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Assessing Yield, Quality and Nutrient Use Efficiency of Rice (*Oryza sativa* L.) with Agronomic Iron Bio-fortification

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

To assess the impact of foliar iron nutrition on the yield, quality, and nutrient utilization of rice variety SR-4 (*Oryza sativa* L.), a field experiment was conducted during the Kharif season of 2019 at the Crop Research Farm of the Division of Agronomy, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Wadura. The experiment involved four levels of iron (0.25%, 0.5%, 0.75%, and 1%) applied through three different sources ($FeSO_4$. $7H_2O$, Na-Fe EDTA, and Ferric chloride), along with a control, arranged in a randomized complete block design with three replicates. Results showed that applying $FeSO_4.7H_2O$ at 1% significantly

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increased crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR), grain yield, straw yield, biological yield, iron content in brown rice and straw, as well as iron uptake. However, iron chelate at 0.75% demonstrated the highest harvest index. Iron chelate at 0.25% exhibited the highest iron use efficiency, while $FeSO_4.7H_2O$ at 1% resulted in the highest protein content. No significant difference was observed among treatments regarding amylose content. Consequently, it was determined that applying $FeSO_4.7H_2O$ at 1% could be a promising strategy for agronomic biofortification with iron to improve both yield and quality of rice in the temperate conditions of Kashmir valley.

Keywords: Biofortification; chelated iron; iron sulphate; iron use efficiency; rice.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is globally recognized as the most vital staple food, catering to over half of the world's population and contributing to 20% of their daily caloric intake [1]. Despite its significance, the rate of rice production growth has slowed down, and with an estimated 3.5 billion people depending on rice, it is projected that by 2030, a 30% increase in production will be necessary [2]. In Jammu & Kashmir, rice holds a significant position with cultivation spanning twelve districts, particularly Anantnag, Jammu, Baramulla, and Pulwama known for their high productivity. The region's total rice production area covers approximately 0.28 million hectares, yielding 0.55 million tonnes with a productivity of 2.1 tonnes per hectare [3]. While rice in its unmilled form provides essential micronutrients and macronutrients, the milling process removes a significant portion of these nutrients, particularly from the rice bran and fat layer, leaving polished rice with only 4-5 mg kg^{-1} of iron, significantly lower than the recommended daily intake of 17-35 milligrams [4,5]. This low iron concentration in rice contributes to widespread malnutrition in rice-consuming populations [6]. Agronomic biofortification presents a promising strategy to enhance the micronutrient content of edible crops, thereby addressing iron deficiency and improving human health [7]. Foliar application of micronutrients presents a practical and cost-effective method for enhancing cereal grains with essential micronutrients, particularly iron, thereby addressing deficiencies that can diminish both nutritional value and crop yields [8]. Iron deficiency is particularly widespread among food grains and contributes significantly to global instances of anemia, with recent research even associating low maternal iron intake with autism spectrum disorder in children. Iron serves as a crucial cofactor for numerous enzymes essential for various physiological functions. In developing nations, iron deficiency ranks as the sixth leading cause of death and disability, resulting in anemia, impaired cognitive development, and disability. Fortifying food with iron is considered a cost-effective and economically viable alternative to supplementation, making the enrichment of rice with iron fertilization a promising solution to iron deficiency [9]. Given the scarcity of research on iron fortification of rice in temperate climates, this study aimed to evaluate the agronomic biofortification of rice variety SR-4 in Kashmir's temperate conditions, with a focus on enhancing yield, quality, and iron content in rice grains.

2. MATERIALS AND METHODS

2.1 Study Location and Experimental Design

A field experiment was conducted during the 2019 kharif season at the Crop Research Farm of the Division of Agronomy, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, located in Wadura Sopore (34°34'N latitude, 74°40'E longitude, and 1584 meters above mean sea level). The soil of the experimental site was silty clay loam with moderate organic carbon content, slightly neutral pH, normal electrical conductivity, and high iron content. Daily weather records were maintained by a nearby meteorological observatory from planting to harvest. Situated in a mid-altitude temperate zone, the experimental site experiences hot summers and extremely cold winters. Over the past 20 years, the average annual precipitation has been 812 mm, primarily occurring between December and April. During the cropping period in question, the cumulative rainfall was 152.5 mm. The minimum temperature ranged from 8.7°C to 17.8°C, and the maximum temperature ranged from 24.2°C to 32.9°C, with average maximum relative humidity varying from 76.4% to 89.0%, and average minimum relative humidity ranging from 46.4% to 70.0% during the kharif 2019 growing season. The experimental design consisted of a Randomized Complete Block Design with 13 treatments, including four iron levels (0.25%, 0.50%, 0.75%, and 1.0%) and three iron sources (FeSO4.7H2O, NaFe-EDTA, and Iron chloride), along with one control treatment, each replicated three times.

2.2 Growth and Yield Observations

The Crop Growth Rate (CGR) was determined by assessing the increase in plant biomass over 30-day intervals, calculated using the formula devised by Redford [10], and expressed as grams per square meter per day (g m^{-2} day^{-1}).

$$\text{CGR (g m}^{-2}\text{ day}^{-1}) = \frac{1}{A} \times \frac{W2-W1}{t2-t1}$$

where W1 represents the dry matter production per plant (in grams) at time t1, W2 denotes the dry matter production per plant (in grams) at time t2, and A represents the area (spacing) of the plant.

The Relative Growth Rate (RGR) was estimated using the formula proposed by Blackman [11], which measures the rate of dry weight gain per unit dry weight, and the results were expressed in milligrams per gram per day (mg g^{-1} day^{-1}).

$$\text{RGR (mg g}^{-1}\text{day}^{-1}) = \frac{\log W2-\log W1}{t2-t1}$$

W_1 and W_2 are dry matter production of plants at time t_1 and t_2 respectively

The Net Assimilation Rate (NAR) was calculated using the formula proposed by Evans [12], representing the rate of increase in total plant dry weight per unit leaf area per unit time, expressed as grams per square centimeter per day. This computation was conducted as per the following formula:

$$\text{NAR (g cm}^{-2}\text{ day}^{-1}) = \frac{W2-W1}{t2-t1} \times \frac{logL2-logL1}{L2-L1}$$

W_1 and W_2 represent the total dry weight of plants at time t_1 and t_2 respectively. L_1 and L_2 represent total leaf area of plants at time t_1 and t_2 respectively.

