UDC 633.18:631.825
doi: 10.5937/AASer2355039G

Acta Agriculturae Serbica, 28 (55), 39–47, 2023

Publisher: Faculty of Agronomy Čačak

www.afc.kg.ac.rs

Effect of iron application methods on grain yield and iron concentration of rice under different nitrogen levels

Usman Ghani[1], Ameer Khan[1,2], Ali Shahzad[3*], Ayesha Fizza[1], Muhammad Shahzad[1,2], Hameed Gul[3], Soufiane Mohamed[4], Shareef Gul[3]

[1] *Department of Botany, University of Agriculture Faisalabad, Faisalabad 38040, Pakistan*
[2] *Department of Agronomy, Zhejiang University, Hangzhou 310058, China*
[3] *College of Agronomy and Biotechnology, Southwest University, Beibei, Chongqing 400715, China*
[4] *College of Resources and Environmental Sciences, Southwest University, Beibei, Chongqing 400715, China*

**Corresponding author: ali.thathyala3212@outlook.com*

Received 3 January 2023; Accepted 6 April 2023

A B S T R A C T

Rice is a staple cereal crop that helps food security and overcomes nutrition problems. The application of synthetic nitrogen (N) fertilizers results in the improvement of nutrient concentration. Therefore, iron (Fe) biofortification in rice can be improved by altering Fe application methods under different N levels. We made a comprehensive assessment on this, analyzing Fe concentration in the root, shoot and grain of the Super Basmati cultivar raised under two N levels (80 kg h-1 and 160 kg h-1) with Fe applied through the soil and/or foliar supply at different growth stages. The results showed that agronomic traits such as plant height, chlorophyll contents, number of productive tillers, panicle length, number of spikelets per panicle, 100-grain weight, grain yield, biological yield, and harvest index were significantly influenced by Fe application methods and N levels. Soil + Foliar application of Fe at 80 kg ha-1 of N level had maximum biological yield (18.70 g/pot), grain yield (7.31 g/pot), and harvest index (60.87%). Results revealed that iron concentration was significantly influenced by Fe application methods under different N levels, and their interaction showed significant influence. The highest values of Fe concentration in the shoot (300.50 ppm), root (446.63 ppm), and grain (141.13 ppm) were observed under 80 kg ha-1 N application. Results suggest that Fe biofortification has the potential to improve the Fe content in rice grain by various application methods with optimal N availability.

Keywords: rice, iron, grain yield, harvest index

И З В О Д

Пиринач је једна од основних житарица која доприноси сигурности хране и превазилажењу проблема с исхраном. Применом вештачких азотних (N) ђубрива побољшава се концентрација хранива. Стога се биофортификација пиринча гвожђем (Fe) може побољшати различитим начинима примене Fe при различитим дозама N. Спровели смо свеобухватно истраживање о томе, анализом концентрације Fe у корену, стабљици и зрну сорте Супер Басмати гајене уз употребу две дозе N (80 kg h-1 и 160 kg h-1) и примену Fe путем земљишта и/или фолијарном прихраном у различитим фазама раста. Резултати су показали да су агрономске особине – висина биљке, садржај хлорофила, број продуктивних бокора, дужина метлице, број класића у метлици, маса 100 зрна, принос зрна, биолошки принос и жетвени индекс – биле под значајним утицајем начинâ примене Fe и доза N. Земљишна + фолијарна примена Fe при концентрацији N 80 kg ha-1 дала је максималан биолошки принос (18,70 g/саксији), принос зрна (7,31 g/саксији) и жетвени индекс (60,87%). Резултати су показали да је концентрација гвожђа била под значајним утицајем начинâ примене Fe при различитим дозама N, а њихова интеракција је показала значајан утицај. Највеће вредности концентрације Fe у стабљици (300,50 ppm), корену (446,63 ppm) и зрну (141,13 ppm) утврђене су при дози N 80 kg ha-1. Резултати указују на то да биофортификација Fe може да побољша садржај Fe у зрну пиринча коришћењем различитих начина његове примене у условима оптималне приступачности N.

Кључне речи: пиринач, гвожђе, принос зрна, жетвени индекс.

1. Introduction

Iron (Fe) is vital for all living things, including plants. Plants require Fe for cellular respiration, oxygen transport, intermediate metabolism, DNA stability and repair, and photosynthesis, among other things (Rout and Sahoo, 2015). Despite Fe abundance in the earth's crust, it is one of the least available elements for plants growing in aerobic soils with neutral to basic pH. Because alkaline soils cover approximately 30% of the world's land, there is a need for bioavailable Fe for plant fitness. As a result, Fe deficiency is a major constraint on crop productivity and quality, which has an influence on human health via the food chain, particularly for people whose diets rely heavily on plant-based foods (Abadia et al., 2011). Fe deficiency in humans causes major health consequences, including anemia, which affects billions of people worldwide

(McLean et al., 2009). In impoverished countries, cereal-based foods are a staple of the diet, yet they have low Fe content and bioavailability (Cakmak, 2008; Gibson et al., 2010). As a result, increasing the concentration and bioavailability of Fe in cereal grains is a major issue and a top research priority (Bouis and Welch, 2010).

