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Genotypic variation in rice tolerance to iron toxicity: insights from Bangladesh

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Abstract. Iron (Fe) toxicity is a major abiotic stress affecting rice production, particularly in acidic soils. Although not well-documented in Bangladesh, Fe toxicity is becoming a significant agronomic concern due to rising concentrations in irrigated soils. This study aimed to assess the tolerance of 18 rice genotypes to Fe toxicity stress based on morphophysiological and yield-related traits. A pot experiment was conducted at Gazipur Agricultural University, Gazipur, using a completely randomized design with three Fe levels: control (0 mg L^{-1}), Fe_1 (600 mg L^{-1}), and Fe_2 (1,200 mg L^{-1}). Results showed that increasing Fe^{2+} concentrations significantly affected plant height, tillering ability, chlorophyll content, and grain yield. At Fe_2, total tillers per hill declined by 65.8%, and effective tillers reduced by 35.5% compared to the control. Panicle length and the number of filled grains decreased, while unfilled grains increased, indicating reproductive stage Fe stress. The SPAD chlorophyll index declined by 21.3% under Fe_2, reflecting Fe-induced chlorosis. Grain yield exhibited a sharp reduction of 46.3% under Fe_2, highlighting the detrimental impact of Fe toxicity on productivity. Genotypic variations were observed, with BU line 5 exhibiting the highest grain yield (0.92), effective tillers hill^{-1} (0.89), and lowest unfilled grains per panicle (1.26), suggesting superior tolerance. Multivariate analysis grouped genotypes based on the stress response, identifying BU line 5, BU line 13, and BRRI Dhan 28 as promising Fe-tolerant candidates. These findings highlight the critical need to breed Fe-tolerant rice varieties to mitigate yield losses in Fe-affected soils. Further research on the genetic and molecular mechanisms of Fe toxicity tolerance will facilitate breeding programs for stress-resilient rice varieties, ensuring food security in regions prone to Fe toxicity stress.

Key words: chlorophyll index, iron toxicity tolerance, multivariate analysis, rice genotypes, yield.

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food that provides essential calories and nutrients for more than half of the world's population. However, its production faces increasing challenges due to many abiotic stresses, with iron (Fe) toxicity being a significant concern. Fe toxicity, caused by excessive iron accumulation in waterlogged and acidic soils, severely hampers rice growth and yield (Nadeem & Farooq, 2019). Although Bangladesh has not officially reported widespread Fe toxicity, research shows that Fe levels in agricultural soils are increasing, which means it could soon be a big problem for rice farming (Sheel et al., 2015; Hassan et al., 2017; Islam et al., 2020). A strong correlation between elevated Fe levels and declining crop yields has been observed (Briat et al., 2015; Liliane & Charles, 2020), further exacerbated by the growing dependence on iron-rich groundwater for irrigation. This situation emphasizes the importance of understanding and reducing Fe toxicity to protect rice production in Bangladesh.

Fe toxicity is a widespread issue globally. Studies show that approximately 18% of cultivated soils experience moderate to severe Fe toxicity (Das & Roychoudhury, 2014; Rout et al., 2014). In West Africa, over 55% of rice-growing areas are affected by excess Fe accumulation, leading to yield losses of 16% to 78% (Audebert & Fofana, 2009; Cherif et al., 2009). High concentrations of Fe^{2+} in flooded soils disrupt photosynthetic activity and root development, resulting in considerable loss of yield and disturbances in nutrient uptake in rice (Wairich et al., 2024). In Bangladesh, the Madhupur tract has long been facing acute Fe toxicity, with increasing Fe concentrations (Miah et al., 2005; Sultana et al., 2015). Climate change could accelerate this process, posing a threat to rice production and food security. Given the global prevalence of Fe toxicity, development of Fe-tolerant rice varieties is essential for sustainable rice production.

Most research on Fe toxicity has focused on Fe^{2+} (ferrous iron), the more soluble and phytotoxic form prevalent under flooded and anaerobic soil conditions (Quinet et al., 2012; Rout & Sahoo, 2015; Andrew et al., 2020; Ullah et al., 2023; Fujimoto et al., 2024). In Bangladesh, however, Fe toxicity may also be influenced by high concentrations of Fe^{3+} (ferric iron), particularly in oxidized surface soils, a mechanism that remains understudied. Elevated levels of soil Fe have been reported in agricultural regions such as Mymensingh, Jamalpur, and Tangail (Sheel et al., 2015; Islam et al., 2020), where Fe accumulation threatens crop productivity and contributes to progressive soil degradation. Despite increasing recognition of Fe toxicity as a constraint to rice production in Bangladesh, limited studies have investigated the physiological and genetic responses of rice genotypes to Fe toxicity stress. Most existing research has focused on soil Fe status rather than plant tolerance mechanisms. This highlights the urgent need for systematic evaluation and development of Fe-tolerant rice genotypes to address the growing challenge of Fe toxicity in rice production systems.

