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Relative Changes in Growth and Recovery Responses of Rice to Fe-Toxicity at Different Growth Stages

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ABSTRACT

Fe-toxicity is a critical and complicated constraint to rice growth that requires simple and quick screening methods for selection of tolerant rice to Fe-stress. This study revealed relative changes in the growth and recovery of rice responding to Fe-toxicity at germination and vegetative stages. Two rice cultivars were exposed to iron concentrations of 0, 50, 100, and 200 ppm at the germination stage and Fe-treatment of 50–100 and 100–200 ppm at the vegetative stage. Increasing Fe-toxicity reduced germination rates, seedling height, length and number of seedling roots. However, relative reduction to control in seedling height and root length at germination could be more dependable to check genotypes tolerant to Fe stress than other parameters. Growth characteristics measured at 6, 12, 18, and 24-day excess Fe indicated that plant height, number of tillers, and leaves were kept slightly relative increasing to control until 18-day and 12-day, respectively. In contrast, root length and root number decreased as soon as plants were exposed to Fe-stress. Relative decrease in growth to control increased with higher iron concentration and longer exposure time. Plants exhibited ability to recover after 24-day under stress with relative increase to the point before stress relieving from 3–12% for plant height, 1–11% for tiller number, and 7–19% for root number. There were significant differences between two cultivars for relative changes in growth and recovery parameters, suggesting a simple and efficient method, and suitable growth parameters for evaluating and selecting tolerant genotypes to Fe-stress at germination and vegetative stages.

Keywords: Fe-toxicity, germination, growth responses, relative increase, relative reduction, vegetative stage.

INTRODUCTION

Iron Fe-toxicity to plant refers to syndrome of disorders associated with high concentration of reduced iron (Fe^{2+}) in the soil solution (Becker and Asch, 2005). About 18% of global soil is Fe toxic. Besides other abiotic stresses such as drought, salinity, heat and cold, Fe-toxicity is also one of the major constraints affecting rice production on acid sulphate and waterlogged soil conditions (Becker and Asch, 2005; Sahrawat, 2005; Panhwar et al., 2016). Especially, a significant proportion of rice-growing area is affected by Fe-toxicity in China, India, Southeast Asia (e.g. the Philippines, Thailand, Malaysia, Indonesia, and Vietnam) (Le, 1981; Attanandana and Vacharotayan 1986; Le and van Mensvoot, 2004; Mahender et al., 2019), west and central Africa, and Brazil (Asch et al., 2005; Gridley et al., 2006; Fageria 2007; Crestani et al., 2009; Kirk et al., 2022). Fe-toxicity impairs the rice growth and development through tissue damage and cellular homeostasis disruption (Moore, 1990; Aung and Masuda 2020), and can cause yield losses of 10–90% or even complete crop failures if Fe-toxicity occurs during the early vegetative stage (Audebert and Sahrawat, 2000; Becker and Asch, 2005; Chérif et al., 2009; Rodenburg et al., 2014; Sikirou et al., 2015).

Iron is an essential microelement for plant growth and survival. Fe participates in important metabolic processes of plants such as photosynthesis, chloroplast development, chlorophyll biosynthesis, electron transport and redox reactions, nucleic acids synthesis and repair, metal homeostasis (Marschner, 1995; Gross et al., 2003; Rout and Sahoo 2015; Bashir et al., 2017). Plants obtain Fe primarily from the soil in the form of Fe^{2+} of which optimum availability is essential for healthy plant growth and development. Iron toxicity occurs as a result of the reduction of insoluble Fe^{3+} into soluble Fe^{2+} under both anaerobic and low pH conditions (Becker and Asch, 2005; Stein et al., 2009). When growing on acidic sulfate soil, rice growth faces the main problems including (i) acidity with adverse efects of H^+, aluminum (Al) toxicity and phosphorus deficiency; (ii) Fe stress with combined effects of Fe-toxicity and deficiencies of other divalent cations; and (iii) impaired microbial activities (Moore et al., 1990). Fe^{2+} ion is also abundant in paddy fields and absorption of them in rice roots causes severe Fe-toxicity. Fe-toxicity damages rice plants with both below-ground and above-ground symptoms. Root growth and lateral root initiation are inhibited through decreased cell division and elongation (Li et al., 2015; reviewed by Kirk et al., 2022). The high Fe uptake and subsequent transport from root to leaves via xylem leads to cellular Fe overload in plant tissues (Briat and Lobréaux, 1997). Excess Fe in cells leads to browning symptoms on leaves due to cell death (Asch et al., 2005). This typical symptom of leaf bronzing and necrotic lesions leading in severe cases even to plant failure (Wu et al., 2014; Rasheed et al., 2020; Kar et al., 2021).

Adverse effects of iron toxicity on rice growth occur at any stages including germination, seedling, vegetative, and reproductive stages and vary with iron levels, genotypes/cultivars and exposure period of time (Quinet et al., 2012; Bresolin et al., 2019; Kar et al., 2021; Theerawitaya et al., 2022; Ahmed et al., 2023). Increasing level of iron excess, especially > 300 mg L^{-1}, causes detrimental for germination and growth of rice (Ahmed et al., 2023). Critical levels of iron can vary from 20–3000 mg L^{-1} depending on sites and genotypes (Becker and Asch 2005; Panhwar et al., 2016). Numerous studies have suggested screening methods in attempts to identify donors for tolerance to iron toxicity which are important sources for rice breeding strategies. These studies have conducted experiments either in the field in iron toxic hotspots (Sikirou et al., 2018; Melandri et al., 2021; Pawar et al., 2021) or in the hydroponic solution adding excess iron at high concentrations for short periods of time (Dufey et al., 2015; Matthus et al., 2015). Various traits have been used for measurement such as germination rates, biomass and leaf symptoms, grain yield, root characteristics and physiological responses (i.e., SPAD, Fv/Fm) and even accumulation of different cations in leaf and shoot (Asch et al., 2005; Quinet et al., 2012; Bresolin et al., 2019; Kar et al., 2021; Theerawitaya et al., 2022; Ahmed et al., 2023). However, most studies have compared the measurements under the control and Fe stress conditions rather than considering the relative reduction of plant responses under the Fe stress conditions to the control and recovery ability after stress released. The exposure period of rice plant to Fe-toxicity is also short-term (≤ 3 days) and long-term (up to 3–4 weeks) (Quinet et al., 2012; Stein et al., 2019). This can result in weak correlation between visible symptoms and other quantitative trait measurements, which also leads to different selection of the best line tolerance to Fe-toxicity from the same breeding population under the field and nutrient solution (Nozoe et al., 2004; Kirk et al., 2022).