Enhancement (biofortification) of food crops with micronutrients by using agricultural methods is one of the most widely employed solutions for reducing the prevalence of Fe deficiency in human populations (Cakmak and Kutman, 2018; Birol and Bouis, 2019). Agronomic biofortification (e.g., fertilizer treatments) is the complementary and cost-effective agricultural approach to address this problem (Zhang et al., 2018). Fe is essential for the biofortification of grains because half of the world's population is fed on Fe deficient cereals (Welch and Graham, 2004). In agronomic practices, fertilizer is directly added to the soil or applied as foliar while breeding techniques need a long period of experimentation. Therefore, the agronomic process can be used as an immediate source for enhancing the concentration of Fe in crops. Fe can be applied to the rice crop through soil application, foliar application and soil plus foliar application (Fageria et al., 2009). Different Fe application practices can be used in the rice crop growing areas which largely depend upon the social and economic conditions (Zuo and Zhang, 2011). Along with the Fe application, the plant nitrogen (N) status is a crucial driver in grain Fe enrichment (Singh et al., 2018). The remobilization of N and Fe from the vegetative tissue and transfer into the seed is maintained by the same genetic pathways, resulting in a positive relationship between grain Fe and N contents (Uauy et al., 2006; Bošković-Rakočević et al., 2018). Increased soil N treatment dramatically increased shoot and grain Fe concentrations in field and greenhouse trials (Cakmak et al., 2010; Kutman et al., 2010; Shi et al., 2010). Foliar urea spraying boosted grain Fe levels in a similar way (Kutman et al., 2010).

Rice is a staple food for almost half of the world's population, providing 21% of their energy and protein requirements. Rice is grown and consumed across South, Southeast, and East Asia, which accounts for around 62.5 percent of the global human population. Rice grains typically have low levels of micronutrients and do not provide enough amounts of these micronutrients to humans (Naeem et al., 2021). The purpose of this study was to see how soil and foliar applied Fe fertilizers affected the yield and Fe content of rice cultivated in soils with varying levels of nitrogen.

2. Materials and methods

2.1. Experimental site, planting material and experimental design

The experiment was carried out to evaluate the influence of nitrogen on iron enrichment in rice (*Oryza sativa* L.) at Field Allelopathy Laboratory, Agronomy Research farm, University of Agriculture, Faisalabad, Pakistan. Rice cultivar Super Basmati was used as the experimental material in this study and its seeds were obtained from Rice Research Institute, Ayub Agriculture Research Institute, Faisalabad, Pakistan.

Table 1.
Meteorological conditions of the experimental site in the 2017 crop growing season.

Months	Average temperature (°C)	Rainfall (mm)	Relative humidity (%)	Average wind speed (Km/h)
June	34.4	19.9	38.9	6.3
July	32	193.5	59.6	6.2
August	31.1	48.1	62.2	4.2
September	31	12	53.6	3.5
October	26.7	22.2	51.3	3.6
November	20.1	0	60.1	2.6
December	16.4	0	68.7	2.8

2.2. Soil analysis

The physicochemical properties of the experimental soil were analyzed by using the method described by Homer and Pratt (1961). The soil was sandy loamy, containing sand (33.78%) and silt (34.12%), and having pH 8.2, EC 0.23 dSm-1, available nitrogen 0.049%, available potassium 177 ppm, available phosphorus 5.9 ppm, and available iron 1.95 ppm.

2.3. Crop husbandry and treatment application

Thirty-day-old rice seedlings were manually transplanted into pots during the mid-week of August 2017; each pot was filled with 6 kg of soil. The full dose of Basal fertilizers, 1100 mg P2O5 (diammonium phosphate, DAP), and 600 mg K2O (sulfate of potash, SOP) per pot, were applied at the time of seedling transplant. The treatments consisted of two factors, nitrogen, and iron. Urea was used for N source and two levels of N (80 kg ha^{-1}, 120 kg ha^{-1}) were applied. Iron sulfate was used for Fe source and four Fe application methods (Control, Soil application, Foliar application, Soil + Foliar application) were used. The N was applied in split doses (3 splits; first dose at transplanting of rice and second dose divided into two equal parts and applied at tillering and panicle initiation stage). Iron sulfate at the rate of 25 kg ha^{-1} was applied to the soil at the time of transplanting rice as soil application. For foliar application, 12 ml iron sulfate solution was sprayed at panicle initiation of rice. A combination of both soil and foliar application of iron sulfate was also applied. The crop was harvested in the mid-week of November and samples were packed into separate plastic bags of each pot.

2.4. Data collection

From each pot, three plants were selected after two weeks of transplanting and their height (cm) was measured with a measuring scale and the plant height (cm) was measured at the stage of maturity. At maturity, the chlorophyll contents of the flag leaf were measured with a spade meter. The number of tillers was counted from each pot at physiological maturity. Three panicles were selected from each plant and their length was measured with a measuring scale and also the number of spikelets and number of grains were counted from each panicle. After threshing, 100-grain weight was measured in grams by using an electrical balance. Rice crop was harvested with the standard procedure at maturity. The harvested crop was tied with thread and left for sun drying. When the plant was fully dried, the biological yield was recorded by using the electrical balance in grams (g) per pot. Grain yields were recorded on a dry weight basis. Grains were harvested from each pot and dried at an optimum temperature (60°C). Then, the grain was cooled at room temperature and weighted by using the electrical balance. Averaged grain yield was calculated after harvesting. The harvest index was calculated by using the following equation:

