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Resistance of four mutant rice (Oryza sativa L.) Varieties to iron toxicity at Njala inland valley swamp ecology in southern Sierra Leone

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ABSTRACT

Four lowland rice varieties were examined for tolerance to Fe toxicity using morphological traits. In 2021, at the Njala Agricultural Research Centre (NARC), we conducted a field trial at the Inland Valley Swamp (IVS) in Sierra Leone. The four rice varieties, Bina 8, 10, 17, and Early Mutant, were planted in a randomized complete block design with three replications. The variance analysis revealed significant differences in all four rice varieties' traits. Elemental analysis of plant stalk revealed that Early Mutants and Bina 17 had the lowest Fe^{2+} concentrations (0.4 mg/kg soil and 0.8 mg/kg soil, respectively). On the other hand, Bina 10 and Bina 8 had higher Fe2+ concentrations (3.5 mg/kg soil and 1.5 mg/kg soil, respectively). In contrast, Bina 8 and Bina 10 had higher Fe^{2+} concentration values in their roots and stalks, indicating a higher accumulation of Fe^{2+}. Thus, these two varieties also had the highest yield, indicating they tolerate Fe toxicity. This finding implies that rice varieties' capacity to withstand Fe toxicity plays a crucial role in sustaining growth and yield in Fe-toxic environments. The study concludes that screening rice varieties for Fe toxicity tolerance is important, but further research is needed to understand the mechanisms underlying Fe toxicity tolerance in rice.

Contribution/Originality: Lowland rice varieties were screened for Fe toxicity under field conditions. Significant differences in growth and yield were noted among the four tested rice varieties. Bina 8 and Bina 10 obtained the highest value of Fe2+ concentration in their roots and stalks, and they were the most tolerant to Fe toxicity. The research's findings will assist farmers in selecting rice varieties that are either tolerant or resistant to iron-toxic soil.

1. INTRODUCTION

More than half of the world's population consumes rice (Oryza sativa L.), one of the most essential cereal crops (Fahad et al., 2019). The world's consumption of rice was more than 488 million tons (MT), with Asia accounting for 90% of the production and utilization in 2018 (USDA, 2019). The increasing importance of rice in consumer diets has declared it a political crop, with its price and availability directly impacting social stability. The price of rice tripled in a matter of months during the food crisis of 2007–2008, from which rice prices never really rebounded to their pre-2007 level (Soullier, Demont, Arouna, Lançon, & Del Villar, 2020). Rice continues to become the main staple food in Sierra Leone, and the demand outpaces local production, resulting in significant imports to make up for the difference. Rice production is an important source of livelihood for over 80% of rice-farming households and for millions of the rural population, whose main economic activity is rice farming in the country. It is a strategic commodity, as the overall economic and political stability are dependent on an adequate, affordable, and stable supply of this staple food. However, its growth and yield are affected by several abiotic stresses, including salt and iron toxicity, especially in low-land ecologies where most of the rice is produced. Iron toxicity is one of the major abiotic stresses that affects rainfed rice production in Sierra Leone. The condition is characterized by elevated levels of the reduced form of iron, Fe^{2+}, in the soil solution and is specific to flooded soils; thus, it affects mainly lowland rice cultivation. The mechanism of iron toxicity in rice and the screening of tolerant cultivars for iron toxicity have been the subject of several studies (Tanaka, Loe, & Lavasero, 1996). Symptoms of iron toxicity in rice are usually small brown spots that originate at the tips of the leaf and extend towards the base of the lower leaves (Ponnamperuma, Bradfield, & Peech, 1955). Using tolerant rice cultivars is the most economical approach to managing iron toxicity. Using tolerant cultivars and improving soil and nutrient management practices can yield better results in areas with severe iron toxicity (Sahrawat et al., 1996). Essential plant nutrients are also important in controlling iron toxicity and expressing iron tolerance in rice cultivars. The Inland Valley Swamp (IVS), an iron-toxic environment in Sierra Leone, cultivates some lowland rice varieties. The National Agricultural Research Coordinating Council (NARCC), now known as the Sierra Leone Agricultural Research Institute (SLARI), has released and recommended two iron-tolerant varieties, Rokupr-rice (ROK), ROK 23, and ROK 24, for cultivation in IVS over the years. This study screened four mutant rice varieties, introduced by Njala University: Bina 8, 10, 17, and Early Mutant, for Fe toxicity tolerance. Bina 8 is a salt-tolerant, high-yielding variety. It is suitable for Inland Valley swamp and mangrove swamp agro ecologies. It is a semi-dwarf, early-maturing, and medium-bright grain variety. Bina 8 takes between 130 and 135 days to mature. Bina 10 is an earlier-maturing variety (127–132 days) than other salt varieties. Bina 17 is also an early-maturing variety, with a duration of 112–118 days from seed to seed and requiring less input. Early Mutant is an early-maturing variety with a crop duration of 110–115 days from seed to seed. Plant height is intermediate (113.4 cm) and has long grains. The four rice varieties (Bina 8, Bina 10, Bina 17, and Early Mutant) are palatable, easy to cook, and have good keeping quality. The Sierra Leone Seed Certification Agency (SLeSCA) in Sierra Leone has officially released the early mutant Bina 8 and Bina 17 varieties, renaming them as Njala University Crop Science (NUCOS) NUCOS 1, NUCOS 2, and NUCOS 3, respectively, after screening by the Crop Science Department, School of Agriculture and Food Sciences, Njala University.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

The study area is located at the Njala Agricultural Research Centre (NARC) experimental site in Njala, Kori Chiefdom, Moyamba District, Southern Sierra Leone (Figure 1).

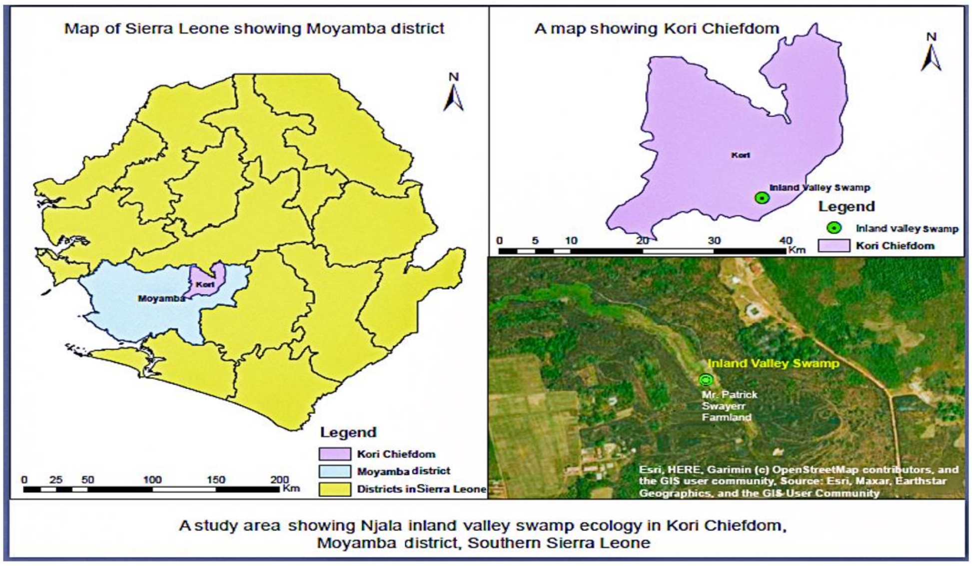

Figure 1. Experimental site in Kori Chiefdom, Moyamba District Southern Sierra Leone.

