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RESEARCH PAPER

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Biomass allocation and leaf trait responses of rice genotypes to iron (Fe^{2+}) toxicity

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ABSTRACT

Iron (Fe) toxicity, particularly in the ferrous form (Fe^{2+}), poses a serious threat to rice productivity in lowland regions of Bangladesh due to waterlogging and the use of Fe-rich irrigation. This study evaluated six rice genotypes-four previously identified as tolerant (BU Line-2, BU Line-5, BU Line-8 and BU Line-12), one moderately susceptible (BU Line-7), and one susceptible (BRRI Dhan-102)—under three Fe^{2+} levels i.e. Fe_0=Control, Fe_{600}=600 mg L^{-1}, and Fe_{1200}=1200 mg L^{-1} in semi-controlled pot conditions. Key physiological traits, including biomass allocation, root and leaf characteristics, leaf bronzing score (LBS), and tissue damage, were measured at 75 days after transplanting. Genotypes responded differentially to Fe stress. At 1200 mg L^{-1} Fe, BU Line-12 showed the lowest total dry weight reduction (28.2%), compared to 55.7% in BU Line-5 and 50.5% in BRRI Dhan-102. BU Line-12 also recorded the lowest LBS (2.7 at Fe_{600}), while BU Line-2 exhibited strong root resilience (only 9.7% root DW reduction at Fe_{600}) but suffered higher shoot damage (LBS 7.84 at Fe_{1200}). Reproductive biomass was especially sensitive, with up to 96.2% reduction in BU Line-5 under severe Fe stress. BU Line-12 consistently maintained shoot-root balance and showed fewer fully damaged leaves, making it the most promising candidate for Fe-toxic environments. The findings highlight the complex nature of iron (Fe) tolerance in rice and emphasize the effectiveness of trait-based screening in identifying tolerant genotypes. These results are valuable for guiding rice breeding efforts and selecting genotypes suitable for Fe-rich lowland ecosystems.

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INTRODUCTION

Iron (Fe) toxicity is a major abiotic stressor affecting rice (*Oryza sativa* L.) production, especially in waterlogged and acidic soils of lowland ecosystems. Under such situations, excess iron is primarily soluble in the form of ferrous (Fe^{2+}), making it readily available for plant uptake at toxic levels. Globally, about 18% of soils are affected by moderate to severe Fe toxicity, which limits crop productivity (Das and Roychoudhury, 2014; Rout *et al.*, 2014). For example, in West Africa, more than 55% of rice-growing areas, particularly in Guinea, Ivory Coast, and Ghana experienced yield losses between 16% and 78% (Audebert and Fofana, 2009). High Fe concentrations disrupt nutrient uptake, reduce plant growth and grain quality, and negatively impact soil redox status and microbial health (Kirk *et al.* 2022; Aung and Masuda 2020; Becker and Asch, 2005).

Although Fe toxicity is widely recognized as a global issue, it has received relatively limited attention in Bangladesh. However, recent evidence indicates its increasing relevance, particularly in areas reliant on iron-rich groundwater for irrigation. Prolonged application of Fe-rich irrigation water results in the accumulation of soluble Fe in surface soil. Several studies have reported high levels of Fe in soils of Mymensingh, Tangail, and Jamalpur districts, where rice yields have also been adversely affected (Sheel *et al.*, 2015; Islam *et al.*, 2020; Ahmed *et al.*, 2018). The Soil Resource Development Institute (SRDI) has further documented widespread iron accumulation across different regions of the country, highlighting an emerging threat to crop productivity in Bangladesh (Sultana *et al.*, 2015; Miah and Uddin, 2005).

Most research on Fe toxicity in rice has focused on the ferrous form (Fe^{2+}), which predominates in anaerobic environments and is highly bioavailable (Aung and Masuda, 2020; Bashir *et al.*, 2017; Müller *et al.*, 2015). Ferrous iron stress causes various physiological disturbances, including oxidative stress, membrane damage, chlorosis, root inhibition, and impaired reproductive development (Khairullah *et al.*, 2021; Li and Lan, 2017). In lowland rice ecosystems in Bangladesh, characterized by declining soil conditions and water stagnation, Fe^{2+} toxicity is particularly relevant and is expected to intensify with climate change, increasing irrigation demand, and declining groundwater quality (Ahmed *et al.*, 2018; Hassan *et al.*, 2017).