This study aims to identify rice genotypes that can tolerate iron (Fe) toxicity by exploring the morphological and physiological traits important for enhancing resistance. By developing Fe-tolerant varieties, this study seeks to provide a practical solution for farmers in Bangladesh and other regions facing similar soil conditions. Ultimately, these initiatives will contribute to developing climate-resilient rice varieties and ensure sustainable rice production even when soil iron levels increase.

MATERIALS AND METHODS

Planting materials

A total of 18 rice genotypes (three released varieties of BARI and 15 BU Lines) were used in the study. A list of genotypes with their accession number is given in Table 2. Seeds of the genotypes were collected from the Genetic Resources Unit of the Department of Agronomy, Gazipur Agricultural University, Gazipur-1706, Bangladesh.

Location, experimental design, and treatments

The study was conducted at the Agronomy Field Research Site of Gazipur Agricultural University, Gazipur, from 18 December 2022 to May 2023 under semi-controlled conditions. The baseline soil properties prior to treatment application were as follows: available Fe = 9.6 mg kg^{-1} soil (DTPA-extractable), pH = 6.1, organic matter = 1.24%, and soil texture classified as silty clay loam (17% sand, 48% silt, and 35% clay). The experiment followed a completely randomized design with a factorial combination of 18 rice genotypes and three Fe levels i.e. Fe_0–control, Fe_1–600 mg L^{-1}, and Fe_2–1,200 mg L^{-1} Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) was used as the iron source for the treatments. However, no iron was added to the control treatment (0 mg L^{-1}), and no chelating agents (e.g., EDTA) were applied to eliminate trace Fe present in the soil.

Plant establishment

Plants were grown in plastic pots (height: 24 cm, top diameter: 27 cm, bottom diameter: 18 cm) filled with 8 kg of soil. Nursery trays were filled with H_2O_2 sterilized seeds and three 20-day old seedlings were transplanted in each pot. Iron treatments were applied through irrigation by maintaining a standing water depth of 3–5 cm until the grain-filling stage. Iron concentration was checked periodically to maintain the appropriate dose. Paraffin oil (5 mL pot^{-1}) was used to suppress evaporation and limit the aeration and oxidation of Fe^{2+}ions. To further inhibit Fe^{2+} oxidation, the pH of the irrigation water was regularly checked and kept within a slightly acidic range (pH 5.8–6.2).

Phenology and morphophysiological characters

Days to first flowering were recorded when a single flower appeared for each genotype. Days to 50% flowering were recorded when 50% of the tillers were fully headed, and days to maturity when 50% of panicles turned brown. Plant height was measured from the base to the tip of the tallest leaf, and the mean of three hills was calculated. Tillers were counted from three hills, and the mean number per plant was determined. SPAD chlorophyll readings (SPAD-502, Minolta, Japan) were taken from the youngest fully expanded leaf at 10-day intervals from 30–90 DAT. The readings were averaged from three measurements per plant. At maturity, plants from ten selected samples were harvested for yield estimation.

Yield and yield-contributing characters

Panicle length was measured from the base of the panicle to the tip, and the mean was calculated. Grains were separated, and filled and unfilled grains per panicle were counted using a Multi Auto Counter (DCI NO: 501102, Ever Well Crop, Tokyo, Japan). Thousand grains were weighed after counting and grain yield (g $plant^{-1}$) was recorded. Samples were threshed, cleaned, dried, and adjusted to 14% moisture content (MC), determined using an infrared moisture meter (Model, F-1A, Kett Electric Laboratory, Tokyo, Japan). Grain yield was adjusted to 14% MC using the formula: Grain yield at 14% MC = (100 - sample MC) / (100 – 14) · grain sample weight.

