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RESEARCH ARTICLE

Elucidating the Response of Rice Genotypes to Iron Toxicity through Hydroponics

A Suma[1*], RM Francies[2], VV Radhakrishnan[2], P Sureshkumar[2] and Eldho Varghese[3]
[1]*ICAR-National Bureau of Plant Genetic Resources, Regional Station, Vellanikkara, Thrissur-680656, Kerala, India*
[2]*College of Agriculture, Kerala Agricultural University, Vellanikkara, KAU P.O, Thrissur-680656, Kerala, India*
[3]*ICAR-Central Marine Fisheries Research Institute, Kochi- 682018, Kerala, India*

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Iron (Fe) toxicity is a major abiotic stress that impedes rice cultivation in many lowland environments worldwide. Although several cultural and management practices are advocated to address this problem, the best and most economic approach to combat this issue is the use of tolerant cultivars. The present study was aimed at elucidating the response of rice genotypes, including local landraces and released varieties to iron toxicity by screening seedling growth in a hydroponic solution containing varying levels of iron. The increasing concentration of iron had significant effect on all characters studied and the severity of symptoms. Significant difference was also observed for concentration × variety interaction for all the characters studied except root and shoot length of the seedlings. Increase in root number was observed at 600 ppm, indicating the repair mechanism of the plants against edaphic stress beyond the threshold level. Varsha, a mid-early, high yielding red-kernelled rice variety and Chuvannamodan, an indigenous landrace of Kerala, showed tolerance to high concentration of iron (> 600ppm), among the ten genotypes screened. It can be inferred that evaluation of genotypes at 600 to 800ppm concentration of iron in a hydroponic solution is a quick and efficient methodology to delineate the tolerance of rice genotypes to iron toxicity.

Key Words: Abiotic stress, Hydroponics, Iron toxicity, *Oryza sativa*, Rice

Introduction

Rice (*Oryza sativa* L.) cultivation is facing a multitude of problems including abiotic as well as biotic stresses. In recent times, abiotic stresses affecting the crop growth are on the rise owing to the changing climatic conditions, the consequences on productivity being either direct or indirect. Abiotic stresses include drought, waterlogging/ flood, salinity, nutrient deficiency/toxicity etc.. Iron (Fe) toxicity is a major stress to rice cultivation in many lowland environments worldwide (Asch *et al*., 2005). It often occurs in rice grown in submerged paddy fields with low pH, leading to dramatic increase in ferrous ion concentration, disrupting cell homeostasis and impairing growth and yield (Aung and Masuda, 2020). The typical symptoms are generally manifested as tiny brown spots starting from the tips and spreading towards the base of the lower leaves (Doberman and Fairhurst, 2000). Subsequently, the whole leaf turns yellowish or orange to brown. Growth and tillering are greatly affected and the root system is coarse, scanty and dark brown. The yield is reportedly reduced by 12-100%, depending on the severity of toxicity and the tolerance of the rice cultivars (Benckiser *et al*., 1982; Audebert and Sahrawat, 2000).

Many cultural practices are adopted to ameliorate iron toxic soil conditions. It includes trenching around the fields, application of dolomite, lime and chalk, and recurrent draining-off of accumulated irrigation water after submersion etc.. Rice plants have developed physiological avoidance and/or tolerance mechanisms to survive under Fe-toxic conditions (Nozoe *et al*., 2008). Molecular studies have shown that there are four defence mechanisms adopted by the rice plants to mitigate the iron toxicity. They include either iron exclusion by suppressing the genes involved in Fe uptake and translocation, or by retaining the excess Fe in the root system itself rather than by translocation to shoots or by compartmentalisation of Fe in the shoot by regulating the vascular Fe transport or by detoxifying reactive oxygen species produced in the plant system in response to Fe toxicity (Aung and Masuda, 2020). It has been reported that an iron tolerant variety absorbs