Although global studies have reported significant genotypic variation in Fe^{2+} tolerance among rice genotypes (Rajonandraina *et al.,* 2023; Stein *et al.,* 2019), local genotype responses remain insufficiently explored. The development and establishment of Fe-tolerant rice genotypes provides a practical solution to reduce yield losses in iron-prone soils. Fe-toxicity tolerance mechanisms in rice involve complex physiological and biochemical adaptations, including exclusion, compartmentalization, and antioxidant defense mechanisms (Das *et al.,* 2017: López-Millán *et al.*, 2016). However, these processes may vary across developmental stages and environmental conditions, requiring targeted screening under context-specific situations.

The aim of this study was to evaluate the performance of six rice genotypes, previously classified based on their responses to Fe^{2+} stress, under three levels of iron toxicity (control, medium and high) in semi-controlled pot conditions. The primary objectives were to assess genotypic variation in biomass allocation, quantify root and leaf morpho-physiological traits, and assess visible indicators of stress such as leaf bronzing and tissue damage. The results of this study are expected to guide the development, breeding and soil management strategies of Fe-tolerant rice varieties and contribute to sustainable rice production in the Fe-affected lowland ecosystems of Bangladesh.

MATERIALS AND METHODS

Planting materials

Six rice genotypes were selected based on prior observations, which have known differential responses to iron (Fe) toxicity. These included four tolerant genotypes (BU Line-2, BU Line-5, BU Line 8 and BU Line-12), one moderately susceptible genotype (BU Line-7), and one susceptible genotype (BRRI Dhan-102). Seeds were obtained from the Genetic Resources Unit, Department of Agronomy, Gazipur Agricultural University (GAU), Gazipur-1706, Bangladesh.

Experimental site, design, and treatments

The experiment was conducted under semi-controlled environmental conditions at the Agronomy Field Research Site, GAU, from February 24, 2023, to July 2024. The soil at the site was classified as silty clay loam. A completely randomized design (CRD) was used with a factorial combination of six rice genotypes and three Fe treatments: control (Fe_0 = 0 mg L^{-1}), moderate (Fe_{600} = 600 mg L^{-1}), and high (Fe_{1200} = 1200 mg L^{-1}). Each treatment was replicated four times to ensure statistical reliability.

Plant establishment

Surface-sterilized seeds were germinated in nursery trays, and 20-day-old seedlings were transplanted in plastic pots (24 cm height, 27 cm top diameter, 18 cm bottom diameter), each pot filled with 8 kg of silty clay loam soil. Three seedlings were transplanted per pot, and Fe treatment was applied through irrigation water. A standing water level of 3–5 cm was maintained until the maximum grain-filling stage to simulate anaerobic field conditions favorable to Fe^{2+} solubilization. To reduce the evaporation and oxidation of Fe^{2+}, 5 mL of paraffin oil was added to each pot.

Measurement of dry matter allocation

At 75 days after transplanting (DAT), plants were harvested to assess the distribution of dry matter in different plant components. Leaves, stems, and roots were carefully washed to remove soil residues, oven-dried at 70°C to a constant weight (approximately 72 hours), and then weighed using an analytical balance.

Measurement of root and leaf traits

Root and leaf characteristics were measured at 75 DAT. Root length was determined by measuring the longest intact root. Root volume was assessed using the water displacement method in a graduated cylinder. The number of fully expanded leaves per plant was recorded manually. Leaf area per plant was measured using a leaf area meter (Model AAM-8, Hayashi Denkoh Co., Ltd., Tokyo, Japan).

