemical and physical properties. The samples were taken at a depth of 0-30cm. The analysis showed that the soil was silty loam in texture, slightly alkaline, non-saline with low level of organic matter, moderate in calcium carbonate and low in nitrogen content as shown in table 2.

Table. 2. The chemical and physical properties of soil sampled before treatment effect

Characteristics	Soil depth (cm) 0-30
Clay (%)	25
Silt (%)	57.5
Sand (%)	17.5
Textural class	Silty loam
EC (dS m^{-1})	0.58
pH	7.41
Organic matter (%)	0.67
Lime content (%)	7.25
Nitrogen (%)	0.07
Iron content (mg kg^{-1})	4.20

Agronomic observation and analyses: The observation was recorded at the time of harvesting, and data was collected based on five selected plants in each repeat, which were randomly selected and labeled. Number of tillers (m^2), Number of grains spike $^{-1}$, 1000-grains weight (g), Grain yield (kg ha^{-1}), N & Fe content in plants (%), Soil N (%) & Soil Fe (mg kg^{-1}) were recorded after crop maturity. Before sowing, composite soil samples were collected randomly from each block at the depth of 0-30cm, and were analyzed for texture, EC (dSm^{-1}), pH, organic matter (%), lime content (%), N (%) content and Fe (mg kg^{-1}) content (Ryan et al., 2007). After harvesting, plant samples were manually separated, first washed with tap water and then with distilled

water. The samples were then dried, ground, and analyzed for nitrogen and iron content (Ryan et al., 2007). Phytic acid levels were determined colorimetric method proposed by Haug & Lantzsch (1983). The proposed amount of wheat flour was added with the predefined volume of 0.2 N HCl and extracted. From that extract 0.5 ml pipetted in a test tube, in which defined quantity of ferric solution was added. The tubes were boiled for $\frac{1}{2}$ an hour and allowed to cool down. Afterward, 2 mL of 2, 2-Bipyridine solution was added into polypropylene tubes and shaken with hand. The samples were analyzed on Spectrophotometer at the wavelength of 510 nm within one minute. Phytate concentration was calculated by the formula (Bhatti et al., 2021).

$$\text{Phytate (mg kg}^{-1}\text{)} = \text{concentration from spectrophotometer} \times 200 / 0.282$$

According to the chemical and physical properties of the soil at the experimental site before planting, the soil was silty loam with a slightly alkaline reaction (7.42), non-saline (0.58 dS m^{-1}), low organic matter (0.067%), low nitrogen (0.07%), and iron (4.20 mg kg^{-1}) content.

Statistical analysis: The collected data was subjected to statistical analysis using Statistix 8.1 version (Statistics, 2006). Four replications with a randomized complete block design (RCBD) were used to determine significant differences among the means were resolved by Analysis of variance (ANOVA) followed by LSD (Least Significant Difference) was applied to compare the treatment effect at alpha 5% ($p \leq 0.05$).

RESULTS AND DISCUSSION

Effect of Fe treatments on agronomic characters and yield attributes: The details of growth and yield parameters are presented in Table 3. One-way

ANOVA analysis revealed that several agronomic traits of wheat were significantly influenced by the application of Fe treatments. These parameters included the number of tillers per square meter (m^2), grains per spike, seed index (g), and grain yield (kg ha^{-1}). The response of these traits to the applied treatments was statistically significant, with iron-coated urea showing superior performance compared to commercial urea. The highest number of tillers (494), grains spike $^{-1}$ (60), 1000-grain weight (47g), and grain yield (5600 kg ha^{-1}) was noticed in T3, where Fe applied as coated urea. Significant differences ($p \leq 0.05$) were observed in thousand grain weight, with the Fe-coated urea (T3) and Fe seed priming (T4) treatments producing higher values compared to the control and Fe broadcasting treatments. While, the lowest traits in terms of growth and yield was recorded in T1 (Control) where no Fe was applied. Mean comparison among treatments shown a significant difference ($p \leq 0.05$) potentially

attributed to the Fe application and fertilization technique specifically with coating urea with Fe.

Table. 3. Agronomic and yield attributes affected by Fe treatments

Treatments	Number of tillers (m ²)	grains spikes ⁻¹	1000 grain weight (g)	Grain yield (kg ha ⁻¹)
T ₁ Straight Fertilizers (control)	338 ± 7.57 c	48.5 ± 0.35 d	38.1 ± 0.66 b	3916.6 ± 44.1 d
T ₂ Fe application with broadcasting	387.3 ± 6.00 b	50.5 ± 0.35 c	40.3 ± 0.88 b	4150 ± 28.8 c
T ₃ Fe application with coated urea	494 ± 8.810 a	60.2 ± 1.31 a	44.6 ± 0.66 a	5533.3 ± 33.3 a
T ₄ Fe application with Seed priming	382 ± 4.16 b	53.4 ± 0.70 b	46.0 ± 0.57 a	4633.3 ± 60.1 b
SE ± =	6.6847	0.6793	1.1219	68.381
LSD 0.05 =	16.357	1.6622	2.7452	167.32

In the present study growth and yield parameters were significantly affected with application of Fe treatments. This was expected as it hypothesized. Cereals manage iron (Fe) deficiency in two steps: first, their roots release phytosiderophores into the rhizosphere to solubilize Fe³⁺; second, specific transporters absorb the resulting Fe³⁺-phytosiderophore complexes (Marschner, 2012).

