RESEARCH ARTICLE

EFFECT OF IRON (Fe^{2+}) TOXICITY ON YIELD AND YIELD COMPONENTS OF SELECTED HIGH-YIELDING RICE VARIETIES UNDER LOW COUNTRY WET ZONE FIELD CONDITIONS

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ABSTRACT

Iron (Fe^{2+}) toxicity is a significant nutritional disorder that reduces rice yields, particularly in Sri Lanka's Low Country Wet Zone. This study aimed to assess the effects of Fe^{2+} toxicity on grain yield and yield components of selected high-yielding rice varieties and analyse their sensitivity to iron toxicity by comparing yield and yield-related parameters obtained under iron stress treatment with those recorded under control conditions. A field screening experiment was conducted during the 2023–2024 *Maha* season at the Regional Rice Research and Development Centre, Bombuwala, Sri Lanka using 15 rice varieties (*BW272-6B, Bg359, BW372, BW302, At311, BW266-7, BW312, BW361, Bg300, BW367, BW364, BW267-3, BW363, Bg374, BW51*). A site with high Fe^{2+} levels (294.45 to 317.10 mg kg^{-1}) was selected to induce toxic effects, while a control site had low levels (78.90 to 112.63 mg kg^{-1}). To evaluate the effect of iron toxicity on grain yield and yield components of 15 rice cultivars, a randomized complete block design (RCBD) with 3 replicates (n=3) was used. Grain yield per plant, along with plant height, tiller number, 1000 grain weight, and fertility (filled and unfilled grains per panicle), were recorded as yield components. A Generalized Linear Model was employed to assess the effects of treatment, variety and their interaction on yield and yield parameters. The results showed that *BW267-3, BW367, BW363, BW372, BW302, and At311* had no significant differences in yield and yield components compared to the control, indicating high tolerance to Fe^{2+} toxicity. Conversely, *BW272-6B* and *Bg359* showed significant reductions in yield (33%), 1000-grain weight (40%), fertility (25%), plant height (18%), and tillering (33%), thereby classifying them as susceptible. Other varieties were moderately affected and classified as moderately tolerant.

Key words: Field screening, High-yielding rice varieties, Iron toxicity, Susceptibility, Tolerance

INTRODUCTION

Iron (Fe) is an essential transitional metal for almost all living organisms. Although Fe is the fourth most abundant element in the Earth's crust, its bioavailability in soil is generally low due to its presence as a low-soluble ferric (Fe^{3+}) form (Wairich *et al*.,2024). Iron toxicity is a major constraint to rice production in many tropical and subtropical regions of the world. In Sri Lanka, prolonged flooding and specific soil conditions in paddy fields can promote the accumulation of soluble Fe2+, posing a potential risk to rice productivity. This toxicity arises from the reduction of insoluble Fe^{3+} to soluble Fe^{2+} under anaerobic, low-pH conditions. The critical concentration of Fe^{2+} for toxicity symptoms is 300 mgL^{-1}. Rice has different mechanisms for the uptake and utilization of iron. Upon excessive iron accumulation in rice plants, an increase in reactive oxygen species (ROS) can be observed, with several detrimental consequences. The increased ROS hampers the biochemical and physiological processes of the typical rice plant. These interruptions lead to yield losses and the death of the rice plant (Harish, *et al*., 2023).

Fe^{2+} toxicity is more pronounced in the inland valleys of the low country than in the mid- and up-country wet zones of Sri Lanka, with the low country being the most affected due to frequent waterlogging.

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Approximately 30,000 ha of land fall within the low country wet zone, out of the estimated 40,000ha of lowland rice cultivation, the total area affected by Fe^{2+} toxicity (Siriwardana *et al.* 2018).

Introduction of tolerant rice varieties to Fe^{2+} toxicity has been identified as the most viable solution to overcome the problem (Senadhira and Akbar, 1991; Wairich *et al.*,2024). Rice breeders and biotechnologists have developed rice varieties with tolerance to Fe^{2+} toxicity through a deeper understanding of the genetics, the molecular basis of Fe^{2+} regulation and transporting mechanisms, identification of responsible genetic traits for Fe^{2+} tolerance, and the interactions of Fe^{2+} with other nutrients such as P, N, K, Zn, Mn, etc. (Mahender *et al.*, 2019).