Leaf bronzing score (LBS) assessment

Leaf bronzing was scored visually using a standardized 0–10 scale, where 0 = no symptoms and 10 = completely dead leaves. Severity scores were categorized following IRRI (2002) and Novianti *et al.* (2020): 0–1 (≤9% affected) = very tolerant; 2–3 (10–29%) = tolerant; 4–5 (30–49%) = moderately tolerant; 6–7 (50–69%) = susceptible; 8–10 (≥70%) = highly susceptible (Fig. 1).

Fig. 1. Visual scores of leaf bronzing

Assessment of leaf damage

The extent of leaf damage due to Fe toxicity was measured by counting both partially and fully affected leaves. Partially damaged leaves were defined as those that had bronzing symptoms over at least 50% of the surface but not completely necrotic. Fully affected leaves were completely bronzed or necrotic. Visual assessment was conducted manually at 75 DAT to provide a comparative measure of Fe^{2+} toxicity between genotypes.

RESULTS

Effects of iron toxicity on plant biomass

Plant components dry weight

Under iron toxicity conditions, all rice genotypes showed varying degrees of dry weight (DW) reduction in different plant components—such as roots, stems, leaves, reproductive parts, and total biomass—when compared to the control treatment (Fe_0).

The reductions were generally more severe at higher Fe1200 concentration, although some genotypes exhibited tolerance at the moderate Fe600 level (Fig. 2).

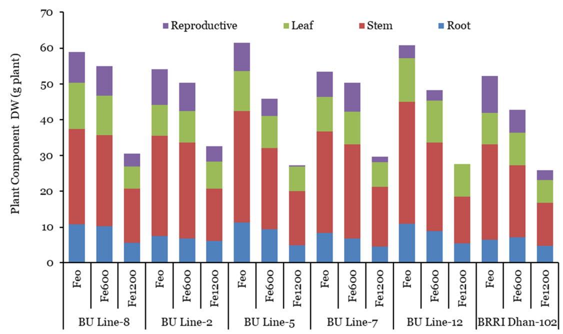

Fig. 2. Dry biomass partitioning across Fe treatments in six rice genotypes

For root dry weight, BU Line-12 and BU Line-2 exhibited relatively small reductions of 8.52% and 9.67%, respectively, under Fe600. In contrast, BU Line-5 and BU Line-7 experienced more substantial decreases, with reductions of 17.17% and 17.59%. Under the more severe Fe1200 stress, BU Line-5 was the most affected, losing 55.53% of its root biomass, followed closely by BU Line-7 and BU Line-8. Meanwhile, BU Line-12 and BU Line-2 maintained relatively better root biomass under this stress level, with reductions of only 12.97% and 18.10%, respectively.

Stem dry weight exhibited a similar trend. BU Line-2 demonstrated strong performance under Fe600, with only a 4.41% decrease. In contrast, BU Line-5 and BRRI Dhan-102 experienced the greatest reductions at this level, both nearing 27%, while the remaining genotypes had moderate reductions ranging from 5% to 13%. Under Fe1200 stress, the largest losses in stem biomass were observed in BRRI Dhan-102 and BU Line-5, both showing reductions exceeding 50%. However, BU Line-12 exhibited better retention of stem biomass, with a comparatively lower reduction of 32.71%.

Leaf dry weight decreased across all genotypes under both stress levels. BU Line-12 had the smallest reduction under Fe600, with a decrease of just 3.69%, while BU Line-2 surprisingly showed a slight increase in leaf dry weight. BU Line-7 also performed well at this level. In contrast, BU Line-8, BU Line-5, and BRRI Dhan-102 experienced more significant reductions, ranging from 13.76% to 19.14%. Under Fe1200, BU Line-8 suffered the most substantial loss in leaf biomass, with a decline of 51.79%, followed by BU Line-7 and BRRI Dhan-102. BU Line-12 had a moderate reduction of 16.61%, while BU Line-2 remained comparatively stable with a decrease of 13.91%.