*Author for Correspondence: Email- Suma.A@icar.gov.in

less Fe or transports less from roots to leaves, indicating the presence of physiological avoidance mechanisms (Tadano, 1975; Audebert and Sahrawat, 2000). However, this problem can be best addressed by identifying and developing tolerant cultivars. Wide inter-varietal variability of iron toxicity tolerance in rice have been reported by Gunawardena *et al*. (1982) and Mohanty and Panda (1991). Thus, exploitation of this variability in iron toxicity reaction among genotypes while combating the stress is realised to be the sustainable and cheap alternative. Heritability of this trait has been detailed by Abifarin (1985) and Wu *et al*. (1997). The circumstances thus warrant, adoption of tolerant genotypes adapted to the specific growing environment, to avoid yield losses. Three approaches were in vogue to screen rice varieties for iron toxicity tolerance viz., field trials, pot trials and hydroponics trials. Shimuzu *et al*. (2005) advocated the use of mass screening using culture solution as the most effective method to identify iron toxicity tolerant cultivars as this allows stress conditions to be efficient, easily controlled and reproducible.

In India, iron toxicity is reported especially in Kerala, Orissa, West Bengal and Andaman Islands (Ponnamperuma, 1978; Sahrawat and Singh, 1998 and Mandal *et al*., 2004). Kerala has agro-ecological zones with wide variability in rice genetic resources and kernelled bold rice varieties over white kernelled ones by the local population (Suma *et al*., 2018). Hence, breeding for rice varieties tolerant to stress conditions like Fe toxicity require resistant genes from native landraces. There are reports that most of the modern semi-dwarf, high-yielding rice cultivars were sensitive to iron toxicity (Wade *et al.*, 1999). Studies by Benckiser *et al*. (1984) and Onaga *et al*. (2013) demonstrated that some traditional cultivars have better tolerance to iron toxicity. Therefore, the present study was aimed at elucidating the response of rice genotypes to iron toxicity under varying levels of iron stress in order to deduce the threshold toxicity level of iron that can help differentiate between tolerant and susceptible genotypes. Further, the attempt could resize the number of accessions to be screened for tolerance in elaborate field experiments to a more manageable level.

Materials and Methods

Ten rice genotypes procured from the germplasm collections maintained at the College of Agriculture, Kerala Agricultural University, Thrissur, Kerala, India constituted the experimental material. Thekkencheera and Chuvannamodan were the landraces and Ptb 40 (Matta Triveni), Ptb 49 (Kairali), Ptb 56 (Varsha), Ptb 60 (Vaisakh), Mo 16 (Uma), Mahsuri, and CR 1009 (Ponmani), were the high yielding varieties. Jarwa, a variety from Andaman & Nicobar Islands was also included as a check to elucidate the response. The experiment was laid out in factorial design with the treatments arranged in a completely randomized fashion with two replications, the 10 genotypes and 5 levels of iron. Varying levels of Fe concentrations (0, 200, 400, 600 and 800 ppm) were imposed. Six days old germinated seeds were sown in holes of a polystyrene plate covered at the bottom with nylon net (Fig. 1). The polystyrene plate was floated on a plastic tray filled with normal-strength Yoshida's solution (Yoshida *et al*., 1976), 10 l in each tray, including 1.78 mM silicon (Si) at pH 5.0. The seedlings were exposed to varying iron concentrations by the addition of different concentrations of ferrous Fe with 0.09 mM Fe-EDTA, following the procedure recommended by Shimuzu *et al*. (2005).

The culture solution was renewed weekly, and adjusted to pH 5.0 twice a day with 1N NaOH/HCl. The seedlings were grown until 15 days (3-4 leaf stage), and Fe toxicity responses were scored by subjective visual assessment of bronzing symptoms on developed leaves by following symptom scoring system adopted by Shimuzu *et al*. (2005), modified as shown in Table 1. Subsequently, the plants were harvested for measuring growth attributes like root length (cm), shoot length (cm), number of roots, iron reversibly adsorbed on root zone (%), root dry weight (g) and shoot dry weight (g). Shoot and root length were measured from the base of the culm to the longest leaf and from culm base to longest root, respectively. The collected plant roots