Since different Fe^{2+} toxicity-tolerant rice varieties have different mechanisms to cope with Fe^{2+} toxicity conditions in wetland paddy soil (Onyango *et al.* 2018), their level of tolerance may vary under field conditions. Therefore, it is highly important to characterize the level of Fe^{2+} toxicity tolerance in newly developed cultivars before recommending them to farmers for cultivation.

The Regional Rice Research and Development Centers (RRRDC) have developed high-yielding rice varieties for the low-country wet zone of Sri Lanka. However, their tolerance to Fe^{2+} toxicity has not been systematically tested under field conditions. Therefore, it is important to characterize the possible effects of iron toxicity on recommended rice varieties for the low-country wet zone of Sri Lanka. Thus, the objectives of the present study were to assess the effects of Fe^{2+} toxicity on rice growth and yield components.

MATERIALS AND METHODS
Study area
A field trial was conducted in the rice fields of the Regional Rice Research and Development Centre (RRRDC), Bombuwala, Sri Lanka (60.57" N, 800.01" E) during the 2023/24 *Maha* season.

Materials
Seeds of lowland rice varieties provided by RRRDC, Bombuwala, were used in the study. There were, *Bg374, Bg300, At311, Bg359, BW312, BW363, BW351, BW367, BW372, BW364, BW272-6B, BW267-3, BW266-7, BW02* and B*W361*.

Experimental design
Randomized complete block design (RCBD) was used to set up the experiment with 3 replicates (n=3). The plot size was 10 m × 10 m, with 15 subplots per variety. Each subplot contained 20 plants spaced 15 cm apart.

Soil sample preparation and soil analysis
Soil samples were collected from the two main plots for initial soil characterization. The collected soil was air-dried. Air-dried samples were ground into a fine powder. Ground soil was sieved with a 2 mm mesh before being used for analyses. Initial soil pH, EC, and total iron content were analyzed from both the 2 main plots (control and treatment). For total iron, 5 g of soil was mixed with 50 ml of ammonium acetate buffer (pH 4.8), shaken for 2 hours, filtered using No. 1 filter paper, and analysed for Fe content by atomic absorption spectrometry (AAS) (ammonium acetate extraction method).

Land preparation, nursery establishment, and crop establishment
The soil was dug out to a depth of 20 cm for all main plots and puddled. Healthy seeds from 15 different rice varieties were collected and sown in the upland dry beds to raise seedlings. Nursery beds were maintained for 21 days. Carefully uprooted 21-day-old seedlings were transplanted in rows in the field. The spacing between plants was 15 cm, and the spacing between rows was 20cm. After transplanting, a 5 cm water depth was maintained in all plots to minimize transplanting shock and control weeds. Plants were maintained until harvest.

Data collection and analysis
Data on yield and yield components were recorded as the average of the five randomly selected plants in each subplot. Grain yield was calculated as grain yield per plant. The

number of tillers per plant, fertility (filled and unfilled grains per panicle), and 1000-grain weight were recorded as yield components. Panicles were harvested, dried, threshed, and weighed to calculate the 1000-grain weight of each variety. Plant height was measured from the ground to the tip of the longest panicle.

Soil and yield parameters were analyzed using ANOVA with Minitab 17.1.0 software. A generalized linear model determined significant differences between treatments and rice varieties. Tukey post hoc comparisons were conducted for all parameters at the 95% confidence level (P < 0.005).

Ethical considerations
The study was conducted in accordance with the ethical standards of the Sri Lankan Agricultural Research Ethics Committee. No human or animal subjects were directly involved in the research.

RESULTS AND DISCUSSION
Initial soil analysis was conducted for both control and treatment plots, revealing distinct differences. The treatment plot soil exhibited a lower pH and lower electrical conductivity than the control site (Table 1).