Plants have developed mechanisms to absorb, transport, store, and accumulate Fe in their tissues, making them an important dietary source for humans (Tasken & Gunes, 2022). Total Fe concentration in plants doesn't always reflect the amount of metabolically active Fe²⁺. Therefore, total Fe analysis may not accurately indicate Fe sufficiency as it reported (Ciccolini *et al.*, 2017). Marschner (2012) emphasized that active Fe is crucial for plant biochemical processes.

In this study, Fe coated urea application significantly increased active Fe levels compared to the control. To date, numerous studies have focused on alleviating chlorosis caused by iron (Fe) deficiency in various plants, while research on Fe enrichment of edible plant parts remains limited (Pal *et al.*, 2021; Zimbovskaia *et al.*, 2020; Bouis *et al.*, 2011). Based on this gap, the present study investigated the effect of Fe-coated urea and other Fe application methods, such as seed priming and broadcasting with urea, on grain Fe biofortification. The results showed that FeSO₄ + urea treatments significantly improved agronomic traits in wheat, including the number of

tillers per plant (494 m²), grains per spike (60), seed index (46 g), and grain yield (5533.3 kg ha⁻¹). Nutrients' accessibility towards plants plays a pivotal role on growth parameters and ultimately returns with healthy yield. Iron availability supposed to be accessible towards plants due to its coating with urea which slowly and gradually synchronize the nutrients (Babar *et al.*, 2025; Babar *et al.*, 2024).

Effect of Fe treatments on Fe & N content in wheat grains: The application of Fe treatments significantly increased iron (Fe) content in wheat grains, as shown in Table 4. The analysis confirmed that Fe-coated urea treatments led to greater Fe accumulation in grains compared to the control ($p \leq 0.01$). Among all treatments, the highest Fe content (48.0 mg kg⁻¹) was recorded in treatment 3, while the lowest (26.3 mg kg⁻¹) was observed in the control (T₁), where no Fe-application was applied. These findings demonstrate the effectiveness of Fe treatments in enhancing Fe concentration in grains and addressing iron deficiency in wheat. Additionally, nitrogen (N) content in wheat grains also improved significantly with the use of various iron fertilization methods, as indicated in Table 3. According to data, Fe-coated urea significantly increased grain N content. The highest N content (2.92%) was recorded in T₄, while the lowest (2.1%) was found in the control treatment (T₁). These results suggest that Fe application in every form along with urea not only improves Fe biofortification but also supports better nitrogen uptake and accumulation in wheat grain

Table. 4. Effect of Fe treatments on Fe and N content in wheat grains

s	Treatments	Fe content (mg kg ⁻¹)	N content (%)
T ₁	Straight Fertilizers (control)	26.3±0.86 d	2.14 ± 0.030 d
T ₂	Fe application with broadcasting	33.0±1.01 c	2.51 ± 0.049 b
T ₃	Fe application with coated urea	48.0±1.47 a	2.90 ± 0.011 a
T ₄	Fe application with Seed priming	39.8±2.54 b	2.35 ± 0.028 c
	S.E ± =	1.0278	0.050
	LSD 0.05 =	4.0401	0.124

The effectiveness of iron sulfate (FeSO₄) in improving iron uptake by plants can be attributed to its simpler chemical properties. Unlike chelated forms of iron, which must be reduced before entering the cell, FeSO₄ can easily pass through the plasmalemma (cell membrane), making it more readily available to plants (Rios *et al.*, 2016). Moreover, due to its low molecular weight and simpler structure, FeSO₄ can

penetrate the leaf surface more efficiently than complex chelated iron compounds (Chakraborty *et al.*, 2014).

Urea also plays a vital role in this process. It is known for its high ability to penetrate leaf tissues and is quickly absorbed by plants. Once inside the plant, urea is hydrolyzed in the cytosol, which supports the movement of carbohydrates within the plant. This

process enhances the photosynthetic activity of leaves, improving the plant's overall health and nutrient transport efficiency (Pal *et al.*, 2021).

