Original Research Article

Rainfed rice genotypes adaptability to excess iron stress in hydroponic culture

Abstract:

Iron (Fe) toxicity is a nutritional disorder that affects lowland rice (*Oryza sativa* L.). Excessive amounts of reduced Fe(II) in the soil solution, its uptake by the rice roots, and its transpiration-driven transport result in elevated Fe(II) concentrations in leaf cells that catalyze the formation of reactive oxygen species. The oxidative stress causes rusty brown spots on leaves (bronzing) and the reduction of biomass and yield. While the use of resistant genotypes is the most promising approach to address the problem, the stress appears to differentially affect rice plants as a function of plant age, climatic conditions, stress intensity and duration, and the dominant adaptation mechanism. We comparatively assessed ten contrasting 4-week-old rice genotypes regarding their response (symptom score, agronomic traits, Fe concentrations in different plant parts) to different iron stress intensities (0, 500, 1000 and 1500 mgL^{-1} Fe(II)) exposed for three days in an experiment conducted during the year 2021 at Assam Agricultural University, Jorhat. Rice varieties *Bahadur* and *Podumoni* were found to tolerate high iron concentrations through an exclusion mechanism. In contrast, *Joymoti*, *Ranjit* and *Mahsuri* could tolerate excess iron concentration even after imbibing iron into the plant system. Moniram and Bahadur could also tolerate high iron conditions by maintaining moderately high leaf iron concentrations. *Kushal* and *Podumoni* were sensitive and extremely sensitive, respectively.

Keywords: iron toxicity, rice, adaptation, sensitive, mechanism

Introduction:

Rice (*Oryza sativa* L.) is globally cultivated on approximately 128 million hectares of irrigated and rainfed lowlands (Maclean *et al*., 2002), and about 55% of the rice-growing area is affected by excess iron (Chérif *et al*., 2009). India is estimated to be the leading global producer of rice with approximately 45 million hectares of rice under the plough. Plants depend on iron for a wide range of biological processes including electron transport—a central component of both photosynthesis and respiration (Zhang *et al*., 2019). However, when present in excess, iron can prove toxic and thus while plants must work hard to scavenge this often-scarce resource, they must do so with caution (Zhang *et al*., 2018). Under flooded conditions, the soil solution's ferrous ion (Fe2+) increases sharply. Iron toxicity in rice also reflects deficiencies of other nutrients resulting in yield reduction (Dobermann & Fairhurst, 2000). Iron toxicity is responsible for yield losses by 12-100 per cent depending on the intensity of the stress and tolerance of the rice cultivars (Sahrawat, 2004).

Although rice cultivars have been screened based on agronomic traits, mostly yield; however, attempts have yet to be made to assess the physiological stress response to overcome toxic iron conditions. Therefore, the present investigation attempts to identify rice genotypes based on their physiological mechanism under variable iron toxic conditions.

Materials and Methods

A laboratory experiment was carried out in the year 2021 at Assam Agricultural University, Jorhat where rice crop was grown in Yoshida medium (hydroponic culture) explicitly developed for rice (Yoshida *et al.*, 1976). The experimental setup consisted of 6 boxes containing 60 PVC cut pipes each. PVC pipes (3.6 cm in diameter) were cut into 9 cm lengths. Such 60 cut pipes were tied (6x10) for one box (6.5 L, 37 cm in length, 26.5 cm in width). A set of tied PVC pipes were inserted in each box. Ten rice cultivars were tested for the study.

Two-week-old seedlings from sand-cultured nursery beds were transplanted into the PVC pipes. Rice plants were fixed in half-split foam plugs (3.5 cm in diameter and 3 cm in thickness so that the stem is above the foam plug) and inserted into the PVC pipes so that only roots were in direct contact with the Yoshida solution. Liquid paraffin oil (low density) was added to each PVC pipe to maintain a 3 mm layer for checking the oxidation process (Kpongor *et al*., 2003). An appropriate amount of $FeSO_4.7H_2O$ was dissolved in Yoshida solution to get a concentration of 500, 1000 and 1500 mg $Fe^{2+}L^{-1}$ in the treatments. Yoshida solution alone was prepared to give 0 mg L^{-1} of Fe^{+2} for the control without iron.