Harvest index % = Grain yield / Biological yield × 100

2.5. Sampling and Fe determination analysis

Samples of rice shoot, root and grains were dried at 70 °C using an oven. Dried samples were ground into fine powder by using a grinder. Samples (0.25 g) of oven-dried plant material (root, shoot and grains) powder were taken and transferred into 100 mL digestion flasks, followed by the addition of 2.5 mL of bi-acid mixture (HNO3-HClO4) with a 2:1 ratio (Jones and Case 1990). After 24 hours, the mixture of samples was digested at 150°C on hot plate for 30 minutes. After the digestion procedure, the sample-containing flasks were cooled down for a few minutes and then distilled water was added to acquire the desired volume (25 mL). Then, these digestion samples were filtered by using Whatman filter paper No. 42 and kept in airtight plastic bottles. After completing the above-mentioned procedure, the atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) was used to determine Fe concentration in the digested samples by following the conditions explained in AOAC (1990).

2.5. Statistical analysis

The data of observed parameters were subjected to analysis of variance (ANOVA) corresponding with the two factorial randomized complete block design (RCBD) using the procedure of Steel et al (1997). The statistical software Statistix 8.1 (Analytical, Tallahassee, FL, USA) was used to perform the analysis, and the treatment means were compared by using the least significant difference (LSD) test at 5% probability level.

3. Results

Results showed that the management of N application and Fe significantly influenced the yield and yield contributing traits of rice along with the enhancement of Fe concentration (Table 2).

Table 2.
Statistical summary of rice traits under various Fe application methods and N levels.

Traits	Nitrogen (N)	Iron (Fe)	N × Fe
Plant height	*	**	NS
Chlorophyll contents	**	*	NS
Number of productive tillers per plant	*	***	NS
Panicle length	NS	**	NS
Number of spikelets per panicle	*	*	NS
100-grain weight	***	**	NS
Grain yield per pot	***	***	**
Biological yield per pot	***	***	***
Harvest index	***	**	***
Iron concentration in the root	***	***	***
Iron concentration in the shoot	***	***	***
Iron concentration in the grain	***	***	***

NS= Non-significant; *= Significant at *P≤0.05*; **= Significant at *P ≤0.01*; ***= Significant at *P ≤0.001*

3.1. Plant growth

Analysis of variance results showed that rice traits such as plant height, chlorophyll contents and number of productive tillers per plant were significantly influenced by the application of N and Fe, but the interaction between them was non-significant (Table 2). Results indicated that N application at 80 kg ha-1 was the best combination with the different Fe application methods (Table 3). Maximum plant height (105.42 cm) was observed in the Foliar application of Fe under 80 kg N ha^{-1}, while minimum plant height (83.67 cm) was observed in control treatment under 80 kg N ha-1 (Table 3.a). Similarly, the highest chlorophyll content (49.33) was obtained in the Foliar application of Fe under 80 kg N ha-1 (Table 3.b). In the case of number of productive tillers per plant, the highest number (3.50) was observed in the Soil application of

Fe under 80 kg N ha^{-1}, while the minimum number (1.58) was observed in control under 160 kg N ha^{-1} (Table 3.c).

Table 3.
Effect of various Fe application methods on growth-related traits in rice under two N levels in the soil.

Treatments	**N levels (kg ha^{-1})**		**Mean**
	80	**160**	
	a. Plant height (cm)		
Control	74.17	93.17	83.67 **D**
Soil application Fe	104.75	88.83	96.79 **C**
Foliar application Fe	105.42	97.92	101.67 **A**
Soil + Foliar application Fe	102.33	95.83	99.08 **B**
Mean	96.67 **A**	93.94 **B**	
	b. Chlorophyll contents (SPAD value)		
Control	48.38	45.45	46.912 **C**
Soil application Fe	48.15	46.89	47.521 **B**
Foliar application Fe	49.33	48.62	48.971 **A**
Soil + Foliar application Fe	48.48	45.35	46.912 **C**
Mean	48.58 **A**	46.58 **B**	
	c. Number of productive tillers per plant		
Control	1.92	1.58	1.75 **D**
Soil application Fe	3.50	2.92	3.21 **A**
Foliar application Fe	3.17	2.59	2.88 **C**
Soil + Foliar application Fe	3.27	2.75	2.96 **B**
Mean	2.94 **A**	2.46 **B**	

Means with different letters are significantly different ($P \leq 0.05$).

3.2. Yield-related traits

Analysis of variance results showed that rice traits such as panicle length, number of spikelets per panicle and 100-grain weight were significantly influenced by N and Fe application, but the interaction between them was non-significant (Table 2). The highest values for panicle length (22.90 cm) and 100-grain weight (2.08 g) were shown by 80 kg ha-1 N application in the combination with the Soil + Foliar application of Fe (Table 4.a, 4.c), while for number of spikelets per panicle the highest value (7.38) was observed under 160 kg ha^{-1} N application in combination with the Soil + Foliar application of Fe (Table 4.b).

Grain yield per pot, biological yield per pot and harvest index were significantly influenced by N and Fe management, and the interaction between them was also significant (Table 2). The highest grain yield per pot and harvest index (7.31 g, 60.87%, respectively) were recorded in the Soil + Foliar application of Fe under 80 kg ha^{-1} N application (Table 4.d, 4.f). In the case of biological yield per pot, the maximum value (18.73 g) was obtained for the Foliar application of Fe under 80 kg ha^{-1} N application (Table 4.e).