The numbers of panicles residing in quadrant 0.25 m^2 were enumerated from individual plots before harvesting followed by its conversion to m^2. Panicle weight estimation was done by selecting randomly labelled plants from each plot in each replication, and the average weight was expressed in grams. The number of grains per panicle was calculated from each panicle, and the mean value was reported as grains per panicle. After harvesting and sun-drying for 3-4 days, the bundle weight of each net plot was measured using an electronic balance and expressed in tonnes per hectare. The produce from each plot was sun-dried, threshed, and cleaned properly. Yield from each plot was measured in kilograms and then converted to tonnes per hectare. Straw yield for each plot was determined by subtracting the grain yield from the respective biological yield and expressed in tonnes per hectare. Harvest index was calculated using the following formula:

$$\text{H.I} = \frac{Economic\ yield}{Biological\ yield} \times 100$$

2.3 Estimation of Protein and Amylose Content

Protein content was determined following the method outlined by Juliano [13], which involved multiplying the nitrogen concentration by a coefficient factor of 5.77 and expressing the result as a percentage. To measure amylose content, a powdered sample of rice was prepared by crushing 1 gram of milled rice grain in a mortar and pestle, then storing it at a consistent 12% moisture level. A 100 mg sample was weighed and transferred to a 100 ml volumetric flask. The flour was soaked by adding 10 ml of 1N NaOH to an Erlenmeyer flask, followed by the gradual addition of 1 ml of ethanol and uniform mixing. After 1 hour of gelatinization, the sample suspension was heated in a water bath for 10 minutes. A 100 ml volume of distilled water was prepared, and 2.5 ml aliquot was placed in a 50 ml volumetric flask with approximately 20 ml of water added. Three drops of phenolphthalein indicator were added and mixed thoroughly. By slowly adding 0.1 N HCL until the pink color disappeared, the contents were acidified. The volume was then brought up to 50 ml, and 1 ml of iodine reagent was added to develop a blue color. The absorbance at 590 nm was measured using a spectrophotometer. A standard curve was generated based on the absorbance values of known concentrations of pure amylose (ranging from 0.2 to 1 mg). This standard curve was then used to determine the sample's amylose content by comparing it to a blank created by diluting 1 ml of iodine reagent to 50 ml of distilled water [14].

Calculation:

Absorbance corresponds to 2.5 ml of test solution = ‘x’ mg amylose in test solution.
100 ml contains = $\frac{x}{2.5}$ x 100% amylase

2.4 Plant Iron Content and Uptake

The grain and straw iron content of rice was assessed using the method described by Prasad *et al*. [15], utilizing an Atomic Absorption Spectrophotometer (AAS), with results expressed in milligrams per kilogram (mg kg-1). Iron uptake was determined by multiplying the iron content of the grain and straw by their respective yields, calculated in kilograms per hectare (kg ha^{-1}).

2.5 Soil Nutrient Studies

After the crop harvest, soil samples were collected from individual plots to a depth of 15 cm and were air-dried under shade, labelled accordingly, and placed on plain white paper for several days. Following drying, the soil samples were sieved through a 2 mm mesh, and a composite sample was created from each representative sample for further laboratory analysis. Soil organic carbon content was determined using the rapid titration method outlined by Walkley and Black [16], with pH measured using a 1:2.5 ratio suspension of soil water and read using a "Blackman's glass electrode pH meter," while electrical conductivity was assessed using a Solubridge conductivity meter, as described by Jackson [17]. Available nitrogen content in each soil sample was determined using the alkaline permanganate method described by Subbiah and Asija [18]. Available phosphorus was measured for each treatment using 0.5 N $NaHCO_3$ at pH 8.5 [19], while available potassium was assessed using the 1 N ammonium acetate extraction method at pH 7 [17]. Additionally, available iron in soil samples from each treatment was determined following the procedure outlined by Lindsay and Norvell [20] using an Atomic Absorption Spectrophotometer.

2.6 Statistical Analysis

The association between growth and yield attributes with yield was worked out using correlation analysis. Employing the Statistical Package for Social Science, regression analysis of yield was fitted to evaluate the response of yield explained by growth and yield features (SPSS).

3. RESULTS

3.1 Crop Growth Rate, Relative Growth Rate and Net Assimilation Rate

CGR, RGR, and NAR are crucial indicators for assessing crop growth. The data depicted in Fig. 1 illustrate that as the crop matures and approaches harvest, its crop growth rate decreases. Among the various iron treatments, the $FeSO_4.7H_2O$ @ 1% treatment showed the highest crop growth rate (23.56 g m^{-2} day^{-1}), which statistically compared favorably with iron chelate @ 1% (20.79 g m^{-2} day^{-1}) at 60-30 DAT and 90-60 DAT intervals. At 90-60 DAT, Iron chelate @ 0.25 % recorded the highest crop growth rate (10.46 g m^{-2} day^{-1}), statistically similar to $FeSO_4.7H_2O$ @ 1% (10.22 g m^{-2} day^{-1}), iron chloride @ 1% (10.22 g m^{-2} day^{-1}), and iron chloride @ 0.75% (10.22 g m^{-2} day^{-1}). Moreover, Iron chloride @ 0.25 % exhibited the highest crop growth rate (1.58 g m-2 day-1) at maturity-90 DAT, statistically comparable to $FeSO^4.7H^2O$ @ 0.5% (1.39 g m^{-2} day^{-1}), followed by $FeSO_4.7H_2O$ @ 0.25% (1.23 g m^{-2} day^{-1}) and iron chloride @ 0.5 % (1.23 g m^{-2} day-1). However, the control treatment recorded the lowest crop growth rate of 13.53, 8.38, and 0.57 at 60-30 DAT, 90-60 DAT, and maturity-90 DAT, respectively.