Rice plants were harvested post-iron treatment at six weeks after transplanting. The roots of the plants subjected to 500, 1000 and 1500 mg L^{-1} Fe^{2+} treatments were washed with 15 mL of 0.5M hydrochloric acid (HCl) to obtain the iron plaque, washed with distilled water, dried with a paper towel, weighed and stored in liquid nitrogen. The HCl washing solutions were stored at 8°C, filtered, diluted and analyzed with atomic-absorption spectrometry to determine the iron plaque formation on the surface of the roots. Roots, stems and leaves from all treatments were collected, the fresh weight determined and stored in liquid nitrogen. To estimate the iron distribution in the plant, leaves, stems and roots were sampled separately in paper bags. The samples were taken to a freeze-drier for drying and preparation for the analysis of iron. Different plant parts were digested with sulphuric acid for iron estimation. Scoring of Fe- toxicity symptoms were done according to the scoring system provided by the International Network for Genetic Evaluation of Rice (*INGER*; *IRRI*, 1996). All data collected were subjected to analysis of variances appropriate to the factorial completely randomized design. If found, significant means were compared using Duncan multiple range test at alpha level 5%.

Result and discussion:

Relationship of the Bronzing Severity with observed character and iron content in different plant parts.

In rice, iron toxicity leads to what has been named 'leaf bronzing', the severity of which is closely correlated with yield losses (Matthus *et al.*, 2015). We found a significant variation in leaf bronzing among genotypes at 6 weeks-exposure with different iron concentrations resulting in a significant correlation between the observed characters and leaf bronzing (Table 1). Further, it was observed that, after 4 weeks, LBS correlated well with traits related to shoot length and the number of leaves, indicating that excess iron inhibited aboveground agronomic traits in sensitive genotypes compared to those in tolerant genotypes. Hence, observing aboveground agronomic traits may be a screening criterion for iron-tolerant and sensitive rice genotype selection. There was no significant correlation between LBS with root length. However, LBS was significantly correlated with the shoot length (r=-0.403**) and the number of leaves (r=-0.226**), denoting shorter root and shoot length. The susceptible genotype *Podumoni* was the most affected seedling observed in this experiment

(19.3 cm), while the tolerant genotype like Ranjit (22.6 cm) had a significantly longer shoot length. High shoot length and biomass accumulation of Ranjit was also reported by other researchers (Satapathy *et al.*, 2015).

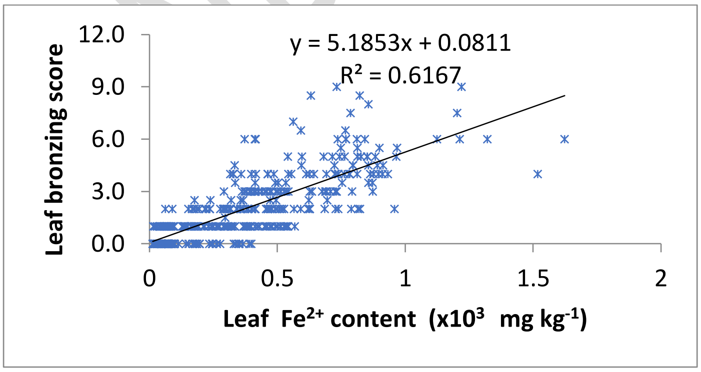
Table 1: Simple correlation between leaf bronzing score (LBS) and each observed character of rice seedlings exposure with 0, 500, 1000 and 1500 mg. L^{-1} of ferrous iron (N=360)

Characters	LBS at four weeks
LBS at 42-d	-
Root length	-0.062
Shoot length	-0.403**
Number of leaves	-0.226**
Leaf iron content	0.759**
Stem iron content	0.556**
Root iron content	0.664**

In this present study, LBS had a significant correlation with root iron content (r= 0.664**), indicating that some tolerant genotypes could store the iron in the roots, and others excluded the iron from the leaves left in the solution. Leaf bronzing symptoms significantly and positively correlated with leaf iron content (r=0.759**). Reddy et al., (2019) found high significant positive correlation between leaf bronzing score (visual scoring for iron-toxicity symptoms) and iron content in the leaf.