K-means clustering and discriminant function analysis

K-means clustering and discriminant function analysis (DFA) were performed to classify 18 rice genotypes based on eight quantitative variables: plant height, total tillers per plant, effective tillers $hill^{-1}$, panicle length, filled grains per panicle, unfilled grains per panicle, SPAD chlorophyll index, and grain yield. Prior to analysis, all these trait values were expressed as the ratio of Fe-stress to control conditions to ensure uniformity across variables and enable meaningful comparisons of genotype responses under stress. K-means clustering grouped genotypes based on their similar responses to Fe toxicity stress, while DFA identified the most useful variables in separating groups and assessed the accuracy of clustering. The stepwise procedures of DFA, including chi-square tests, structure matrix analysis, and tests of equality of group means were performed following the procedure described by Islam et al. (2007). Phenological traits were excluded from the DFA due to their very weak correlation with the eight selected variables and limited contribution to group separation.

RESULTS AND DISCUSSION

Effect of iron toxicity levels on rice genotypes

Iron (Fe) toxicity significantly influences the growth and yield-related parameters of rice genotypes. The impact of increasing Fe concentrations (600 mg L^{-1} and 1,200 mg L^{-1}) is evident across various plant characteristics, with notable reductions in plant height, tillering ability, grain yield, and chlorophyll content (Table 1).

Growth and morphological responses

The plant height of the 18 rice genotypes under control conditions ranged from 72.5 cm to 126.1 cm, with a mean of 89.02 cm. When subjected to Fe toxicity at 600 mg L^{-1} (Fe_1), the range extended from 64.67 cm to 131.6 cm, and the mean value slightly decreased to 87.14 cm. At 1,200 mg L^{-1} (Fe_2), plant height further declined, with a range of 58.43 cm to 127.1 cm and a mean of 82.6 cm (Table 1). The relative reduction in mean plant height due to Fe_1 stress was 2.1%, while Fe_2 caused a greater reduction of 7.2% compared to the control. The reduction in plant height under elevated Fe levels suggests that excessive Fe disrupts cell elongation and impairs vegetative growth (Sikirou et al., 2015; Viana et al., 2017; Zahra et al., 2021). The mean height decreased from 89.02 cm (control) to 82.6 cm (Fe_2), showing a decline in plant vigor.

Under control conditions, total tillers hill^{-1} ranged from 9.67 to 18.67, with a mean of 13.16. Exposure to Fe_1 stress resulted in a reduced range of 8.00 to 14.00 and a lower mean of 10.96. The decline was more pronounced at Fe_2, where total tillers ranged from 8.42 to 11.50, with a mean of 4.50 (Table 1). The reduction in total tillers hill^{-1} under Fe_1 stress was 16.7%, whereas under Fe_2 stress, it was significantly higher at 65.8% compared to the control. The effective tillers hill^{-1} ranged between 8.33 and 17.67 under control conditions, with an average of 10.98. Under Fe_1 stress, the range narrowed to 6.33-12.00, with a mean of 9.02, reflecting a reduction. Under Fe_2 stress, the mean further dropped to 7.08, with a range of 3.50 to 9.50 (Table 1). The relative reduction in effective tillers hill^{-1} was 17.9% under Fe_1 stress and 35.5% under Fe_2 stress compared to the control. Tillering capacity was markedly impacted, with prnounced reductions in both total and effective tillers under Fe toxicity stress. At Fe_2, total tillers hill^{-1} dropped to 4.50 from 13.16 in control, and effective tillers also reduced from 10.98 to 7.08, suggesting that Fe stress disrupts tiller initiation and development (Onyango et al., 2019; Diedhiou et al., 2020; Page et al., 2022).

Table 1. Range and mean for quantitative characteristics of 18 rice genotypes subjected to iron toxicity stress

Plant characters	Control		Fe$_1$: 600 mg L^{-1}		Fe$_2$: 1,200 mg L^{-1}	
	range	mean^1	range	mean	range	mean
Plant height (cm)	72.5–126.1	89.02 ± 11.7^a	64.67–131.6	87.14 ± 15.8^a	58.43–127.1	82.6 ± 15.5^b
Total tillers hill^{-1} (no.)	9.67–18.67	13.16 ± 2.42^a	8.00–14.00	10.96 ± 1.79^b	4.50–11.50	8.42 ± 1.45^c
hill^{-1} (no.)	8.33–17.67	10.98 ± 2.43^a	6.33–12.00	9.02 ± 1.60^b	3.50–9.50	7.08 ± 1.29^c
Panicle length (cm)	21.47–26.06	23.86 ± 1.46^a	18.22–25.36	22.43 ± 1.81^{ab}	17.36–24.47	21.25 ± 2.04^b
Filled grains panicle^{-1} (no.)	43.90–94.22	72.63 ± 13.17^a	27.79–85.09	58.59 ± 14.94^b	23.22–71.23	47.22 ± 15.92^c
Unfilled grains panicle^{-1} (no.)	13.75–40.05	26.91 ± 7.61^c	17.44–59.13	37.52 ± 10.22^b	25.06–69.95	44.91 ± 11.37^a
SPAD Chlorophyll index	38.2–45.0	41.6 ± 2.25^a	34.7–43.8	37.5 ± 2.25^b	26.3–37.1	32.8 ± 2.63^c
Grain yield (g plant^{-1})	11.8–29.23	16.92 ± 4.42^a	5.43–18.80	11.65 ± 3.59^b	4.73–13.50	9.09 ± 2.65^c