Source: ICTD Njala University

It has a landscape characteristic of an upland area, with several swamps in between. The study area experiences two seasons: the dry season and the rainy season. Each of the seasons lasts for six months. The dry season spans from November to April, and the rainy season lasts from May to October, with relatively high humidity coupled with heavy downpours of rain. As shown in Figure 2, the study area is characterized by iron toxicity, which was also visibly evident before the study.

Figure 2. Iron toxic IVS site at Njala in Kori Chiefdom in Moyamba district prior to the study.

2.2. Plant Materials

This study used four mutant rice varieties from the School of Agriculture and Food Sciences: Bina 10, 17, 8, and Early Mutant.

2.3. Water Sample Collection

We collected water samples from the research site to determine the physiochemical properties. The Njala University Quality Control Laboratory conducted sampling to ascertain the iron concentration. Due to the nature and topography of the land, we used non-isokinetic samplers to collect the sampled water for analysis. The water was still in good shape, with a low flow rate from the source. A non-isokinetic sampler obtained from the laboratory was cleaned with tap water and liquid soap and rinsed with deionized water. It was taken across the plots horizontally and immersed halfway down the water. Composite samples were taken across the field to ensure homogeneity and representation of the entire plot. The composite sample represents a cross-section of the water body. We collected three representative water samples, one from the main drain and the other two from the proposed experimental plot location.

2.4. Soil Sample Collection

Composite soil samples were collected across the field to ensure homogeneity and representation of the entire field using a zigzag pattern. The samples were collected at a depth of 0–15 cm using an auger. Samples were labelled accordingly for laboratory analysis to determine the iron concentration, pH level, and Electrical Conductivity (EC).

2.5. Experimental Design

The trial was laid out using a randomized complete block design with three replications (Table 1). Twenty-one-day-old seedlings were transplanted in a plot size of 5 m x 1.5 m with a spacing of 20 cm x 20 cm between and within rows, using two seedlings per hill. Each of the three blocks contained four test entries (rice varieties), which were subjected to iron toxicity. The total land area covered was 17 m long x 7.5 m wide; 1 m between blocks and 0.5 m between plots were used. One application of nitrogen, phosphorus, and potassium (NPK) fertilizer was done at the vegetative stage using a recommended rate of 60 kg N, 40 kg P_2O_5, and 40 kg K_2O per hectare. Each plot received a 0.200 kg application of NPK 15:15:15 fertilizer. The fertilizer applied was not used as a treatment but to boost the growth and development of the four rice varieties.

Table 1. Experimental design with treatment.

Block A (Replication 1)	Block B (Replication 2)	Block C (Replication 2)
Bina 10	Bina 17	Bina 8
Bina 8	Early mutant	Bina 10
Early mutant	Bina 8	Bina 17
Bina 17	Bina 10	Early mutant

2.6. Data Collection

The following data was collected during the study:

2.6.1. Plant Height (cm)

Using a calibrated ruler, we measured the height of five representative plants randomly selected from each plot from the soil surface to the tip of the tallest panicle in centimeters (cm). The first data on plant height was collected four weeks after transplanting. The average of representative plant samples was recorded for each block across replications.

2.6.2. Days to 50% Flowering

Field observation was recorded when 50% of the plants reached the heading stage. The dates for each of the plots were recorded and converted to days.

2.6.3. Days to 80% Maturity

Field observation was recorded from seedling to the time when 80% of the grains on panicles attained maturity and the ripening stage. The date for each of the plots was recorded and converted to days.

2.6.4. Tillers /Plant

Five samples were randomly selected to observe the number of effective tillers. The number of tillers was determined by counting the number of effective tillers per hill four weeks after transplanting. The average of representative samples was recorded for each block across replications.

2.6.5. Effective Panicles/ Plant

Data was collected from five randomly selected plants. The number of effective panicles was determined by counting the number of effective panicles in each of the sample plants four weeks after transplanting. The average number of tillers per plant bearing panicles from the five representative samples was recorded.

2.6.6. Tillers/Metre Square

The number of tillers per metre square was determined by counting the total number of effective tillers in a 1 m by 1 m square plot. The average of the representative samples was recorded for each block across replications.

2.6.7. Panicles/Meter Square

This was determined by counting the total number of effective panicles in a 1 m by 1 m square plot. The average of the representative samples was recorded for each block across replications.

2.6.8. Panicle Length

After harvest, we determined the panicle length of the five tagged plants. The panicle length was measured from the base of the panicle to the highest tip of the panicle.

2.6.9. Grain Yield

Dried grains obtained from each plot were weighed and their moisture content recorded.

2.6.10. 1000 Grain Weight (gram)

One thousand (1000) random samples of well-developed whole grains dried to 14% moisture content were counted and weighed on a precision balance for all the varieties across replications.

2.6.11. Above Biomass (Kg)

Rice plants harvested from within a 1 m^2 area of each replication were cut from the surface of the soil and weighed using an electronic hanging scale to determine the weight. The average weight from each replication was recorded.

2.7. Data Analysis

To determine the significant growth differences among the four rice varieties, all collected data underwent One-way analysis of variance (ANOVA) using STATISTICA software version 12 (Stat Soft Inc., Tulsa, OK, USA). Post-hoc analysis was conducted with the DUNCAN MULTIPLE RANGE TEST (DMRT) at a significance level of 0.05 to separate the means.

3. RESULTS

3.1. Chemical Composition of Soil and Water

Table 2 presents laboratory results from water and soil samples collected from the experimental field prior to trial establishment. The results of the analysis indicate that the pH of the soil samples was highly acidic (4.3–4.5). The results further revealed that Fe^{2+} concentration in the water prior to trial establishment ranged from 1.5–2.9 mg/kg soil and Fe^{2+} toxicity in the soil ranged from 2.8 mg/kg to 10.3 mg/kg. The analysis results indicate that the soil samples' pH was highly acidic in nature (4.3 – 4.5). The results further revealed that Fe^{2+} concentration in the water prior to trial establishment ranged from 1.5 – 2.9 mg/kg soil and Fe^{2+} toxicity in the soil ranged from 2.8 mg/kg – 10.3 mg/kg. According to Table 2, Fe^{2+} concentration in the water sample from Block 1 is the highest (2.9 mg/kg soil), followed by Block 2 (2.5 mg/kg soil), while Fe^{2+} concentration in the water sample from the main drain is the lowest (1.5 mg/kg soil). The soil sample from plot 1 had the highest Fe^{2+} concentration (10.3 mg/kg soil), followed by plot 2 (6.3 mg/kg soil), compared to the soil sample from the main drain, which had the lowest Fe^{2+} concentration (2.8 mg/kg soil).