As illustrated in Fig. 2, the relative growth rate decreases notably as the crop progresses in age. Among the various iron treatments, the highest relative growth rate was observed in $FeSO_4.7H_2O$ @ 1% (27.46 mg g^{-1} day^{-1}), followed by iron chelate @ 1% (25.27 mg g^{-1} day^{-1}) at 30-60 DAT. During 60-90 DAT, Iron chelate @ 0.25% exhibited the highest relative growth rate (8.82 mg g^{-1} day^{-1}), followed by iron chloride @ 0.25% (8.77 mg g^{-1} day^{-1}), and from 90 DAT to maturity, Iron chloride @ 0.25 % displayed the highest relative growth rate (1.18 mg g^{-1} day^{-1}). Conversely, across different time intervals, the lowest relative growth rate was observed in the control treatment.

Furthermore, as depicted in Fig. 3, the net assimilation rate decreased as the crop aged, exhibiting a downward trend. Among the various levels and sources of iron, $FeSO_4.7H_2O$ @ 1% displayed the highest net assimilation rate (0.37 g cm^{-2} day^{-1}) at 30-60 DAT and (0.21 g cm^{-2} day^{-1}) at 60-90 DAT in rice, statistically comparable to iron chelate @ 1%, which recorded values of 0.35 g cm-2 day-1 and 0.19 g cm^{-2} day^{-1} at the respective intervals. $FeSO_4.7H_2O$ @ 0.75% also exhibited relatively high values (0.33 g cm^{-2} day^{-1} and 0.19 g cm^{-2} day^{-1}) at these intervals, with the control showing the lowest net assimilation rate (0.20 g cm^{-2} day^{-1} at 30-60 DAT and 0.17 g cm^{-2} day^{-1} at 60-90 DAT). There were no significant differences among the different sources and levels of iron with respect to net assimilation rate at 90 DAT to maturity. Iron sulphate @ 1% demonstrated the highest values for CGR, RGR, and NAR among the different sources and levels of iron.

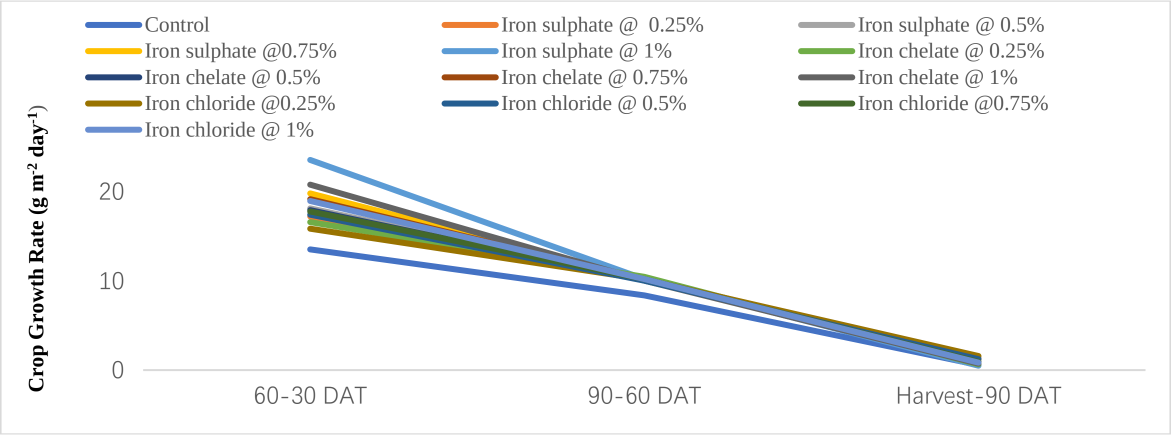

Fig. 1. Effect of different sources and levels of iron on crop growth rate (g m^{-2}day^{-1}) of rice

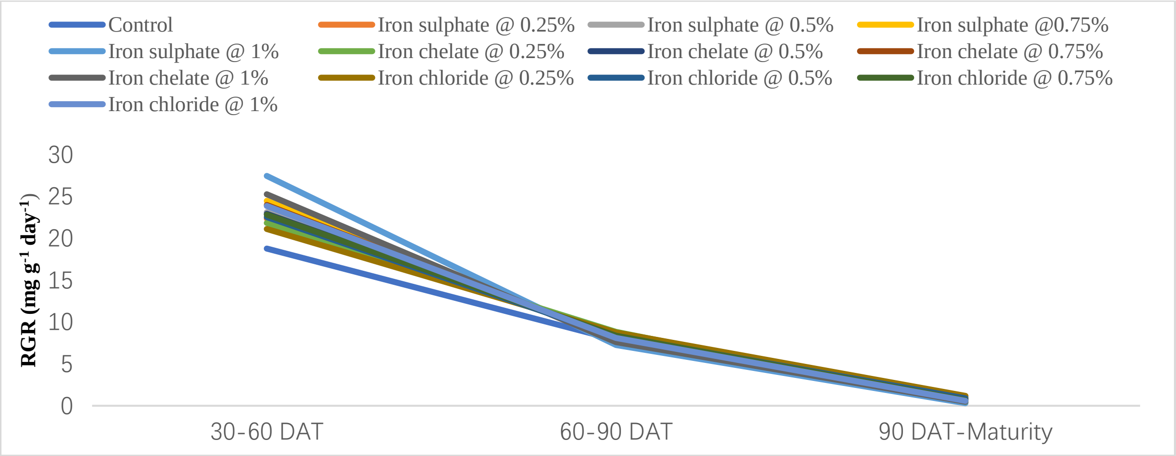

Fig. 2. Effect of different sources and levels of iron on relative growth rate (mg g^{-1} day^{-1}) of rice

