NARDI FUNDULEA, ROMANIA ROMANIAN AGRICULTURAL RESEARCH, NO. 42, 2025
www.incda-fundulea.ro; doi.org/10.59665/rar4239 First Online: March, 2025. DII 2067-5720 RAR 2025-138

Sustainable Iron Enrichment in Wheat: Leveraging Fertilization and Microbial Solubilization for Higher Yield and Nutrient Density

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ABSTRACT

Globally, micronutrient deficiencies in diets are widespread, posing a significant health concern for over 2 billion people. Addressing malnutrition has become a primary objective for agriculture due to the emphasis on productivity. Field experiments conducted at the Agricultural Research Institute (ARI) Tarnab, Peshawar, Pakistan, employed a factorial randomized block design to assess the impact of wheat genotypes (TRB-29-116 - Advance line, TRB-02-103 - Advance line, and Pirsabak-19 - approved variety), iron (Fe) application methods (no Fe application, seed priming with 0.5% Fe, soil application of 10 kg ha^{-1} Fe, and foliar application of 0.5% Fe), and the use of Iron-Solubilizing Bacteria (FeSB) (with bacteria and without bacteria) to combat Fe deficiency. The study revealed significant effects on wheat's Fe content, uptake, and grain yield, influenced by genotypic variance, Fe application approaches, and FeSB. The TRB-29-116 (Advance line) genotype, with 0.5% foliar Fe and FeSB, excelled, enhancing grain yield, grain and straw Fe content, and increasing grain, straw, and total Fe uptake in wheat. These findings prompt further discussion on optimizing agricultural practices. In conclusion, utilizing the TRB-29-116 (Advance line) genotype with 0.5% foliar Fe application and FeSB enhances wheat's Fe content, uptake, grain quality, and addresses malnutrition.

Keywords: iron agronomic biofortification, FeSB, genotypes, yield, Fe content, Fe uptake.

INTRODUCTION

Globally, deficiencies in dietary micronutrients are widespread, presenting a significant health concern for over 2 billion people (Velu et al., 2014; Zhao et al., 2020; Zulfiqar et al., 2020). Following the Green Revolution, scientists shifted their primary focus from improving the quality of edible crop parts to enhancing productivity (Cakmak et al., 2010; Yang et al., 2019). This shift in focus towards productivity is a key reason why addressing malnutrition has become a primary objective for agricultural scientists (Ramzan et al., 2020). Fe and zinc (Zn) deficiencies are the most prevalent micronutrient disorders globally. Fe deficiency can lead to anemia and complications during pregnancy (Black et al., 2008; Han et al., 2024), Fe deficiency can manifest in various ways, including tiredness and a weakened immune system, diminished work capacity and intellectual performance, impaired cognitive development, slowed growth, and compromised

Received 18 February 2025; accepted 17 March 2025.

reproductive performance (Bouis, 2002). The monotonous and excessive consumption of wheat-based products has contributed significantly to the rise of malnutrition. Wheat, being one of the staple crops, is consumed as a primary food source by 1.2 billion people worldwide (Iqbal et al., 2018). In 2019, wheat cultivation globally covered an area of approximately 214.7 million hectares (M ha), making it the second-highest cereal crop. The production of wheat reached 749 million tons (MT), trailing only behind maize (Prospects, 2020; Peng et al., 2025). In Pakistan in the year 2018, wheat was cultivated on an area of 8.79 million hectares, resulting in a production of 25.076 million tons. This production surplus exceeded the country's demand (GOP Agriculture, 2019). In rural regions, 70% of daily caloric intake is derived from wheat, with 60% of the population considering wheat as a staple in their basic dietary food (Raza et al., 2019). The demand for wheat to sustain the growing global population is projected to rise by up to 40% by the year 2050, emphasizing the need to enhance food security measures (Khaled et al., 2018; Akhtar et al., 2019). According to a survey conducted by (Vose, 1982), 25-30% of soil is characterized as calcareous and deficient in Fe. Around the world, it is estimated that approximately 50% of soils under wheat cultivation exhibit Zn deficiency (Zhao et al., 2014; Lin et al., 2025). Zn and Fe deficiencies are more prevalent in Pakistani soil characterized by high pH, free $CaCO_3$, and HCO_3^-, which hinder the accessibility of Fe and Zn to plants (Imtiaz et al., 2010; Akhtar et al., 2019). The prevalence of micronutrient-deficient soils is on the rise, attributed to the frequent cultivation of higher-yielding crops and the intensive application of fertilizers, including nitrogen, potassium, and phosphorus (Salim and Raza, 2020). In plants Fe plays a crucial role in chlorophyll synthesis as it serves as a component of cytochromes and is integral to electron transport processes (Soetan et al., 2010; Wang et al., 2024). Fe deficiency in plants leads to a reduction in the activity of various enzymes, including catalase and peroxidase, which contain porphyrin as a prosthetic group (Hsu and Miller, 1968). Fe chlorosis is also induced by bicarbonate ions (HCO_3^-), which impairs the mechanism of Fe uptake in plants (Coulombe et al., 1984). Among various fortification techniques, agronomic biofortification stands out as the most cost-effective, rapid, and sustainable strategy to enhance the micronutrient content in wheat grains. This approach is particularly crucial in addressing widespread Zn and Fe deficiencies in human populations (Cakmak, 2008). Fe sulphate ($FeSO_4$) is one of the most widely used inorganic fertilizers for providing Zn and Fe to crops. This is attributed to its high solubility and cost-effectiveness, making it a popular choice in agricultural applications (Aciksoz et al., 2011; Wang et al., 2012; Zhang et al., 2025). There is compelling evidence supporting the effectiveness of Zn and Fe fertilizers in enhancing wheat grain concentrations and economic yield, particularly in regions characterized by Zn and Fe deficiencies (Yilmaz et al., 1997; Ramzan et al., 2020). The application of zinc sulphate ($ZnSO_4$) and Fe sulphate ($FeSO_4$) has been reported as an efficient method for enhancing the quality of wheat grains (Rengel et al., 1999; Wang et al., 2012; Yao et al., 2014). Given the highlighted concerns, this study aims to assess the impact of Fe fertilization and the application of FeSB on grain yield, Fe content, uptake across different wheat genotypes and the goal is to address and mitigate malnutrition.

MATERIAL AND METHODS

Field experiments were carried out during the winter seasons at the Agricultural Research Institute (ARI) Tarnab, Peshawar, located in Khyber Pakhtunkhwa, Pakistan. The first experiment was conducted from 2021 to 2022 and was repeated in the 2022 to 2023 season. The average monthly temperatures and rainfall during the growing periods are depicted in Figure 1. The experimental site soil, classified as Alfisol, had a composition of 2% sand, 88% silt, and 10% clay. The soil's pH was recorded at 8.1, with an electrical conductivity (EC) of 0.48 dS m^{-1}. Additionally, the soil contained 0.46% organic matter, 6.75% calcium carbonate ($CaCO_3$), 0.086 mg kg^{-1} nitrogen, 9.03 mg kg^{-1} phosphorus, 87 mg kg^{-1} potassium, and 0.98 mg kg^{-1} iron.