The leaf bronzing severity is aggravated by the increased leaf iron content (Figure 2). Further, a unit change in leaf iron content influenced leaf bronzing severity by 5.26 units (Figure 1). In the present study, highly significant positive correlation between leaf bronzing index and iron content in leaf was observed. Similar findings were also reported by Asch et al., (2005) and Nyamangyoku and Bertin (2013). All the above correlations, confirms the utility of LBI as criterion for differentiating between susceptible and tolerant genotypes. Several earlier workers (Dufey et al., 2012; Wu et al., 2014) had relied on LBI scoring to identify genotypes tolerant to Fe stress.

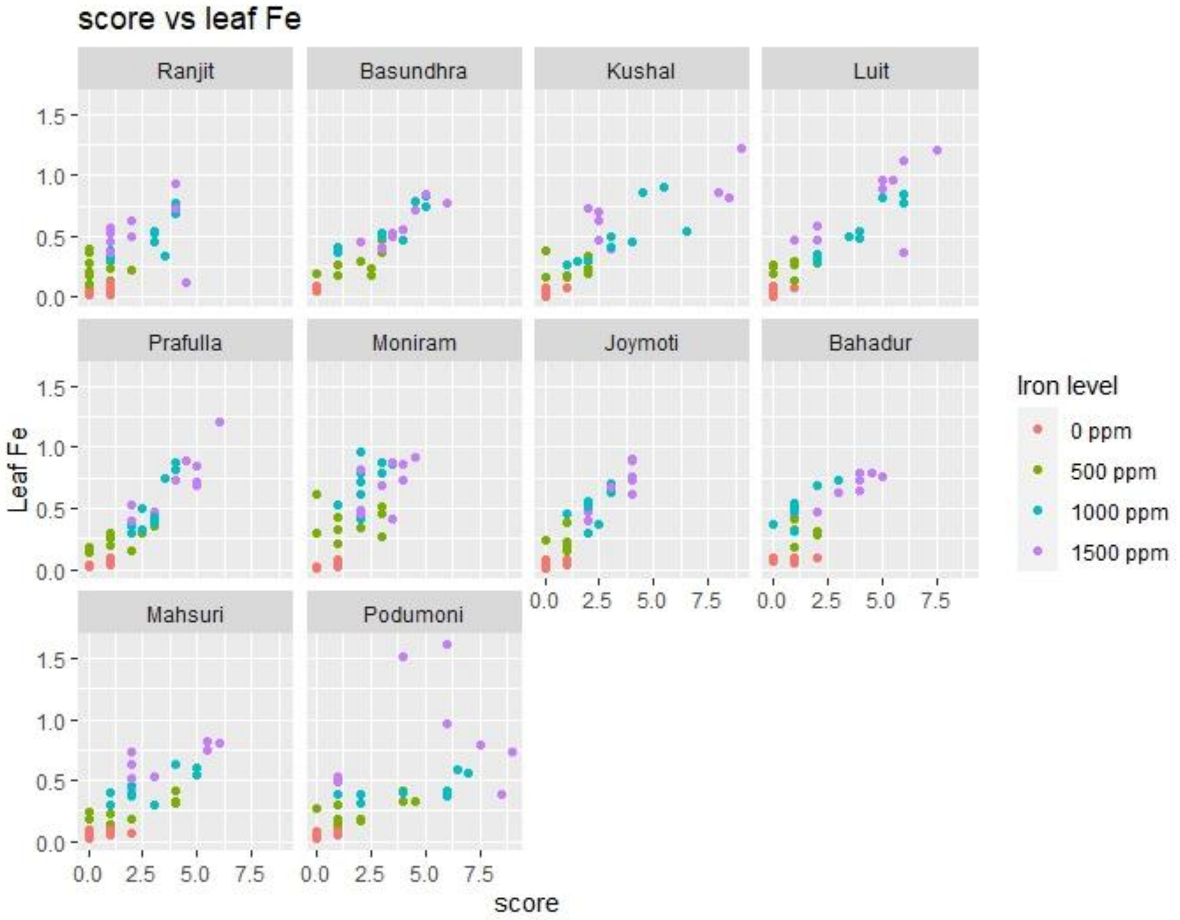
Figure 1: The relationship between symptom scores and leaf-tissue Fe concentrations of rice cultivars (3 days after exposure to external Fe(II) 0, 500, 1000 and 1500 mg L^{-1} in nutrient solution at 4-week-old seedling stage)