Reproductive dry weight, an important indicator of yield potential, showed the most drastic declines under iron toxicity, especially at the Fe1200 level. BU Line-5 experienced a dramatic 96.18% reduction, effectively losing almost all of its reproductive biomass. Similarly, BU Line-8 and BRRI Dhan-102 also faced severe declines under the same conditions. Interestingly, BU Line-7 exhibited a slight increase in reproductive biomass under Fe600; however, this advantage was short-lived, as values dropped sharply under Fe1200. Both BU Line-12 and BU Line-2 were heavily affected, with reproductive dry weight losses of 71.56% and 55.88%, respectively, under high iron stress.

Total dry weight

Total dry weight decreased across all genotypes as iron (Fe) stress intensified. Under Fe600 conditions, BU Line-7 and BU Line-8 maintained relatively stable biomass, while BU Line-2 also performed well. In contrast, BU Line-5 experienced the most significant reduction at 25.41%, and both BU Line-12 and BRRI Dhan-102 recorded moderate losses. Under Fe1200 conditions, the most severe reductions in total biomass were observed in BU Line-5 and BU Line-8. BRRI Dhan-102 and BU Line-7 experienced moderate reductions, whereas BU Line-12 demonstrated better biomass retention at 28.22%, closely followed by BU Line-2.

Among the genotypes, BU Line-12 and BU Line-2 exhibited the most consistent tolerance in terms of root, stem, leaf, and total dry weight, particularly under Fe_{600} conditions, and retained a reasonable level of biomass under Fe_{1200} as well. BU Line-7 also performed moderately well under Fe_{600} and showed some resilience in total and leaf biomass under Fe_{1200}, despite a sharp decline in reproductive weight. BU Line-5 and BU Line-8 were highly susceptible to Fe_{1200}, especially concerning root and reproductive biomass. Although BRRI Dhan-102 showed slight increases in root and leaf biomass under Fe_{600} conditions, it was highly sensitive under Fe_{1200}, particularly in reproductive and stem dry weight.

Table 1. Effects of iron (Fe) toxicity on root and leaf characteristics of six rice genotypes

Genotypes	Fe toxicity stress	Root length (cm)	Root volume (cc)	Number of leaves (no.)	Leaf area (cm² plant⁻¹)
BU Line-8	Fe_0	33.50^{b-d}	77.25^{a}	80.00^{a}	105.51^{a-c}
	Fe_{600}	31.68^{b-e}	71.75^{a}	77.50^{ab}	102.68^{a-c}
		(5.43)	(7.12)	(3.13)	(2.68)
	Fe_{1200}	22.90^{gh}	37.75^{e}	46.00^{d-f}	57.74^{d-h}
		(31.64)	(51.13)	(42.50)	(45.28)
BU Line-2	Fe_0	41.00^{a}	49.50^{b-e}	76.00^{ab}	98.47^{a-c}
	Fe_{600}	36.00^{a-c}	45.25^{c-e}	71.00^{ab}	83.40^{a-f}
		(12.20)	(8.59)	(6.58)	(15.30)
	Fe_{1200}	27.75^{d-g}	46.30^{c-e}	60.00^{d-e}	38.68^{h}
		(7.93)	(6.46)	(21.05)	(40.41)
BU Line-5	Fe_0	36.00^{a-c}	65.00^{a-c}	67.75^{a-c}	115.28^{a}
	Fe_{600}	36.25^{a-c}	63.00^{a-d}	63.50^{a-d}	103.88^{a-c}
		(2.08)	(3.08)	(6.27)	(9.89)
	Fe_{1200}	27.00^{d-h}	60.50^{a-d}	38.75^{f}	46.18^{gh}
		(25.00)	(6.92)	(42.80)	(59.94)
BU Line-7	Fe_0	30.75^{b-f}	50.50^{b-e}	65.00^{a-c}	108.79^{ab}
	Fe_{600}	32.50^{b-e}	43.00^{de}	63.75^{a-d}	105.30^{a-c}
		(4.07)	(14.85)	(1.92)	(3.21)
	Fe_{1200}	25.00^{f-h}	30.00^{e}	41.25^{f}	50.67$_{f-h}$
		(18.70)	(40.59)	(36.54)	(53.42)
BU Line-81	Fe_0	31.15^{b-f}	79.75^{a}	76.50^{ab}	90.81^{a-d}
	Fe_{600}	35.30^{a-c}	68.50^{ab}	71.25^{ab}	85.92^{a-e}
		(2.73)	(14.11)	(6.86)	(5.38)
	Fe_{1200}	21.00^{h}	43.75^{de}	43.50^{ef}	55.90^{e-h}
		(32.58)	(45.14)	(26.14)	(38.44)
BRRI Dhan-102	Fe_0	36.75^{ab}	50.25^{b-e}	75.75^{ab}	75.61^{b-g}
	Fe_{600}	29.95^{c-f}	47.25^{c-e}	72.25^{ab}	73.63^{c-g}
		(18.50)	(5.97)	(4.62)	(2.62)
	Fe_{1200}	26.25^{e-h}	31.50^{e}	51.00^{c-f}	37.00^{h}
		(28.57)	(37.31)	(32.67)	(51.06)