^{1}Mean±Standard deviation. Values in each row followed by different letters are significantly different at $p < 0.05$ according to Tukey's *HSD* test.

Reproductive traits

Panicle length varied between 21.47 cm and 26.06 cm in control conditions, with an average of 23.86 cm. Fe$_1$ stress led to a reduction, with a range of 18.22 to 25.36 cm and a mean of 22.43 cm. Under Fe$_2$ stress, panicle length further declined, ranging from 17.36 cm to 24.47 cm, with a mean of 21.25 cm (Table 1). The reduction in mean panicle length was 6.0% under Fe$_1$ and 10.9% under Fe$_2$ compared to the control. There was a decline in panicle length under Fe stress, though the effect was less pronounced than in tillering traits. The mean length decreased from 23.86 cm (control) to 21.25 cm (Fe$_2$), which could influence grain production (Streck et al., 2019).

The number of filled grains per panicle ranged from 43.90 to 94.22 under control conditions, with a mean of 72.63. Under Fe$_1$ stress, the range decreased to 27.79–85.09, and the mean reduced to 58.59. A further decline was observed under Fe$_2$ stress, with a range of 23.22–71.23 and a mean of 47.22 (Table 1). The reduction in filled grains per panicle was 19.4% under Fe$_1$ and 35.0% under Fe$_2$ relative to the control. The unfilled grains per panicle in control conditions ranged from 13.75 to 40.05, with an average of 26.91. Under Fe$_1$ stress, the number increased, ranging from 17.44 to 59.13, with a mean of 37.52. Under Fe$_2$ stress, the unfilled grains further increased to a range of 25.06–69.95, with a mean of 44.91 ± 11.37 (Table 1). The increase in unfilled grains per panicle was 39.4% under Fe$_1$ and 66.9% under Fe$_2$ compared to the control. A significant reduction in filled grains per panicle was observed, dropping from 72.63 (control) to 47.22 (Fe$_2$), while unfilled grains increased, indicating that Fe toxicity impairs grain filling and reproductive success (Rout et al., 2014; Aratani et al., 2023). When too much iron is absorbed, it can cause oxidative damage to cells by disrupting redox status, which changes morphophysiological and yield-related characteristics (Kar & Panda, 2020).

Physiological and yield parameters

The SPAD chlorophyll index, an indicator of chlorophyll content and photosynthetic efficiency, ranged from 38.2 to 45.0 under control conditions, with a mean of 41.6. Under Fe_1 stress, the range narrowed to 34.7–43.8, and the mean declined to 37.52. A more pronounced reduction was observed under Fe_2, with values ranging from 26.3 to 37.1 and a mean of 32.8 (Table 1). The relative reduction in chlorophyll index was 9.9% under Fe_1 and 21.3% under Fe_2 compared to the control. Mahender et al. (2019) reported a significant reduction in the chlorophyll index from 41.6 (control) to 32.8 (Fe_2 treatment) under Fe toxicity, primarily due to Fe-induced chlorosis and disruption of photosynthetic processes. Elevated Fe concentrations in flooded soils promote the formation of hydroxyl radicals and reactive oxygen species (ROS), which degrade chlorophyll and damage cellular components, thereby impairing photosynthesis (Pereira et al., 2013; Onaga et al., 2016). Moreover, Fe toxicity may induce secondary deficiencies of essential micronutrients such as zinc (Zn), which plays a critical role in chlorophyll biosynthesis (Armatmontree et al., 2023; Trajano de Oliveira et al., 2023). Therefore, future research should include quantification of leaf Fe, Zn, and Mn concentrations to elucidate the physiological mechanisms underlying Fe-induced reductions in chlorophyll content. Grain yield per plant ranged from 11.80 g to 29.23 g under control conditions, with a mean of 16.92 g. Under Fe_1 stress, grain yield declined, ranging from 5.43 g to 18.80 g, with a mean of 11.65 g. Fe_2 stress further reduced grain yield to a range of 4.73–13.50 g and a mean of 9.09 g (Table 1). The relative reduction in grain yield was 31.1% under Fe_1 and 46.3% under Fe_2 compared to the control. The most affected parameter was grain yield, which declined sharply under Fe toxicity. The mean grain yield per plant decreased from 16.92 g (control) to 9.09 g (Fe_2), showing a substantial yield loss due to Fe stress.