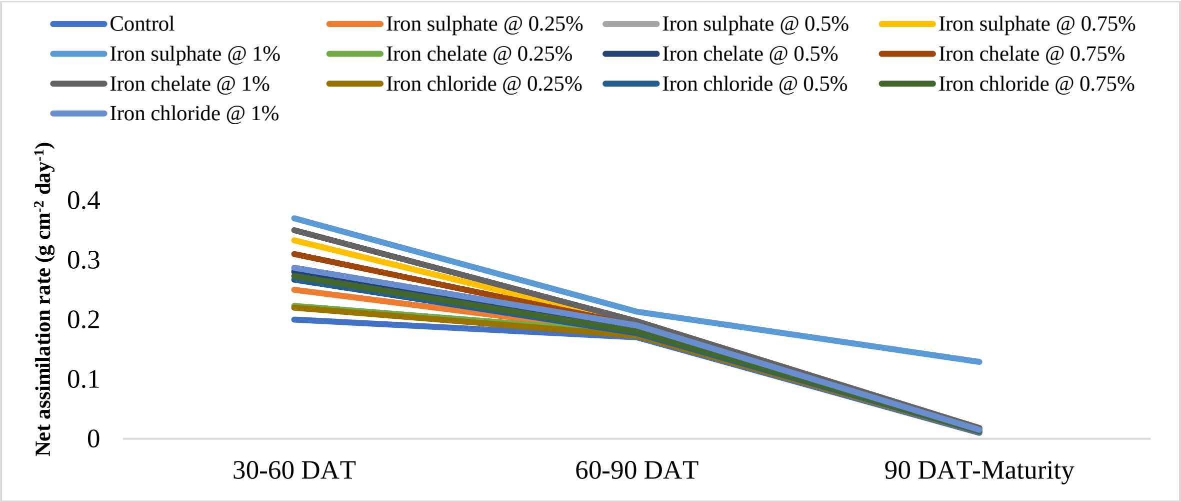

Fig. 3. Panicle Density (m^{-2}), Panicle Weight (g) and Grains Panicle^{-1}

Various iron treatments had a significant impact on panicle density, a crucial yield attribute in rice (Table 1). The Iron sulfate @ 1% treatment showed the highest Panicle m^{-2} (365.20), statistically comparable to iron chelate @ 1% (365.87), $FeSO_4.7H_2O$ @ 0.75% (365.20), and iron chelate @ 0.75% (364.87), respectively. In contrast, the control treatment had the lowest panicle m^{-2} value (344.87). Analysis revealed significant differences among treatments for panicle weight (Table 1). The Iron sulfate @ 1% treatment exhibited the highest panicle weight (2.97g), statistically similar to iron chelate @ 1% and iron sulfate @ 0.75%, with panicle weights of 2.80g and 2.77g, respectively. The control treatment recorded the lowest panicle weight (1.83 g). Regarding grains per panicle (Table 1), significant variations were observed under different iron concentrations. Iron chloride @ 1% had the highest number of grains per panicle (113.6), followed by iron chloride @ 0.25% (110.73). However, $FeSO_4.7H_2O$ @ 1% showed the highest number of filled grains per panicle, while the control treatment had the lowest number of grains per panicle (106.53).

3.2 Yield (t ha^{-1}) and Harvest Index (%)

The application of different iron treatments, with varying levels and sources, led to a significant improvement in rice yield. The highest grain yield (7.68 t ha^{-1}), straw yield (9.87 t ha^{-1}), and biological yield (17.55 t ha^{-1}) were achieved with Iron sulfate @ 1%, while the control treatment yielded the lowest (Table 2). However, the yield obtained with Iron sulfate @ 1% was statistically similar to that of iron chelate @ 1%, suggesting that both sources are equally effective for foliar iron application in rice.

Various iron treatments had a significant impact on the harvest index. The highest harvest index was observed with the application of iron chelate @ 0.75%, recording a value of 43.90%, which was statistically comparable to iron sulfate @ 0.75% (43.88%) and iron sulfate @ 1% (43.76%). Conversely, the control treatment exhibited the lowest harvest index value (43.08%). Harvest index represents the function of grain yield to the total biological yield (grain + straw), and different levels and sources of iron significantly influenced it. Iron chelate @ 0.75% demonstrated the highest harvest index, while the control treatment showed the lowest values (Table 2).

3.3 Iron Content, Uptake in Grain and Straw and Iron Use Efficiency

Significant variations were observed among different iron treatments regarding iron content and its uptake in grain and straw (Table 3). The application of iron sulfate @ 1% through foliar spraying notably increased its content (48.96 mg kg^{-1}) and uptake (0.38 kg ha^{-1}) in brown rice, as well as its content (205.96 mg kg^{-1}) and uptake (1.94 kg ha^{-1}) in straw. The iron uptake in rice straw notably increased with iron fertilization, particularly with foliar sprays of 1% $FeSO_4.7H_2O$ application, which was highest compared to other treatments. Among the various sources and levels of iron, the highest iron use efficiency (33.33%) was observed with iron chelate @ 0.25%, while the lowest (4.36%) was recorded with iron chloride @ 1% (Table 3).

Table 1. Effect of different sources and levels of iron on yield attributes of rice

Treatment	Panicle m^{-2}	Panicle weight (g)	Grains $panicle^{-1}$
Control	344.87	1.83	106.53
Iron sulphate @ 0.25%	350.53	2.27	109.66
Iron sulphate @ 0.5%	358.20	2.43	109.73
Iron sulphate @ 0.75%	365.20	2.77	109.80
Iron sulphate @ 1%	367.20	2.97	108.73
Iron chelate @ 0.25%	349.53	2.17	110.27
Iron chelate @ 0.5%	358.20	2.33	109.34
Iron chelate @ 0.75%	364.87	2.48	106.93
Iron chelate @ 1%	365.87	2.80	110.00
Iron chloride @ 0.25%	348.20	2.13	110.73
Iron chloride @ 0.5%	356.53	2.27	109.20
Iron chloride @ 0.75%	356.87	2.33	108.47
Iron chloride @ 1%	361.20	2.43	113.60
SEm ±	5.24	0.13	0.15
C.D($p \leq 0.05$)	15.72	0.40	0.42

Table 2. Effect of different sources and levels of iron on grain, straw, biological yield and harvest index of rice

Treatment	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Biological yield (t ha^{-1})	Harvest index (%)
Control	5.70	7.53	13.23	43.08
Iron sulphate @ 0.25%	6.87	8.95	15.82	43.42
Iron sulphate @ 0.5%	7.06	9.21	16.27	43.39
Iron sulphate @ 0.75%	7.32	9.36	16.68	43.88
Iron sulphate @ 1%	7.68	9.87	17.55	43.76
Iron chelate @ 0.25%	6.81	8.94	15.75	43.23
Iron chelate @ 0.5%	7.04	9.06	16.10	43.72
Iron chelate @ 0.75%	7.27	9.29	16.56	43.90
Iron chelate @ 1%	7.34	9.49	16.83	43.61
Iron chloride @ 0.25%	6.71	8.76	15.47	43.37
Iron chloride @ 0.5%	6.91	8.99	15.90	43.45
Iron chloride @ 0.75%	6.95	9.02	15.97	43.51
Iron chloride @ 1%	7.16	9.21	16.37	43.73
SEm ±	0.12	0.17	0.31	0.10
C.D($p \leq 0.05$)	0.36	0.52	0.90	0.31