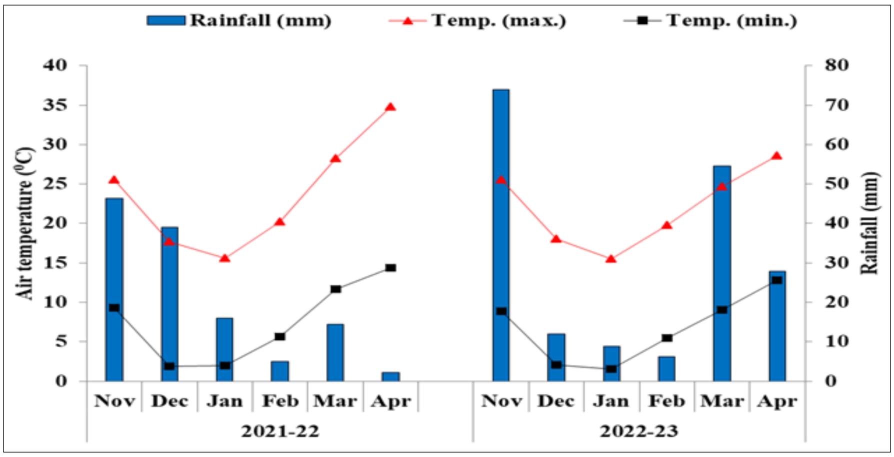


Figure 1. Agro-meteorological conditions during winter seasons 2021-2022 and 2022-2023

The experiments were conducted using a randomized complete block design (RCBD) with a factorial arrangement and were replicated three times. The study examined three variables:

(A) Three wheat genotypes - G1: TRB-29-116 advanced line, G2: TRB-02-103 advanced line, and G3: Pirsabak-19 approved variety.

(B) Four methods of Fe application - AM1: no Fe application (control); AM2: Fe seed priming, which involved soaking wheat seeds in an aerated Fe solution at a concentration of 0.5% for 12 hours, with artificial aeration provided by an aquarium pump. The seeds were then rinsed with distilled water, dried, and returned to their original weight; AM3: Fe soil application at 10 kg ha^{-1} during seed bed preparation; AM4: foliar Fe spray at a concentration of 0.5% applied using a manually operated knapsack sprayer at the crop's heading stage. $FeSO_4 \cdot 7H_2O$ was used as the Fe source in all treatments.

(C) Two methods of FeSB application - BF1: application of FeSB (Bacillus sp. strain MN54, accession number KT375574) and BF0: no FeSB application. The FeSB solution was prepared from strains provided by the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, and applied to the soil at a rate of 5 L ha^{-1} before sowing.

The wheat crop was manually sown using a hand hoe, with rows spaced 30 cm apart and each row measuring 3 meters in length, at a seeding rate of 120 kg ha^{-1}. Fertilization followed the recommended N: P: K ratio of 120-90-60 kg ha^{-1}, utilizing urea, di-ammonium phosphate (DAP), and sulphate of potash. Half of the recommended nitrogen (N) dose, along with all of the phosphorus (P) and potassium (K), was applied at the time of sowing. The remaining nitrogen was divided into two equal portions and applied during the first and second irrigation sessions. Field preparation included two passes with a cultivator, followed by a rotavator to break up clods and achieve a fine soil texture. Each plot measured 3 × 1.8 m, consisting of six rows. The iron (Fe) content in both grain and straw was determined using atomic absorption spectroscopy, as described by (Wright and Stuczynski, 1996). Fe uptake and associated parameters contributing to its utilization were subsequently calculated using specific equations.

Statistical Analysis

The data were analyzed using Statistics 8.1 software (Analytical Software, Statistix; Tallahassee, FL, USA, 1985–2003). Post hoc comparisons were performed using Fisher's least significant difference (LSD) test at a significance level of $p \leq 0.05$ to differentiate and compare the means of various treatments. Additionally, multivariate analysis was conducted to identify strong Pearson correlations among different variables, with Biplot and correlation plots generated using the Origin-Pro 2023b software.

RESULTS AND DISCUSSION

Grain yield (t ha^{-1})

In a two-year study (2021-2022 and 2022-2023), the performance of various genotypes, application methods, and the use of biofertilizer was evaluated for their impact on crop yield (Table 1). The genotypes TRB-29-116 and TRB-02-103, both advanced lines, demonstrated higher mean yields of 4.47 and 4.03, respectively, compared to the approved variety Pirsabak-19, which had a mean yield of 3.92. Among application methods, foliar application of Fe at 0.5% yielded the highest mean of 4.72, significantly outperforming other methods such as soil application (4.27), seed priming (3.94), and control (3.63). The use of biofertilizer (FeSB) also resulted in a higher mean yield (4.47) compared to the treatment without FeSB (3.81). Notably, genotype and application method interactions were significant (P=0.000), as were genotype and biofertilizer interactions (P=0.016), indicating that these factors play crucial roles in optimizing yield. Yearly differences and other interactions were not statistically significant (Figure 2).

Table 1. Mean phenotypic data values

Treatment	Year		Mean
	2021-22	2022-23	
Genotypes (G)			
Genotypes (G)			
TRB-29-116 (Advance line)	4.42^{a}	4.53^{a}	4.47^{a}
TRB-02-103 (Advance line)	3.98^{b}	4.07^{b}	4.03^{b}
Pirsabak-19 (Approved variety)	3.92^{c}	3.92^{c}	3.92^{c}
LSD $_{(0.05)}$	0.12	0.16	0.12
Application methods (AM)			
Control	3.61^{d}	3.64^{d}	3.63^{d}
Seed Priming of Fe @ 0.5%	3.92^{c}	3.97^{c}	3.94^{c}
Soil application of Fe @ 10 kg ha^{-1}	4.21^{b}	4.33^{b}	4.27^{b}
Foliar application of Fe @ 0.5%	4.70^{a}	4.75^{a}	4.72^{a}
LSD $_{(0.05)}$	0.14	0.18	0.14
Biofertilizer (BF)			
With FeSB	3.78^{b}	3.85^{b}	3.81^{b}
Without FeSB	4.44^{a}	4.50^{a}	4.47^{a}
LSD $_{(0.05)}$	0.10	0.13	0.10
Year means	4.10^{a}	4.17^{a}	NS
Interactions effects	P value		P value
G × AM	0.000	Y × AM	NS
G × FeSB	0.016	Y × G × AM	NS
AM × FeSB	NS	Y × FeSB	NS
G × AM × FeSB	NS	Y × AM × FeSB	NS
Y × G	NS	Y × G × FeSB	NS
		Y × G × AM × FeSB	NS

The data represent the replicated mean, and the different letters within columns indicate significant differences at p≤0.05, as determined by the least significant difference test (LSD).