Distribution of Fe within plant tissues

Four weeks after sowing, seedlings of 10 genotypes were exposed to 0, 500, 1000 and 1500 mg L^{-1} Fe(II) in the nutrient solution for 3 days. Ten cultivars' roots, stem, and leaves were sampled separately and analyzed for Fe concentrations in plant tissues to determine Fe distribution within plant tissues. In addition, roots (n = 3) were washed with 0.5 N HCl, and the washing solutions were analyzed to determine the amount of Fe excluded at the root surface (Fe plaque) and how large the amount is retained in the root tissue.

Figure 2: Relation between Leaf scoring and leaf Fe content (mg/g) at different Fe (II) concentrations.



Data of mean values, their standard error of mean and the Least significant difference value (stem, leaf and root-tissue Fe concentrations) for 10 genotypes are presented in Table 2. One of the problems in field screening large numbers of genotypes for tolerance to Fe-toxic conditions is to provide sufficiently homogenous and elevated Fe levels in the soil, thus providing comparable stress levels to all materials (Audebert & Sahrawat, 2000). Therefore, differentiation of genotypes according to their sensitivity or tolerance to iron toxicity was achieved with the adapted setup.

As compared to the mechanistic screening method (early vegetative stage) developed by Asch *et al*. (2005), the adapted setup allowed access to the root for the analysis of Fe plaque at the root surface and, consequently, analyze the root Fe Plaque and the root-tissue Fe concentrations that allowed to differentiate the exclusion of Fe(II) at the root surface (oxidation power of rice root) and the root cells (root retention) of all tested cultivars. In such a way, genotype differentiation was possible based on exclusion potential for Fe on the root surface, iron partitioning in different tissues and toxicity symptom expression on the leaf. There were significant differences ($p = 0.05$) in root iron plaque among the tested genotypes (Table 2). differential iron plaque formation suggests that the oxidation power of tested genotypes differed. However, the amount of Fe plaque formed at the root surface in all tested genotypes was approximately three times higher than the tissue-Fe concentrations of rice plants. This indicates that the oxidation power of rice root plays an essential role in coping with Fe stress as an avoidance mechanism. Reduced iron, having passed the oxidative barrier of the rhizosphere, enters the root apoplast. Reduced iron can be excluded at the root cell membranes (Tadano, 1976). Significantly higher ($p = 0.05$) root iron plaques were recorded in *Bahadur* and *Podumoni*.

Table 2: Fe distribution within plant tissues of 10 cultivars under external Fe (II) stress (3 days after exposure to Fe (II) 0, 500, 1000 and 1500 mg L^{-1} at the 4-week-old seedling stage)

	Leaf Fe (mg g^{-1})				Stem Fe (mg g^{-1})				Root Fe (mg g^{-1})				Fe-plaque (mg g^{-1})			
	0	500	1000	1500	0	500	1000	1500	0	500	1000	1500	0	500	1000	1500
Ranjit	0.05	0.23	0.48	0.53	0.05	0.34	0.54	0.87	0.06	2.60	5.12	6.38	1.79	5.93	8.07	9.2
Basundhara	0.07	0.28	0.55	0.57	0.05	0.41	0.56	0.87	0.05	2.57	5.22	6.88	1.92	5.89	7.96	9.58
Kushal	0.05	0.24	0.50	0.69	0.04	0.42	0.61	0.84	0.06	2.55	5.34	6.72	1.93	6.59	8.35	9.22
Luit	0.06	0.22	0.54	0.78	0.04	0.39	0.58	0.62	0.06	2.65	5.31	6.94	2.04	4.83	6.65	8.87
Prafulla	0.04	0.25	0.52	0.72	0.06	0.49	0.66	1.00	0.06	2.64	5.26	7.00	1.89	6.47	8.30	9.45
Moniram	0.03	0.38	0.72	0.69	0.05	0.51	0.65	1.02	0.04	2.69	5.36	7.05	2.02	5.96	8.26	8.81
Joymoti	0.05	0.25	0.50	0.65	0.07	0.25	0.42	0.61	0.07	4.16	5.28	7.19	1.86	6.52	8.10	9.37
Bahadur	0.07	0.29	0.51	0.63	0.06	0.35	0.72	0.83	0.05	1.62	5.23	6.65	2.04	7.02	8.80	10.05
Mahsuri	0.05	0.24	0.43	0.62	0.06	0.47	0.67	0.92	0.05	2.56	5.36	7.19	2.00	6.53	8.10	9.42
Podumoni	0.05	0.25	0.42	0.83	0.05	0.42	0.44	0.61	0.06	2.65	5.42	6.91	2.2	6.55	8.93	10.09
LSD(0.05)	0.16				0.18				0.65				1.89			
Sem(±)	0.05				0.06				0.23				0.68			