Table 1: **Soil characteristics of soil in the experimental sites**

Soil Parameter	Treatment Range	Mean	± SD	Control Range	Mean	±SD
Total iron(mg/L)	294.45-317.10	315.775	±31.25	78..90-112.63	105.77	±16.45
pH	3.83-5.56	4.597	±2.21	5.90-8.01	6.55	±2.46
EC (mS/cm)	79.10-86.10	85.375	±10.32	84.11-112.30	98.55	±24.36

* SD: Standard deviation

Fe^{2+} concentrations in the treatment plot ranged from 294.45 to 317.10 mg kg^{-1} throughout the trial period, levels known to induce toxicity in certain rice varieties (Saharawat, 2005). Therefore, the results indicate the presence of favourable conditions, as highlighted above, for the development of iron toxicity in the treatment plots. However, one of the problems in field screening is that, when screening large numbers of varieties for tolerance to Fe^{2+} toxicity, it is important to provide sufficiently homogeneous, elevated Fe^{2+} levels in the soil to ensure comparable stress levels for all varieties (Audebert and Sahrawat, 2000).

Iron toxicity negatively impacts rice growth and development (Gadal *et al*., 2019). In this study, certain varieties exhibited significant height reductions in the treatment plot compared to the control (p < 0.05). Notably, the *BW272-6b, BW359,* and *BW312* varieties declined in plant height during this period (Figure 1). Therefore, results indicate the susceptibility of these varieties to Fe^{2+} stress. The tested varieties, *BW267-3, BW266-7, BW367, BW363,* and *At311,* exhibited heights similar to the control, indicating moderate tolerance.
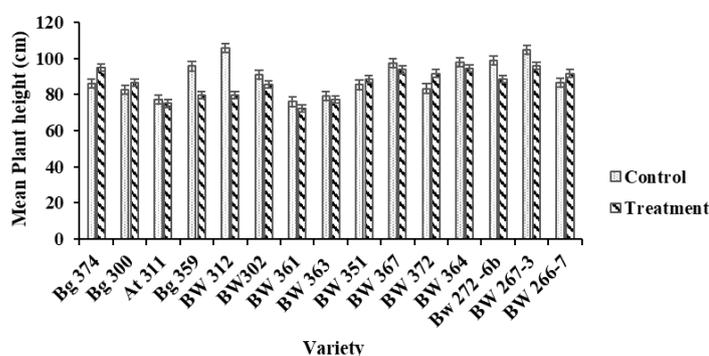

Figure 1: Variation in mean plant height in 15 rice varieties between control and treatment plots

Fe^{2+} stress has reduced the number of tillers per plant in certain varieties. The maximum reduction was observed in *Bg359, BW272-6B, and BW361, while BW267-3, BW302, BW363,* and *BW351* showed minimal reduction. Vromman *et al*. (2013) reported the negative impact of Fe^{2+} toxicity on tillering, especially during the vegetative stage. The observed decrease in tillers (Figure 2) might be due to impaired growth and photosynthesis. Further, Fe^{2+} toxicity-induced leaf bronzing due to the production of reactive oxygen species (ROS) might be one of the major reasons for the reported reduction in plant height and tillering (Rout and Sahoo, 2015).
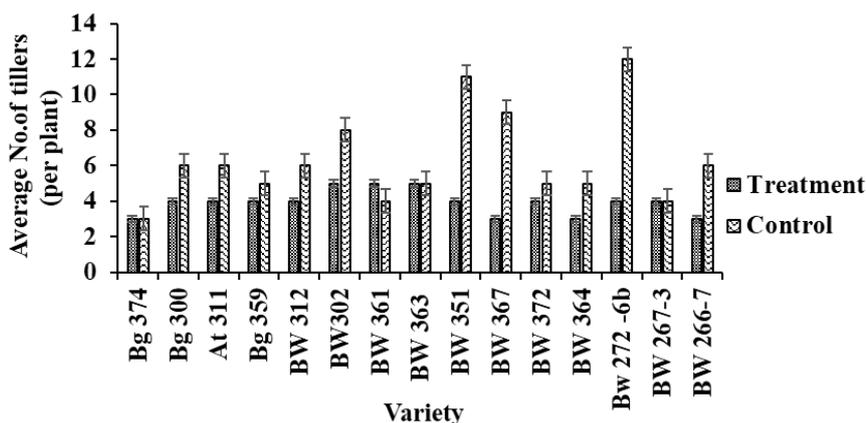