Table 1. Bronzing score classified into nine ranks according to inspection of leaf blades

Score	Leaf order			
	1st	2nd	3rd	4th
1	N	N	N	N
2	T	N	N	N
3	T	T	N	N
4	P	T	N	N
5	P	T	T	N
6	P	P	T	N
7	D	P	P/T	T/N
8	D	D	P/T	T
9	D	D	D/P	No Leaf/GS

N: normal; T: discoloration of leaf tip; P: partly discoloured; D: rolled or dead leaf; GS: Growth stunted

Fig. 1. Seedlings grown in polystyrene plates under different Fe concentrations

were washed thoroughly with deionized water without dislodging the iron plaques on the root surface. The roots were then immersed in 25 ml 0.01M calcium chloride solution for 5 min to release the adsorbed iron. Calcium chloride solution containing iron was treated with concentrated hydrochloric acid to dissolve the ferric iron and 5 ml of this solution was made up to 50 ml and the Fe content was estimated using Atomic Absorption Spectrophotometer (Model: Analyst-400 Perkin-Elmer). The iron adsorbed on the roots was correlated with the performance of different varieties under varying concentrations of iron. Further, root and shoot samples were wrapped in Aluminium foil separately and oven dried at 80 ^{o}C for 48 h for measuring shoot and root dry weight separately. Statistical analysis was done using SAS (9.3 version).

Results and Discussion

Analysis of variance indicated that the increasing Fe concentration had significant effect on all characters studied (Table 2). Significant difference was also observed for concentration × variety interaction for all the characters except root and shoot length. Grouping of treatments based on the significant difference of mean values of the characters studied (Table 3) indicated that, irrespective of the genotypes there was significant reduction in root and shoot length beyond 400 ppm. It was earlier reported that excess iron can lead to reduction in shoot length, which can be a useful characteristic for screening of tolerant genotypes (Bresolin *et al*., 2019). Ferrous toxicity inhibits cell division and elongation of the primary roots and subsequently the growth of lateral roots (Li *et al*., 2016). However, there was an increase in root number at 400 ppm, indicating the triggering of inherent defence mechanism in plants against a stress beyond a threshold level. Shoot length difference was not significant beyond 600 and 800 ppm concentrations, indicating that 600 ppm is the threshold level above which the effect of toxicity was more pronounced. Most of the varieties studied exhibited an increase in growth parameters up to 200 ppm revealing the beneficial effect of iron availability and uptake by the plants up to this concentration. This is consistent with the earlier reports that, adequate Fe concentration in the plant tissue is

Table.2. Analysis of Variance for effect of varying Fe concentrations on growth attributes of ten selected rice varieties

		Mean square values					
	Df	Root length (cm)	Shoot length (cm)	No. of roots	Fe adsorbed on roots (mgL^{-1})	Root dry weight (g)	Shoot dry weight (g)
Concentration	4	11.715**	102.356**	10.465**	39.199**	.00128**	.00195**
Variety	9	8.286**	65.467**	5.690**	2.113**	.00229**	.00144**
Concentration × variety interaction	36	0.454	3.014	1.4816**	0.833**	.000017**	.000095**

**significantly different at 1% level

Table 3. Grouping of treatments based on the significant difference of mean values of the characters studied

Treatment	Root length (cm)		Shoot length (cm)		No.of roots		Fe adsorbed on roots (mgL^{-1})		Root dry weight (g)		Shoot dry weight (g)	
	Mean	Letter group	Mean	Letter group	Mean	Letter group	Mean	Letter group	Mean	Letter group	Mean	Letter group
Control	6.069	AB	18.35	A	5.7	B	0.57	E	0.0805	C	0.0795	A
200ppm	6.579	A	18.32	A	5.55	BC	1.51	D	0.09	B	0.067	B
400ppm	6.196	A	17.60	A	6.60	A	2.76	C	0.103	A	0.0635	B
600ppm	5.439	B	14.59	B	5.15	C	3.593	B	0.091	B	0.0575	C
800ppm	4.626	C	13.53	B	4.65	D	3.868	A	0.09	B	0.054	C

in the range of 70-300 mg kg^{-1} (Wells *et al*., 1993). Iron deficiency or toxicity occurs at concentrations below or above this sufficiency range (Fageria *et al*., 1981). Changes in shoot length, root length and nutrient accumulation in tissues during early developmental stages have been reported to constitute an objective form of evaluation that can be used in conjunction with bronzing scores (Bresolin *et al*., 2019).