Note: Fe_0=Control (0 mg L^{-1}), Fe_{600}=600 mg L^{-1}, Fe_{1200}=1200 mg L^{-1}. Figures in parenthesis indicate percent reduction compared to Fe0-control)

Effects of iron toxicity on root and leaf traits

Root characteristics

BU Line-5, BU Line-12, and BU Line-7 displayed the smallest reductions in root length when exposed to Fe_{600} stress, indicating a strong tolerance at moderate levels (Table 1). In contrast, BRRI Dhan-102 experienced the highest reduction at 18.50%, demonstrating its sensitivity even at this lower concentration of iron. Under severe stress (Fe_{1200}), BU Line-2 showed excellent resilience, with root length decreasing by only 7.93%. Conversely, BU Line-8 and BU Line-12 were more adversely affected, experiencing reductions of 31.64% and 32.58%, respectively. In terms of root volume, BU Line-5 and BU Line-2 again exhibited superior tolerance across both stress levels. BU Line-5 only faced a 3.08% reduction under Fe_{600} stress, while BU Line-2 had a slightly higher reduction of 8.59%. Both genotypes continued to perform well under Fe_{1200} stress. In contrast, BU Line-8 and BU Line-12 showed significant losses in root volume at Fe_{1200}, with reductions of 51.13% and 45.14%, respectively, indicating lower adaptability to high iron toxicity.

Leaf characteristics

The number of leaves remained largely unaffected under Fe600 stress across most genotypes (Table 1). Among them, BU Line-7 demonstrated the greatest stability, with only a 1.92% decrease in leaf count, followed closely by BU Line-5 (6.27%) and BU Line-12 (6.86%).

However, under Fe1200 conditions, there was a more significant decline in leaf numbers, particularly in BU Line-8 and BU Line-5. In contrast, BU Line-2 and BU Line-12 maintained better stability, experiencing moderate reductions of 21.05% and 26.14%, respectively. Leaf area showed only minor decreases under Fe600, but a substantial drop was noted under Fe1200 across all genotypes. BU Line-5 and BU Line-8 were the most affected, with sharp reductions of 59.94% and 45.28%, respectively. On the other hand, BU Line-2 and BU Line-12 performed relatively well under high iron stress, retaining a greater proportion of their leaf area.

BU Line-5 demonstrated consistent performance across various traits under Fe600 conditions, effectively sustaining root length, root volume, and leaf number. However, it exhibited a marked decline in leaf area when exposed to Fe1200. BU Line-2 also exhibited strong tolerance, especially in root traits and leaf number, with moderate reductions across all measured parameters. BU Line-12 performed well under moderate stress but proved more vulnerable under severe conditions, particularly concerning root volume. BU Line-7 demonstrated excellent stability in leaf traits under Fe600 but was adversely affected by higher iron levels. BU Line-8 performed adequately under moderate stress but displayed clear sensitivity at Fe1200. Among all genotypes, BRRI Dhan-102 was the most sensitive, especially in terms of root length.