Table 3. Effect of different sources and levels of iron on iron content, uptake in rice grain and straw and iron use efficiency

Treatment	Grain iron content (mg/kg)	Grain uptake (kg/ha)	Straw iron content (mg/kg)	Straw uptake (kg/ha)	Iron use efficiency (%)
Control	27.00	0.15	176.33	1.35	__
Iron sulphate @ 0.25%	43.66	0.30	200.66	1.73	26.32
Iron sulphate @ 0.5%	45.80	0.32	202.80	1.78	14.91
Iron sulphate @ 0.75%	47.70	0.35	204.70	1.84	11.70
Iron sulphate @ 1%	48.96	0.38	205.96	1.94	10.09
Iron chelate @ 0.25%	43.03	0.29	200.03	1.71	33.33
Iron chelate @ 0.5%	45.30	0.32	202.30	1.77	20.24
Iron chelate @ 0.75%	47.46	0.35	204.46	1.80	15.87
Iron chelate @ 1%	48.66	0.36	205.66	1.84	12.50
Iron chloride @ 0.25%	42.66	0.29	199.66	1.67	13.57
Iron chloride @ 0.5%	44.63	0.31	201.63	1.75	7.75
Iron chloride @ 0.75%	44.96	0.31	201.96	1.76	5.18
Iron chloride @ 1%	46.23	0.33	203.23	1.79	4.36
SEm ±	1.86	18.96	2.57	30.00	1.61
C.D($p \leq 0.05$)	5.47	55.69	7.56	88.08	4.83

3.4 Protein and Amylose Content

Protein content exhibited significant variability across different iron sources and levels. Conversely, amylose content did not demonstrate notable variations among the various treatments, although lower iron application rates tended to yield slightly higher amylose content values. The highest protein content (6.45%) was observed in $FeSO_4.7H_2O$ @ 1%, while the lowest protein content (5.93%) was detected in the control treatment (Table 4).

3.5 Soil Nutrient Studies

The soil nutrient status following crop harvest reflects the efficiency of nutrient mobilization and utilization by the crop. At harvest, there were no significant differences observed among iron sources and levels concerning pH, EC, organic carbon, P, and K. However, the lowest soil available N (244.22 kg ha^{-1}) was observed in iron sulfate @ 1%, while the highest soil available N (264.73 kg ha^{-1}) was found in the control treatment (Table 5). This suggests that iron sulfate @ 1% efficiently utilized nitrogen compared to other treatments. Our study findings indicate significant variations in soil iron content with different iron sources and levels.

3.6 Correlation Studies

The correlation analysis presented in Table 6 demonstrated a strong and positive correlation of panicle length with various yield-related traits, including panicle weight (0.96), test weight (0.37), grain yield (0.82), straw yield (0.80), biological yield (0.81), harvest index (0.74), grain iron uptake (0.78), and straw iron uptake (0.77). Similarly, panicles m-2 exhibited a significant and positive relationship with panicle weight (0.92), grain yield (0.87), straw yield (0.83), biological yield (0.85), harvest index (0.88), grain iron uptake (0.85), and straw iron uptake (0.83). Panicle weight also showed positive and significant correlations with grain yield (0.91), straw yield (0.89), biological yield (0.90), harvest index (0.76), grain iron uptake (0.87), and straw iron uptake (0.87).

Furthermore, grain yield displayed strong and positive correlations with biological yield (0.99), harvest index (0.81), grain iron uptake (0.99), and straw iron uptake (0.99), while straw yield showed similar relationships with biological yield (0.99), harvest index (0.74), grain iron uptake (0.98), and straw iron uptake (0.99). Biological yield exhibited positive and significant

Table 4. Effect of different levels and sources of iron on grain quality parameters of rice

Treatment	Protein content (%)	Amylose content (%)
Control	5.93	13.80
Iron sulphate @ 0.25%	6.10	13.76
Iron sulphate @ 0.5%	6.14	13.63
Iron sulphate @ 0.75%	6.31	13.83
Iron sulphate @ 1%	6.45	13.84
Iron chelate @ 0.25%	6.20	13.60
Iron chelate @ 0.5%	6.14	13.64
Iron chelate @ 0.75%	6.20	13.83
Iron chelate @ 1%	6.37	13.66
Iron chloride @ 0.25%	6.08	13.72
Iron chloride @ 0.5%	6.12	13.76
Iron chloride @ 0.75%	6.14	13.77
Iron chloride @ 1%	6.16	13.79
SEm ±	0.06	0.063
C.D(p≤0.05)	0.19	NS

Table 5. Nutrient status of soil at harvest as affected by different sources and levels of iron

Treatment	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Fe (mg kg^{-1})	Organic carbon (%)	pH	EC (ds m^{-1})
Control	264.73	16.35	179.88	54.66	0.87	6.50	0.85
Iron sulphate @ 0.25%	253.62	16.10	173.12	57.53	0.85	6.45	0.83
Iron sulphate @ 0.5%	249.41	16.01	169.28	59.13	0.84	6.43	0.84
Iron sulphate @ 0.75%	246.47	15.91	166.34	61.30	0.84	6.40	0.81
Iron sulphate @ 1%	244.22	15.72	164.70	63.60	0.84	6.41	0.86
Iron chelate @ 0.25%	254.10	16.14	173.18	57.36	0.87	6.42	0.82
Iron chelate @ 0.5%	249.51	16.03	171.09	59.03	0.85	6.37	0.86
Iron chelate @ 0.75%	247.34	15.94	168.21	60.46	0.85	6.35	0.81
Iron chelate @ 1%	245.13	15.90	165.94	62.10	0.84	6.52	0.83
Iron chloride @ 0.25%	255.14	16.23	174.90	56.83	0.86	6.48	0.84
Iron chloride @ 0.5%	251.93	16.8	171.55	58.36	0.85	6.33	0.85
Iron chloride @ 0.75%	250.88	16.07	171.30	59.00	0.84	6.39	0.83
Iron chloride @ 1%	248.62	15.95	168.63	60.10	0.84	6.46	0.84
SEm ±	3.03	0.43	1.83	0.56	0.007	0.09	0.08
C.D(p≤0.05)	9.08	NS	NS	1.66	NS	NS	NS