This study assessed rice growth responses to Fe excess in different stages including germination and vegetative stages and recovery ability, with long-term exposure (24 days) and short term recovery (15 days) to serve as a basis for screening rice varieties under similar conditions. Additionally, relative changes of traits measured under the Fe stress to the normal condition should be used as indicators for quick screening and effective selection.

MATERIALS AND METHODS

Plant materials and Fe-toxicity imposition

Seeds of two rice cultivars were used in this study, BC15 (improved cultivar, G1) and Phu An cultivar (a landrace, G2). These two cultivars present different ability for adaptation in terms of geographical regions in general and soil types in particular. BC15 is a pure cultivar which was bred from IR17494 and is adapted in wide geographical range of Vietnam, including the Mekong Delta in the south and Red River Delta in the north. Especially, acid sulfate soils distributed over 1.6 million hectares in the Mekong Delta and over 200,000 hectares in the Red River Delta (Le, 1981; Attanandana and Vacharotayan 1986; Le and van Mensvoot, 2004). Phu An cultivar is a red-seeded rice which is grown in the central coast of Vietnam. Fe-toxicity was imposed at germination and vegetative growth stages. Concentrations of $FeSO_4.7H_2O$ solution (50, 100, and 200 ppm; pH = 4.0 ± 0.1) were prepared following the phenotyping protocols for abiotic stress tolerance in rice (IRRI, 2021) (Table 1). The germination test was conducted with three replicates, 30 seeds per replicate and four levels of iron, 0 ppm (control), 50 ppm, 100 ppm, and 200 ppm. Seeds were germinated on petri dishes with 10 seeds/petri dish. The Fe-solution of appropriate levels was applied directly to the filter paper and the seeds submerged in the solution (Figure 1a). Seeds were checked every day and added Fe-solution if necessary for 7 days.

A preliminary test using the hydroponic solution and 10 plants per treatment was conducted to determine appropriate Fe-treatment (i.e., slowly increasing Fe-concentration), appropriate growth stages, and duration to apply Fe-toxicity (data not shown). Based on the preliminary results, experiments for Fe-toxicity imposed at vegetative stage were conducted in the greenhouse from April to July 2022 (summer season) using hydroponic solution. Experimental design was split-plot with three replicates and two factors (2 cultivars and 3 levels of Fe-toxicity including non-Fe-toxicity as the control). Fifteen plants were used for each replication. Seeds germinated for 5 days before placing on the plugs which were attached to flat boards (Figure 1b). The flat boards were placed on trays of 55 cm in length × 33 cm in width × 12 cm in height having capacity of 20 L liquid solution. Plants grew until 7-fully developed leaves before applying Fe treatments. Based on our preliminary test (data not shown), Fe should be applied gradually to avoid the shock for plants. So, for the experiment, initially, Fe levels of 50 ppm and 100 ppm were applied for 12 days so that plants were not sock. Then, the levels of Fe increased to 100 ppm and 200 ppm respectively and lasted for another 12 days (24 days in total for Fe imposition and hereafter referred as Fe treatments of 50–100 ppm and 100–200 ppm). Solution was changed every three days. After 24 days, Fe-toxicity was removed by applying normal hydroponic solutions to plants for recovery measurement after 5, 10 and 15 days.

Hydroponic solution was diluted from Grow master A and B consisting of macro and micronutrients (Table 2) with ratio of 1:10000. This growth solution with defined components was used to ensure no nutrient stress to plants. Additionally, leaf bronzing and stained leaf edge (Asch et al., 2005) which are the typical symptoms of Fe-toxicity were also observed (Figure 1d). These ensured that rice plants underwent Fe stress and not other stress.

Evaluation of plant growth characters

Measurement at germination test included germination rates (%), seedling length, and number of seedling roots at three time-points of 4, 6 and 8-day. The measured growth characters included plant height, number of tillers, stem diameter, leaf number, leaf size, number of roots, and root length at four time-points of 6, 12, 18 and 24-day after Fe-toxicity imposition. The recovery measurements after 10-day removing Fe-toxicity included plant height, number of tillers, and number of roots.

Statistical analysis

ANOVA analysis was used to evaluate the differences in measured traits between cultivars exposed to Fe-toxicity at different stages (germination and vegetative) and at different times (6, 12, 18 and 24-day Fe-toxicity). The mean values were calculated from replicates. Means were compared by using the least significant difference test with a 0.05 level of significance

The relative reduction of evaluated traits under the Fe-toxicity treatment in compared with the control was calculated as follows:

Table 1. Fe stress levels and the corresponding concentrations (IRRI, 2021)

Stress level (ppm = mg Fe·L^{-1} working solution)	Concentration (mM)	g·L^{-1} solution ($FeSO_4·7H_2O$)
50	0.890	0.2497 g
100	1.795	0.4990 g
200	3.591	0.9983 g
500	8.977	2.4957 g
1000	17.953	4.9911 g