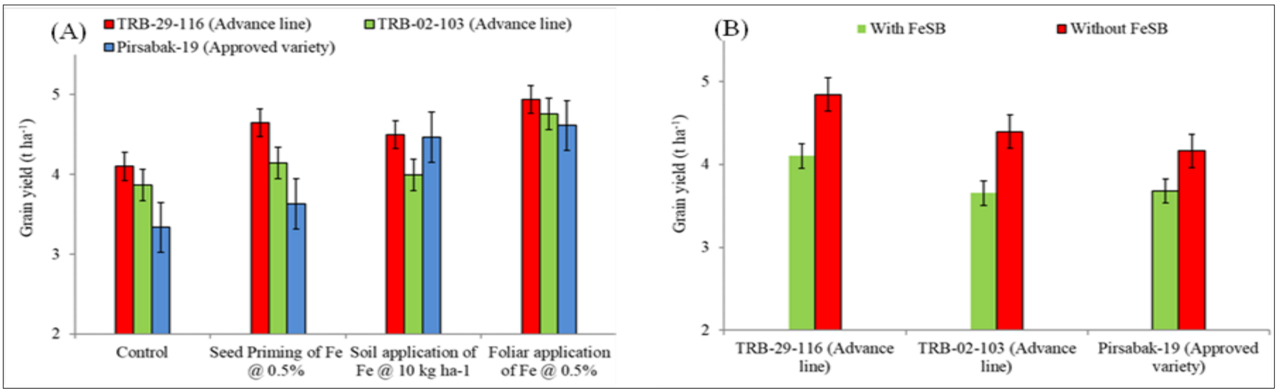


Figure 2. Interaction effects of genotypes and Fe application methods (A), genotype and FeSB (B), on grain yield. The data represent the replicated mean with standard error bar, and the different letters within columns indicate significant differences at p≤0.05, as determined by the least significant difference test.

Grain and Straw Fe Content PPM

Table 2 shows the wheat grain Fe content (PPM) affected by genotypes (G), Fe application methods (AM), and Fe-solubilizing biofertilizer (BF). Among genotypes, TRB-29-116 (Advance line) had the highest mean Fe content (46.15 PPM), followed by TRB-02-103 (44.31 PPM) and Pirsabak-19 (44.32 PPM). Foliar Fe application at 0.5% resulted in the highest Fe content (46.00 PPM), compared to soil application (45.01 PPM), seed priming (44.49 PPM), and control (44.21 PPM). Crops without FeSB showed higher Fe content (46.13 PPM) than those with FeSB (43.73 PPM). Significant interactions were found between genotype and application method (P=0.007), genotype and biofertilizer (P=0.000), and application method and biofertilizer (P=0.000), indicating these factors influence grain Fe content. No significant interactions were observed for G × AM × BF (Figure 3).

Table 2. Grain Fe content PPM of wheat as affected by genotypes, Fe application methods and FeSB

Treatment	Year		Mean
	2021-22	2022-23	
Genotypes (G)			
Genotypes (G)			
TRB-29-116 (Advance line)	45.21^{a}	47.10^{a}	46.15^{a}
TRB-02-103 (Advance line)	43.31^{b}	45.31^{b}	44.31^{b}
Pirsabak-19 (Approved variety)	43.32^{b}	45.32^{b}	44.32^{b}
LSD $_{(0.05)}$	0.59	0.84	0.67
Application methods (AM)			
Control	43.28^{bc}	45.14b	44.21^{bc}
Seed Priming of Fe @ 0.5%	43.49^{b}	45.49b	44.49^{b}
Soil application of Fe @ 10 kg ha^{-1}	44.01^{b}	46.01b	45.01^{b}
Foliar application of Fe @ 0.5%	45.00^{a}	47.00a	46.00^{a}
LSD $_{(0.05)}$	0.68	0.97	0.77
Biofertilizer (BF)			
With FeSB	42.77^{b}	44.70^{b}	43.73^{b}
Without FeSB	45.13^{a}	47.13^{a}	46.13^{a}
LSD $_{(0.05)}$	0.48	0.68	0.55
Year means	43.94	43.91	0.000
Interactions effects	P value		P value
G × AM	0.007	Y × AM	NS
G × FeSB	0.000	Y × G × AM	NS
AM × FeSB	0.000	Y × FeSB	NS
G × AM × FeSB	NS	Y × AM × FeSB	NS
Y × G	NS	Y × G × FeSB	NS
		Y × G × AM × FeSB	NS

The data represent the replicated mean, and the different letters within columns indicate significant differences at p≤0.05, as determined by the least significant difference test (LSD).

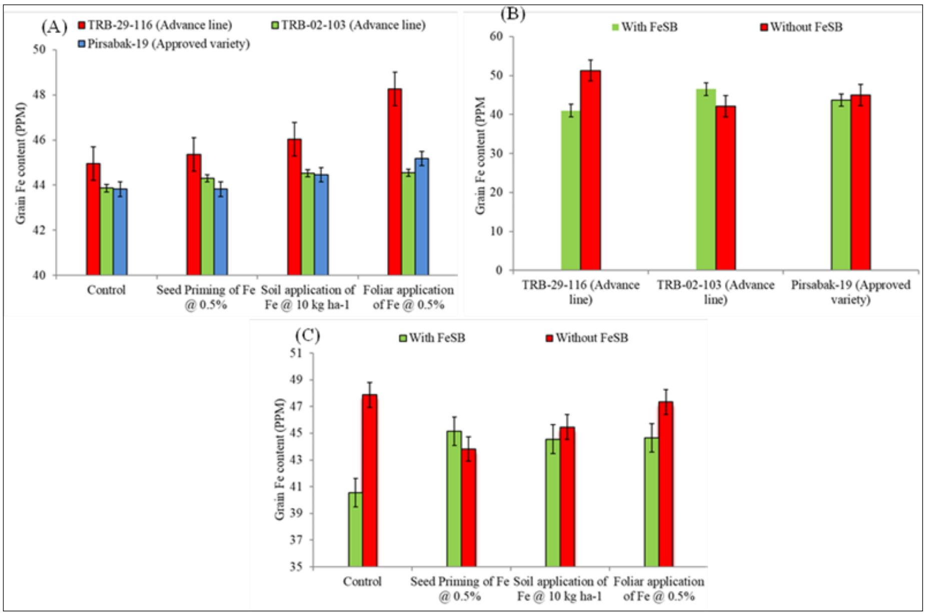


Figure 3. Interaction effects of genotypes and Fe application methods (A), FeSB and genotype (B), and Fe application methods and FeSB (C) on grain Fe content.
The data represent the replicated mean with standard error bar, and the different letters within columns indicate significant differences at $p \leq 0.05$, as determined by the least significant difference test.