The soil sample from Plot 1 had the highest pH (4.5), followed by the soil sample from the main drain (4.4), and the soil sample from Plot 2 had the lowest pH (4.3).

According to the laboratory results, the soil sample from plot 2 has a high electrical conductivity (190 S/cm), followed by the soil sample from a water source (176 S/cm), compared to plot 1, which has the lowest EC (167 S/cm).

Table 2. Laboratory results on soil and water under Fe^{2+} toxic IVS conditions.

Lab label	Client label	Sample type	Fe^{2+} (mg/kg soil)	pH	EC (µS/cm)
1746	(Block 1)	Water	2.9		
1747	(Block 2)	Water	2.5		
1748	(Main drain)	Water	1.5		
1840	(Plot 1)	Soil	10.3	4.5	167
1841	(Plot 2)	Soil	6.3	4.3	190
1842	(Main drain)	Soil	2.8	4.4	176

3.2. Accumulation of Fe^{2+} in the Shoot and Root

According to Table 3, the results revealed that there was more Fe^{2+} accumulated in the roots of the four rice varieties compared to the stalks analyzed. Bina 8 and Bina 10 had more Fe^{2+} in the roots and stalks compared to Bina 17 and Early mutant after harvest.

According to the results, Bina 8 had the highest Fe^{2+} concentration in the root (9.0 mg/kg soil), followed by Bina 10 (6.1 mg/kg soil), compared to Bina 17, which had the lowest Fe2+ concentration (1.6 mg/kg soil).

The shoot analysis also revealed that Bina 10 had the highest Fe^{2+} concentration (3.5 mg/kg soil), followed by Bina 8 (1.5 mg/kg soil), while Early Mutant had the lowest Fe^{2+} concentration (0.4 mg/kg soil) (Table 3).

Table 3. Fe^{2+} concentration in the roots and stalks of the four varieties tested.

Lab label	Client label	Sample type	Fe^{2+} (mg/kg soil)
2045	(Root soil)	Plant root	2.3
2045	(Plant stalk)	Plant stalk	0.4
2046	(Root soil)	Plant root	6.1
2046	(Plant stalk)	Plant stalk	3.5
2047	(Root soil)	Plant root	1.6
2047	(Plant stalk)	Plant stalk	0.8
2048	(Root soil)	Plant root	9.0
2048	(Plant stalk)	Plant stalk	1.5

3.3. Shoot/Root Fe^{2+} Concentration Ratio

Table 4 shows variation in the shoot/root Fe^{2+} concentration ratio among four rice varieties at 89 days after planting (harvest) under Fe-toxic IVS conditions. Bina 10 further showed a high shoot/root Fe^{2+} concentration (0.57 mg/kg soil), followed by Bina 17 (0.50 mg/kg soil) compared to Bina 8, which showed the lowest shoot/root Fe^{2+} concentration (0.16 mg/kg soil).

Table 4. Shoot/Root Fe^{2+} concentration ratio among four rice varieties after harvest.

Rice variety	Fe^{2+} concentration (mg/kg soil)		Shoot/Root Fe^{2+} concentration ratio (mg/kg soil)
	Root	Shoot	
Bina 10	6.1	3.5	0.57
Bina 8	9.0	1.5	0.16
Bina 17	1.6	0.8	0.50
Early mutant	2.3	0.4	0.17

3.4. Growth Parameters of Four Rice Varieties Under Iron Toxic IVS

3.4.1. Number of Leaves per Plant

The results in Figure 3 revealed a significant ($P \leq 0.05$) difference in the number of leaves per plant among the four rice varieties at different growth stages under Fe-toxic IVS conditions. At week 4 (28 DAP), the B8 variety produced the highest number of leaves (28.00), followed by the B17 variety with the second highest number of leaves (26.33) compared to the early mutant with the least number of leaves (14.00). At week 6 (48 DAP), the B10 variety produced the highest number of leaves (40.66), followed by the B8 variety with the second highest number of leaves (39.00), compared to the early mutant variety with the least number of leaves (20.33). Also, at week 8 (58 DAP), the B10 variety produced the highest number of leaves (56.33), followed by B17 (42.33), while the early mutant produced the least number of leaves (27.66) (Figure 3).

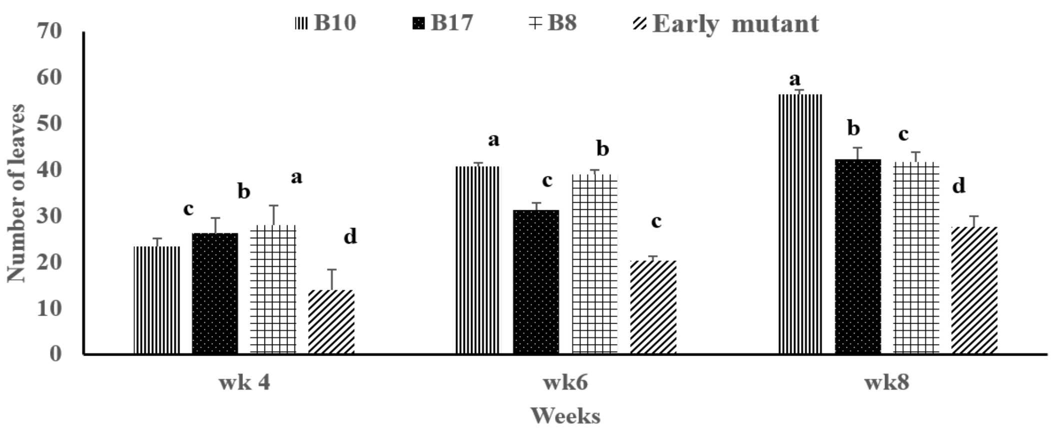

Figure 3. Number of leaves per plant among four rice varieties at different growth stages under Fe toxic IVS conditions.

3.4.2. Plant Height (cm)

There was a statistically significant (P≤0.05) difference in plant height among the four types of rice at different stages of growth when exposed to Fe-toxic IVS (Fig. 4). At week 4 (28 days after planting (DAP), plants of the early mutant variety were the tallest (64.33 cm), while the B8 variety showed the second tallest plants (56.33 cm) compared to B10 with the shortest plants (42.0 cm). At week 6 (42 DAP), the early mutant variety produced taller plants (74.33 cm), while the B17 variety showed the second tallest plants (71.33 cm) compared to B10, which showed the shortest plants (62.0 cm). Similarly, at week 8 (56 DAP), the early mutant variety had the tallest plants (94.33 cm), followed by the B8 variety with the second tallest plants (86.33 cm), compared to the B10 variety with the shortest plants (72.00 cm) (Figure 4).