Figure 2: Variation of the average number of tillers in 15 rice varieties between the control and the treatment

As reported by Audebert and Fofana (2009), Fe^{2+} tolerance varieties tend to increase shoot volume as a mechanism to reduce Fe^{2+} toxicity by diluting excess Fe^{2+} in the xylem.

The results showed significant yield reduction (average panicles per plant) in *Bg359, BW364, BW 272-6B,* and *BW361* under iron stress compared to controls (Figure 3). These varieties exhibited symptoms of iron toxicity, such as leaf bronzing and chlorosis, which impact photosynthetic efficiency and biomass production. Tolerant varieties maintained higher grain yields under stress (Sikirou *et al*., 2018). The verities, BW267-*3, BW266-7, BW351, BW363,* and *BW374* did not show yield reduction, confirming their tolerance to Fe^{2+} stress. Previous studies have shown that severe iron toxicity can hinder overall plant growth, reducing yield in sensitive varieties.
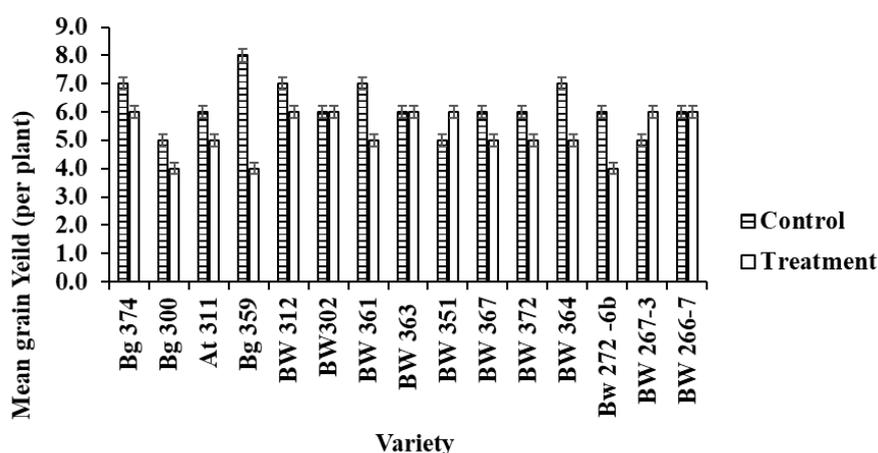

Figure 3: Variation of the mean grain yield per plant in 15 rice varieties between the control and treatment plots at the harvesting stage