Furthermore, different nitrogen (N) compounds such as amino acids, peptides, and nicotinamide have been shown to aid in the loading of iron into the phloem, which is the tissue responsible for long-distance nutrient transport. These compounds support the movement of iron from the leaves to the developing grains, contributing to better Fe accumulation in edible parts like wheat grains (Zulfiqar *et al.*, 2020). Yang and Zhang (2006) reported that adequate nitrogen availability slows down plant aging and extends the grain-filling period, allowing more time for essential nutrients like Fe and Zn to be transported to the grains. Similarly, Kutman *et al.* (2011) found that when plants receive sufficient nitrogen, up to 60% of the total Fe content can be successfully transferred to the grains. In contrast, under nitrogen-deficient conditions, only about 38% of Fe is mobilized to the grains. In summary, both FeSO₄ and nitrogen individually and in combination play a key role in improving iron uptake, transport, and accumulation in wheat. Their combined use not only enhances nutrient efficiency but also supports better grain quality and yield. The nutrient uptake is consequently enhanced in the current research.

Reduced dietary intake of iron is a major contributor to iron deficiency, which affects a

substantial portion of the global population (Mushtaq *et al.*, 2024). Enhancing the iron concentration in food crops has therefore become an urgent priority to mitigate health issues associated with this deficiency. The results of this research indicate that advanced fertilization techniques such as the application of Fe-coated urea can play a critical role in improving the nutritional quality of crops. This approach significantly increased the Fe concentration in wheat grains (119.3 ± 0.52 mg kg⁻¹) and nitrogen content ($2.9 \pm 0.011\%$) in wheat. These findings underscore the potential of Fe-coated urea as a valuable agronomic practice for addressing both crop productivity and nutritional challenges in a sustainable manner.

Effect of Fe treatments on phytic acid (PA) concentration in wheat grains: The effects of Fe-coated urea on phytic acid (PA) concentration (mg g⁻¹) in wheat grains are presented in Table 5. The data indicate that PA concentration in wheat grains was significantly reduced by the fertilization treatments compared to the control. The highest PA concentration was recorded in T1 (control) at 5.116 mg g⁻¹, while the lowest was observed in T3 at 1.807 mg g⁻¹. Mean comparison among treatments revealed a highly significant difference ($p \leq 0.01$), which was most likely due to the fertilization technique involving the coating material.

Table 5. Effect of Fe treatments on phytic acid (PA) concentration (mg g⁻¹) in wheat grains

	Treatments	Means $\pm$ S.E
T ₁	Straight Fertilizers (control)	5.117 $\pm$ 0.12 a
T ₂	Fe application with broadcasting	4.325 $\pm$ 0.23 b
T ₃	Fe application with coated urea	1.807 $\pm$ 0.12 d
T ₄	Fe application with Seed priming	3.635 $\pm$ 0.25 c
	S.E $\pm$ =	0.2373
	LSD _{0.05} =	0.5808

Phytic acid reduced under T3 (Fe coated urea) than under control (Table 5), indicating that availability should decrease under available N conditions. The highly reduced phytic acid concentration under Fe-coated urea was probably due to the fact that most N availability would lead to reduced phytic acid. It has been reported that high N treatment reduced phytic acid and increased Fe content in rice (Zhao *et al.*, 2007). Similarly in pearl millet, cultivars with low phytic acid had high Fe and Zn contents (Krishnan *et al.*, 2007). This finding is in accordance with our study. Significant correlations between N fertilization and Fe and Zn concentration in wheat grains has been previously reported (Bouis & Welch, 2010). Phytic acid was reduced by increasing N concentration in barley. Phytic acid possesses anti-nutritional characteristics (Vashishth *et al.*, 2017) which are associated with decreased bioavailability of Fe and Zn (Eagling *et al.*, 2014). Therefore, the current results indicate that in areas experiencing sufficient Fe and N make these nutrients more available, which can combat malnutrition and improve human health. It has

been reported that 55% of Fe was absorbed when the phytic acid:Fe ratio was less than 5, while 35% of Fe was absorbed when the ratio was between 5 and 15, and only 15% was absorbed when the ratio was higher than 15 (Magallanes *et al.*, 2017).

CONCLUSION

The current study concluded that the application of Fe-coated urea was more effective in improving growth, yield, and Fe content (mg kg⁻¹) in wheat grains. Grain yield and Fe concentration significantly increased with the use of the coated material. Moreover, the coating material also reduced nitrogen (N) losses. Phytic acid concentration decreased as iron application was efficiently accessed towards grains. These findings suggest that the application of Fe-coated urea fertilizer not only increases crop yields but helps to alleviates human Fe malnutrition and minimizes environmental pollution and fertilization costs.

RECOMMENDATIONS

Future studies should be refined to include multi-location and multi-season trials across diverse wheat varieties to ensure broader applicability and validation of the findings.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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AUTHORS' CONTRIBUTION

Babar, S. K. conceptualized the study and designed the experiments. Mashori, Z. performed data collection and analysis. Babar, S.K., and Mashori, Z. wrote the original draft, and Depar, N.D., and Shujrah, H. reviewed and edited the manuscript. Aydin Gunes contributed expertise in soil fertility and plant nutrition, assisted in refining the experimental methodology, and provided critical input in data interpretation and strengthening the discussion. All authors read and approved the final manuscript.

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