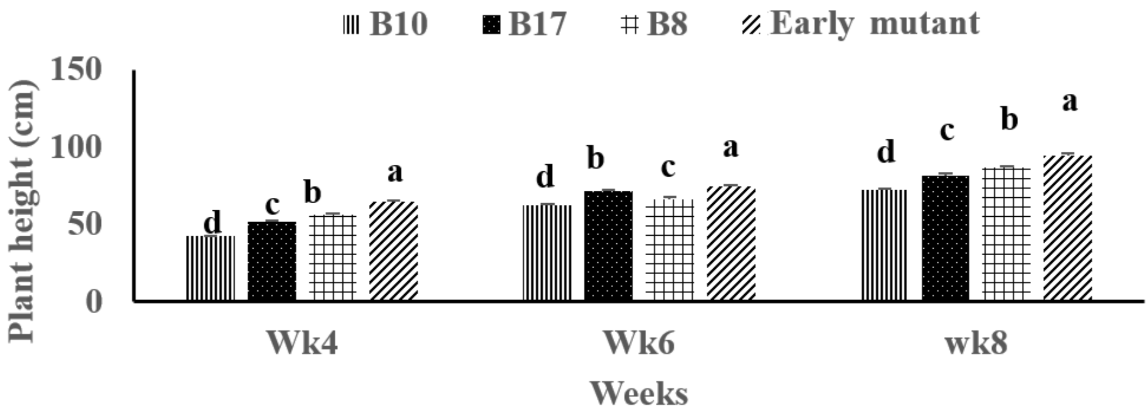

Figure 4. Plant height among four rice varieties at different growth stages under Fe Toxic IVS conditions.

3.4.3. Number of Tillers Per Plant

There was a significant (P≤0.05) difference in the number of tillers among the four rice varieties at different growth stages under Fe-toxic IVS conditions. At week 4 (28 DAP), the B8 variety produced the highest number of tillers (7.26), followed by the B10 variety with the second highest number of tillers (6.73), compared to the Early mutant with the least number of tillers (4.20). At week 6 (48 DAP), the B8 variety produced the highest number of tillers (9.53) compared to the early mutant variety, which had the least number of tillers (5.66). Also, at week 8 (58 DAP), the B8 variety produced the highest number of tillers (22.23), followed by B10 (14.33), compared to B17 with the least number of tillers (7.66) (Figure 5).

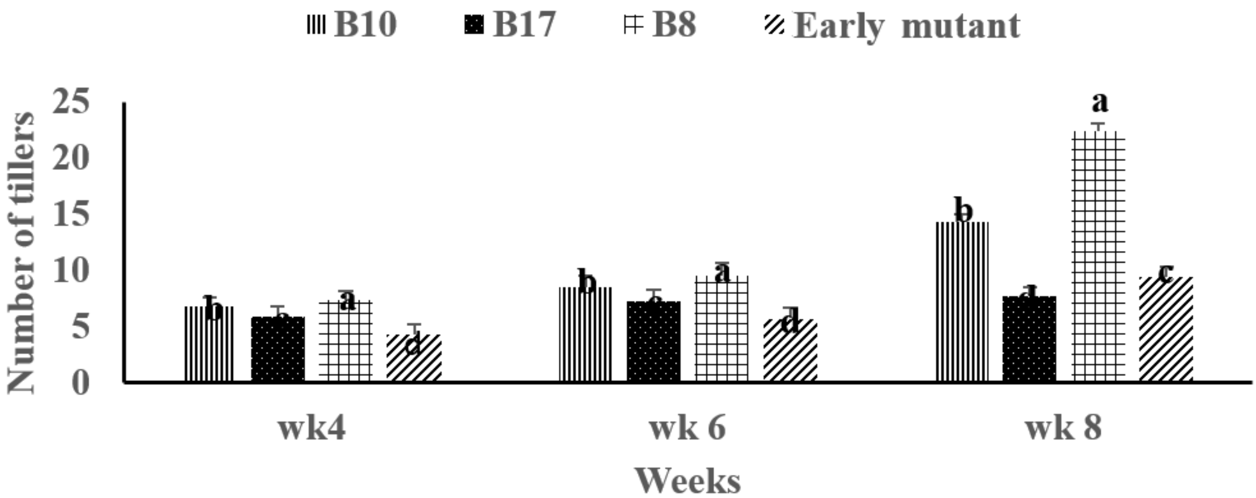

Figure 5. Number of tillers per plant among four rice varieties at different growth stages under Fe toxic IVS conditions.

3.5 Yield and Yield Components

3.5.1 Number of Effective Panicles Per Hill (EP. Hill)

The analysis of variance showed that there was a significant (P≤0.05) difference among the four types of rice at the panicle stage when grown in Fe-toxic IVS conditions in the number of effective panicles per hill. According to the results, the B8 variety produced the most effective panicles per hill (9.00), followed by B10 (7.00) compared to the early mutant variety (6.00), and B17 had the least effective panicles per hill (5.33) (Figure 6).

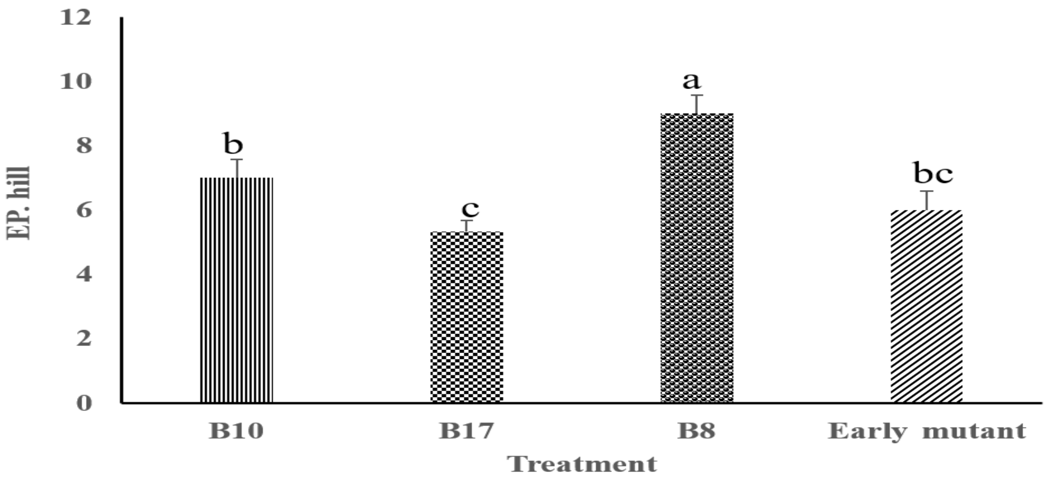

Figure 6. Number of effective panicles per hill among four rice varieties at panicle stage under Fe toxic IVS conditions.