Iron toxicity stress negatively impacts rice genotypes, with higher Fe concentrations leading to stunted growth, reduced tillering, lower chlorophyll content, and significant yield losses. The most sensitive traits under Fe toxicity are tillering ability, chlorophyll index, and grain yield, which should be key targets for developing Fe-tolerant rice genotypes. A drastic reduction in tillering ability at Fe_2 suggests that Fe toxicity severely inhibits vegetative growth. The decline in chlorophyll content indicates Fe-induced stress affecting photosynthesis. A marked decrease in yield highlights the severe impact of Fe toxicity on productivity. These facts are supported by many literatures (Müller et al., 2015; Rasheed et al., 2020; Ahmed et al., 2023).

Genotypic performance of rice under Fe toxicity stress

The performance of rice genotypes under Fe toxicity stress (1,200 mg L^{-1} Fe) was evaluated based on various agronomic and physiological traits (Table 2). The findings reveal significant variation among genotypes in their responses to excess Fe, highlighting differences in tolerance levels. Among the tested genotypes, BU line 2 exhibited the highest relative plant height (1.06), indicating a strong ability to maintain growth under Fe stress. Likewise, BU line 5 excelled in effective tillers $hill^{-1}$ (0.89) and grain yield (0.92), demonstrating its resilience.

Regarding tillering capacity, BRRI Dhan 29 recorded the highest total tillers hill^{-1} (0.86), while BU line 5 maintained the highest effective tillers hill^{-1} (0.89), signifying its ability to sustain productive tillers under stress. Since high tillering capacity often correlates with stress tolerance (Lubis et al., 2022; Rajonandraina et al., 2023), this trait may help compensate for potential reductions in individual plant productivity. On the other hand, BU line 4 exhibited the highest number of unfilled grains per panicle (2.80), followed by BRRI Dhan 29 (2.05) and BU line 10 (2.20), suggesting a higher sterility rate under Fe toxicity. An increased in the number of unfilled grains indicates disruption of reproductive development, potentially due to oxidative damage caused by Fe toxicity (Aung & Masuda, 2020; Harish et al., 2023). In contrast, BU line 5 recorded the lowest unfilled grains per panicle (1.26), reinforcing its superior stress tolerance and reproductive resilience.

Table 2. Relative performance of rice genotypes as affected by F_2 (1,200 mg L^{-1}) level of iron

	Relative value[1]							
Genotypes	plant height	total tillers hill^{-1}	effective tillers hill^{-1}	panicle length	filled grain per panicle	unfilled grain per panicle	SPAD Chlorophyll index	grain yield
BU line 1	0.94	0.54	0.51	0.79	0.73	1.61	0.75	0.46
BU line 2	1.06	0.56	0.59	0.95	0.89	1.41	0.74	0.57
BU line 3	0.81	0.75	0.72	0.95	0.87	1.79	0.80	0.53
BU line 4	0.92	0.55	0.50	0.89	0.80	2.80	0.81	0.58
BU line 5	0.91	0.72	0.89	0.95	0.83	1.26	0.81	0.92
BU line 6	0.96	0.60	0.61	0.89	0.42	1.85	0.77	0.61
BU line 7	0.90	0.61	0.64	0.95	0.67	2.48	0.80	0.60
BU line 8	1.00	0.73	0.68	0.95	0.76	1.29	0.74	0.56
BU line 9	0.86	0.72	0.70	0.80	0.57	1.32	0.76	0.50
BU line 10	1.01	0.76	0.82	0.94	0.45	2.20	0.83	0.39
BU line 11	1.04	0.32	0.29	0.83	0.60	1.49	0.76	0.29
BU line 12	0.94	0.69	0.65	0.90	0.81	1.23	0.85	0.64
BU line 13	0.93	0.63	0.72	0.86	0.76	1.29	0.86	0.68
BRRI Dhan 28	0.95	0.68	0.72	0.88	0.44	1.35	0.84	0.65
BRRI Dhan 29	0.73	0.86	0.76	0.91	0.27	2.05	0.83	0.41
BRRI Dhan 102	0.83	0.55	0.79	0.79	0.48	1.89	0.67	0.35
BU line 14	0.92	0.68	0.62	0.86	0.70	1.82	0.73	0.55
BU line 15	0.92	0.83	0.70	0.93	0.69	2.17	0.80	0.55
Min	0.73	0.32	0.29	0.79	0.27	1.23	0.67	0.29
Max	1.06	0.86	0.89	0.95	0.89	2.80	0.86	0.92
Mean	0.92	0.65	0.66	0.89	0.65	1.74	0.79	0.55
SD	0.08	0.13	0.14	0.06	0.18	0.46	0.05	0.14