The severity of symptoms increased linearly with the increase in Fe concentration (Fig. 2). However, the varieties expressed varying degrees of tolerance to different Fe concentrations, providing scope for breeding for iron toxicity tolerance. Bronzing score did not increase in variety Kairali from 200 to 600 ppm concentration. Variety Varsha expressed the lowest bronzing score (4.00) at 800 ppm. Maximum scoring was exhibited by the variety Mahsuri.

Varietal Response to Fe Toxicity

Root and shoot elongation was affected significantly beyond 400 ppm, except in Kairali and Vaishak, which exhibited more root elongation at 200 ppm (Table 4a). All the growth attributes were severely affected at 800 ppm. Chuvannamodan, an upland landrace showed increased root length and Varsha, showed an increase in shoot length at 600 ppm than the immediate lower concentration (400 ppm). Kuraev (1966) reported that the initial toxic effect of high iron inhibits root development, and this was more pronounced at higher iron concentrations (200 ppm), which may have been due to possible toxicity mechanisms such as the iron-induced production of superoxide (O_2^-). However, Kairali, Jarwa, Vaishak,

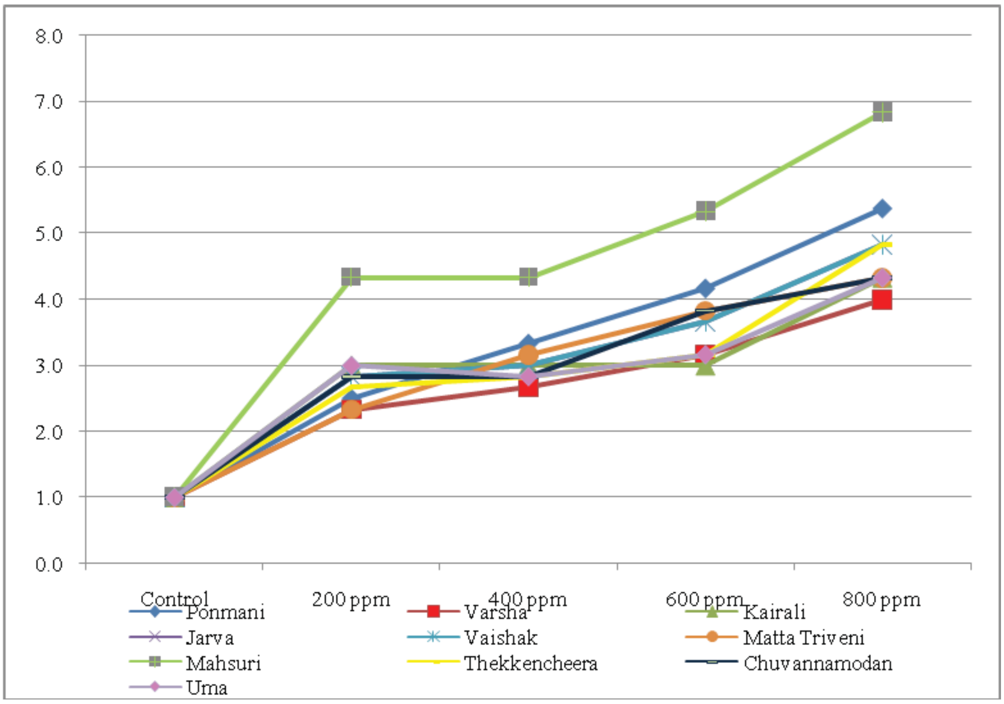

Fig. 2. Bronzing score for ten rice cultivars at varying Fe concentrations

Table 4a. Effect of different iron concentration on root length, shoot length and number of roots in 10 varieties of rice