Table 4.
Effect of various Fe application methods on yield-related traits in rice under two N levels in the soil.

Treatments	**N levels (kg ha^{-1})**		**Mean**
	80	**160**	
	a. Panicle length (cm)		
Control	15.10	16.70	15.92 **C**
Soil application Fe	21.40	18.60	20.00 **B**
Foliar application Fe	20.70	21.40	21.04 **A**
Soil + Foliar application Fe	22.90	20.50	21.71 **A**
Mean	20.01 **A**	19.32 **B**	

b. Number of spikelets per panicle			
Control	5.96	5.21	5.58 **C**
Soil application Fe	6.42	6.46	6.44 **B**
Foliar application Fe	6.08	6.79	6.44 **B**
Soil + Foliar application Fe	7.21	7.38	7.29 **A**
Mean	6.42 **B**	6.46 **A**	
c. 100-grain weight (g)			
Control	1.80	1.66	1.73 **B**
Soil application Fe	1.89	1.65	1.77 **B**
Foliar application Fe	2.06	1.84	1.95 **A**
Soil + Foliar application Fe	2.08	1.76	1.92 **A**
Mean	1.96 **A**	1.73 **B**	
d. Grain yield per pot (g)			
Control	5.42 **d**	5.54 **d**	5.48 **D**
Soil application Fe	6.34 **bc**	5.55 **d**	5.94 **C**
Foliar application Fe	6.71 **b**	6.41 **bc**	6.56 **B**
Soil + Foliar application Fe	7.31 **a**	6.18 **c**	6.74 **A**
Mean	6.44 **A**	5.92 **B**	
e. Biological yield per pot (g)			
Control	12.61 **b**	11.92 **b**	12.26 **C**
Soil application Fe	12.46 **b**	12.20 **b**	12.33 **C**
Foliar application Fe	18.73 **a**	12.76 **b**	15.74 **A**
Soil + Foliar application Fe	18.30 **a**	10.15 **c**	14.22 **B**
Mean	18.30 **A**	10.15 **B**	
f. Harvest index (%)			
Control	46.64 **bc**	43.58 **cd**	45.11 **BC**
Soil application Fe	50.68 **b**	35.77 **e**	43.23 **C**
Foliar application Fe	50.92 **b**	45.38 **bc**	48.15 **AB**
Soil + Foliar application Fe	60.87 **a**	39.93 **de**	50.40 **A**
Mean	52.28 **A**	41.16 **B**	

Means with different letters are significantly different ($P \leq 0.05$).

3.3. Fe concentration in the root, shoot and grain

Results showed that iron concentration in the shoot, root and grain was significantly influenced by N and Fe management and the interaction between them was also significant (Table 2). The highest Fe concentration in the shoot and root was observed in the Soil + Foliar application of Fe under 80 kg ha^{-1} N application (300.50, 393.44 ppm, respectively), while minimum concentration was recorded in control under 160 kg ha-1 N application (128.31, 313.75 ppm, respectively). The maximum grain Fe concentration (141.13 ppm) was recorded in the Soil + Foliar application of Fe under 80 kg ha^{-1} N application and the least (58.63 ppm) was observed in control under 160 kg ha^{-1} N application (Table 4).

Table 4.
Effect of various Fe application methods on Fe concentration in the root, shoot and grain of rice under two N levels in the soil.

Treatments	N levels (kg ha^{-1})		Mean
	80	**160**	
	a. Iron concentration in the root		
Control	356.75 **c**	313.75 **e**	335.25 **D**
Soil application Fe	383.75 **b**	338.25 **d**	361.00 **C**
Foliar application Fe	446.63 **a**	322.92 **e**	384.77 **B**
Soil + Foliar application Fe	393.44 **b**	388.75 **a**	391.09 **A**
Mean	395.14 **A**	340.92 **B**	
	b. Iron concentration in the shoot		
Control	152.16 **d**	128.31 **e**	140.24 **C**
Soil application Fe	228.00 **b**	194.75 **c**	211.38 **B**
Foliar application Fe	235.94 **b**	201.13 **c**	218.54 **B**
Soil + Foliar application Fe	300.50 **a**	235.13 **b**	267.82 **A**
Mean	229.15 **A**	189.83 **B**	
	c. Iron concentration in the grain		
Control	67.81 **e**	58.63 **f**	63.22 **C**
Soil application Fe	112.50 **b**	98.17 **c**	105.33 **B**
Foliar application Fe	141.50 **a**	86.00 **d**	113.75 **A**
Soil + Foliar application Fe	140.13 **a**	81.94 **d**	111.03 **A**
Mean	115.48 **A**	81.85 **B**	

Means with different letters are significantly different (*P≤0.05*).

4. Discussion

Iron is an important mineral for human health. Its content in rice is lowered more than any other mineral as a result of post-harvest processing. Paddy (raw rice) has 38 parts per million of iron, which is decreased to 8.8 parts per million in brown rice after processing and to 4.1 parts per million in milled rice (Dexter, 1998). In another study, iron concentration in brown rice was lowered from 19 ppm to roughly 4 ppm in polished grains (a 4.75-fold drop) (Masuda et al., 2009). Iron biofortification for milled rice was created in response to the apparent loss of iron in consumable rice grain.