[1] The relative values were calculated as the ratio of the parameter value under iron stress (F_2: 1,200 mg L^{-1} Fe^{2+}) to the corresponding value under the non-stressed control condition (F_0: 0 mg L^{-1} Fe^{2+}).

The relative SPAD chlorophyll index ranged from 0.67 (BRRI Dhan 102) to 0.86 (BU line 13). Higher SPAD index values in BU line 13 (0.86), BU line 12 (0.85), and BRRI Dhan 28 (0.84) suggest a better ability to maintain chlorophyll content under Fe stress, which is essential for sustaining metabolic functions. BU line 5 also exhibited a high SPAD chlorophyll index (0.81), further emphasizing its potential tolerance to Fe toxicity. In terms of grain yield, BU line 5 (0.92) outperformed all other genotypes, showing a strong capacity to sustain productivity under Fe stress. This was followed by BU line 13 (0.68) and BRRI Dhan 28 (0.65). The ability of these genotypes to sustain grain yield under Fe toxicity indicates the presence of efficient Fe homeostasis mechanisms, including Fe compartmentalization and the regulation of essential physiological processes, as highlighted by Rout & Sahoo (2015). Conversely, BRRI Dhan 102 (0.35) and BU line 11 (0.29) exhibited the lowest relative grain yields, indicating high susceptibility to Fe stress.

The observed variation among genotypes suggests that Fe toxicity tolerance is associated with multiple physiological and morphological traits, including improved tillering ability, reduced sterility, enhanced chlorophyll retention, and efficient Fe detoxification precesses. Among the genotypes tested, BU line 5, BU line 13, and BRRI Dhan 28 exhibited promising tolerance to Fe toxicity, making them potential candidates for breeding programs aimed at enhancing Fe stress resilience in rice. Similar findings in recent literature have emphasized the importance of genotypic screening based on traits such as SPAD chlorophyll values, effective tillering, reduced spikelet sterility, and sustained chlorophyll content as key indicators of Fe tolerance (Ahmed et al., 2023; Aratani et al., 2023; Harish et al., 2023). A genome-wide association study (GWAS) involving 239 rice accessions further identified shoot height, SPAD chlorophyll index, leaf bronzing score (LBS), and root biomass as key traits for differentiating tolerant and sensitive genotypes under Fe stress conditions (Theerawitaya et al., 2022). In addition, several Fe-tolerant lines, including CSSLOG254-256, have been proposed as valuable resources for breeding programs (Sikirou et al., 2025). Future studies should focus on uncovering the genetic and molecular mechanisms underlying Fe toxicity tolerance among these genotypes to support breeding strategies aimed at developing stress-resilient rice varieties.

Grouping of genotypes through multivariate analysis

To group the genotypes, multivariate analysis was employed to identify desirable plant traits associated with iron toxicity tolerance. In this context, it was essential to form statistically homogeneous groups based on the evaluated variables. To achieve this, K-means non-hierarchical cluster analysis was performed using eight quantitative plant traits that exhibited high correlation coefficients (Table 3). These traits included plant height (cm), total tillers per plant, and effective tillers per hill, panicle length (cm), filled grains and unfilled grains per panicle, SPAD chlorophyll index, and grain yield.

Table 3. List of 18 rice genotypes under four clusters classified by K-means cluster analysis based on eight plant characters

Clustering	No. of genotypes	Genotypes
Cluster 1	7	BU line 3, BU line 6, BU line 10, BRRI Dhan 29, BRRI Dhan 102, BU line 14 and BU line 15
Cluster 2	7	BU line 2, BU line 5, BU line 8, BU line 9, BU line 12, BU 13 and BRRI Dhan 28
Cluster 3	2	BU line 1 and BU line 11
Cluster 4	2	BU line 4 and BU line 7

Based on the analysis, the 18 rice genotypes were categorized into four distinct clusters. The genotypes within each cluster displayed a high degree of similarity. The largest number of genotypes (7) was grouped into cluster 1 and clusters 2, while cluster 3 and cluster 4 each contained 2 genotypes. A detailed list of the four clusters of rice genotypes is provided in the Table 3.