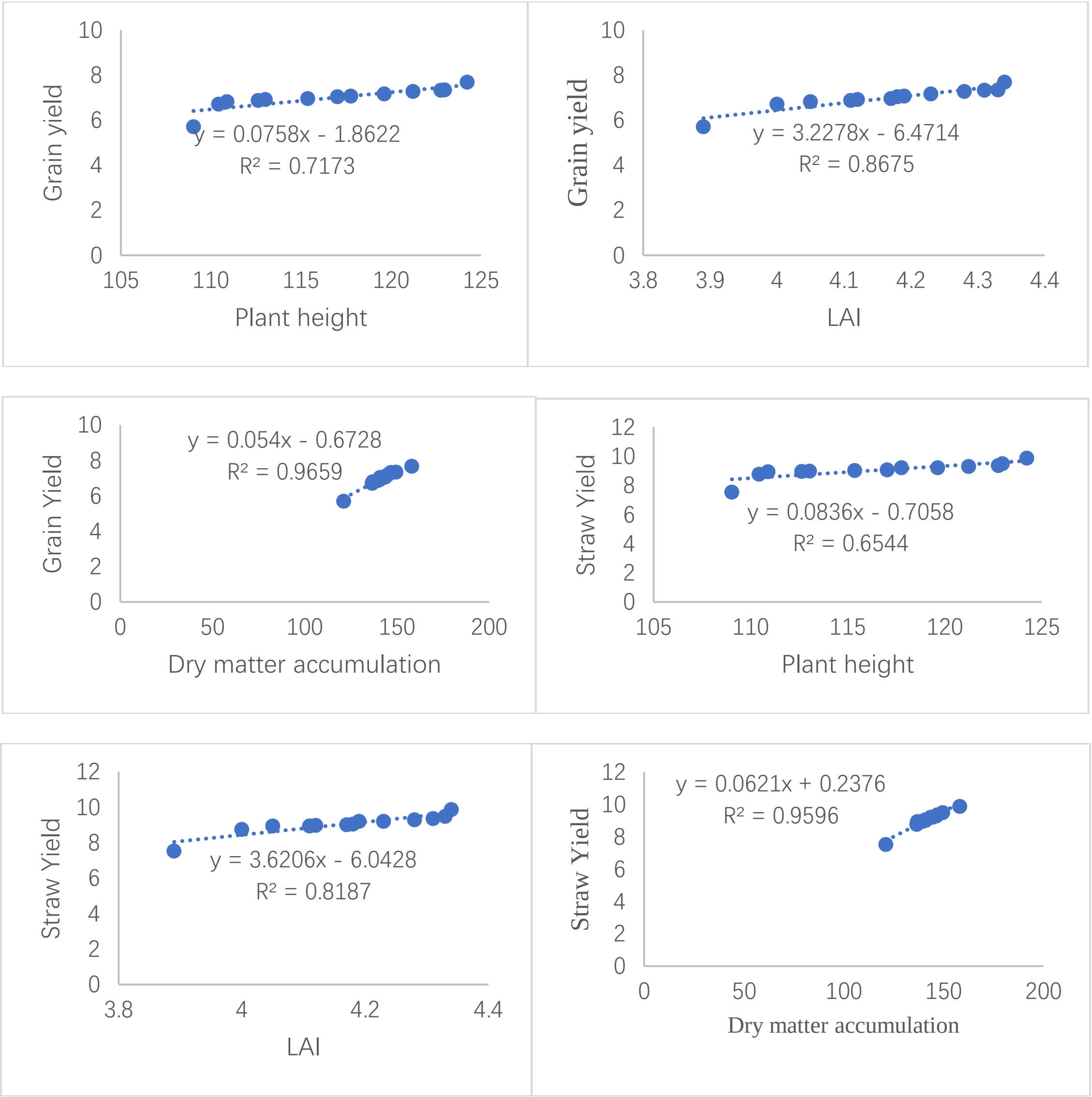

Fig. 4. Regression analysis of different growth parameters and yield

associations with harvest index (0.78), grain iron uptake (0.99), and straw iron uptake (0.99). Moreover, harvest index had positive and significant correlations with grain iron uptake (0.80) and straw iron uptake (0.76), while a strong positive correlation was observed between grain iron uptake and straw iron uptake (0.99). In Table 7, plant height was found to have positive and significant correlations with leaf area index (0.96), dry matter accumulation (0.87), grain yield (0.84), and straw yield (0.80). Leaf area index showed significant and positive relationships with dry matter accumulation (0.93), grain yield (0.93), and straw yield (0.90). Additionally, dry matter accumulation exhibited significant and positive associations with grain yield (0.98) and straw yield (0.98).

3.7 Regression between Growth and Yield Attributes with Grain Yield

Regression analysis was conducted to examine the relationship between growth parameters and yield. A positive correlation was observed between plant height at harvest and grain yield (R^2= 0.71), indicating that 71% of the variability in grain yield could be accounted for by plant height. Similarly, leaf area index exhibited a positive association with grain yield (R^2= 0.86), explaining 86% of the variation in grain yield. Dry matter accumulation at harvest also showed a positive correlation with grain yield (R^2= 0.96), indicating that 96% of the variability in grain yield could be explained by dry matter accumulation.

Regarding straw yield, a positive relationship was found with plant height (R^2= 0.65), leaf area index at flowering (R^2= 0.81), and dry matter accumulation at harvest (R^2= 0.95). This suggests that 65%, 81%, and 95% of the variability in straw yield can be explained by plant height, leaf area index at flowering, and dry matter accumulation at harvest, respectively.