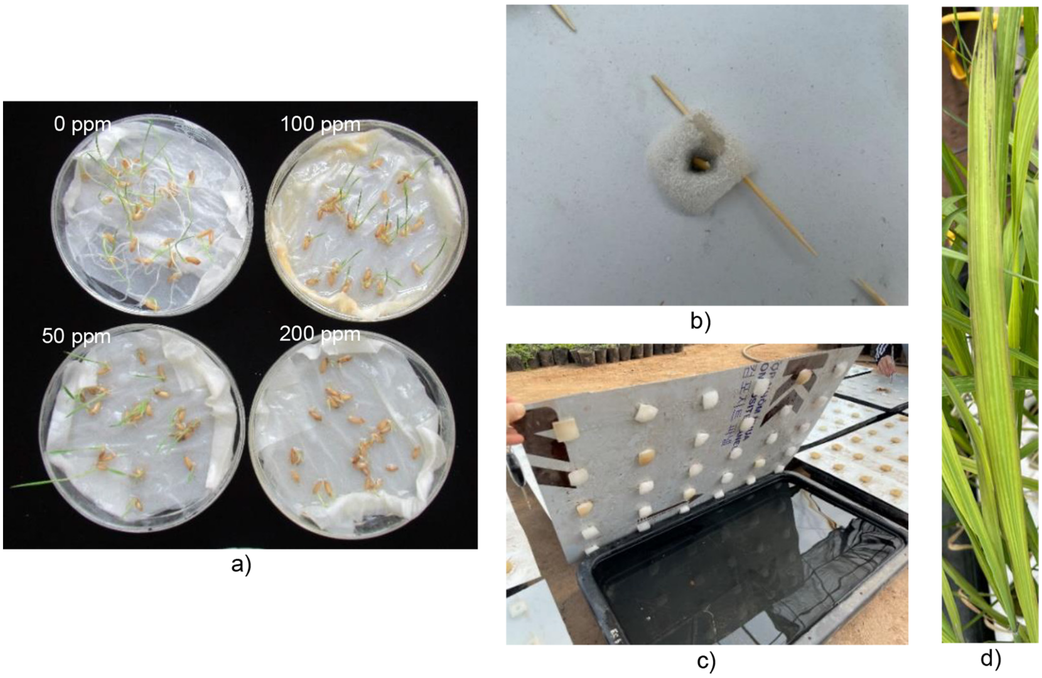

Figure 1. Experimental setup for two rice cultivars (BC15 and Phu An cultivars) under different levels of Fe-toxicity imposition: a) germination test: 10 seeds/petri dish, 30 seeds/replication and seeds were applied with 0, 50 ppm, 100 ppm and 200 ppm of iron concentration; b) Seeds were placed on plug that attached to a board; c) a board floating on hydroponic solution which was applied with treatment of 0, 50–100 ppm and 100–200 ppm of Fe; d) leaf bronzing and stained leaf edge as typical symptoms of Fe-toxicity in rice plant were observed

Table 2. Components of hydroponic solution grow master

GROUP A	Concentration	GROUP B	Concentration
Nitrogen (NO_3^-, NH_4^+)	60 g l^{-1}	Photphoric acid (P_2O_5)	42.5 g l^{-1}
Calcium CaO	88.33 g l^{-1}	Postasium (K_2O)	66.0 g l^{-1}
Iron (Fe)	0.925 g l^{-1}	Magnesium (MgO)	28.5 g l^{-1}
		Manganumt (Mn)	0.0341%
		Borum (B)	0.0294%
		Zincum (Zn)	0.004%
		Cuprum (Cu)	0.001%
		Molybdenum (Mo)	0.002%

$$Relative\ reduction\ (\%) = \frac{P_t - P_c}{P_c} \times 100 \quad (1)$$

where: P_t and P_c – the trait under Fe-toxicity and control treatment, respectively.

For the recovery, relative change of evaluated traits to the point before removal of Fe-toxicity (i.e. after 24-day Fe-toxicity imposition) was calculated as follows:

$$Relative\ change\ (\%) = \frac{P_r - P_{bf}}{P_{bf}} \times 100 \quad (2)$$

where: P_r and P_{bf} are the trait under Fe-toxicity and at the point before removal of Fe-toxicity, respectively.

RESULTS

Responses of rice to Fe-toxicity at germination stage

There were significant differences at the germination stage between two rice cultivars responding to different levels of Fe-toxicity (Table 3; Figure 2). Germination rates of BC15 and Phu An cultivars were both high, 97.33% and 96.0% respectively at control. However, as iron concentration increased, the germination rates decreased from 95.33% to 92.67% for BC15 and from 92.67% to 84.67% for Phu An cultivar. This

Table 3. Responses of two rice cultivars in germination rate, seedling height, seedling root length and number of seedling roots to different iron concentration

Iron concentration (ppm)	Germination rate (%) ± SE	Seedling height (cm) ± SE	Length of seedling root (cm) ± SE	Number of seedling root ± SE	Relative reduction in comparison to control (%)	
					Seedling height (%) ± SE	Length of seedling root (%) ± SE
BC15						
0	97.33^{a} ± 1.33	4.25^{bc} ± 0.52	4.44^{a} ± 0.22	7.08^{a} ± 0.33	-	-
50	95.33^{a} ± 1.23	3.89^{cd} ± 0.08	1.67^{b} ± 0.07	6.03^{b} ± 0.29	-3.34^{a} ± 0.14	-61.73^{a} ± 2.84
100	94.67^{a} ± 0.84	3.16^{de} ± 0.09	0.61^{c} ± 0.05	3.07^{d} ± 0.19	-23.53^{b} ± 2.38	-86.09^{b} ± 1.12
200	92.67^{ab} ± 2.40	1.49^{f} ± 0.07	0.19^{c} ± 0.02	1.00^{e} ± 0	-62.96^{e} ± 1.96	-95.44^{d} ± 0.43
Phu An cultivar						
0	96.0^{a} ± 2.07	5.14^{a} ± 0.11	4.88^{a} ± 0.24	7.62^{a} ± 0.27	-	-
50	92.67^{ab} ± 0.67	4.78^{ab} ± 0.09	1.84^{b} ± 0.07	4.57^{c} ± 0.25	-6.90^{ab} ± 1.70	-62.10^{a} ± 2.40
100	89.33^{ab} ± 3.37	4.50^{abc} ± 0.06	0.64^{c} ± 0.08	1.76^{e} ± 0.05	-12.19^{b} ± 1.33	-87.23^{bc} ± 1.51
200	84.67^{b} ± 1.91	2.71^{e} ± 0.09	0.28^{c} ± 0.02	1.18^{e} ± 0.06	-47.28^{d} ± 1.73	-93.94^{cd} ± 0.60

Note: means followed by the different letters are significantly different (Tukey's pairwise comparisons, $P \leq 0.05$).