Leaf bronzing symptoms as an indicator of iron toxicity tolerance

Under conditions of iron (Fe) toxicity, all genotypes displayed increased values for the measured trait under both Fe600 and Fe1200 treatments compared to the control (Fe0), where the values remained at zero (Fig. 3). This pattern suggests that the trait—likely the leaf bronzing score (LBS), a common symptom of iron stress—was absent under non-stress conditions and became apparent only with exposure to iron toxicity. The severity of symptoms intensified as the stress level increased from Fe600 to Fe1200. At the Fe600 level, BRRI Dhan-102 recorded the lowest bronzing score (3.6), indicating relatively better tolerance under moderate stress, closely followed by BU Line-12 (3.7). BU Line-7 had a score of 4.45, while BU Line-2 and BU Line-8 exhibited slightly higher values. When the iron stress increased to Fe1200, BU Line-12 continued to show the lowest score (4.65), demonstrating strong resistance to severe iron toxicity. BU Line-2, BU Line-5, and BU Line-8 displayed moderate increases in symptom severity. Conversely, BRRI Dhan-102 recorded the highest value (7.45), indicating the greatest susceptibility under high-stress conditions.

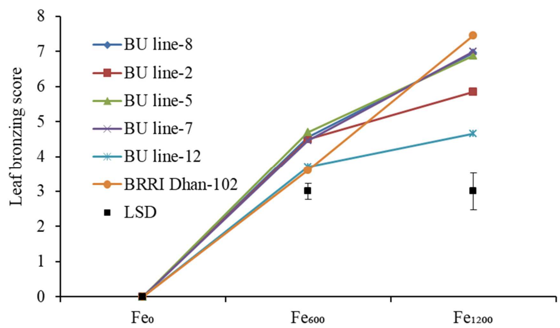

Fig. 3. Fe toxicity impact on leaf bronzing of six rice genotypes. Note: Fe_0=Control (0 mg L^{-1}), Fe_{600}=600 mg L^{-1}, Fe_{1200}=1200 mg L-1; Leaf bronzing score: 0-1 = Very tolerant; 203 = Tolerant; 4-5 Moderate; 6-7 = Susceptible and 8-10 = very susceptible. Bars indicate standard error (SE).

Overall, BU Line-12 consistently exhibited the lowest symptom expression across both Fe600 and Fe1200 treatments, indicating strong inherent tolerance to iron toxicity. While BRRI Dhan-102 performed well under Fe600, it showed a significant increase in stress symptoms at Fe1200, highlighting a considerable drop in tolerance. BU Line-2 maintained a moderate and steady increase in symptom scores, suggesting stable performance as stress increased. BU Lines-5, BU Line-7 and BU Line-8 exhibited higher levels of symptom severity, particularly under Fe_{1200}. In summary, BU Line-12 and BU Line-2 emerged as the most tolerant genotypes, while BRRI Dhan-102 proved to be the most sensitive under severe iron toxicity.

Leaf damage due to iron toxicity

The effects of iron toxicity on various rice genotypes were evident in the number of leaves exhibiting partial and full damage under Fe_{600} and Fe_{1200} treatments, compared to the control condition (Fe_0). Under Fe_0, no visible leaf damage was observed in any of the genotypes, indicating that all maintained healthy leaf conditions in the absence of excess iron. However, as iron concentration increased, distinct variations among genotypes emerged in terms of leaf damage severity (Table 2).

Table 2. Fe toxicity impact on the number leaves partially and fully damage of six rice genotypes

Genotypes	Number of leaves partially (at least 50%) affected due to Fe toxicity		
	Fe toxicity level		
	Fe_0	Fe_{600}	Fe_{1200}
BU Line-8	0.00d	11.25bc	11.50bc
BU Line-2	0.00d	11.00bc	14.50b
BU Line-5	0.00d	9.50c	20.50a
BU Line-7	0.00d	9.50c	19.25a
BU Line-12	0.00d	9.00c	10.50bc
BRRI Dhan-102	0.00d	9.25c	22.75a
Genotypes	Number of leaves fully (100%) affected due to Fe toxicity		
	Fe toxicity level		
	Fe_0	Fe_{600}	Fe_{1200}
BU Line-8	0.00e	14.50d	18.50cd
BU Line-2	0.00e	16.00cd	19.25cd
BU Line-5	0.00e	24.75a	26.25a-c
BU Line-7	0.00e	22.25ab	28.00ab
BU Line-12	0.00e	12.75cd	16.50d
BRRI Dhan-102	0.00e	21.50a-c	30.00a

Note: Fe_0=Control (0 mg L^{-1}), Fe_{600}=600 mg L^{-1}, Fe_{1200}=1200 mg L^{-1}.