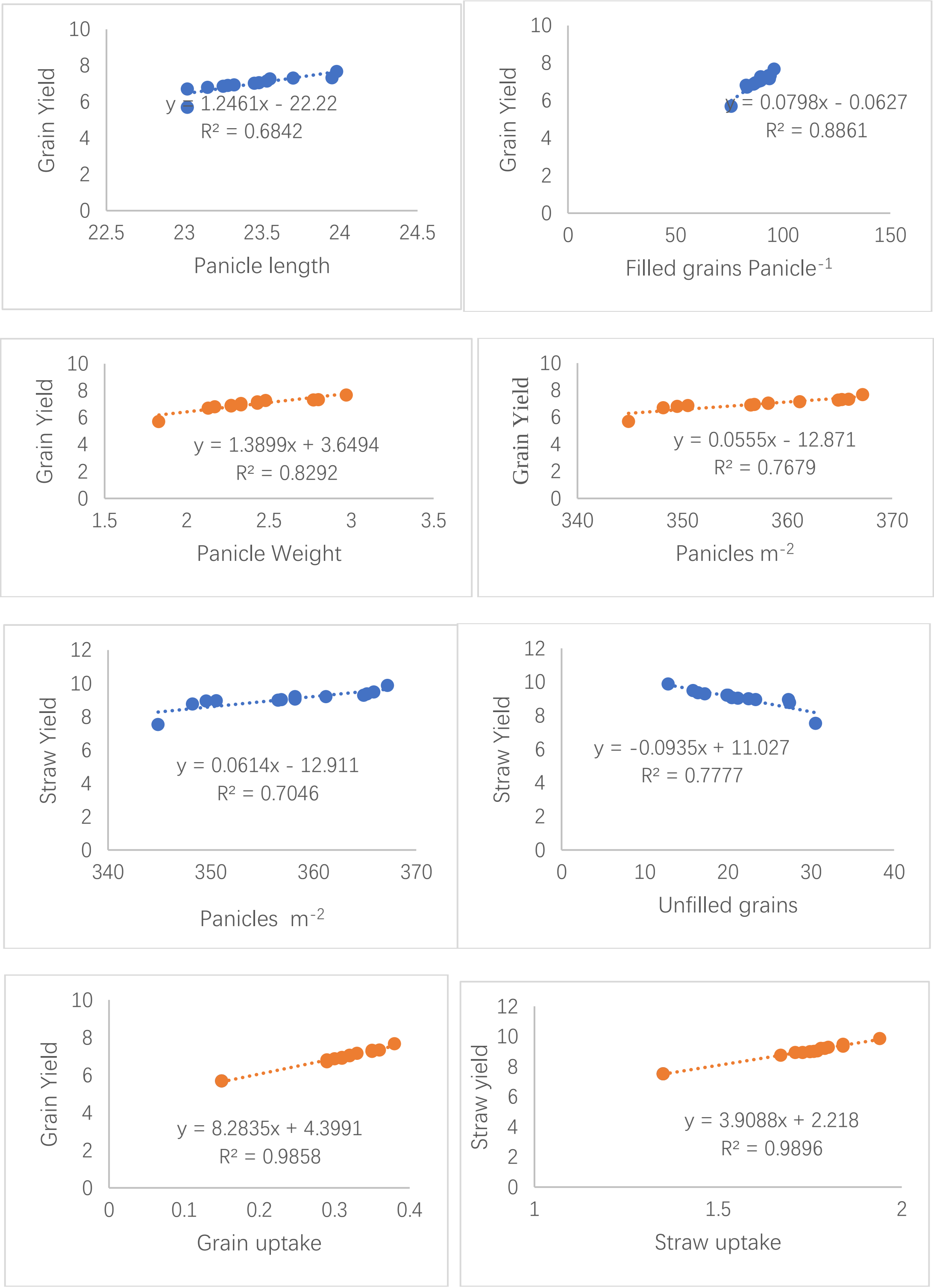

Fig. 5. Regression analysis of different yield attributes, yield and iron uptake

Table 6. Correlation studies of yield and yield parameters with grain and straw iron uptake

	Panicle length	**Panicle m^{-2}**	**Panicle weight (g)**	**Grains $panicle^{-1}$**	**Test weight**	**Grain yield ($t\ ha^{-1}$)**	**Straw yield ($t\ ha^{-1}$)**	**Biological yield**	**Harvest index (%)**	**Grain iron uptake (kg/ha)**	**Straw iron uptake (kg/ha)**
Panicle length	1										
Panicle m^{-2}	0.93**	1									
Panicle weight (g)	0.96**	0.92**	1								
Grains $panicle^{-1}$	0.10	0.09	0.16	1							
Test Weight	0.37	0.41	0.32	0.12	1						
Grain yield ($t\ ha^{-1}$)	0.82**	0.87**	0.91**	0.31	0.20	1					
Straw yield ($t\ ha^{-1}$)	0.80**	0.83**	0.89**	0.34	0.15	0.99**	1				
Biological yield	0.81**	0.85**	0.90**	0.33	0.17	0.99**	0.99**	1			
Harvest index (%)	0.74**	0.88**	0.76**	0.13	0.45	0.81**	0.74**	0.78**	1		
Grain uptake (kg/ha)	0.78**	0.85**	0.87**	0.33	0.20	0.99**	0.98**	0.99**	0.80**	1	
Straw uptake (kg/ha)	0.77**	0.83**	0.87**	0.35	0.17	0.99**	0.99**	0.99**	0.76**	0.99**	1

***. Correlation is significant at the 0.01 level (1-tailed).*

Table 7. Correlation studies of growth parameters with yield

	Plant Height	Leaf Area Index	Dry Matter Accumulation	Grain Yield	Straw Yield
Plant Height	1				
Leaf area index	0.96**	1			
Dry matter accumulation	0.87**	0.93**	1		
Grain Yield	0.84**	0.93**	0.98**	1	
Straw Yield	0.80**	0.90**	0.98**	0.99**	1

***. Correlation is significant at the 0.01 level (1-tailed).*

Additionally, regression analysis revealed associations between yield and yield attributes such as panicles m^{-2}, panicle weight, and grains panicle $^{-1}$. A positive correlation was observed between grain yield and panicles per sq. m (R^2= 0.76), indicating that 76% of the variability in grain yield could be explained by panicles per sq. m. Similarly, a positive relationship was found between grain yield and panicle weight (R^2= 0.82), indicating that 82% of the variability in grain yield could be explained by panicle weight. Additionally, panicle length (R^2= 0.68) and filled grains per panicle (R^2= 0.88) showed positive associations with grain yield.