4.1. Plant growth

In the current study, we found that Fe application under nitrogen management promotes the plant height and chlorophyll contents. Because Fe is involved in the photosynthetic activity, it helps to improve plant height (Rout and Sahoo, 2015). Moreover, nitrogen supply at a critical point enhances vegetative growth, cell elongation and cell division, and eventually improves plant height (Beres et al., 2019). Iron plays a vital role in enzyme activity, the synthesis of chlorophyll, the chlorophyll contents of the chloroplast, and the maintenance of chloroplast function and structure (Eskandari, 2011; Yadegari 2014; Rout and Sahoo, 2015; Sherefu and Zewide, 2021). The application of nitrogen enhanced ATP synthesis, chlorophyll contents, amino acid, and nucleic acid in rice (Ali et al., 2000). Optimum application of nitrogen is very important for the enhancement of the leaf area index of rice, which results in improved chlorophyll a and chlorophyll b contents in the chloroplast (Nasser, 2002).

The soil application of Fe significantly influences the productive number of tillers in rice. Our findings were identical with the findings of Singh and Singh et al. (2018), who concluded that the application of Fe at the initial stage of growth significantly influenced the productive tillers. Optimum application of nitrogen fertilizer enhances the micronutrient uptake in plants and helps to improve the productive number of tillers along with other yield contributing traits (Zhang et al., 2020). In the current study, almost all observed traits decreased under 160 kg N ha^{-1} as compared to 80 kg N ha^{-1}, indicating that excessive application of nitrogen fertilizer adversely affects rice quality and production (Rahman and Zhang, 2018).

4.2. Yield-related traits

Grain yield represents the economic yield of rice crop. It is considered to be an important yield contributing parameter in rice which determines the yield potential of rice crop. It is the final product of different mechanisms in which physiological and morphological processes are included during plant growth and development. Both Fe and N are the most important nutrients for the growth and development of crop. Increasing N supply usually improves vegetative growth through enhanced plant N status; Fe is a necessary element involved in enzyme activity, and its effect on plant pigments ultimately increased grain yield (Singh et al., 2018). Fageria et al. (2009) reported that the soil plus foliar application of Fe is a more effective method, which is used to enhance plant yield and yield-related parameters.

In the current study, the rice crop showed positive responses along with both Fe and N application. Panicle length is an important morphological yield contributing parameter which ultimately affects the number of spikes per panicle and grain yield. Therefore, a large panicle length brings a high grain yield. Panicle length gives better performance by the application of micronutrients. The increase in the content of critical micronutrients can substantially increase the panicle length, number of spikes per panicle and grain yield. Both soil and foliar applied Fe significantly increased the panicle length, number of spikes per panicle, 1000 grain weights, grain and straw yield, as well as its concentration in the seed and straw (Singh and Singh, 2018; Zulfiqar et al., 2021). The application of N at tillering stage significantly increased the panicle length (Raza et al., 2003). Optimal N application is important for highest productivity (Gul et al., 2021). Ali et al. (2000) observed that the morphological parameter was significantly affected by the N application such as number of spikelets per panicle, grain yield, and panicle length.

Fe application increased the 100-grain weight of rice because of the high concentration of Fe moving in the phloem system through the root, shoot, stem and developing grain. The maximum grain yield, number of spikelets per panicle and 100-grain weight with increasing N supply at 80 kg N ha^{-1} with either of the Fe application methods used were also evident in the present study. N is the most important element involved in the process of photosynthesis and it ultimately promotes 100-grain weight (Stangoulis and Knez, 2022).

4.3. Fe concentration in the root, shoot and grain

Foliar fertilizer application is commonly thought to be a technique used to overcome low nutrient concentrations in grains induced by mineral immobilization in the soil (Fageria et al., 2009). Plants absorb Fe through their leaves, and better absorption should be possible at the site of use, in growing tissues (Stangoulis and Knez, 2022). The transformation of nutrients then happens via the phloem or xylem inside the stem and leaf tissue (Wu et al., 2010), and treatment during appropriate plant growth stages can improve grain Fe content and yield under deficit conditions (Farooq et al., 2012; Kiran et al., 2021). The use of animal dung and plant wastes to change the soil characteristics has also been demonstrated to boost the availability of Fe (Prasad et al., 2014). However, in terms of increasing grain nutrient concentrations, foliar application looks to be far more successful than non-foliar, soil nutrient administration. The success of unloading adequate amounts of fertilizer into grains is determined by the time of foliar spray (Cakmak et al., 2010). N enhanced the Fe concentration in plants because N application increased shoot and root growth and ultimately promoted the ability to absorb the nutrients (Kutman et al., 2010; Shi et al., 2010).

With respect to shoot, root and grain Fe concentration, the current study revealed that it was significantly influenced by the application of Fe under different N managements. The foliar application of Fe enhances the nutritional value and Fe concentration in grain and shoot parts (Aciksoz et al., 2011). The milk to grain filling phases of crop development are most effective for biofortifying the grains with micronutrients (Ramzan et al., 2020; Xia et al., 2020) and this enrichment is primarily taken in the aleurone and embryo (Cakmak et al., 2010). At the grain maturation stage, the foliar application of fertilizer appears to be most effective because at this stage starch endosperm absorption capacity nears up to three-fold (Aisbitt et al., 2008; Bhatt et al., 2020). According to Das et al., (2019), a combination of soil mineral application and foliar fertilization spray after anthesis resulted in an opportune timing for enhancing grain mineral concentration. Because grain nutrient concentrations are intimately linked to the soil's inherent Fe capacity, the adequate grain nutrient concentrations require a sufficient amount of minerals and water in the soil throughout the plant's reproductive stage (Cakmak and Kutman, 2018). Fe and the soil's cation exchange capacity have an indirect relationship (Yoo and James, 2002) corresponding with the various genotypes (Kastori et al., 2021).