Table 3 presents the straw Fe content (PPM) of wheat, influenced by genotypes (G), Fe application methods (AM), and Fe-solubilizing biofertilizer (BF). Among genotypes, TRB-29-116 (Advance line) had the highest mean Fe content (122.28 PPM), followed by Pirsabak-19 (121.61 PPM) and TRB-02-103 (116.02 PPM). For Fe application methods, foliar application at 0.5% resulted in the highest mean Fe content (129.26 PPM), followed by soil application at 10 kg ha^{-1} (126.37 PPM), seed priming at 0.5% (123.32 PPM), and control (100.93 PPM). Treatments without FeSB had higher mean Fe content (122.66 PPM) compared to those with FeSB (117.27 PPM). Significant interaction effects were observed for genotype and application method (P=0.000), genotype and biofertilizer (P=0.000), application method and biofertilizer (P=0.000), genotype, application method, and biofertilizer (P=0.000), and year, genotype, application method, and biofertilizer (P=0.009). These interactions indicate that the choice of genotype, application method, and biofertilizer significantly impacts straw Fe content (Figure 4).

Table 3. Straw Fe content PPM of wheat as affected by genotypes, Fe application methods and FeSB

Treatment	Year		Mean
	2021-22	2022-23	
Genotypes (G)			
Genotypes (G)			
TRB-29-116 (Advance line)	120.84[a]	126.71[b]	122.28[a]
TRB-02-103 (Advance line)	112.17[b]	119.86[c]	116.02[b]
Pirsabak-19 (Approved variety)	116.93[b]	123.28[a]	121.61[a]
LSD $_{(0.05)}$	6.21	2.80	3.91
Application methods (AM)			
Control	96.10[b]	105.75[c]	100.93[c]
Seed Priming of Fe @ 0.5%	119.75[a]	126.89[b]	123.32[ab]
Soil application of Fe @ 10 kg ha^{-1}	124.11[a]	128.62[b]	126.37[a]
Foliar application of Fe @ 0.5%	126.65[a]	131.88[a]	129.26[a]
LSD $_{(0.05)}$	7.17	3.23	4.52
Biofertilizer (BF)			
With FeSB	112.68b	121.87b	117.27[b]
Without FeSB	120.62a	124.70a	122.66[a]
LSD $_{(0.05)}$	5.07	2.28	3.19
Year means	116.64	123.28	0.000
Interactions effects	P value		P value
G × AM	0.000	Y × AM	NS
G × FeSB	0.000	Y × G × AM	0.006
AM × FeSB	0.000	Y × FeSB	NS
G × AM × FeSB	0.000	Y × AM × FeSB	NS
Y × G	NS	Y × G × FeSB	NS
		Y × G × AM × FeSB	0.009

The data represent the replicated mean, and the different letters within columns indicate significant differences at p≤0.05, as determined by the least significant difference test (LSD).

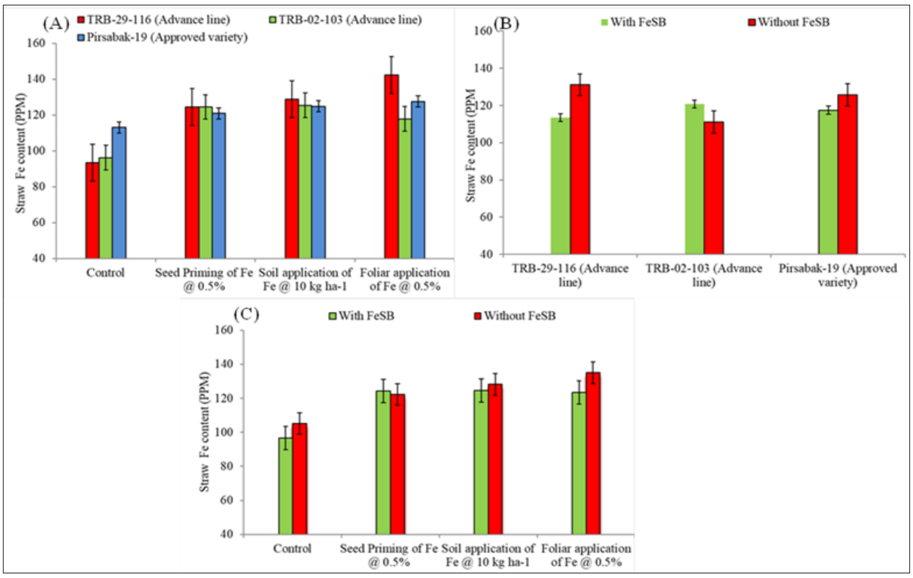


Figure 4. Interaction effects of genotypes and Fe application methods (A), FeSB and genotype (B), and Fe application methods and FeSB (C) on straw Fe content.
The data represent the replicated mean with standard error bar, and the different letters within columns indicate significant differences at p≤0.05, as determined by the least significant difference test.

Grain, Straw and Total Fe Uptake g ha^{-1}

Table 4 presents the grain Fe uptake (g ha^{-1}) of wheat as influenced by genotypes (G), Fe application methods (AM), and Fe-solubilizing biofertilizer (BF). Among genotypes, TRB-29-116 (Advance line) showed the highest mean Fe uptake (208.45 g ha^{-1}), followed by TRB-02-103 (177.45 g ha^{-1}) and Pirsabak-19 (174.48 g ha^{-1}). For Fe application methods, foliar application at 0.5% resulted in the highest mean Fe uptake (217.31 g ha^{-1}), followed by soil application at 10 kg ha^{-1} (192.61 g ha^{-1}), seed priming at 0.5% (175.62 g ha^{-1}), and control (161.62 g ha^{-1}). Treatments without FeSB had a higher mean Fe uptake (206.96 g ha^{-1}) compared to those with FeSB (166.62 g ha^{-1}). Significant interaction effects were observed for genotype and application method (P=0.000), genotype and biofertilizer (P=0.000), application method and biofertilizer (P=0.000), and various year-related interactions, indicating that the combination of genotype, application method, and biofertilizer significantly impacts grain Fe uptake (Figure 5).

Table 4. Grain Fe uptake g ha^{-1} of wheat as affected by genotypes, Fe application methods and FeSB

Treatment	Year		Mean
	2021-22	2022-23	
Genotypes (G)			
Genotypes (G)			
TRB-29-116 (Advance line)	201.79^{a}	215.11^{a}	208.45^{a}
TRB-02-103 (Advance line)	171.45^{b}	183.44^{b}	177.45^{b}
Pirsabak-19 (Approved variety)	170.52^{b}	178.43^{b}	174.48^{b}
LSD $_{(0.05)}$	6.06	7.78	6.46
Application methods (AM)			
Control	157.54^{c}	165.69^{d}	161.62^{d}
Seed Priming of Fe @ 0.5%	170.57^{b}	180.68^{c}	175.62^{c}
Soil application of Fe @ 10 kg ha^{-1}	185.59^{b}	199.64^{b}	192.61^{b}
Foliar application of Fe @ 0.5%	211.31^{a}	223.31^{a}	217.31^{a}
LSD $_{(0.05)}$	6.99	8.98	7.46
Biofertilizer (BF)			
With FeSB	161.28^{b}	171.96^{b}	166.62^{b}
Without FeSB	201.22^{a}	212.70^{a}	206.96^{a}
LSD $_{(0.05)}$	4.95	6.35	5.27
Year means	181.25	192.33	0.000
Interactions effects	P value		P value
G × AM	0.000	Y × AM	0.000
G × FeSB	0.000	Y × G × AM	0.007
AM × FeSB	0.000	Y × FeSB	0.000
G × AM × FeSB	0.000	Y × AM × FeSB	NS
Y × G	0.000	Y × G × FeSB	0.000
		Y × G × AM × FeSB	0.000

The data represent the replicated mean, and the different letters within columns indicate significant differences at p≤0.05, as determined by the least significant difference test (LSD).