Analysis also revealed positive associations of straw yield with panicle length (R^2= 0.65), panicle weight (R^2= 0.80), panicles per square meter (R^2= 0.70), and unfilled grains per panicle (R^2= 0.77). Moreover, a positive relationship was observed between grain yield and grain iron uptake (R^2= 0.98), indicating that 98% of the variability in grain yield could be explained by grain iron uptake. Similarly, a positive association was found between straw yield and straw iron uptake (R^2= 0.98), suggesting that 98% of the variability in straw yield could be explained by straw iron uptake.

4. DISCUSSION

CGR, RGR, and NAR serve as crucial indicators for assessing crop growth. The data illustrates that as the crop nears maturity and approaches harvest, its crop growth rate decelerates, while the relative growth rate and net assimilation rate decline notably with the progression of crop age. Among the various iron treatments, higher growth rates were observed in the $FeSO_4.7H_2O$ @ 1% treatment, which, statistically, was comparable to the iron chelate @ 1% treatment. Within different sources and levels of iron, iron sulphate @ 1% exhibited the highest CGR, RGR, and NAR. Adequate iron supply aids plants in enhancing various metabolic processes by facilitating the uptake and availability of other essential nutrients. Consequently, this promotes improved crop growth, leading to enhanced yield attributes. External application of iron contributes to increased photosynthesis, net assimilation, and relative growth in rice by supporting the metabolism of chlorophylls. Similarly, Iqbal *et al*. [21] also observed a significant impact of iron on forage crops.

Iron sulphate @ 1% and iron chelate @ 1% exhibited the highest values for panicles per square meter, panicle weight, and grains per panicle, whereas the control treatment showed the lowest yield attributes. The improved yield attributes observed in rice are attributed to the stimulation of crop growth and the promotion of tiller production as iron levels increase. The increased availability of essential nutrients due to iron application contributes to overall crop growth, leading to a higher number of panicles per square meter. Kumar et al. [7] similarly reported a beneficial impact of iron on rice. During the post-flowering phase, enhanced photosynthesis and assimilate transfer result in increased panicle and grain weights. Foliar iron spray enhances various plant activities, including membrane integrity, stomatal regulation, chlorophyll formation, and energy utilization during early growth stages, which ultimately contribute to increased grain size and weight at later stages [22,23]. The rise in grains per panicle with the application of different iron treatments is attributed to iron's facilitation of nutrient accessibility, enhanced nutrient uptake, and improved photosynthate transfer from source to sink, thereby increasing panicle fertility and leading to a higher number of grains per panicle [24,25].

The data reveals that applying iron via foliar spray at a higher concentration (1%) using iron sulfate and iron chelate significantly enhanced the grain, straw, and biological yield of rice. Increased grain yield in any crop is often associated with enhanced vegetative growth and greater accumulation of dry matter. The greater the vegetative growth, the higher the dry matter accumulation, leading to increased grain yield. However, grain yield is influenced by various yield-contributing factors. In rice, the higher grain yield resulting from foliar iron application can be attributed to the increased number of panicles per square meter and grains per panicle. Additionally, iron plays a crucial role in initiating reproductive organs and in the biosynthesis of Indole Acetic Acid (IAA). The higher straw yield observed with iron applications compared to the control can also be attributed to iron's support for overall vegetative growth by improving nutrient availability and uptake. Kumar et al. [8] also reported increased rice yields with iron fortification. Harvest index, which represents the proportion of grain yield to total biological yield (grain + straw), was highest with iron chelate @ 0.75%, possibly due to improved carbohydrate translocation from source to sink [8].

The application of iron sulphate (1%) through foliar spray notably enhanced both the iron content and uptake in grains and straw. This increase in iron content in brown rice may be attributed to the high mobilization of iron sulphate within plants, facilitating its rapid absorption and translocation to various plant parts. This enhanced availability and mobility of iron contribute to its increased uptake by the plants. The micronutrient enrichment through foliar micronutrient application proportionally influences the components of yield, effectively enriching rice flour. There was a positive correlation observed between crop yield and iron uptake in grains, indicating that higher nutrient uptake, facilitated by foliar iron application, contributes to increased yield. Additionally, the higher iron content in straw compared to grains may be due to the increased availability, absorption, and limited mobility of iron in different plant parts. The uptake of iron in rice straw was significantly increased with iron fertilization, particularly with foliar sprays of 1% $FeSO_4.7H_2O$ application, compared to other treatments, likely due to the higher micronutrient content and rice yield from this treatment. However, iron use efficiency decreased with higher rates of iron application, with maximum efficiency observed in chelated iron. This decrease in iron use efficiency with increasing iron content from a particular source or level is consistent with existing literature [26].

The notable role of iron as a crucial cofactor, particularly in enhancing nitrite and nitrate reductase enzymes, could explain the observed increase in protein content following foliar iron application. Iron's presence in chloroplasts facilitates sulphur and nitrogen metabolism, while its involvement in heme and non-heme protein formation further contributes to the elevated protein levels [27,28].

Despite variations in iron sources and levels, no significant differences were observed in soil pH, electrical conductivity (EC), organic carbon, phosphorus (P), and potassium (K) at harvest. However, the lowest soil available nitrogen was detected in plots treated with iron sulphate at 1%, whereas the highest nitrogen availability was noted in the control treatment. This suggests that iron sulphate at 1% efficiently utilized nitrogen compared to other treatments. Our findings also indicated significant variations in soil iron levels across different iron treatments and levels. Specifically, the application of iron sulphate at 1% led to a significant increase in soil iron availability at harvest.

5. CONCLUSION

The findings of this study suggest that foliar iron fertilization is a valuable agricultural strategy for improving rice yield, iron content, bioavailability of iron, and protein content. Among the various sources and levels of iron evaluated, the application of $FeSO_4.7H_2O$ at a concentration of 1% proved to be the most effective in enhancing crop growth rate, relative growth rate, net assimilation rate, yield, protein content, iron content, and uptake in both brown rice and straw. Additionally, it resulted in an increase in soil iron content and a decrease in soil nitrogen levels at harvest. However, iron chelate at a concentration of 0.25% exhibited the highest iron use efficiency, making it a favorable option for agronomic fortification of rice with iron in the temperate conditions of Kashmir.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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