3.5.2. Number of Non-Effective Panicles per Hill (NEP. Hill)

Figure 7 shows that there was a significant (P≤0.05) difference among the four types of rice at the panicle stage when it came to the number of ineffective panicles per hill. The B10 variety produced the highest number of NEP. hills (3.66), followed by the Early Mutant (3.00), while the B17 and B8 varieties produced the least number of NEP. hills (2.33) (Figure 7).

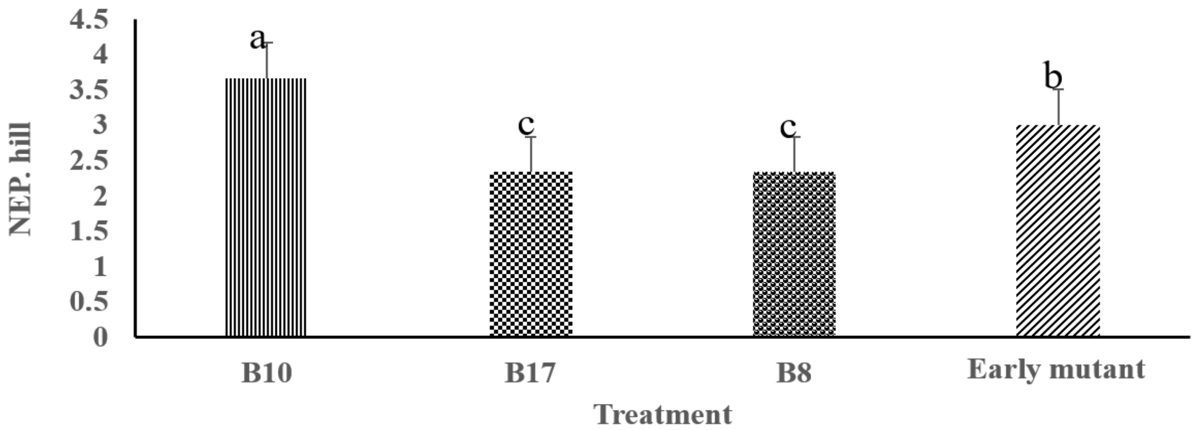

Figure 7. Number of non-effective panicles per hill among four rice varieties at reproductive growth stage under Fe toxic IVS conditions.

3.5.3. Panicle Length (cm)

Figure 8 shows that there was a significant (P≤0.05) difference in the length of the panicles of the four types of rice that were harvested in Fe-toxic IVS conditions. The B10 variety produced the tallest panicles (10.00 cm), followed by the B8 variety (9.66 cm), compared to the early mutant, which had the shortest panicles (5.66 cm).

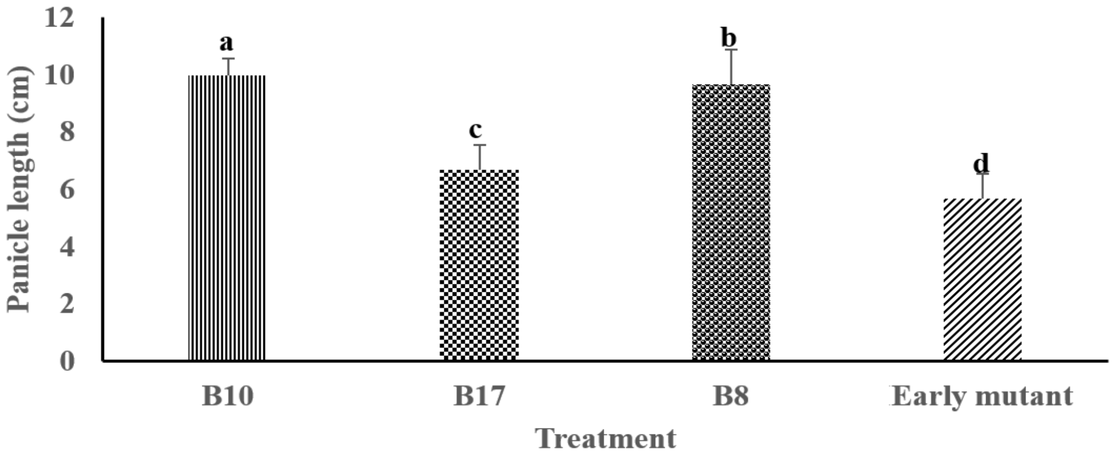

Figure 8. Panicle length (cm) among four rice varieties after harvest under Fe toxic IVS conditions.

3.5.4. Yield Traits of Four Rice Varieties Under Iron Toxic IVS Conditions

Yield traits, which include flowering and maturity, grain yield, and 1000 grain weight and above biomass, were evaluated to determine the tolerance of four mutant rice varieties to iron toxicity in the IVS ecology.

3.5.5. Days to 50% Flowering (X50 FW)

There was a big difference ($P \leq 0.05$) in the number of days it took for each of the four types of rice to flower 50% when they were in the reproductive stage and the soil was Fe-toxic IVS. According to the findings, B10 had the highest number of days to 50% flowering (96.00), followed by B17 and B8, which had the same number of days to 50% flowering (94.00), compared to the early mutant variety with the lowest number of days (73.66) (Figure 9).

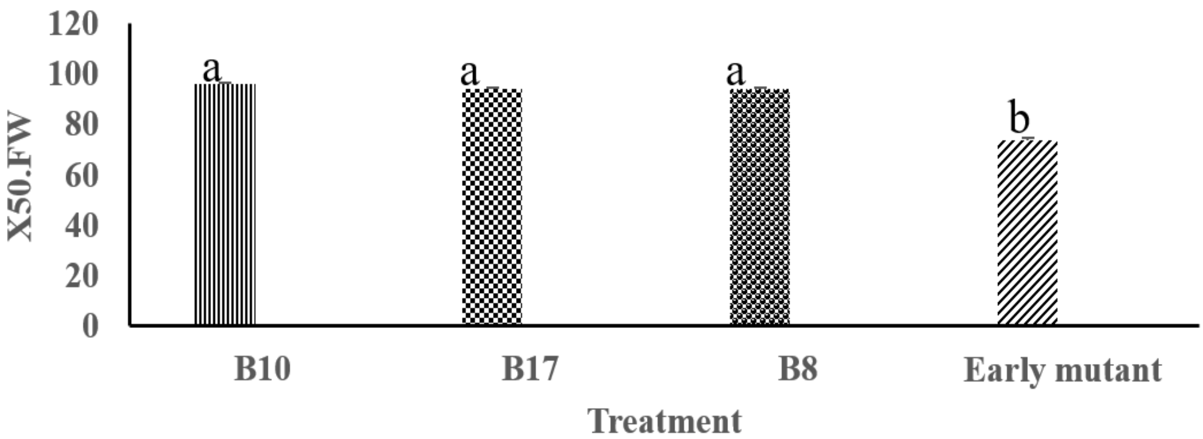

Figure 9. Number of days to 50% flowering among four rice varieties at reproductive growth stage under Fe toxic IVS conditions.