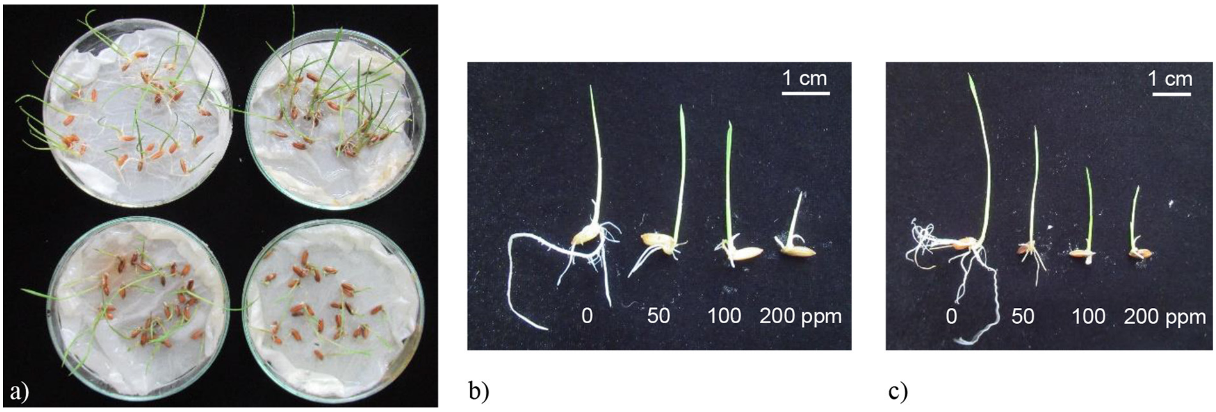

Figure 2. Germination of two rice cultivars under 0.50 ppm, 100 ppm and 200 ppm of iron concentration after 7 days of Fe-toxicity imposition: (a) Germination rates of BC15; (b) Germination of BC15 cultivar; (c) Germination of Phu An cultivar

trend was also observed for seedling height, seedling root length, and number of seedling root. At 50–200 ppm iron concentration, seedling height of BC15 varied from 1.49–3.89 cm, while that of Phu An cultivar varied from 2.71–4.78 cm. Length and number of seedling roots decreased more significantly at high iron concentration of 200 ppm (Figure 2b, c). Length and number of seedling roots of BC15 respectively were 0.19 cm and 1.0 roots, while those of Phu An were 0.28 cm and 1.18 roots at 200 ppm but there were no significant differences. However, numbers of seedling roots in BC15 at 50 ppm and 100 ppm (6.03 and 3.07 roots respectively) were significantly higher that those in Phu An cultivar (4.57 and 1.76 roots respectively). In comparison to control, relative decreases in seedling height and length of seedling root were respectively in the range of 3.34–62.96% and 61.73–95.44%.

Growth responses to Fe-toxicity at vegetative stages

Significant differences in relative changes of two rice cultivars under Fe-toxicity compared to the control were observed for growth characters (Table 4; Figure 3). Plant height, number of tillers and number of leaves still increased when exposed to Fe-excess for at least first 12 days. Initially, plant height of two cultivars increased from 4.52–13.0% compared to the control in the first 12 days, then the relative increases were only 0.31–5.30% in 18-day. Plant height decreased significantly at 24-day for 3.17–14.36% compared to the control.

However, initially, tiller number and leaf number relatively increased when exposed to Fe-toxicity compared to control. Relative increases of both cultivars to the control for number of tillers and of leaves ranged from 2.84–10.85% and 1.55–6.06% respectively in 6- and 12-day. While tiller number slightly increased up to 12-day exposure, leaf number slightly increased until 24-day exposure for BC15 (4.36% and 1.85%) and up to 18-day exposure for Phu An cultivar (1.90% and 0.72%). BC15 had relative reduction for tiller number higher than Phu An at 18- and 24-day correspondence to iron concentration but there were no significant differences within the same evaluated day. Especially for each cultivar, the relative reduction in the tiller number at 100–200 ppm was 2–3 times more than that at 50–100 ppm. Relative reduction in number of leaves to the control was only observed for Phu An cultivar in 24-day at 50–100 ppm Fe (1.03%), and 100–200 ppm Fe (4.29%). The relative reduction to the control was observed for root length and root number as soon as the rice plants were exposed to iron and increased with the days of exposure. Over two Fe-treatment, relative reductions of BC15 root length from 6–24-day exposure changed from 5.70 to 27.41%, of which the relative reduction at 100–200 ppm treatment was more double than that at 50–100 ppm treatment. Phu An cultivar had root length reduction compared to the control ranged from 6.0–25.36% with quite similar reduction level between two Fe-treatments within the evaluated day. There were more significantly relative reductions in root number between Fe-treatment and among days of exposure. Initially, in 6-day, the root number slightly decreased about 0.87–3.15% (BC15) and 5.0–5.32% (Phu An cultivar) compared to the control, then significantly reduced to about more than 10%, 20% and 30% in the next 12, 18 and 24-day in comparison to the control. The patterns of plant growth characteristics for BC15 and Phu An cultivars under Fe-treament were clearly shown

Table 4. Relative changes (%) of plant height, number of tillers, number of leaves, length and number of roots in two rice cultivars under different iron concentration at 6, 12, 18, 24-day compared to control at vegetative stage