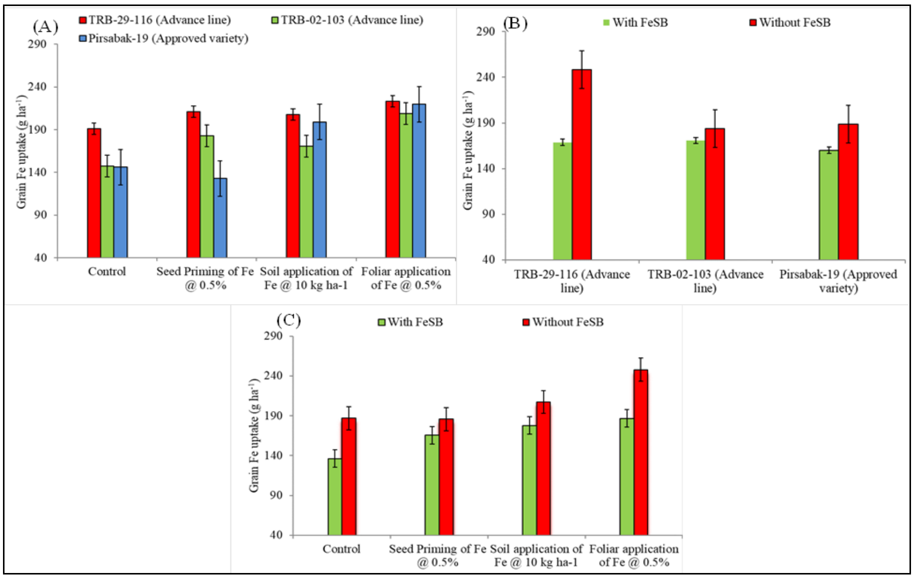


Figure 5. Interaction effects of genotypes and Fe application methods (A), FeSB and genotype (B), and Fe application methods and FeSB (C) on grain Fe uptake.
The data represent the replicated mean with standard error bar, and the different letters within columns indicate significant differences at p≤0.05, as determined by the least significant difference test.

Table 5 depicts the straw Fe uptake (g ha^{-1}) of wheat influenced by genotypes (G), Fe application methods (AM), and Fe-solubilizing biofertilizer (BF). Among genotypes, TRB-29-116 (Advance line) exhibited the highest mean Fe uptake (748.75 g ha^{-1}), followed by TRB-02-103 (621.25 g ha^{-1}) and Pirsabak-19 (610.42 g ha^{-1}). Regarding Fe application methods, foliar application at 0.5% resulted in the highest mean Fe uptake (812.61 g ha^{-1}), followed by soil application at 10 kg ha^{-1} (716.50 g ha^{-1}), seed priming at 0.5% (650.17 g ha^{-1}), and control (461.28 g ha^{-1}). Treatments without FeSB had a higher mean Fe uptake (693.99 g ha^{-1}) compared to those with FeSB (626.30 g ha^{-1}). Significant interaction effects were observed for application method and biofertilizer (P=0.001), as well as year and genotype with FeSB (P=0.009). These interactions suggest that the combination of application method and biofertilizer, as well as the presence of FeSB, significantly influences straw Fe uptake (Figure 6).

Table 5. Straw Fe uptake g ha^{-1} of wheat as affected by genotypes, Fe application methods and FeS

Treatment	Year		Mean
	2021-22	2022-23	
Genotypes (G)			
Genotypes (G)			
TRB-29-116 (Advance line)	738.17^{a}	759.33^{a}	748.75^{a}
TRB-02-103 (Advance line)	601.88^{b}	640.61^{b}	621.25^{b}
Pirsabak-19 (Approved variety)	572.01^{c}	648.84^{b}	610.42^{b}
LSD $_{(0.05)}$	45.85	27.55	31.39
Application methods (AM)			
Control	437.54^{d}	485.03^{d}	461.28^{d}
Seed Priming of Fe @ 0.5%	618.08^{c}	682.26^{c}	650.17^{c}
Soil application of Fe @ 10 kg ha^{-1}	699.90^{b}	733.10^{b}	716.50^{b}
Foliar application of Fe @ 0.5%	793.89^{a}	831.34^{a}	812.61^{a}
LSD $_{(0.05)}$	52.95	31.81	36.25
Biofertilizer (BF)			
With FeSB	604.91^{b}	647.69^{b}	626.30^{b}
Without FeSB	669.80^{a}	718.17^{a}	693.99^{a}
LSD $_{(0.05)}$	37.44	22.49	25.63
Year means	637.35	682.93	0.000
Interactions effects	P value		P value
G × AM	0.000	Y × AM	NS
G × FeSB	0.000	Y × G × AM	NS
AM × FeSB	0.001	Y × FeSB	NS
G × AM × FeSB	0.001	Y × AM × FeSB	NS
Y × G	NS	Y × G × FeSB	0.009
		Y × G × AM × FeSB	NS

The data represent the replicated mean, and the different letters within columns indicate significant differences at p≤0.05, as determined by the least significant difference test (LSD).

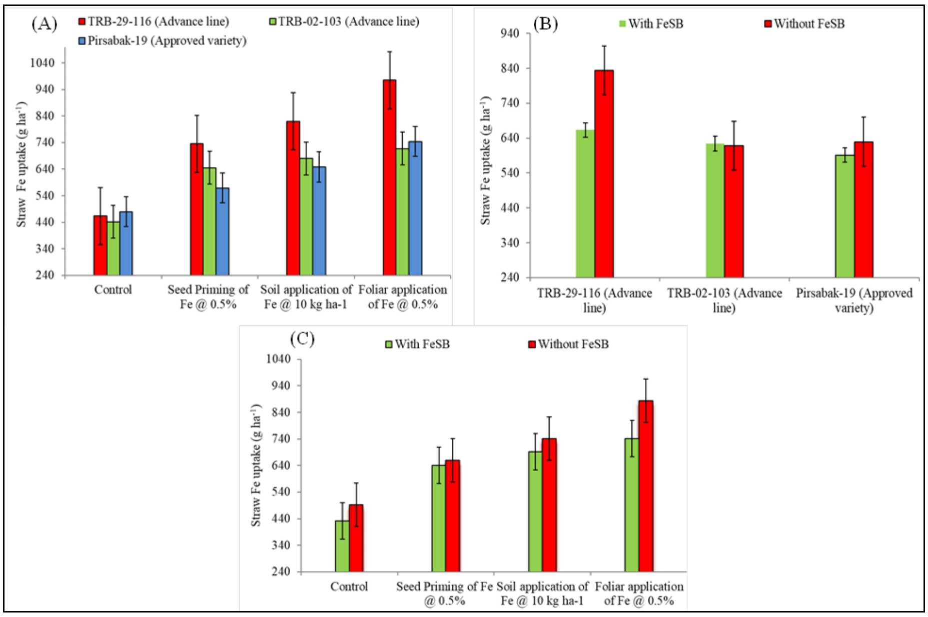


Figure 6. Interaction effects of genotypes and Fe application methods (A), FeSB and genotype (B), and Fe application methods and FeSB (C) on straw Fe uptake.
The data represent the replicated mean with standard error bar, and the different letters within columns indicate significant differences at $p \leq 0.05$, as determined by the least significant difference test.