3.5.6. Number of Days to 80% Maturity (X80.MTY)

According to the results shown in Figure 10, the analysis of variance revealed a significant ($P \leq 0.05$) difference in the number of days to 80% maturity among the four rice varieties at the maturity stage under Fe-toxic IVS conditions. B10 had the longest number of days to 80% maturity (126.0), followed by B17 and B8, which had the same number of days to 80% maturity (124.0), compared to the early mutant variety with the least number of days (105.6).

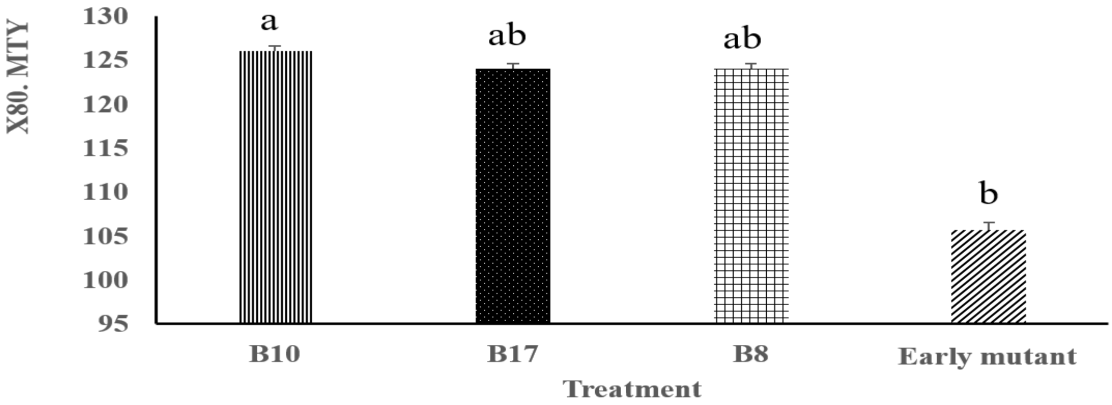

Figure 10. Days to 80% maturity among four rice varieties at reproductive growth stage under Fe toxic IVS conditions.

3.5.7. Number of Grains

The analysis of variance showed significant (P≤0.05) differences in the number of grains among the four rice varieties after harvest. From the results (Figure 11), B10 had the highest number of grains (156.0), followed by B8 (146.0), and B17 had the least number of grains (104.0).

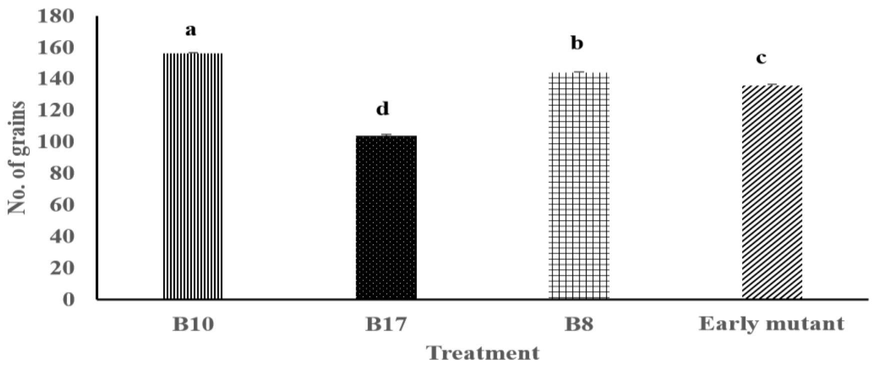

Figure 11. Number of grains among four rice varieties after harvest.

3.5.8. Grain Yield per Plant (G)

After harvest, a significant (P≤0.05) difference was observed in the grain yield per plant among the four rice varieties. The results showed that the B8 variety had the highest grain yield (409.33 g), followed by the B10 variety (352.0 g), and the early mutant variety had the lowest grain yield (245.66 g) (Figure 12).

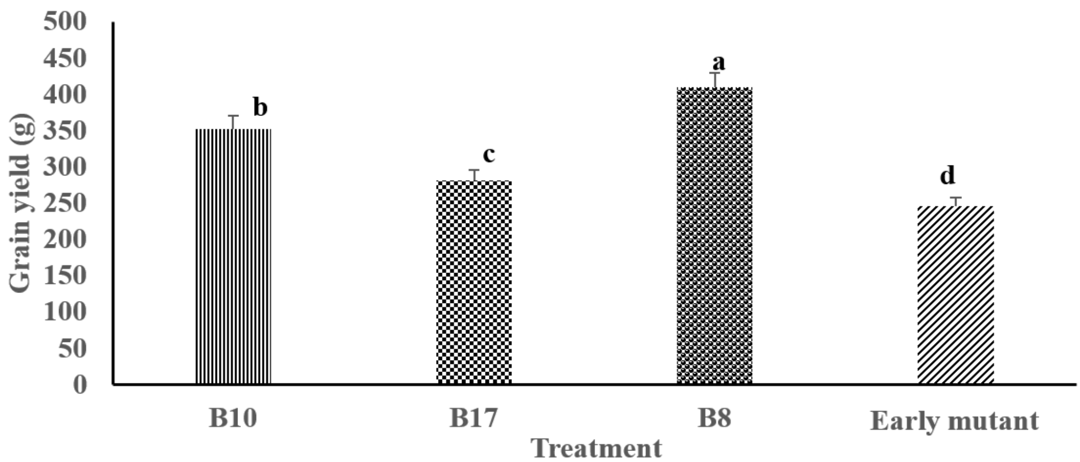

Figure 12. Grain yield (g) per plant among four rice varieties after harvest.

3.5.9. 1000 Grain Weight Per Plant (G)

The analysis of variance showed a significant (P≤0.05) difference in grain weight per plant (g) among the four rice varieties after harvest. According to the results, B8 had a heavier 1000 grain weight (wgt) (24.20 g), followed by the B10 variety (21.83 g), compared to the early mutant variety with a lighter 1000 grain weight (18.03 g) (Figure 13).