LSD test. The probable significance level for the mean differences between leaf bronzing score, root and shoot length at 0, 500, 1000 and 1500 mg Fe(II) L^{-1}. Sem(±)- standard error mean significant at $p = 0.05$.

The root-tissue Fe concentration was relatively low in *Bahadur*, and *Ranjit* showed the lowest root-tissue Fe concentrations. These data suggest that the genotypes vary in their root ion selectivity and retaining power.

Joymoti, *Podumoni* and *Mahsuri* Genotypes have high root membrane selectivity and retention power. In contrast, the genotypes *Bahadur* and *Ranjit* has extremely low root membrane selectivity and root retention power.

The Fe^{2+} entering the xylem stream will follow the transpiration-driven acropetal long-distance transport. However, some of this iron may be immobilized and deposited at specific "dumping sites" within the plant. However, it is impossible to quantify how much iron is excluded at the root apoplast and retained inside root tissues in this study. The analytical data of stem, leaf and root-tissue Fe concentrations (Table 2) showed significant differences ($p = 0.05$) among tested genotypes. In contrast to root-Fe concentrations of tested genotypes, lower stem and leaf tissue-Fe concentrations were recorded in *Luit*, *Podumoni*, *Ranjit* and *Joymoti*, whereas *Moniram* and *Bahadur* showed higher stem and leaf tissue-Fe concentrations.

These data showed that *Luit*, *Podumoni*, *Ranjit* and *Joymoti* retained a more significant amount of Fe in the root and transported less to the stem and leaf tissue than *Moniram* and *Bahadur*. The leaf-tissue concentrations increased as a function of increased stem-tissue concentrations in genotypes *Moniram* and *Bahadur*.

The increase in stem tissue-Fe concentration increased the leaf tissue-Fe concentration in tested genotypes in this study, indicating that the stem tissue may act as a mediator, unlike root tissues. These results suggest that the retention power of rice root plays a significant role in inclusion/avoidance mechanisms rather than stem tissue retention. The leaf damage symptoms were significantly higher ($p = 0.05$) in *Podumoni*, *Basundhara*, *Luit* and *Kushal* compared with *Ranjit* and *Bahadur* (Table 2).

Conclusion:

According to the investigation of root Fe plaque (as an indicator of rhizospheric oxidation power), root and stem-tissue Fe concentrations (retention power), leaf-tissue Fe concentration and toxicity symptom expressions (as indicators for leaf tissue tolerance) in tested genotypes as described above, the tolerance or sensitivity of genotypes to iron toxicity were differentiated as follows: *Bahadur* and *Podumoni* (Tolerant-excluder/avoidance), *Joymoti*, *Ranjit* and *Mahsuri* (Tolerant-includer/avoidance), Moniram and Bahadur (tolerant-includer/avoidance and moderately high leaf tissue tolerance), *Kushal* (sensitive) and *Podumoni* (Extremely sensitive). Therefore, region with high soil iron level (500 to 1500 mg Fe^{2+}/ kg soil) may go for growing any of Bahadur, Joymoti, Ranjit, Mahsuri and Moniram variety of rice cultivar. This would save farmers of the region from 15 to 100 per cent crops loss that happens due to iron toxic condition and could improve rice production from hitherto crop damage so far.

References

Asch, F., Becker, M and Kpongor, D. S. (2005). A quick and efficient screen for resistance to iron toxicity in lowland rice. Journal of Plant Nutrition and Soil Science 168, 1-10.

Audebert, A., Sahrawat, K. L. (2000). Mechanisms for iron toxicity tolerance in lowland rice. J. Plant Nutr. 23, 1877-1885.