Measured characters	Cultivar	Fe-treatment (ppm)$^{\gamma}$	Fe-toxicity exposure days*			
			6-day	12-day	18-day	24-day
a. Plant height ± SE	BC15	50–100	9.06^{b} ± 0.64	11.84^{a} ± 0.39	4.04^{c} ± 0.39	-11.03^{g} ± 0.60
		100–200	8.89^{b} ± 0.46	13.0^{a} ± 0.42	3.73^{c} ± 0.30	-14.36^{h} ± 0.50
	Phu An	50–100	9.28^{b} ± 0.69	12.44^{a} ± 0.49	4.83^{c} ± 0.36	-3.17^{e} ± 0.31
		100–200	4.52^{c} ± 0.53	8.91^{b} ± 0.47	0.80^{d} ± 0.14	-8.52^{f} ± 0.46
b. Number of tillers ± SE	BC15	50–100	10.85^{a} ± 0.52	5.70^{bc} ± 0.13	-8.33^{e} ± 0.45	-24.79^{h} ± 0.75
		100–200	8.20^{b} ± 0.57	5.60^{bc} ± 0.18	-22.07^{g} ± 0.70	-40.14^{i} ± 0.79
	Phu An	50–100	5.99^{bc} ± 0.27	4.39^{cd} ± 0.15	-6.70^{e} ± 0.30	-17.34^{f} ± 0.67
		100–200	6.08^{bc} ± 0.34	2.84^{d} ± 0.16	-19.02^{f} ± 0.87	-42.13^{i} ± 0.75
c. Number of leaves ± SE	BC15	50–100	6.06^{a} ± 0.08	5.37^{b} ± 0.06	4.81^{c} ± 0.02	4.36^{cd} ± 0.02
		100–200	2.56^{e} ± 0.06	2.30^{ef} ± 0.05	2.17^{ef} ± 0.07	1.85^{fg} ± 0.07
	Phu An	50–100	3.97^{d} ± 0.08	2.08^{ef} ± 0.05	1.90^{fg} ± 0.01	-1.03^{i} ± 0.03
		100–200	4.84^{c} ± 0.09	1.55^{g} ± 0.06	0.72^{h} ± 0.01	-4.29^{j} ± 0.09
d. Root length ± SE	BC15	50–100	-5.70^{a} ± 0.11	-7.47^{ab} ± 0.76	-9.25^{bc} ± 0.41	-12.02^{cd} ± 0.30
		100–200	-6.99^{ab} ± 0.28	-16.71^{ef} ± 0.91	-20.05^{g} ± 0.54	-27.41^{i} ± 0.77
	Phu An	50–100	-6.0^{a} ± 0.23	-14.41^{de} ± 0.95	-18.56^{fg} ± 0.86	-24.11^{h} ± 0.82
		100–200	-13.61^{de} ± 0.35	-15.01^{de} ± 0.79	-20.31^{g} ± 0.83	-25.36^{hi} ± 0.46
e. Number of root ± SE	BC15	50–100	-0.87^{a} ± 0.08	-11.78^{c} ± 0.97	-20.02^{ef} ± 1.24	-29.63^{gh} ± 0.90
		100–200	-3.15^{ab} ± 0.08	-17.46^{de} ± 0.94	-27.69^{g} ± 1.21	-39.18^{i} ± 0.79
	Phu An	50–100	-5.0^{ab} ± 0.09	-14.18^{cd} ± 0.91	-21.85^{f} ± 1.15	-32.33^{h} ± 0.54
		100–200	-5.31^{b} ± 0.08	-16.84^{de} ± 0.91	-22.91^{f} ± 1.14	-37.57^{i} ± 0.88

Note: γ – two treatments of iron were applied as follows: Iron concentrations of 50 ppm and 100 ppm were applied for the first 12 days, then increased to 100 ppm and 200 ppm respectively up to 24 days. * – positive and negative values indicated relative increase and decrease in comparison to the control, respectively. Means followed by the different letters are significantly different across cultivars, iron concentration and exposure days (Tukey's pairwise comparisons, $P \leq 0.05$).

in Figure 3. All measured traits expressed lower values under higher Fe-toxicity levels (100–200 ppm). Phu An cultivar had higher plant height than BC15 in both control and Fe-treatment conditions (Figure 3a). Other traits including number of tillers and leaves, root length and number were quite similar between two cultivars when comparing under the same conditions. Plant height of both cultivars increased slightly at 6, 12 and 18-day compared to the control and started to be reduced at 24-day of Fe-toxicity exposure. Number of tillers and leaves had similar pattern with increases in 6, 12-day, then decreases from 18-day of exposure (Figure 3b, c). Root length and root number under the control increased with the growth day, but slightly reduced at 6-day and significantly reduced from 12-day under the Fe-treatments (Figure 3d, e).

Recovery of rice under Fe-toxicity

Both cultivars exhibited recovery ability after 10 days relieving Fe-toxicity in terms of plant height, number of tillers and roots (Table 5). In comparison to the point prior to recovery, significant recovery was observed between BC15 and Phu An cultivars at different Fe-treatment. Generally, BC15 showed higher relative increases compared to the point prior to recovery in all traits than Phu An did at the same Fe-treatment.

However, compared with the control, both cultivars were still lower from 6.14–15.81% for plant height, 21.43–45.57% for tiller number and 23.73–37.40%. Consequently, plant height, number of tillers and roots were also lower than the control. For example, plant height of BC15 were