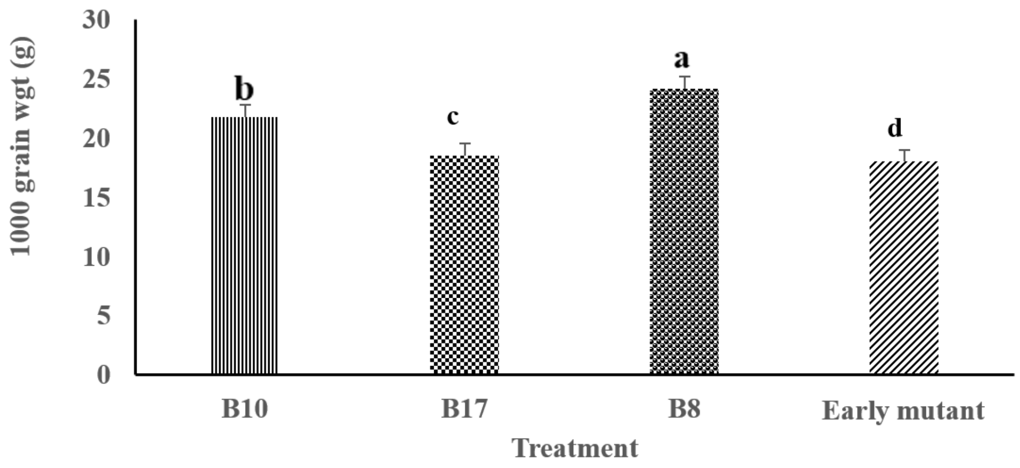

Figure 13. 1000 grain weight (g) per plant among four rice varieties after harvest.

3.6. Above Biomass

The analysis of variance showed a significant (P≤0.05) difference in the above biomass among the four rice varieties after harvest. The results in Figure 14 show that the B10 variety had the heaviest above biomass (3.73 g), followed by the B8 variety (3.53 g), while the early mutant variety had the lightest above biomass (2.56 g).

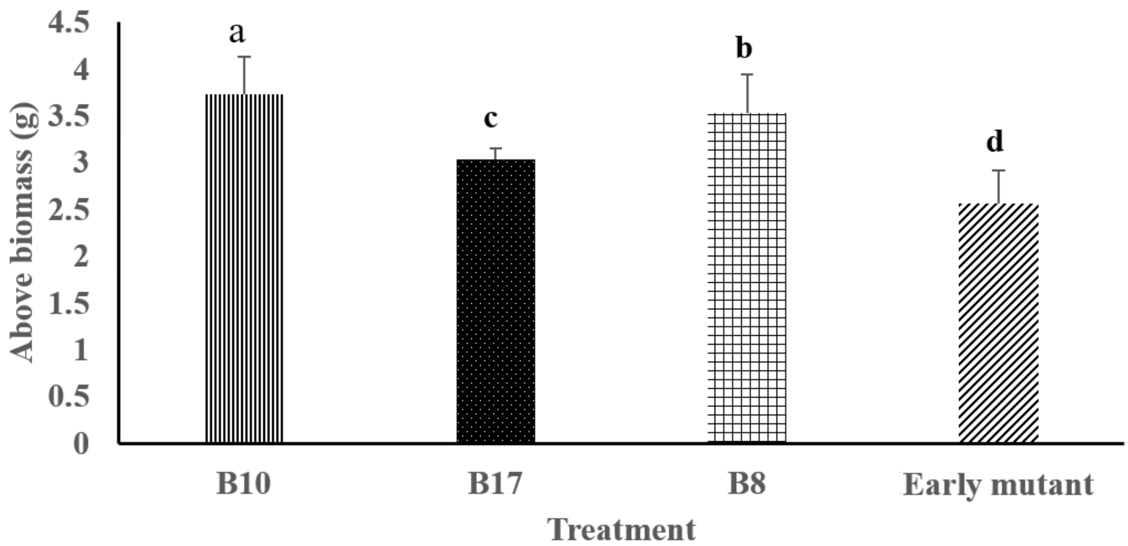

Figure 14. Above biomass (g) among four rice varieties after harvest.

4. DISCUSSION

Iron toxicity is a prevalent nutrient disorder that impacts rice cultivation in tropical regions like Asia and Africa, particularly lowland rice varieties. This element plays a significant role in the manifestation of toxicity symptoms in paddy fields. Increased absorption and translocation of iron in rice plants contribute to toxicity, which is a key limiting factor in the yield performance of wetland rice. The goal of this study was to assess the resistance of four mutant rice varieties to iron toxicity in Kori Chiefdom, Moyamba District, Southern Sierra Leone's Njala inland valley swamp agro-ecology. This part talks about the results we got from testing how well these four types of mutant rice can handle iron toxicity in Fe-toxic IVS conditions.

4.1. Growth Parameters of Four Rice Varieties Under Fe Toxic IVS

In this study, growth parameters (number of leaves, plant height, tillers, and effective panicles per plant) were evaluated to determine the tolerance level of the four rice varieties grown under Fe-toxic IVS conditions. The growth parameters were significantly affected by exposure to Fe-toxic IVS, and some varieties (Early mutant, Bina 8, and Bina 17) were noted to be tolerant with higher values of the measured growth parameters.

4.2. Varietal Tolerance to Fe^{2+} on Number of Leaves

Flag leaf area is a very important trait because it plays a significant role in the grain filling period. All varieties tested exhibited a significant increase in this trait at 4, 6, and 8 weeks after transplanting, except for the early mutant variety, which did not show a significant increase compared to the other three varieties (Bina 8, Bina 10, and Bina 17). Bina 10 recorded the highest number of leaves at 6 and 8 weeks, while the early mutant variety recorded the lowest. This result suggests that the Fe2+ concentration had a significant impact on the number of leaves counted in the Early mutant variety, potentially due to its genetic predisposition (tolerance mechanisms) to mitigate Fe-toxicity (Lantin & Neue, 1989; WARDA, 1995; Yamanouchi & Yoshida, 1981).

4.3. Varietal Tolerance to Fe^{2} on Plant Height

Varieties also significantly influenced plant height at 4, 6, and 8 weeks after transplanting. Considering the plant at 4, 6, and 8 weeks, the early mutant variety recorded the tallest plant height, whereas Bina 10 had the shortest plant height. The results show that plant height for all the varieties varied from 4 weeks to 6 weeks to 8 weeks after transplanting. The significant differences noted were an indication of variation in tolerance levels among the varieties evaluated. According to Abu, Tucker, Harding, and Sesay (1989), toxicity during the vegetative stage is associated with reduced plant height and dry-matter accumulation, with the shoot being more affected by the root biomass (Fageria, 1988). Cheema, Chaudhary, Takkar, and Sharma (1990) reported that both the tiller formation and the share of productive tillers can be severely reduced with the severity of $Fe^{2+ \text{ toxicity}}$ dependent on the varietal tolerance mechanism.

4.4. Varietal Tolerance to Fe^{2+} on Tillers and Effective Panicles Per Plant

The number of tillers per plant showed significant differences for all the varieties tested at 4, 6, and 8 weeks after transplanting. Based on the performance of the four varieties tested under iron-toxic conditions, at week 8, Bina 8 and Bina 10 varieties with the highest tillers also obtained the highest number of effective panicles per plant, whereas Bina 17 and the Early Mutant variety had the least effective panicles per plant and the lowest tiller count. This revealed genetic variability among all the varieties in terms of the number of tillers per plant.

4.5. Varietal Tolerance to Yield Traits of Four Rice Varieties Under Iron Toxic IVS Conditions

The study evaluated four rice varieties' yield traits (flowering, maturity, grain yield, 1000 grain weight, and above biomass) to determine their tolerance levels when grown under Fe-toxic IVS conditions.