Table 6 presents the total Fe uptake (g ha^{-1}) of wheat influenced by genotypes (G), Fe application methods (AM), and Fe-solubilizing biofertilizer (BF). Among genotypes, TRB-29-116 (Advance line) showed the highest mean Fe uptake (957.20 g ha^{-1}), followed by TRB-02-103 (798.69 g ha^{-1}) and Pirsabak-19 (784.90 g ha^{-1}). For Fe application methods, foliar application at 0.5% resulted in the highest mean Fe uptake (1029.93 g ha^{-1}), followed by soil application at 10 kg ha^{-1} (909.11 g ha^{-1}), seed priming at 0.5% (825.79 g ha^{-1}), and control (622.90 g ha^{-1}). Treatments without FeSB had a higher mean Fe uptake (900.95 g ha^{-1}) compared to those with FeSB (792.92 g ha^{-1}). Significant interaction effects were observed for genotype and application method (P=0.000), genotype and biofertilizer (P=0.000), application method and biofertilizer (P=0.001), and genotype, application method, and biofertilizer (P=0.001). These interactions suggest that the combination of genotype, application method, and biofertilizer significantly influences total Fe uptake. Additionally, a significant interaction effect was found for year, genotype, and application method with FeSB (P=0.012), indicating a complex interplay among these factors influencing total Fe uptake (Figure 7).

Table 6. Total Fe up g ha^{-1} of wheat as affected by genotypes, Fe application methods and FeSB

Treatment	Year		Mean
	2021-22	2022-23	
Genotypes (G)			
Genotypes (G)			
TRB-29-116 (Advance line)	939.96a	974.45	957.20
TRB-02-103 (Advance line)	773.33b	824.06	798.69
Pirsabak-19 (Approved variety)	742.53c	827.28	784.90
LSD $_{(0.05)}$	48.00	29.50	33.26
Application methods (AM)			
Control	595.08d	650.72d	622.90d
Seed Priming of Fe @ 0.5%	788.65c	862.94c	825.79c
Soil application of Fe @ 10 kg ha^{-1}	885.49b	932.73b	909.11b
Foliar application of Fe @ 0.5%	1005.20a	1054.65a	1029.93a
LSD $_{(0.05)}$	55.42	34.07	38.41
Biofertilizer (BF)			
With FeSB	766.19b	819.64b	792.92b
Without FeSB	871.03a	930.87a	900.95a
LSD $_{(0.05)}$	39.19	24.09	27.16
Year means	818.60	875.25	0.000
Interactions effects	P value		P value
G × AM	0.000	Y × AM	NS
G × FeSB	0.000	Y × G × AM	0.012
AM × FeSB	0.001	Y × FeSB	NS
G × AM × FeSB	0.001	Y × AM × FeSB	NS
Y × G	NS	Y × G × FeSB	NS
		Y × G × AM × FeSB	NS

The data represent the replicated mean, and the different letters within columns indicate significant differences at p≤0.05, as determined by the least significant difference test (LSD).

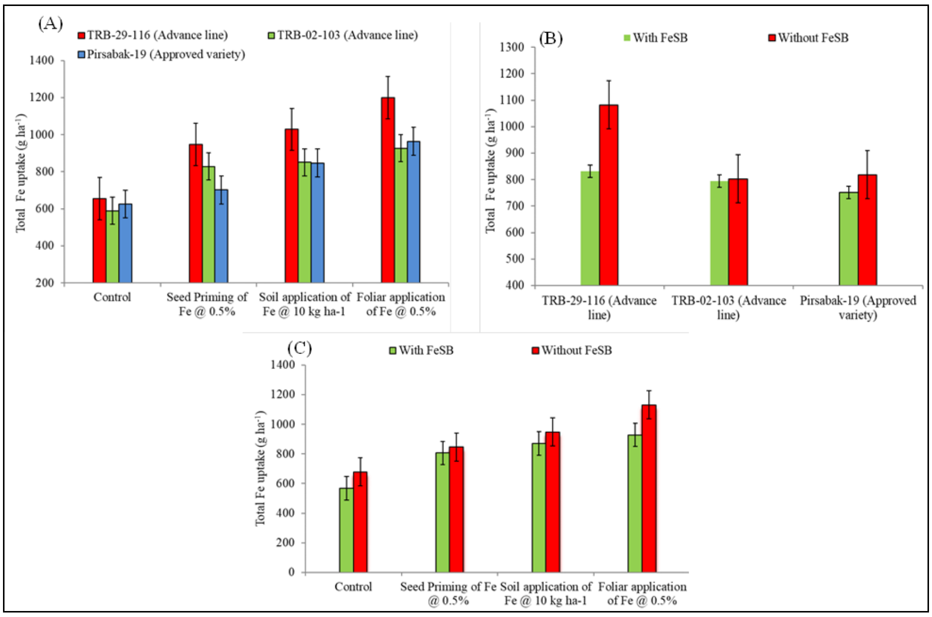
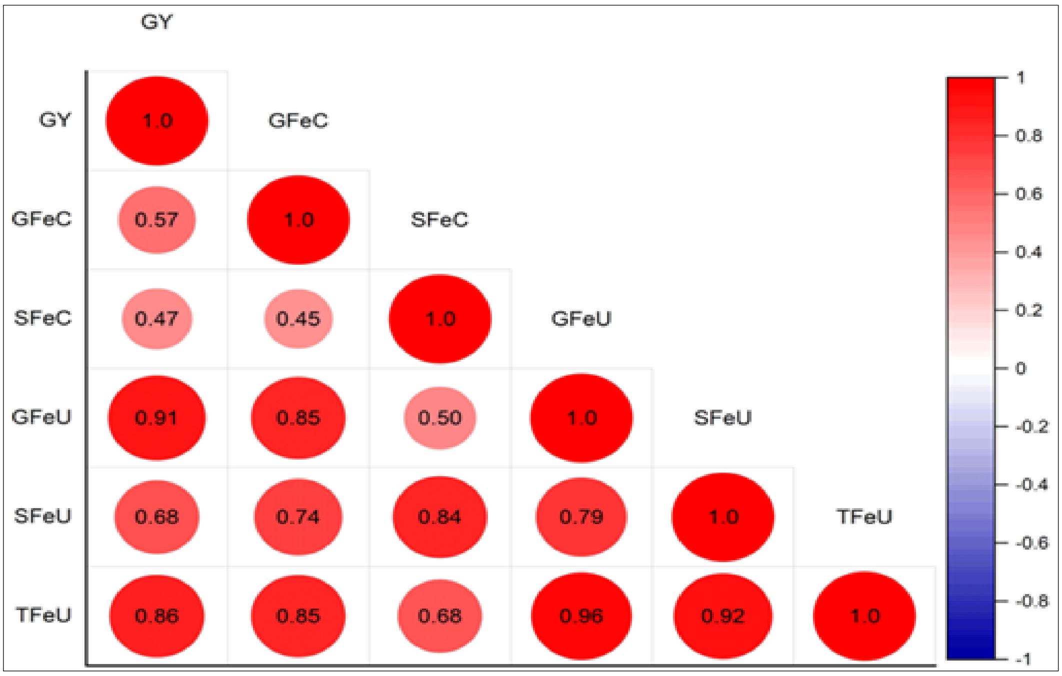