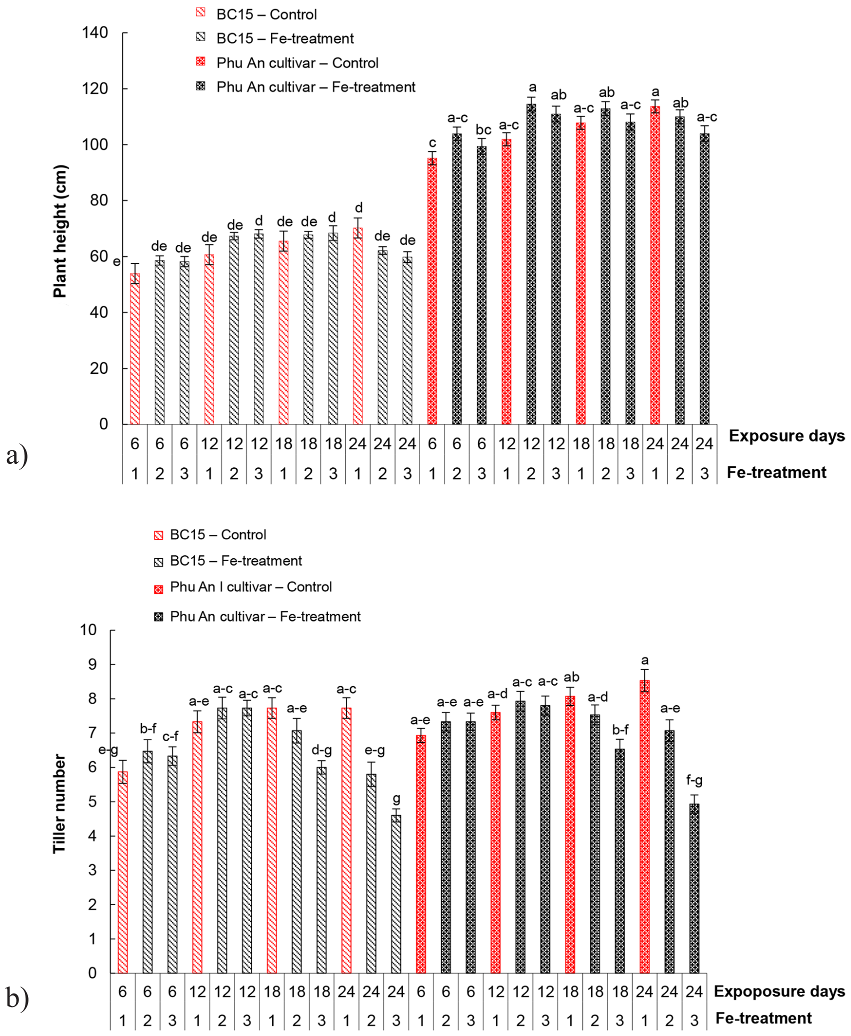

Figure 3. Growth responses of two rice cultivars, BC15 and Phu An cultivars under different levels of Fe-treatment (1 = 0 ppm, control; 2 = 50–100 ppm; 3 = 100–200 ppm) after 6, 12, 18 and 24-day exposure: (a) plant height (cm); (b) tillter number; (c) leaf number; (d) root length (cm); (e) root number. Different small letters indicate significant differences at P ≤ 0.05. Error bars indicate ± SE (n = 15). Hyphen (-) is used for significant differences of more than two letters (a-c = abc; b-d = bcd; d-g = defg)

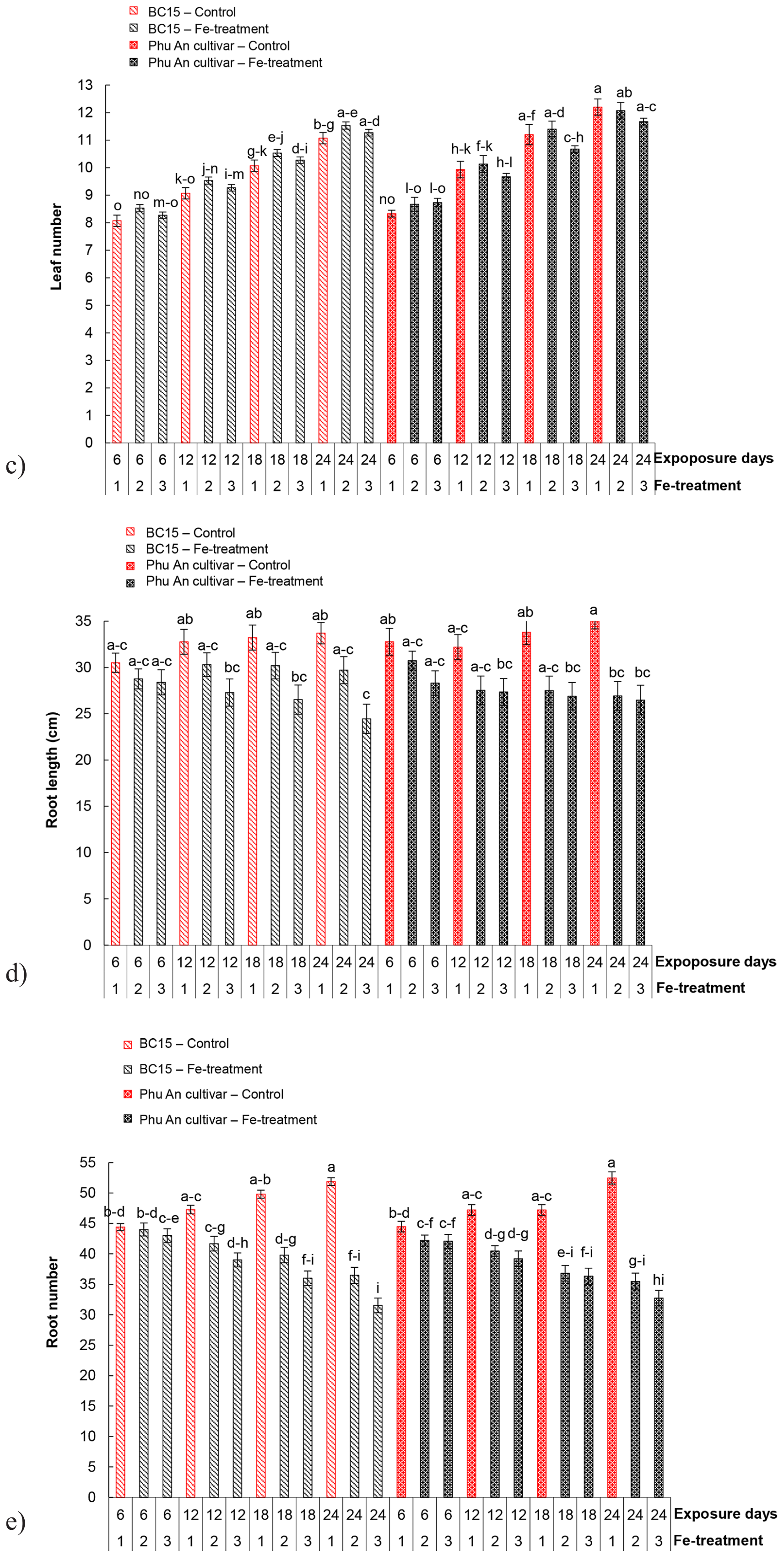

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Table 5. Relative change of growth characters (%) in comparison to point prior recovery and control, and recovery 10 days after releasing Fe-toxicity in two rice cultivars