In acidic soil, plant uptake of mineral nutrients such as phosphorus (P), magnesium (Mg), and calcium (Ca) is reduced due to competition from excessive H+ (Poschenrieder *et al*., 1995). As these mineral nutrients are crucial for panicle development, nutritional deficiencies may lead to shorter panicles in sensitive varieties, resulting in reduced mean grain yield per plant. Significant differences were observed in mean 1000 grain weight between control and treatment for *BW359, BW272-6B,* and *BW364* under iron toxicity (Figure 4). Other varieties, including *Bg374, Bg300, At311, BW312, BW302, BW361, BW363, BW351, BW367, BW372, BW267-3,* and *BW266-7,* did not show significant differences in grain weight. Previous studies indicated that Fe^{2+} toxicity causes a significant reduction in tiller number and spikelet fertility, thereby decreasing rice grain yields (Santos *et al*., 2020). Further, excess Fe may impair root system development, leading to nutrient deficiencies in plants and reduced growth and yields (Wairich *et al*., 2024). Tolerant varieties are more resistant to iron toxicity and could maintain relatively higher grain yield under stress compared to others (Sikirou *et al*., 2018). Hence, previous findings are in agreement with the results of the present study.
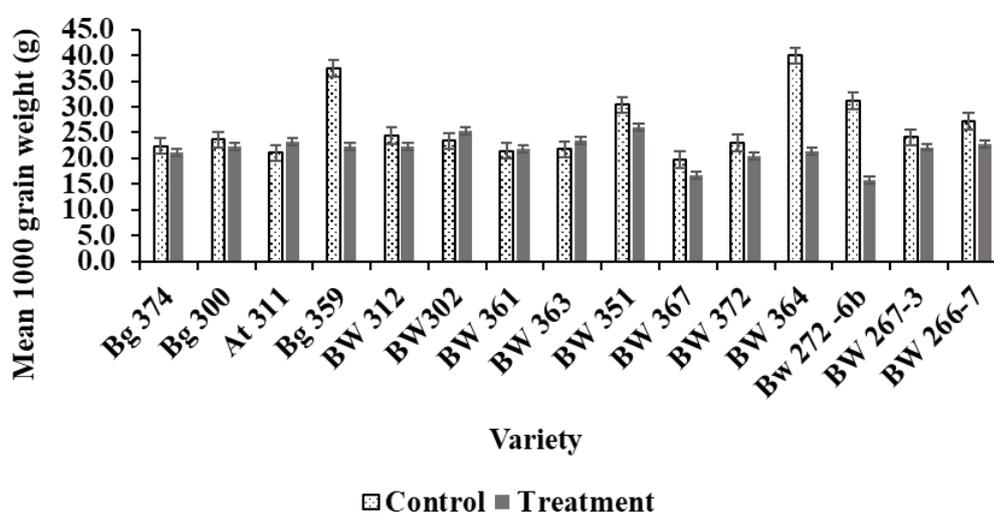

Figure 4: Variation of the mean 1000-grain weight of 15 rice varieties between the control and the treatment plot

Fertility is the ability of a plant to produce viable seeds. It can be quantified as spikelet fertility, expressed as the percentage of filled grains relative to the total number of spikelets (filled grains + unfilled grains). As a mineral nutrient, rice plants require P for vegetative growth, promote tillering, root growth, and flowering, but K is required through reproductive growth (Dobermann and Fairhurst, 2000).

During the grain-filling stage, the demand for rice plants for mineral nutrients, especially P and K, is high. In Fe^{2+} excess soil, under the existing low pH, cation exchange capacity tends to decrease (Rajonandraina *et al*.,2023). This condition may increase the number of unfilled grains in sensitive varieties due to plant nutritional deficiencies. The results showed significant reductions in fertility for At311 (40%), Bg300 (23%), Bg359 (35%), and *BW272-6B* (20%). Therefore, these varieties are susceptible to Fe^{2+} toxicity. The varieties, including *BW312, BW367, BW372, BW266-7, Bg374, BW363,* and *BW267-3,* exhibited no significant reduction in fertility, which may be due to defence mechanisms against Fe^{2+} toxicity.

CONCLUSIONS
Taking into account both overall yield and yield components, the *BW 272-6b* and *Bg359* varieties were significantly affected by iron-rich conditions (294.45 to 317.10 mg/L) and

are therefore categorized as susceptible to iron toxicity. In contrast, the *BW267-3, BW367, BW363, BW372, BW302,* and *At311* varieties showed no significant differences in overall yield and yield components and exhibited nearly normal growth, indicating that they remain unaffected by iron stress. These varieties can be categorized as highly tolerant to iron toxicity. *BW272-6b* and Bg359 varieties are significantly affected by iron-rich conditions in the field and would be categorized as susceptible to iron toxicity. The remaining seven varieties, *BW266-7, BW312, BW361, Bg300, Bg374, BW351* and *BW364,* are moderately affected by iron stress under low-country wet-zone field conditions and can be categorized as moderately tolerant to excess iron. Overall, these findings may provide valuable insights for varietal selection and rice cultivation in regions characterized by iron-rich soils.

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AUTHOR CONTRIBUTION

KM and DMW jointly conceptualized the study. DSSR conducted both fieldwork and laboratory analyses. All authors contributed to data analysis and draft the manuscript. The final version was prepared by KM and it was approved by all other authors.

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