4.6. Varietal tolerance to Fe^{2+} on Flowering and Maturity

The capacity to tolerate Fe^{2+} is a key factor in plant productivity (Momayezi, Zaharah, & Hanafi, 2009). However, in the presence of Fe^{2+} stress, the varieties showed variation in terms of flowering and maturity. The days to heading are 73.66 days in the early mutant variety compared to Bina 10, which attained 96.00 days. The Bina 17 and Bina 8 varieties were significantly later in maturity (94.00 days) compared to the early mutant variety. Early maturing is desirable as they fit well in multiple cropping systems (Neelam, Ramesha, Reddy, & Sankar, 2009). The analysis results classified Bina 8, 10, and 17 as medium varieties, while the early mutant variety stands out as the only early maturing variety, potentially suitable for multiple cropping in farmers' fields.

4.7. Varietal Tolerance to Fe^{2+} on Grain Yield and 1000 Grain Weight

A significant ($P \leq 0.05$) difference exists among the four rice varieties for grain yield and grain weight. The results revealed that greater variability existed among the varieties tested for these traits. Under iron-toxic conditions, the four rice varieties showed wider variation in yield and yield-contributing characters. 1000 grain weight is an important parameter in grain quality. It provides information on grain density and size. Based on performance under iron toxicity, Bina 8 and Bina 10 varieties obtained higher yields and 1000 grain weight values, while the early mutant variety and Bina 17 obtained lower grain weights and 1000 grain weights, respectively. These variations in grain weight were due to differences in grain size and shape among the varieties evaluated. The low yields obtained could be attributed to the acidic and Fe^{2+} nature of the soil, thus limiting the uptake of available nutrients by plants. Rice varieties with high yield potential likely possess good yield components, including several tillers per hill and several panicles per hill (Department of Rice Thailand, 2006).

4.8. Varietal Tolerance to Fe^{2+} on Above Biomass

Differences were found in the above biomass for the four varieties tested. In general, the results indicate the existence of genetic variability among the varieties for this trait. The analysis revealed that Bina 10 had the highest biomass, followed by Bina 8, with the early mutant variety recording the lowest biomass. This resulted in an increase in yield and yield-related traits (number of grains, grain yield, and 1000 grain weight) for the two varieties.

4.9. Fe^{2+} Accumulation among Four Rice Varieties

Based on their performance, Bina 8 and Bina 10 were observed as iron toxicity-tolerant varieties. The study found that iron toxicity stress had less of an effect on tolerant varieties than on susceptible varieties (Bina 17 and early mutant). This was true for agronomic traits like the number of tillers per plant, the number of effective panicles per plant, and the number of grains per panicle. The strong phytoferritin activity (Baruah, Das, & Das, 2007) might have contributed to iron tolerance in rice. A resistant variety may accumulate higher amounts of phytoferritin, forming a complex with Fe2+ and reducing Fe toxicity damage. A large amount of Fe^{2+} in plants has been associated with protein degradation (Saikia & Baruah, 2012). It is proposed that Fe^{2+} may lead to the formation of reactive oxygen radicals, which are highly phytotoxic and cause protein degradation. This could potentially explain why Bina 8 and Bina 10 exhibit tolerance to iron toxicity. The results show that the two susceptible varieties, Bina 17 and Early Mutant, had the lowest number of tillers per plant, effective panicle per plant, number of grains per panicle, 1000 grain weight, and least grain weight values. It was surprising to note that the tolerant varieties had the highest iron accumulation in the plant stalk and root compared to the susceptible varieties.

Various methods have been suggested to provide resistance against the harmful effects of iron (Becker & Asch, 2005). One of the root-based tolerance mechanisms is the physical barrier (root plaque) that is created when oxidized Fe3+ precipitates, which stops additional excessive Fe absorption (Becker & Asch, 2005). This tolerance mechanism, which is supported by enzymatic Fe2+ oxidation, lateral fine root development, and root aerenchyma, can include root oxygen diffusion (Becker & Asch, 2005; Wu et al., 2014). Retaining iron in particular at "dumping sites" inside roots, which results in metabolically inactive iron, is another root-based tolerance mechanism (Becker & Asch, 2005).

Researchers have linked the ferritin protein in plastids, which can store up to 4000 Fe atoms, to rice's ability to withstand Fe toxicity (Briat, Duc, Ravet, & Gaymard, 2010). The scavenging of ROS by antioxidants (ascorbate, glutathione, and phenolics) or antioxidant enzymes (superoxide dismutase, or SOD) and ascorbate peroxidase (APX) is another mechanism for shoot-based tolerance that has been hypothesized (Majerus, Bertin, & Lutts, 2007).

Soil pH is one characteristic that has a considerable influence on the availability of soil nutrients. Low soil pH might have affected the availability of iron in the plants. Iron concentrations rise with increasing pH values. Low pH conditions increase the availability of Fe, leading to an excessive accumulation of Fe in the leaves. Iron availability is lowest between pH 7.5 and 8.5. The availability of iron increases as the pH of the soil decreases.

The high electrical conductivity of the soil, which ranged from 167 to 190 µS/cm predisposed the availability of Fe^{2+} in high concentration.

5. CONCLUSIONS

The analysis of variance showed that all of the traits compared were significantly different between the four varieties, except for the number of tillers at 4 weeks and the number of panicles that didn't work on each hill. The results indicate that variability existed amongst all the traits studied except for non-effective panicles and some tillers at four weeks after transplanting. Two varieties (Early Mutant and Bina 17) recorded the lowest Fe^{2+} concentration values: 0.4 mg/kg soil and 0.8 mg/kg soil, while the highest concentration was obtained in Bina 10 (3.5 mg/kg soil) and Bina 8 (1.5 mg/kg soil), respectively, in the plant stalk. Bina 8 (9.0) had the highest Fe concentration in the plant root, followed by Bina 17 (1.6). The early mutant had the lowest Fe concentration value (2.3. It was interesting to note that even though Bina 8 and Bina 10 recorded the highest Fe concentration in their roots and stalks, they emerged as the most outstanding varieties in terms of yield compared to the other two varieties (Bina 17 and Early Mutant). In conclusion, we evaluated Bina 8 and Bina 10 varieties as the most tolerant to iron toxicity, while Bina 17 and Early Mutant are less tolerant.

According to the research findings, the varieties that performed well in the IVS under conditions of iron toxicity are potential sources of tolerant varieties and could serve as donor parents in breeding programs for varietal improvement.

Early mutants can serve as donors for multiple cropping in breeding programs because they took the fewest days to reach 80% maturity (105.6). Early mutant varieties can be a useful, sensitive check in further studies.

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APPENDIX

Appendix 1 photo showing the researcher observing the plant materials at the iron toxic experimental site.

Appendix 1. Photo showing data collection at the experimental site.