The application of nitrogen in the soil improves the uptake of micronutrients in the root and shoot of rice, which enhances the growth and metabolic processes that occur within plants (Shi et al., 2010).

Increased nutritional deposition in grains is a result of nitrogen application. The N status of a plant regulates nutrient uptake, transport, and placement within the plant tissue (Kumar et al., 2021); thus, the grain quality is strongly linked with the higher availability of N in the late reproductive plant growth stage. A relationship has been established between N and phytosiderophore release, with increased N supply being particularly beneficial in enhancing phytosiderophore release from roots, as well as improving Fe mobilization (Aciksoz et al., 2011; Shi et al., 2012). The presence of N promotes the activity of nutrient transporter proteins, and also increases the synthesis of long-distance transporter molecules (Palmer and Guerinot, 2009).

5. Conclusions

Iron biofortification has the potential to improve Fe content in the grain along with improving the grain yield of rice. Maximum crop growth was noticed when Fe was applied as foliar application under 80 kg N level ha-1. The Fe application had a significant effect on plant height, chlorophyll content, number of productive tillers per plant, panicle length, 100-grain weight, grain yield, biological yield, harvest index, shoot iron concentration, root iron concentration and grain iron concentration. Therefore, the foliar application of Fe and 80 kg N level ha-1 resulted in optimum yield and higher iron contents in the grain of rice.

Declaration of competing interests

The authors declare that they have no competing interests.

References

Abadia, J., Vazquez, S., Rellan-Alvarez, R., El-Jendoubi, H., Abadia, A., Alvarez-Fernandez, A., López-Millán, A.F. (2011). Towards a knowledge-based correction of iron chlorosis. *Plant Physiology and Biochemistry*, 49, 471-482. https://doi.org/10.1016/j.plaphy.2011.01.026

Aciksoz, S.B., Ozturk, L., Gokmen, O.O., Römheld, V., Cakmak, I. (2011). Effect of nitrogen on root release of phytosiderophores and root uptake of Fe (III)-phytosiderophore in Fe-deficient wheat plants. *Physiologia Plantarum*, 142(3), 287-296. https://doi.org/10.1111/j.1399-3054.2011.01460.x

Aisbitt, B., Caswell, H., Lunn, J. (2008). Cereals–current and emerging nutritional issues. *Nutrition Bulletin,* 33(3), 169-185. https://doi.org/10.1111/j.1467-3010.2008.00704.x

Ali, A., Choudhry, M.A., Malik, M.A., Ahmad, R., Saifullah, (2000). Effect of various doses of nitrogen on the growth and yield of two wheat cultivar. *Pakistan Journal of Biological Sciences*, 3, 1004-1005.

AOAC. (1990). In K. Helrich (Ed.), Official Methods of Analysis (15th ed.). Arlington, VA, USA: Association of Official Analytical Chemists, Inc.

Beres, J., Becka, D., Tomasek, J., Vasak, J. (2019). Effect of autumn nitrogen fertilization on winter oilseed rape growth and yield parameters. *Plant, Soil and Environment*, 65(9), 435-441. https://doi.org/10.17221/444/2019-PSE

Bhatt, R., Hossain, A., Sharma, P. (2020), Zinc biofortification as an innovative technology to alleviate the zinc deficiency in human health: A review. *Open Agriculture*, 5(1), 176-187. https://doi.org/10.1515/opag-2020-0018

Birol, E., Bouis, H.E. (2019). Leveraging biofortified crops and foods: R4D perspective. *Encyclopedia of Food Security and Sustainability*, 2, 181-188. https://doi.org/10.1016/B978-0-08-100596-5.22450-5

Bošković-Rakočević, L., Dinić, Z., Dugalić, G., Dugalić, M., Mladenović, J., & Đurić, M. (2018). Effect of different rates and methods of application of NPK-fertilizers on the quality of potato tubers. *Acta Agriculturae Serbica,* 23(45), 101-110. https://doi.org/ 10.5937/AASer1845101B

Cakmak, I. (2008). Enrichment of cereal grains with zinc: agronomic or genetic biofortification? *Plant Soil*, 302, 1-17. https://doi.org/10.1007/s11104-007-9466-3

Cakmak, I., Kalayci, M., Kaya, Y., Torun, A.A., Aydin, N., Wang, Y., Arisoy, Z., Erdem, H., Gokmen, O., Ozturk L, Horst WJ (2010) Biofortification and localization of zinc in wheat grain. *Journal of Agricultural and Food Chemistry*, 58, 9092-9102. https://doi.org/10.1021/jf101197h

Cakmak, I., Kutman, U.B. (2018). Agronomic biofortification of cereals with zinc: a review. *European Journal of Soil Science*, 69, 172-180. https://doi.org/10.1111/ejss.12437

Das, S., Chaki, A.K.C., Hossain, A. (2019). Breeding and agronomic approaches for the biofortification of zinc in wheat (Triticum aestivum L.) to combat zinc deficiency in millions of a population: a Bangladesh perspective. *Acta Agrobotanica*, 7(2), 1770. https://doi.org/10.5586/aa.1770

Dexter, P.B. (1998). Rice Fortification for Developing Countries; OMNI/USAID: Washington, DC, USA.