Varieties	Root Length (cm)					Shoot Length (cm)					Number of roots				
	Control	200 ppm	400 ppm	600 ppm	800 ppm	Control	200 ppm	400 ppm	600 ppm	800 ppm	Control	200 ppm	400 ppm	600 ppm	800 ppm
Ponmani	4.33	5.07	4.67	4.45	3.75	15.83	14.95	15.67	12.33	11.91	4.50	7.00	5.50	5.00	5.00
Varsha	6.66	6.80	6.00	5.50	4.91	22.08	21.83	24.16	19.03	17.25	6.50	5.50	6.00	5.50	5.50
Kairali	6.42	6.38	6.66	5.33	5.00	19.17	18.41	17.10	14.08	13.98	6.50	5.50	7.50	7.00	5.00
Jarwa	4.58	6.18	5.97	5.17	4.60	16.58	19.52	20.00	15.91	16.42	4.00	4.00	7.00	4.00	4.00
Vaishak	5.50	5.75	5.93	4.25	3.50	16.92	15.20	13.93	12.17	11.66	4.50	4.50	6.50	5.00	3.00
MT	7.55	7.45	6.93	5.83	4.92	19.90	19.10	18.45	15.58	14.25	7.50	6.50	7.00	4.00	4.00
Mahsuri	6.28	7.15	5.90	5.67	5.33	16.68	16.06	13.93	11.73	10.83	6.00	4.50	4.50	3.50	3.50
Thekkencheera	5.25	5.78	5.16	4.37	3.67	18.27	22.65	18.35	15.35	14.17	6.00	6.00	7.50	6.50	5.50
Chuvannamodan	7.57	7.85	7.83	8.62	5.80	22.62	21.20	21.20	16.93	13.85	5.50	4.50	7.50	5.00	5.00
Uma	6.57	7.40	6.93	5.23	4.80	15.52	14.33	13.22	12.78	10.95	6.00	7.50	7.00	6.00	6.00

Table 4b.Effect of different iron concentration on iron adsorbed on roots , dryweight of roots and dry weight of shoots in 10 varieties of rice

Varieties	Iron adsorbed on roots (mg L^{-1})					Dry weight of roots (g)					Dry weight of shoots (g)				
	Control	200 ppm	400 ppm	600 ppm	800 ppm	Control	200 ppm	400 ppm	600 ppm	800 ppm	Control	200 ppm	400 ppm	600 ppm	800 ppm
Ponmani	0.979	1.699	2.089	3.185	3.397	0.07	0.09	0.09	0.09	0.09	0.09	0.08	0.07	0.07	0.05
Varsha	0.906	1.942	2.144	3.508	3.626	0.10	0.10	0.12	0.10	0.10	0.11	0.08	0.09	0.07	0.07
Kairali	0.140	1.469	4.098	2.392	2.573	0.09	0.09	0.12	0.11	0.11	0.09	0.08	0.06	0.06	0.06
Jarwa	0.816	1.697	2.753	3.181	3.370	0.08	0.08	0.09	0.09	0.08	0.07	0.07	0.06	0.05	0.05
Vaishak	0.718	1.219	1.661	2.843	3.222	0.08	0.11	0.12	0.09	0.09	0.07	0.06	0.06	0.06	0.06
MT	0.206	1.499	4.062	4.296	4.537	0.09	0.10	0.12	0.11	0.11	0.10	0.09	0.08	0.06	0.06
Mahsuri	0.470	1.498	3.216	4.074	4.372	0.07	0.06	0.06	0.06	0.06	0.06	0.03	0.06	0.04	0.03
Thekkencheera	0.881	1.395	3.268	5.817	5.968	0.08	0.08	0.10	0.09	0.09	0.08	0.07	0.05	0.06	0.06
Chuvannamodan	0.515	1.718	1.986	3.334	3.755	0.08	0.10	0.14	0.10	0.10	0.09	0.09	0.08	0.07	0.07
Uma	0.059	0.930	2.289	3.306	3.859	0.11	0.12	0.11	0.11	0.11	0.08	0.06	0.07	0.06	0.06