Figure 7. Interaction effects of genotypes and Fe application methods (A), FeSB and genotype (B), and Fe application methods and FeSB (C) on total Fe uptake.
The data represents the replicated mean with standard error bar, and the different letters within columns indicate significant differences at p ≤ 0.05, as determined by the least significant difference test.

Correlation PCA

The Pearson correlation results reveal significant associations among the variables in the provided (Figure 8). Notably, "grain yield" exhibits strong positive correlations with "Grain Fe uptake" (r=0.911) and "Total Fe uptake" (r=0.863), indicating that higher grain yield is associated with increased uptake of Fe in both grain and total plant tissues. Additionally, a moderate positive correlation is observed between "grain yield" and "grain Fe content" (r=0.568). "Grain Fe uptake" and "Total Fe uptake" demonstrate a remarkably high positive correlation (r=0.963), suggesting a close relationship in Fe uptake between grain and total plant components. Furthermore, "Straw Fe uptake" exhibits positive correlations with "Grain Fe uptake" (r=0.787) and "Total Fe uptake" (r=0.923), indicating coordinated Fe uptake in straw and total plant tissues. These correlation coefficients provide insights into the interdependencies among the variables, shedding light on potential patterns and connections within the studied system.



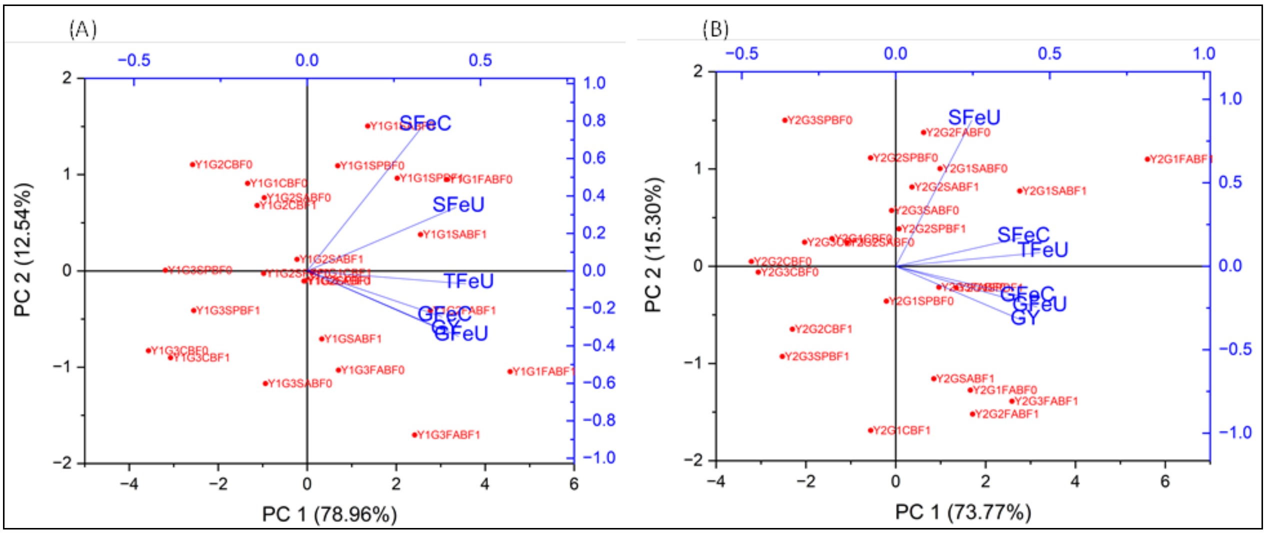
GFeC: grain FE content; SFeC: straw Fe content; GFeU: grain Fe uptake; SFeU: straw Fe uptake; TFeU: total Fe uptake; GY: grain yield.

Figure 8. Pearson correlations method analysis between various Fe-related variables of wheat; correlation is significant at the 0.05 level.

The Principal Component 1 (PC_1) and Principal Component 2 (PC_2) for the variables of first year indicated the contribution of each variable to these principal components in a Principal Component Analysis (PCA) (Figure 9). For "grain yield" the positive coefficient for PC_1 suggests a positive impact on this component, while the negative coefficient for PC_2 implies a negative influence. Similarly, "grain Fe content" has positive contributions to both PC_1 and PC_2. Notably, "Straw Fe content" shows a positive impact on PC_1 and a substantially greater positive influence on PC_2. The variables related to Fe uptake, including "Grain Fe uptake" "Straw Fe uptake," and "Total Fe uptake," all exhibit positive contributions to PC_1 and varied impacts on PC_2. These coefficients provide insights into how each variable contributes to the principal components, aiding in the understanding of their relationships and importance in the overall dataset.

The coefficients for Principal Component 1 (PC_1) and Principal Component 2 (PC_2) of each variable of second indicated "Grain yield" contribute positively to both PC_1 and PC_2, with coefficients of 0.41164 and -0.32507, respectively. Similarly, "grain Fe content" positively influences both components, while "Straw Fe content" has a positive impact on PC_1 and a smaller positive influence on PC_2. "Grain Fe uptake" positively contributes to PC_1 and negatively to PC_2. Notably, "Straw Fe uptake" strongly influences PC_2 positively, suggesting a significant role in this component. "Total Fe uptake" shows a positive impact on both

PC_1 and PC_2. These coefficients provide insights into the direction and strength of each variable's influence on the principal components, aiding in the interpretation of the underlying patterns in the data.



G_1: TRB-02-103; G_2: TRB-29-116; G_3: Pirsabak-19; C: control; SP: seed priming; SA: soil applied; FA: foliar applied; BF_0: without FeSB; BF_1: with FeSB; GY: grain yield; GFeC: grain Fe content; SFeC: straw Fe content; GFeU: grain Fe uptake; SFeU: straw Fe uptake; TFeU: total Fe uptake; of grain.