Eskandari, H. (2011). The importance of iron (Fe) in plant products and mechanism of its uptake by plants. *Journal of Applied Environmental and Biological Sciences,* 1(10), 448-452.

Fageria, N.K., Filho, M.B., Moreira, A., Guimarães, C.M. (2009). Foliar fertilization of crop plants. *Journal of Plant Nutrition*, 32(6), 1044-1064. https://doi.org/10.1080/01904160902872826

Farooq, M., Wahid, A., Siddique, K.H.M. (2012). Micronutrient application through seed treatments: a review. *Journal of Soil Science Plant Nutrition*, 12, 125-142. https://doi.org/10.4067/S0718-95162012000100011

Gibson, R.S., Bailey, K.B., Gibbs, M., Ferguson, E.L. (2010). A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food and Nutrition Bulletin*, 31, 134-146. https://doi.org/10.1177/15648265100312S206

Gul, H., Rahman, S., Shahzad, A., Gul, S., Qian, M., Xiao, Q., & Liu, Z. (2021). Maize (*Zea mays* L.) productivity in response to nitrogen management in Pakistan. *American Journal of Plant Sciences,* 12(8), 1173-1179. https://doi.org/10.4236/ajps.2021.128081

Homer, D.C., Pratt, P.F. (1961). Methods of Analysis for Soils, Plants and Waters. Davis: University of California, Davis.

Jones, J.R., Case, V.W. (1990). "Sampling, handling, and analysing plant tissue samples," in Soil Testing and Plant Analysis, ed. R. L. Westerman (Madison, WI: Soil Science Society of America), pp. 389-428. https://doi.org/10.2136/sssabookser3.3ed.c15

Kastori, R., Maksimović, I., Momčilović, V., Mirosavljević, M., Putnik-Delić, M., Daničič M. (2021). Differences in iron accumulation in the grain between and within Aegilops and Triticum species. *Acta Agriculturae Serbica,* 26(52), 103-110. https://doi.org/10.5937/AASer2152103K

Kiran, A., Wakeel, A., Sultana, R., Khalid, A., Qurrat-Ul-Ain, M.R., Shahzad, A.N., Khan, S., Noor, M. (2021). Concentration and localization of Fe and Zn in wheat grain as affected by its application to soil and foliage. *Bulletin of Environmental Contamination and Toxicology,* 106(5), 852-858. https://doi.org/10.1007/s00128-021-03183-x

Kumar, S., Kumar, S., Mohapatra, T. (2021). Interaction between macro-and micro-nutrients in plants. *Frontiers in Plant Science*, 12, 753. https://doi.org/10.3389/fpls.2021.665583

Kutman, U.B., Yildiz, B., Ozturk, L., Cakmak, I. (2010). Biofortification of durum wheat with zinc through soil and foliar applications of nitrogen. *Cereal Chemistry*, 87, 1-9. https://doi.org/10.1094/CCHEM-87-1-0001

Masuda, H., Usuda, K., Kobayashi, T., Ishimaru, Y., Kakei, Y., Takahashi, M., Higuchi, K., Nakanishi, H., Mori, S., Nishizawa, N.K. (2009). Overexpression of the barley nicotianamine synthase gene HvNAS1 increases iron and zinc concentrations in rice grains. *Rice*, 2, 155-166. https://doi.org/10.1007/s12284-009-9031-1

Mclean, E., Cogswell, M., Egli, I., Wojdyla, D., De-Benoist, B. (2009). Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993-2005. *Public Health Nutrition*, 12, 444-454. https://doi.org/10.1017/S1368980008002401

Naeem, A., Aslam, M., Ahmad, M., Asif, M., Yazici, M.A., Cakmak, I., Rashid, A. (2021). Biofortification of Diverse Basmati Rice Cultivars with Iodine, Selenium, and Zinc by Individual and Cocktail Spray of Micronutrients. *Agronomy*, 12(1), 49. https://doi.org/10.3390/agronomy12010049

Nasser, L.E.A. (2002). Interactive effects of nitrogen starvation and different temperatures on senescence of sunflower (Helianthus annus L.) leaves associated with the changes in RNA protein and activity of some enzymes of nitrogen assimilation. *Journal of Biological Sciences,* 2, 463-469.

Palmer, C.M., Guerinot, M.L. (2009). Facing the challenges of cu, Fe and Zn homeostasis in plants. *Nature Chemical Biology*, 5(5), 333-340. https://doi.org/10.1038/nchembio.166

Prasad, R., Shivay, Y.S., Kumar, D.J. (2014). Agronomic biofortification of cereal grains with iron and zinc. *Advances in Agronomy*, 125, 55-91. https://doi.org/10.1016/B978-0-12-800137-0.00002-9

Rahman, K.M., Zhang, D. (2018). Effects of fertilizer broadcasting on the excessive use of inorganic fertilizers and environmental sustainability. *Sustainability*, 10(3), 759. https://doi.org/10.3390/su10030759

Ramzan, Y., Hafeez, M.B., Khan, S., Nadeem, M., Batool, S., Ahmad, J. (2020). Biofortification with zinc and iron improves the grain quality and yield of wheat crop. *International Journal of Plant Production*, 14(3), 501-510. https://doi.org/10.1007/s42106-020-00100-w

Raza, M., Khan, H., Karim, F., Tahir, M.J. (2003). Nitrogen use efficiency as affected by time of application in rice (IRRl-6). *Sarhad Journal of Agriculture,* 19(4), 453-457.