Fe-treatment$^{\gamma}$	Relative change*						Plant height (cm) ± SE	Tiller number ± SE	Root number ± SE
	In comparison to point prior to recovery (%)			In comparison to control (%)					
	Plant height ± SE	Tiller number ± SE	Root number ± SE	Plant height ± SE	Tiller number ± SE	Root number ± SE			
BC15									
0	-	-	-	-	-	-	79.06^{c} ± 3.61	8.60^{a} ± 0.19	54.27^{a} ± 1.02
50-100	12.38^{a} ± 1.71	11.64^{a} ± 1.57	19.51^{a} ± 3.45	-11.16^{ab} ± 2.69	-24.74^{a} ± 4.41	-23.73^{a} ± 3.88	69.81^{cd} ± 1.24	6.47^{b} ± 0.38	41.33^{bc} ± 2.04
100-200	10.23^{ab} ± 1.05	6.96^{ab} ± 1.62	12.58^{ab} ± 3.17	-15.81^{b} ± 3.31	-42.52^{b} ± 3.25	-37.40^{c} ± 2.75	66.16^{d} ± 2.11	4.93^{c} ± 0.07	33.93^{d} ± 1.45
Phu An cultivar									
0	-	-	-	-	-	-	123.2^{a} ± 2.33	9.07^{a} ± 0.32	57.73^{a} ± 0.98
50-100	5.44^{bc} ± 0.09	2.94^{ab} ± 0.05	12.37^{ab} ± 2.05	-6.14^{a} ± 1.22	-21.43^{a} ± 3.53	-25.61^{ab} ± 2.43	115.75^{ab} ± 2.47	7.13^{b} ± 0.34	42.87^{b} ± 1.27
100-200	3.64^{c} ± 0.01	1.38^{b} ± 0.06	7.96^{b} ± 1.70	-12.74^{ab} ± 2.33	-45.57^{b} ± 1.95	-36.0^{bc} ± 2.09	107.46^{b} ± 2.83	4.93^{c} ± 0.08	36.87^{cd} ± 1.07

Note: γ – two treatments of iron were applied as follows: Iron concentrations of 50 ppm and 100 ppm were applied for the first 12 days, then increased to 100 ppm and 200 ppm respectively up to 24 days. * – positive and negative values indicated relative increase and decrease in comparison to point prior to recovery and to the control, respectively. Means followed by the different letters are significantly different at 10-day recovery (Tukey's pairwise comparisons, $P \leq 0.05$).

69.81 cm and 66.16 cm at 50–100 pm and 100–200 pm respectively, while that were 79.06 cm for the control. Phu An cultivar had higher plant height than BC15 due to its higher initial high. However, there were no significant differences in tiller and root number between two cultivars after 10-day recovery under the same Fe-treatment. But overall, there were significant differences between Fe-treatment, with better recovery at Fe-treatment of 50–100 ppm.

DISCUSSION

Iron toxicity is one of the widely prevalent soil-related micronutrient disorders in rice (Mahender et al., 2019). Iron toxicity can affect rice growth at any stages including germination, seedling, vegetative, and reproductive stages (Bresolin et al., 2019; Kar et al., 2021; Theerawitaya et al., 2022; Ahmed et al., 2023). This study applied Fe-toxicity levels of 50, 100 and 200 ppm at germination and slowly increased iron concentration levels from 50 ppm to 100 ppm and from 100 ppm to 200 ppm at vegetative stages for rice growth responses and recovery assessment. These level ranges of Fe-toxicity, although lower than some other studies such as 300–1000 $mg \cdot L^{-1}$ (Onaga et al., 2013; Mahender et al., 2019; Ahmed et al., 2023), 1000 $mg \cdot L^{-1}$ (Wu et al., 2014), 2000 $mg \cdot L^{-1}$ (Bresolin et al., 2019) or 1000–3000 $mg \cdot L^{-1}$ (Asch et al. 2015), were also used in many previous studies to effectively assess effects of Fe-toxicity, screen genotypes and analyze transcriptome; such as 0.1–0.5 mM (< 50 ppm) (Bashir et al., 2014), 0.36–5.36 mM (< 50–300 ppm) (Aung et al., 2018), 50–250 ppm (Nguyen and Tran, 2020), 125 $mg \cdot L^{-1}$ (Quinet et al., 2012), 150 $mg \cdot L^{-1}$ (Ahmed et al., 2023), and 80–160 $mg \cdot L^{-1}$ (Crestani et al., 2009) (Referred to Table 1 for equivalent concentration). Additionally, the Fe^{2+} concentrations in the soil solution reportedly affect rice from 10 to > 2000 $mg \cdot L^{-1}$. Amounts of soluble Fe^{2+} may be found in acid soils at concentrations of 100–1000 $mg \cdot L^{-1}$, or even up to 5000 $mg \cdot L^{-1}$ (Becker and Asch 2005). Also, depending on the site and the cultivars used, reported critical iron concentrations can range from 20 to 3000 $mg \cdot L^{-1}$ (Becker and Asch 2005; Panhwar et al., 2016). Seed germination is one of the most crucial phases in plant growth and development which is also the most susceptible stage to all types of environmental stresses. Poor germination results in poor seedling establishment and consequently leads to weak vegetative growth and significantly reduction in potential yield.

Reduction in germination rates, seedling height, length and number of seedling roots as iron concentration increased were observed in our studies. Seed germination rates reduced significantly from 96.0–97.33% at the control to 84.67–92.67% at 200 ppm (Table 3). This phenomena were also observed in Ahmed et al. (2023), with germination rates reduced from 92.9% (control) to 88.4% (300 $mg \cdot L^{-1}$) and to 72.1% (900 $mg \cdot L^{-1}$). Significant reduction in root length and shoot length at germination stages at higher iron concentrations can be indicators for iron detrimental effects, as also suggested by Ahmad et al. (2023) for iron level of 300 $mg \cdot L^{-1}$ threshold. However, these characteristics should still not be used in confidence for screening of tolerant genotypes at early germination stage. As observed in our study, Bresolin et al. (2019) and Ahmad et al. (2023), tolerant genotypes can have both significantly shorter or longer shoot and root length than susceptible genotypes and vice versa. For example, RD85 was tolerant to iron excess but had similar root length with RD31 – a sensitive genotype (Ahmad et al., 2023). Sensitive genotypes, Nipponbare and BR-IRGA 409 had higher shoot length at the control and iron excess condition than the tolerant genotypes (Bresolin et al., 2019). Mean performance of shoot length and root length was not negatively impacted by increasing levels of Fe-treatment (Ahmad et al., 2023). Additionally, local or landrace varieties often have higher plant height than the improved cultivars as is the case in our study where Phu An local variety exhibited much higher height (Figure 3a). However, the relative reduction compared to the control can be more dependable for screening. Although there were no significant differences, BC15 had lower relative reduction in seedling height and root length than Phu An at 50 ppm. Relative decrease in shoot and root dry weight after 2 day of excess Fe were used by Kar et al. (2021) to select sensitive and tolerant varieties among 16 rice varieties. Bresolin et al. (2019) also suggested other characteristics including Fe, Zn and Mn accumulation in shoot were useful for screening rice genotypes under iron toxicity condition. Kar et al. (2021) indicated Fe-content in leaves of tolerance plants were lower than that of susceptible plants.