For partially affected leaves under Fe_{600}, all genotypes showed a noticeable increase in damage, with counts ranging from 9.00 to 11.25 leaves (Table 2). BU Line-12 recorded the lowest number (9.00), suggesting it has a stronger tolerance to moderate iron stress. Slightly higher values were observed in BU Line-5, BU Line-7, and BRRI Dhan-102 (ranging from 9.25 to 9.50), while BU Line-2 and BU Line-8 were more affected, with 11.00 and 11.25 leaves, respectively. When exposed to Fe_{1200}, the severity of partial damage increased across all genotypes. BRRI Dhan-102 showed the highest number of partially affected leaves (22.75), followed by BU Line-5 and BU Line-7. In contrast, BU Line-12 again demonstrated greater tolerance, with the fewest affected leaves (10.50), while BU Line-8 exhibited a moderate level of damage (11.50).

Regarding fully damaged leaves, BU Line-12 had the lowest count (12.75) under Fe_{600}, reflecting a stronger defense against moderate toxicity. BU Line-8 and BU Line-2 followed, with 14.50 and 16.00 leaves, respectively. Under severe stress (Fe_{1200}), BRRI Dhan-102 showed the most pronounced damage, with 30.00 fully affected leaves, indicating its high sensitivity. BU Line-2 and BU Line-8 experienced moderate damage levels, recording 19.25 and 18.50 fully affected leaves, respectively. Once again, BU Line-12 maintained the lowest count at 16.50, reaffirming its superior tolerance to iron toxicity.

Overall, BU Line-12 consistently outperformed other genotypes under both Fe_{600} and Fe_{1200} conditions, showing the lowest levels of both partially and fully affected leaves. BU Line-8 and BU Line-2 showed moderate susceptibility. In contrast, BRRI Dhan-102 was the most vulnerable, consistently exhibiting the highest degree of leaf damage under elevated iron stress.

DISCUSSION

The results of the study show that different rice genotypes respond uniquely to Fe^{2+} toxicity, which is a growing problem in flooded and iron-rich lowland environments. The six genotypes evaluated showed significant changes in both biomass distribution and visible stress symptoms, such as leaf bronzing and damage.

Among the genotypes, BU Line-12 consistently emerged as the most tolerant across a variety of traits. It had the least reduction in both root and shoot dry weight and showed minimal signs of leaf bronzing and damage. These features indicate that BU Line-12 possesses effective mechanisms for eliminating excess iron and detoxifying it internally. Its sustained root function under stress may indicate the development of iron plaques or suberized root layers that limit iron uptake (Ullah *et al.*, 2023; Becker and Asch, 2005). In contrast, BU Line-5 and BRRI Dhan-102 exhibited significant sensitivity under Fe1200 stress, characterized by sharp reductions in biomass and extensive leaf damage—symptoms typically associated with excess iron accumulation leading to oxidative injury (Franklin *et al.*, 2015; Winterbourn, 1995).

BU Line-2 and BU Line-8 exhibited moderate tolerance, providing insights into genotype-specific responses to stress. BU Line-2 was able to maintain root biomass and leaf dry weight relatively well. However, it was more adversely affected during the reproductive phase. This indicates that Fe tolerance in this Line may depend on developmental stage (Stein *et al.,* 2019). BU Line-8 performed well under Fe600 but struggled with Fe1200, reflecting patterns observed in some upland rice genotypes (Vu *et al.*, 2024; Müller *et al.*, 2017). The reproductive stage is particularly sensitive to Fe toxicity, which highlights the importance of considering the timing of development and the ability to reduce oxidative stress during panicle formation. This process relies on hormonal regulation and carbohydrate transport, both of which can be compromised by iron-induced damage (Sperotto *et al.*, 2012; Wu *et al.*, 2016). The significant reduction in reproductive biomass observed in BU Line-5 (96.18%) illustrates this sensitivity.