Rout, G.R., Sahoo, S. (2015). Role of iron in plant growth and metabolism. *Reviews in Agricultural Science,* 3, 1-24. https://doi.org/10.7831/ras.3.1

Sherefu, A., Zewide, I. (2021). Review paper on effect of micronutrients for crop production. *Journal of Nutrition and Food Processing*, 4(7), 063. https://doi.org/10.31579/2637-8914/063

Shi, R., Zhang, Y., Chen, X., Sun, Q., Zhang, F., Romheld, V., Zou, C. (2010). Influence of long term nitrogen fertilization on micronutrient density in grain of winter wheat (*Triticum aestivum*). *Journal of Cereal Science*, 51, 165-170. https://doi.org/10.1016/j.jcs.2009.11.008

Shi, R., Weber, G., Köster, J., Reza-Hajirezaei, M., Zou, C., Zhang, F., Von-Wirén, N. (2012). Senescence-induced iron mobilization in source leaves of barley (Hordeum vulgare) plants. *New Phytologist,* 195(2), 372-383. https://doi.org/10.1111/j.1469-8137.2012.04165.x

Singh, A.K., Singh, V. (2018). Effect of foliar application of iron, zinc and age of seedlings on growth and yield of rice (*Oyza sativa* L.). *International Journal of Current Microbiology and Applied Sciences*, 7(8), 1062-1068. https://doi.org/10.20546/ijcmas.2018.708.120

Singh, B.R., Timsina, Y.N., Lind, O.C., Cagno, S., Janssens, K. (2018). Zinc and iron concentration as affected by nitrogen fertilization and their localization in wheat grain. *Frontiers in Plant Science*, 9, 307. https://doi.org/10.3389/fpls.2018.00307

Stangoulis, J.C., Knez, M. (2022). Biofortification of major crop plants with iron and zinc-achievements and future directions. *Plant and Soil.* https://doi.org/10.1007/s11104-022-05330-7

Steel, R.G.D., Torrie, J.H., Dickey, D.A. (1997). Principles and Procedures of Statistics: A biometrical Approach, 3rd edition, McGraw-Hill, New York.

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., Dubcovsky, J. (2006). A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science*, 314, 1298-1301. https://doi.org/10.1126/science.1133649

Welch, R.M., Graham, R.D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*, 55, 353-364. https://doi.org/10.1093/jxb/erh064

Wu, C.Y., Lu, L.L., Yang, X.E., Feng, Y., Wei, Y.Y., Hao, H.L., Stoffella, P.J., He, Z.L. (2010). Uptake, translocation, and remobilization of zinc absorbed at different growth stages by rice genotypes of different Zn densities. *Journal of Agricultural and Food Chemistry*, 58(11), 6767-6773. https://doi.org/10.1021/jf100017e

Xia, H., Wang, L., Qiao, Y., Kong, W., Xue, Y., Wang, Z., Kong, L., Xue, Y., Sizmur, T. (2020). Elucidating the source–sink relationships of zinc biofortification in wheat grains: A review. *Food and Energy Security*, 9(4), e243. https://doi.org/10.1002/fes3.243

Yadegari, M. (2014). Irrigation periods and Fe, Zn foliar application on agronomic characters of medicinal plants. *Advances in Environmental Biology*, 8(4), 1054-1062.

Yoo, M.S., James, B.R. (2002). Zinc extractability as a function of Ph in organic waste amended soils. *Soil Science*, 167(4), 246-259. https://doi.org/10.1097/00010694-200204000-00002

Zhang, C.M., Zhao, W.Y., Gao, A.X., Su, T.T., Wang, Y.K., Zhang, Y.Q., Zhou, X.B., He, X.H. (2018). How could agronomic biofortification of rice be an alternative strategy with higher cost-effectiveness for human iron and zinc deficiency in China?. *Food and Nutrition Bulletin*, 39(2), 246-259. https://doi.org/10.1177/0379572117745661

Zhang, L., He, X., Liang, Z., Zhang, W., Zou, C., Chen, X. (2020). Tiller development affected by nitrogen fertilization in a high-yielding wheat production system. *Crop Science*, 60(2), 1034-1047. https://doi.org/10.1002/csc2.20140

Zulfiqar, U., Hussain, S., Maqsood, M., Zamir, S.I., Ishfaq, M., Ali, N., Ahmad, M., Maqsood, M.F. (2021). Enhancing the accumulation and bioavailability of iron in rice grains via agronomic interventions. *Crop and Pasture Science*, 73(2), 2-43. https://doi.org/10.1071/CP21140

Zuo, Y., Zhang, F. (2011). Soil and crop management strategies to prevent iron deficiency in crops. *Plant and Soil*, 339(1), 83-95. https://doi.org/10.1007/s11104-010-0566-0