Thekkencheera and Chuvannamodan exhibited increased root number at 400ppm, a mechanism to overcome the toxicity. These results corroborated with the results of Reddy *et al*. (2019) that higher values of number of fresh roots, iron adsorbed on root surface and shoot weight are mechanisms observed in rice plants to overcome iron toxicity. Onyango *et al.* (2019) also reported that the number of new lateral roots, under both moderate and severe stress levels, increased in stressed plants of all the varieties compared with control plants. Root architectural traits like formation of an aerenchyma and a large number of lateral fine roots, facilitate the diffusion of oxygen into the rhizosphere, thereby increasing the redox potential above the threshold for Fe oxidation (Wu *et al.,* 2014). Fe exclusion, a root-based tolerant mechanism through inhibition of root-Fe uptake, is achieved by forming Fe plaques on the root surface due to Fe^{3+} precipitation, Fe^{3+} in turn formed by rhizospheric oxidation of Fe^{2+} (Becker and Asch, 2005). In the present study also, the excluded Fe estimated as Fe reversibly adsorbed on roots have increased significantly above 400 ppm. Matta Triveni (MT) showed higher magnitude of Fe exclusion at 400 ppm (4.06 mg/g) and beyond. It is reported that iron uptake and transport related genes such as *OsIRT1*, *OsIRT2*, *OsYSL2*, *OsYSL15*, and *OsNRAMP1* are highly suppressed in roots under Fe toxic conditions (Quinet *et al*., 2012; Finatto *et al*., 2015; Aung *et al.*, 2018). The growth of the plants was affected severely as indicated by the reduction in root and shoot dry weight. The trend of reduction in shoot dry weight with increasing Fe toxicity also supports the results of Nugraha *et al*. (2016). The variety Mahsuri exhibited least tolerance though this variety was acclaimed to be an Fe toxicity tolerant variety (Nugraha *et al*., 2016). This variety recorded a decrease in root weight with slight increase in iron concentration (200 ppm) and least value for both dry root and shoot weight at 800 ppm. In a similar study using four levels of lime and three levels of fertilizer using three rice varieties at RARS, Kumarakom, Kerala, it was reported that integration of genetic tolerance and nutrition management could reduce the intensity of iron toxicity in acid sulfate soils (Thampatti *et al*. 2005).

Varsha, a high yielding variety and Chuvannamodan, an upland landrace of Kerala exhibited comparative tolerance to iron toxicity at higher concentrations (600 and 800 ppm) over all other varieties. This is evident from the high values for length of shoot and root and dry weight of root and shoot. High amount of adsorbed iron on root surface (5.96 mg/L) in variety Thekkencheera at higher Fe concentration reveals that this variety has a higher capability of iron exclusion mechanism to combat iron toxicity stress. This may be due to increased adaptability of the variety indigenous to the problematic zone. However, this requires further confirmation. In addition, significant tolerance level was expressed by the varieties Uma, Mattatriveni and Kairali.

Conclusion

The major problem 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, to elucidate comparable stress levels to all materials. Screening genotypes in hydroponics can help negate this problem. Evaluation of genotypes at 600 to 800ppm concentration of iron would help identifying the difference in varietal reaction to iron at toxic levels. The varieties differed in their response to varying concentrations of Fe. Varsha and Chuvannamodan showed tolerance to high concentration of iron (> 600ppm) among the ten varieties studied. In a previous study conducted by Suma *et al*. (2018), the high yielding variety Varsha had registered high value for elongation ratio and volume expansion ratios of grains on cooking in comparison to other landraces and varieties of rice. The iron absorbed by the plant parts *viz*., roots and older leaves and molecular studiesare needed to understand the mechanism of defence adopted by individual varieties to combat iron toxicity stress. A thorough evaluation of germplasm field testing in hotspot areas after an initial screening through hydroponics will help elucidate the genotypes with high tolerance to this abiotic stress.

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