Generally, iron toxicity at later growth stages (vegetative and reproductive) results in poor growth, development and significant yield loss (Audebert and Sahrawat 2000; Fageria et al., 2008; Audebert and Fofana 2009; Keita et al., 2013; Bresolin et al., 2019). In this study, at the vegetative stage, the higher levels of iron toxicity and the longer exposure to the stress, the more severe impacts and the higher relative reductions compared to the control were observed for growth characteristics including plant height, number of tillers and leaves, root length and number (Table 4). Interestingly, the relative increase in plant height, tiller and leaf number were recorded respectively for the first 18-, 12- and 18-day plants exposed to Fe-treatments of 50–100 ppm and 100–200 ppm (Table 4; Figure 3 a-c). This phenomena were not reported in other studies which focused on seedling stages after 2-day (Kar et al., 2021), 3-day (Bresolin et al., 2019), 14-day (Theerawitaya et al., 2022), on vegetative stages after 7-day (Theerawitaya et al., 2022), at panicle initiation/reproductive and harvesting stage (Theerawitaya et al., 2022; Ahmad et al., 2023) for shoot height, fresh and dry shoot and root weight, leaf bronzing scores, and yield component traits. Quinet et al. (2012) although made observation of rice growth up to 3 weeks under Fe stress, the visible reduction in shoot length and tiller number occurred after 2 weeks (14 days) which was shorter than our observation. This could be due to the fact that increase in iron concentration in this study was applied gradually, from 50 to 100 ppm and from 100 to 200 ppm, so that rice plant had times to adjust itself for stress. In addition, plants had certain number of leaves and tillers (i.e. around average of 5 leaves and 7.4 tillers – results not shown) before exposed to Fe-treatment. These both conditions can allow plants to maintain and even slightly increase in height, number of leaves and tillers for some days until plants cannot stand the stress. Interestingly, Ahmad et al. (2023) also reported performance of growth characteristics (plant height, root length, shoot and root dry weight and tiller number), yield components (panicle number, filled grain ratio, 1000-grain weight), and individual grain yield for rice plants were higher under Fe levels of 150 and 300 $mg \cdot L^{-1}$ than under the control. Accumulative leaf areas were also observed during 2 days of excess Fe, indicating continuous leaf growth (Kar et al., 2021). Besides, a study on common reed (*Phragmites australis*) – a helophytic grass found no direct causal relationship between iron content in aerial tissues and growth inhibition, which strongly suggests that iron toxicity cannot explain growth reduction (Batty and Younger, 2003). This implies complexities in responses of plants in general and rice in particular to Fe-toxicity, not only related to below- and above ground morphological and agronomical changes, physiological changes, but also to many genes (Quinet et al., 2012; Kar et al., 2021; Kirk et al., 2022; Theerawitaya et al., 2022).

In contrast, relative decrease in root length and root number to control were immediately observed at 6-day excess of Fe (0.87–13.61%), and becoming more significantly reduced in 12-, 18- and 24-day between two cultivars and Fe-treatments. These findings were in the agreement with Theerawitaya et al. (2022) in extent that fresh and dry root weights decreased by 7.4–28.2% after 7-day Fe-treatment at vegetative stage. Recovery ability of plants after stress relieve is important since plants will recovery their growth, development and productivity to minimize loss. This study indicated rice plants can endure 24-day excess Fe and were able to recover after relieving Fe-toxicity. Plants increased height, root length and number by 5–10% compared to prior to Fe stress relieve but were still relative lower than the control by 6–37% (Table 5). At seedling stage, leaf length and accumulative leaf areas showed increase immediately when Fe-stress removed (Kar et al., 2021). Ahmad et al. (2023) applied Fe-toxicity conditions until harvest and concluded that genotypes with better vegetative growth in terms of plant height and shoot dry matter exhibited poor yield performance, which was in contrast to genotypes with higher root length and root dry matter had comparatively better yield. Applying Ahmad et al. (2023) to this study and together with lower relative reduction in growth characteristics at 6 – 24-day excess Fe, and higher relative increases when plants recovered, BC15 is more tolerant and than Phu An cultivar under Fe-stress condition.

Thus, this study evaluated relative changes in growth responses and short-term recovery of rice under Fe-toxicity at the germination and vegetative stage. Besides, other studies also revealed adverse effects of iron toxicity on rice plants at the reproductive stage such as Theerawitaya et al. (2022) and Ahmed et al. (2023). Bresolin et al (2019) revealed Fe, Cu, Zn, and Mn accumulation in shoots or five rice genotypes at the seedling stage under Fe-excess conditions. Kar et al. (2021) analyzed Fe and other metal contents in leaves under Fe-toxicity also at the seedling stage. There are still lacking studies on long-term effects of Fe-toxicity on rice plants, in terms of relative changes in yield components and yield at reproductive growth stages and seed quality.

The study results indicate this approach can be used as a quick screening method at early growth stages for rice under Fe-toxicity. Especially, this approach would be useful and dependable when screening a large number of genotypes and selecting tolerant genotypes at the early vegetative growth stage (7-fully developed leaves). Therefore, time, effort, and resources are saved compared to evaluating the effects of Fe-excess at the reproductive stage or waiting until harvest for grain yield (~ 3–4 months). In practice or the field condition, observed symptoms or growth responses of rice plants exposed to Fe-toxicity at germination and the vegetative stage can also help growers to diagnose and have early prevention or management (Attanandana and Vacharotayan 1986; Becker and Asch 2005; Panhwar et al., 2016) to minimize yield losses.

CONCLUSIONS

In conclusion, Fe-toxicity is detrimental to rice plant growth and development at both germination and vegetative stages. The higher level of excess Fe and the longer exposure, plants would suffer more severe damage and growth reduction. Rice plants are able to recover after 24 days exposed to Fe-toxicity. Relative reduction under Fe stress condition compared to the control and relative increase in compared to the point prior to point stress release should be used with more dependable for determining Fe-toxicity response for growth characteristics at germination and vegetative stages. Long-term effects of Fe-toxicity on rice plants, in terms of reproductive success and seed quality are suggested for future studies.

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