B.S. Satapathy, T. Singh, K.B. Pun and S.K. Rautaray (2015). Evaluation of rice (Oryza sativa) under double transplanting in rainfed lowland rice ecosystem of Asom. Indian Journal of Agronomy 60 (2): 245-248

Becker, M., Asch, F. (2005). Iron toxicity in rice – conditions and management concepts. J. Plant Nutr. Soil Sci. 168, 558–573 https://doi.org/10.1002/jpln.200520504.

Bennett, J. (2001). Status of breeding for tolerance of abiotic stresses and prospects for using molecular techniques. TAC Secretariat, Food and Agriculture Organization-FAO, Rome.

Chérif, M., Audebert, A., Fofana, M., Zouzou, M. (2009). Evaluation of iron toxicity on lowland irrigated rice in West Africa. Tropicultura 27, 88–92.

Dobermann, A., Fairhurst, T. (2000). Rice: Nutrient disorders and nutrient management. The International Rice Research Institute, Manila, The Philippines, p. 191.

Dufey, I., Hiel, M. P., Hakizimana, P., Draye, X., Lutts, S., Kone, B., Drame, K. N., Konate, K. A., Sie, M., and Bertin, P. 2012. Multi-environment Quantitative Trait Loci mapping and consistency across environments of resistance mechanisms to ferrous iron toxicity in rice. Crop Sci. 52: 539-550

Government of India, Ministry of Agriculture and Cooperation. (1996-2012). Directorate of Rice Development: Notified Rice Varieties in India. Rice-Varieties-1996-2012.pdf (bih.nic.in).

IRRI INGER (1996). A standard evaluation system for rice. 4th ed., International Rice Research Institute, Manila, The Philippines.

Kpongor, D.S.; Vlek, P.L.G. and Becker, M. (2003). Developing a standardized procedure to screen lowland rice (*Oryza sativa*) seedlings for tolerance to iron toxicity, in University of Göttingen and ATSAF: Technological and Institutional Innovations for Sustainable Rural Development. Klartext GmbH, Göttingen, Germany, p. 103.

M. Amaranatha Reddy, Rose Mary Francies, P.S. Abida and P. Suresh Kumar (2019). Correlation among Morphological, Biochemical and Physiological Responses under Iron Toxic Conditions in Rice. International Journal of Current Microbiology and Applied Science. 8(1). https://doi.org/10.20546/ijcmas.2019.801.005

Maclean, J. L., Dawe, D. C., Hardy, B., Hettel, G. P. (2002). Rice Almanac. CABI Publishing, Wallingford, UK, p. 253.

Matthus E, Wu LB, Ueda Y, Höller S, Becker M, Frei M. (2015). Loci, genes, and mechanisms associated with tolerance to ferrous iron toxicity in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 128, 2085–2098.

Nyamangyoku, I. O. and Bertin, P. (2013). Mechanisms of resistance to ferrous iron toxicity in cutivated rices: Oryza sativa L., Oryza glaberrima Steud and Interspecific Hybrids. J. Plant Nutr. Soil Sci. 168: 764-773.

Tadano, T. (1976). Studies on the method to prevent iron toxicity in lowland rice. Mementoes of the Faculty of Agriculture, Hokkaido University 10, pp. 22-68

Wu, L., Shhadi, M. Y., Gregorio, G., Matthus, E., Becker, M., and Frei, M. 2014. Genetic and physiological analysis of tolerance to acute iron toxicity in rice. Rice 7: 8-9.

Yoshida, S.; Forno, D.A.; Cock, J.H. and Gomez, K.A. (1976). Laboratory manual for physiological studies of rice. (3rd edn.). International Rice Research Institute, Los Baños, The Philippines.

Zhang L, Li G, Wang M, Di D, Sun L, Kronzucker HJ, Shi W. 2018. Excess iron stress reduces root tip zone growth through nitric oxide-mediated repression of potassium homeostasis in Arabidopsis. *New Phytologist* 219, 259–274.

Zhang X, Zhang D, Sun W, Wang T. 2019. The adaptive mechanism of plants to iron deficiency via iron uptake, transport, and homeostasis. *International Journal of Molecular Science* 20, 2424.