The total number of genotypes in each cluster, along with the mean values of the eight plant traits for each cluster, is presented in Table 4. The clusters were characterized as follows: Cluster 2: This cluster is distinguished by the highest relative grain yield (0.65), the high number of effective tillers per hill, and the lowest unfilled grains per panicle. However, all other relative plant traits exhibited moderate values (Table 4). Cluster 4: Genotypes in this cluster exhibited the highest relative values for SPAD chlorophyll index (0.81), filled grains per panicle (0.73), and panicle length (0.92). However, they also had the highest number of unfilled grains per panicle. Cluster 1: Genotypes in this cluster recorded the highest total and effective tillers per plant (0.72). However, other plant traits showed moderate performance, resulting in a moderate relative yield performance (0.48). Cluster 3: This cluster had the lowest relative values for nearly all plant traits and recorded the lowest yield performance (0.38). Despite this, genotypes in this group had the highest relative plant height but produced very few effective tillers per hill.

Table 4. Comparison profile of the four groups of rice genotypes classified by K-means clustering

Characters	Clusters			
	1	2	3	4
No. of genotypes	7	7	2	2
Plant height	0.88	0.95	0.99	0.91
Total tillers hill^{-1}	0.72	0.67	0.43	0.58
Effective tillers hill^{-1}	0.72	0.71	0.40	0.57
Panicle length	0.90	0.90	0.81	0.92
Filled grains per panicle	0.55	0.72	0.66	0.73
Unfilled grains per panicle	1.97	1.31	1.55	2.64
SPAD chlorophyll index	0.78	0.80	0.75	0.81
Grain yield	0.48	0.65	0.38	0.59

Discriminant function analysis

Stepwise discriminant function analysis (DFA) was conducted to identify the set of discriminatory functions responsible for differentiating 18 genotypes into four distinct clusters. This statistical method aims to determine how well predefined groups can be separated based on measured variables and is particularly useful for validating group classifications and graphical visualizing their seperation (Singh et al., 1991).

Table 5. Discriminant functions that analyze with Eigen values

Function	Eigen value	% of variance	Cumulative %	Canonical Correlation
1	13.76^{a}	90.3	90.3	0.97
2	1.48^{a}	9.7	100	0.77

a. First 4 canonical discriminant functions were used in th analysis.

The stepwise DFA identified two significant discriminant functions that contributed to the differentiation among clusters. Table 5 summarizes the contribution of these function in explaining variance, along with their corresponding Eigen values and canonical correlation coefficients. Function 1accounted for 90.3% of the total variance, with a high canonical correlation coefficient of 0.97, indicating a strong relationship between the discriminant scores and the grouping. Function 2 explained the remaining 9.7% of the variance with a canonical correlation of 0.77. Two functions together explain 100% of the variance among the clusters. The statistical significance of the discriminant functions was confirmer by Wilk'sd Lambda test (Table 6), where both functions were significant at the 0.001 probability level, supporting their effectiveness in differentiating genotype clusters.

Table 6. Discriminant functions that analyze by Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 4	0.03	50.41	6	0
through 4	0.40	12.73	2	0.002

Among the evaluated traits, unfilled grains per panicle had the highest standardized coefficient (1.002) in Function 1, and also showed the strongest corelation (0.987) with this function (Table 7 and Table 8). This indicates that this trait was most influential in separating genotypes with function 1. From an agronomic perspective, this suggests that variation in grain filling efficiency plays a dominant role in separating genotype clusters, potentially reflecting differences fertility, stress tolerance, or grain development characteristics. Conversely, the effective tillers per hill had the highest standardized coefficient (0.991) in Function 2, indicating that this trait contributed more to the differentiation explained by Function 2. Although Function 2 accounts for a small portion of the total variation, it still plays an important role in further refining the separation between groups. From an agronomic perspective, differences in tillering ability are important for overall productivity and may reflect genotype-specific adaptations to Fe stress conditions.

Table 7. Standardized Canonical Discriminant Function coefficients of the plant characters mostly contributed in grouping rice genotypes

Discriminating variables	Discriminating Function	
	1	2
Effective tillers per hill	-0.162	0.991
Unfilled grains per panicle	1.002	0.069

Table 8. Structure Matrix representing correlations between eleven discriminating variables and standardized canonical discriminant functions of 18 rice genotypes

Discriminating variables	Discriminating Function	
	1	2
Unfilled grain per panicle	0.987*	0.161
Grain yield	-0.286*	0.244
SPAD chlorophyll index	0.232*	0.189
Panicle length (cm)	0.109*	0.031
Effective tillers per hill	-0.068	0.998*
Total tiller per plant	0.265	0.491*
Plant height (cm)	0.302	-0.415*
Filled grain per panicle	-0.148	-0.162*

*Largest absolute correlation between each variable and any discriminant function.