Traits such as root volume and leaf area were important in differentiating the levels Fe tolerance in rice (Sonu *et al.*, 2024). BU Line-2 was remarkable in retaining these features, supporting the idea that root morphology can serve as an early and reliable indicator of Fe stress (Zhang *et al.*, 2017). BU Line-12 and BU Line-8 also performed better in preserving leaf area under stress, suggesting that mechanisms like controlled Fe translocation and antioxidant defense helped maintain photosynthetic activity (Aung and Masuda, 2020). The most prominent symptom of Fe toxicity in rice, leaf bronzing, has proven to be a reliable secondary trait for evaluation and breeding for tolerance, as its severity consistently correlates with genotypic tolerance rankings (Sikirou *et al.*, 2016). Genotypes exhibiting high scores for leaf bronzing, such as BRRI Dhan-102 and BU Line-5, displayed more damage and Fe accumulation, while BU Line-12 consistently scored low, reinforcing its status as a tolerant variety.

These results emphasize the importance of using multi-trait screening strategies in research on iron (Fe) toxicity. Aung *et al.* (2020) found that rice genotypes tolerant to Fe toxicity are influenced by a complex interaction of morphophysiological and biochemical traits. Relying on screening only at early stage may overlook genotypes that exhibit tolerance at later growth stages or become more susceptible during reproduction. Therefore, to effectively breed rice for tolerance to iron toxicity, a comprehensive selection approach is essential. This method should consider factors such as root structure, the amount of iron absorbed by the plant, signs of stress or cellular damage, and overall plant growth.

Furthermore, the study highlights the importance of semi-controlled pot screening systems. While field testing is crucial for verifying performance in real-world conditions, controlled environments provide the precision needed to investigate the underlying mechanisms underlying tolerance. Future application of molecular tools, such as quantitative trait locus (QTL) mapping and transcriptomic analysis, could greatly improve the identification and utilization of key traits associated with iron toxicity tolerance in rice (Miao *et al.,* 2024; Bashir *et al.,* 2014).

This study reveals significant genotypic variation in response to iron toxicity, with BU Line-12 emerging as a promising candidate for future breeding efforts. To increase the accuracy of Fe-tolerant genotype identification, it is important to adopt a comprehensive screening method that integrates morphological, physiological, and biochemical traits. Given the increasing incidence of iron toxicity in South Asia—largely due to greater reliance on groundwater irrigation—the development and widespread adoption of Fe toxicity tolerant genotypes like BU Line-12 will be crucial for maintaining rice productivity in affected regions.

CONCLUSION

This study highlights significant genotypic variation in rice response to iron (Fe^{2+}) toxicity. Key differences were observed in biomass allocation, leaf bronzing, and tissue damage as iron levels increased. Among the genotypes tested, BU Line-12 stood out as the most tolerant, showing the lowest reduction in total dry weight (28.2%), the lowest reproductive loss, and the lowest scores for bronzing and tissue damage. BU Line-2 also exhibited strong root performance but was less effective in maintaining shoot health under severe iron stress. BU Line-7 and BU Line-8 showed moderate performance at 600 ppm of iron but performance declines at 1200 ppm. In contrast, BU Line-5 and BRRI Dhan-102 were highly susceptible, with biomass losses exceeding 50%. These findings suggest that a coordinated tolerance between shoots and roots is crucial for overcoming iron toxicity. Furthermore, no single trait can fully capture the potential for tolerance. Trait-based screening that includes both morphological and physiological indicators has proven to be an effective method for selecting Fe-tolerant rice genotypes. These insights are valuable for breeding programs aimed at developing resilient varieties suitable for the Fe-affected lowland regions of Bangladesh. Further validation under field conditions is recommended to ensure consistent performance across diverse agro-ecological zones.

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