Figure 9. PCA analysis showing correlation among different Fe-related traits variables for first year (A) and for second year (B) influenced by genotypes, Fe fertilization with Fe SB

This field study aimed to assess the impact of Fe sulfate fertilization on both crop yield and grain Fe contents across various wheat genotypes. The investigation unveiled a significant enhancement in wheat grain yield attributed to the application of Fe sulfate in conjunction with FeSB across different wheat genotypes, as detailed in Table 1. These findings align with previous studies (Yilmaz et al., 1997; Hou et al., 2018; Ramzan et al., 2020; Wu et al., 2024) that underscore the efficacy of Fe fertilization in augmenting wheat grain concentrations and overall economic yield, particularly in regions facing Fe deficiency. A higher grain yield was observed with the application of 40 kg FeSO4 ha^{-1} (Satwadhar et al., 2024; Wu et al., 2024). Notably, the escalating prevalence of micronutrient-deficient soils, exacerbated by the continuous cultivation of exhaustive crops and intensive fertilizer use, including nitrogen, potassium, and phosphorus, has become a growing concern (Salim and Raza, 2020; Zhang et al., 2024). According to (Yao et al., 2024; Khalili et al., 2023), calcareous soils are often deficient in Fe due to their high pH levels. The soil in Pakistan, characterized by high pH, free calcium carbonate ($CaCO_3$), and bicarbonate (HCO_3^-), tends to be more prone to Zn and Fe deficiencies. This inhibits the accessibility of these essential nutrients to plants, as observed in studies by (Imtiaz et al., 2010; Yao et al., 2017; Akhtar et al., 2019). Given these soil conditions, the present study indicated a potential deficiency of Zn and Fe. Consequently, there was an anticipation of improved ion contents in grains and enhanced yields through the soil application of zinc sulfate and Fe sulfate. Among micronutrients, Fe plays a crucial role in the dynamics of crop growth, yield, and produce quality. This study aimed to investigate the impact of various Fe application methods, including seed priming, soil application, foliar spray, and their integrated use, on the growth, yield, and grain quality of different wheat genotypes. The results revealed a significant improvement in Fe accumulation in the grains of wheat genotypes when treated with foliar applications. Notably, the cultivar Pirsabak-19 (Approved variety) exhibited greater efficiency in Fe accumulation compared to TRB-02-103 (Advance line) and TRB-29-116 (Advance line), as indicated in Table 2. This emphasizes the importance of selecting appropriate Fe application methods and considering wheat genotypic variations for optimal Fe enrichment in grains. Fe accumulation was notably enhanced through foliar application treatments in this study. The most substantial improvement was observed with the foliar application of Fe at a 0.5% concentration, followed by soil application at a rate of 10 kg Fe ha^{-1} and seed priming at a 0.5% concentration. Additionally, the use of FeSB resulted in a higher accumulation of Fe in grains compared to treatments without FeSB application. These findings align with the work of Zulfiqar et al. (2020), who similarly reported that Fe soil application led to increased Fe contents in wheat grains. The results underscore the significance of foliar application and the potential benefits of biofertilizers in enhancing Fe accumulation in wheat grains. The concentration and uptake of micronutrients were also notably higher with the application of 40 kg $FeSO_4$ ha^{-1} (Yao et al., 2014; Satwadhar et al., 2024). The observed outcomes find support in the research of Ramzan et al. (2020), who noted that soil application of Zn and Fe resulted in increased concentrations of Cu, Mg, and Ca in wheat grains. Additionally, Wright and Stuczynski (1996) reported a positive impact of foliar application of Zn contents in wheat grains. A study by (Zhang et al., 2010; Yao et al., 2014; Yao et al., 2019) investigating 265 wheat genotypes identified significant variations in grain Fe concentration, further emphasizing the complex dynamics of micronutrient uptake and accumulation in wheat. These collective findings contribute to the broader understanding of the intricate relationships between various nutrient applications and their effects on the elemental composition of wheat grains. The divergent findings in the study by (Zhang et al., 2010) could potentially be ascribed to the greater genetic diversity present among the extensive number of genotypes they investigated. It is plausible that the complexity of micronutrient uptake patterns becomes more evident when studying a larger pool of genotypes, contributing to the observed variations. In contrast, the present study focused on a more limited set of three improved wheat genotypes, potentially reducing the influence of genetic diversity on the outcomes. Notably, the application of Fe significantly resulted in the highest levels of grain, straw, and total Fe uptake in the examined wheat genotype, as indicated in Table 3. This underscores the substantial impact of Fe application on enhancing the overall Fe content in both the grain and straw components of the wheat plants. The results underscore the multifaceted nature of factors influencing Fe uptake in wheat, as detailed in Tables 4, 5 and 6. The study reveals intricate interactions among various variables, highlighting the necessity for holistic agricultural practices and meticulous crop management strategies to enhance Fe uptake in wheat cultivation. Significantly, observed variations in grain, straw, and total Fe uptake across diverse wheat genotypes point to genetic disparities in the absorption and utilization of Fe. Notably, the wheat genotype Pirsabak-19, when subjected to a foliar spray of 0.5% Fe along with FeSB application, exhibited superior efficiency in grain, straw, and total Fe uptake compared to other genotypes. These discrepancies in Fe uptake likely stem from the distinct genetic traits and characteristics inherent to each wheat genotype, contributing to enhanced Fe accumulation in both grain and straw components (Yao et al., 2019; Hafeez et al., 2021). The study noted noteworthy enhancements in grain, straw, and total Fe uptake relative to the control, attributing these increases to diverse application methods influenced by factors such as uptake mechanisms, timing, nutrient mobility, soil conditions, and genotype specificity. The application of Fe fertilizer played a pivotal role in significantly improving Fe accessibility, consequently facilitating increased absorption of Fe and augmenting its uptake by plants (Rengel et al., 1999; Cakmak, 2008; Wang et al., 2012; Hafeez et al., 2021).

CONCLUSIONS

The improvements observed in grain and straw Fe content, Fe uptake in grain and straw, total Fe uptake, grain yield, suggest that when wheat genotypes are subjected to Fe fertilization with FeSB application, they can enhance the bioavailability of Fe up to a certain threshold. The genotypes exhibited the most significant response to Fe fertilization at a 0.5% concentration in foliar spraying, 10 kg Fe ha^{-1} soil application, and 0.5% seed priming with the presence of FeSB when compared to without FeSB application in the control group. Additionally, this combination reduced wheat grain phytic acid. The strongest positive correlations were found in Fe related traits both Fe content and uptake. The TRB-02-103 genotype, when subjected to 0.5% foliar Fe spray along with FeSB application, exhibited higher grain yield, maximum Fe content in both grain and straw, increased grain, straw, and total Fe uptake compared to TRB-02-103 (Advance line) and TRB-29-116 (Advance line) without Fe application and FeSB application. Thus, we recommend the use of TRB-02-103 in conjunction with 0.5% foliar Fe application and FeSB to enhance crop Fe content, uptake Fe biofortification.

ACKNOWLEDGEMENTS

This project was supported by Researchers Supporting Project Number (RSP2025R230) King Saud University, Riyadh, Saudi Arabia.

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