Overall, function 1 captures the main variation related to grain filling characteristics, while function 2 adds further differentiation based on tillering ability. These findings provide both statistical validation of the cluster groupings and meaningful agronomic insight into the trait-based variation among the rice genotypes.

Fig. 1 visually represents the classification of genotypes into four groups based on the first two discriminant functions. Genotypes positioned on the right side of the diagram exhibited a higher number of unfilled grains per panicle, whereas those on the left side had fewer unfilled grains per panicle, as indicated by the X-axis. Genotypes in the other clusters showed intermediate values for this trait. Similarly, genotypes located in the upper part of the diagram had a higher number of effective tillers hill^{-1}, while those in the lower part had fewer effective tillers hill^{-1}, as represented on the Y-axis. Consequently, Function 1 primarily differentiated Group 2 from Group 4 based on the relative number of unfilled grains per panicle, while Function 2 distinguished Group 3 from the other three groups based on the number of effective tillers hill^{-1}.

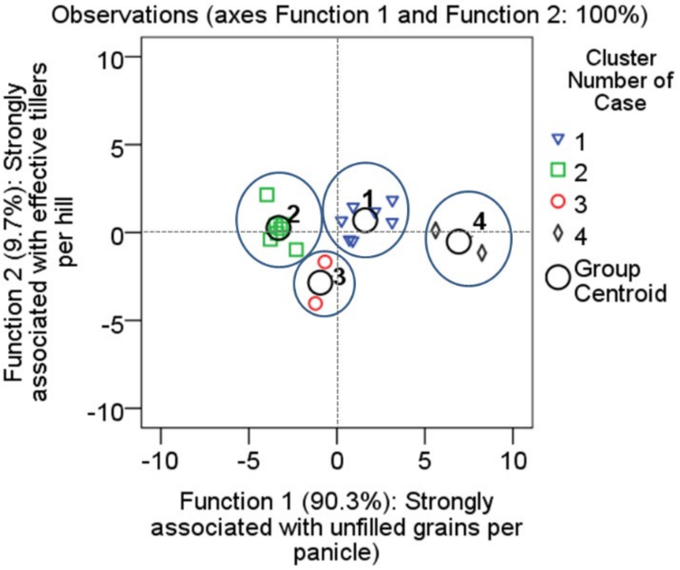

Figure 1. Canonical discriminant function plot showing the separation of 18 rice genotypes into four groups. Function 1 (X-axis) and function 2 (Y-axis) are linear combinations of traits, primarily influenced by unfilled grains per panicle and effective tillers per hill, respectively. Each point reprtesent a genotype, color-coded by group, with group centroids marked by larger black-edged circles. Axes are unitless and standardized.

CONCLUSION

This study shows that iron (Fe) toxicity soignificantly impairs rice growth, physiological function, and grain yield, with substantial genotypic variation in tolerance levels. These results emphasize the necessity of targeted breeding strategies for developing Fe-tolerant rice varieties, particularly in areas affected by excessive soil iron accumulation. Notably, genotypes such as BU line 5, BU line 13, and BRRI Dhan 28 showed superior performance under Fe stress conditions, indicating their potential as valuable parental lines in rice improvement programs. Their advantagious morphological traits, such as, reduced spikelet sterility, enhanced chlorophyll retention and increased effective tillering, serve as practical phenotypic indicators for selection in the breeding pipelines. Additionally, the SPAD chlorophyll index proved to be promissing screening tool for identifiying tolerant genotypes under field contitions.

Future research should prioritize molecular characterization and gene expression profiling to validate the observed tolerance traits at the genomic level. However, ensuring stable rice yields in Fe-affected regions will require a holistic approach that integrates genetically resilient cultivars with sustainable agronomic practices. Combining genetic improvement with field-based interventions, such as soil amendments, optimized water management and the use of Fe-chelating agents will be crucial to reducing the adverse effects of Fe toxicity. Ensuring sustainable rice production in Fe-affected ecosystems will require the strategic integration of phenotypic selection, molecular breeding, and climate-resilient agronomic practices, which together will strengthen national food security and enhance the adaptive capacity of rice-based production systems to cope with climate variability and future environmental stresses.

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