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SCREENING OF IRON TOXICITY IN RICE GENOTYPES ON THE BASIS OF MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL ANALYSIS

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ABSTRACT

Fifty one varieties of upland and lowland rice were tested for their tolerance to different levels of iron (0, 50 mM, 100 mM and 200 mM) in nutrient solution at pH 6.8. Seeds were grown in the nutrient culture with different concentrations of iron under controlled environmental conditions. LC_{50} value was calculated after exposure to iron. Different physiological parameters such as germination percentage, root & shoot growth, shoot and root tolerant index and leaf bronzing symptoms were taken into consideration to screen the tolerant, medium tolerant and susceptible genotypes. Further, the selected high tolerant and susceptible varieties were taken further experiment at lower concentration of iron (0, 10, 20 and 40 mM) to study the physiological and biochemical analysis. Total chlorophyll, proline, total phenol, total protein and total carbohydrate content showed variation in both tolerant and susceptible ones. The oxidative enzymes also showed variation among the tolerant and non-tolerant genotypes. The tolerant, medium tolerant and susceptible to iron were classified on the basis of relative root and shoot growth and biochemical analysis. Based on observations, it is concludes that out of 51 varieties, 16 varieties are tolerant (> 200mM Fe), 11 varieties are medium tolerant (<200 mM Fe) and 24 varieties are susceptible (<100 mM) to selected iron concentration. This study can be employed for quick screening of rice varieties for Fe tolerance for breeding programme.

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1 Introduction

Rice is a widely grown crop in the world. Iron toxicity is one of the most important abiotic stress limiting rice production in lowland systems (Dobermann & Fair-Hurst,2000). It has been reported a major constraint of lowland acidic soils, swamps, coastal swamps and irrigated lands of Asia and Africa. About 128 million hectares of the irrigated and rainfed lands of the world are under rice cultivation. Due to nutrient deficiency or toxicity, rice production has been reduced in about 100 million hectares of these lands (Becker & Asch, 2005; Asch et al., 2005). In India, total 11.7 million hectare of land is affected by iron toxicity. Iron toxicity interfere a range of nutrient disorders and deficiencies particularly potassium, phosphorus, calcium, magnesium and zinc in plant metabolism (Ottow et al., 1983; Mehraban et al., 2008). These elements were reported to play major roles in manifesting toxicity symptoms in paddy. More absorption and translocation of iron in the rice plants led to the toxicity, which has been a major limiting factor in wetland rice with regard to yield performance. Iron toxicity of wetland rice is associated with a high concentration of ferrous iron in soil condition (Ponnamperuma et al., 1955). The stress occurs in reduced soils when a toxic amount of ferrous iron is mobilized in soil *in situ* or when inflow brings in soluble iron from upper slopes (van Breemen & Moormann, 1978).

The symptoms of iron toxicity vary with rice genotypes on the basis of growth and leave-bronzing characteristics. Typically, iron toxicity symptoms are manifested as tiny brown spots starting from the tips and spreading toward the base of lower leaves. With progress of iron toxicity, the brown spot are on the inter-veins of the leaves. With increasing iron toxicity stress, the entire affected leaves look publish brown, followed by drying of the leaves. The roots are also affected by iron toxicity and it became short, coarse and blunted and dark brown in color. Other nutrients may play an important role not only in reducing the effect of iron toxicity but also in the expression of iron tolerance by different rice varieties. (Sahrawat,1979; Sahrawat et al., 1996; Sahrawat, 2000). Deficiencies of P, K, Ca, Mg and Mn decrease the iron-excluding power of rice roots and affect the rice tolerance of iron toxicity (Yoshida, 1981; Sahrawat, 2005; Pooladvand et al., 2012; Wu et al., 2014). The objective of this study was to evaluate the morphological, physiological and biochemical responses as well as the level of tolerance of upland and lowland rice genotypes at the early stage to iron toxicity.

2 Materials and Methods

2.1 Plant material and culture condition

Nutrient culture experiments were conducted under controlled climate growth chamber of the Department of Agricultural Biotechnology, Orissa University of Agriculture & Technology (OUAT), Bhubaneswar. The growth chamber was adjusted to temperature (25 ± 2^0C) with 3000 lux light intensity with 16h photoperiod. Seeds of 51 varieties were collected from rice research station maintained by Department of Plant Breeding & Genetics, College of Agriculture, OUAT, and Bhubaneswar. The varieties are enlisted in Table 1. The seeds were treated with fungicide (Bavistin, w/v) for 20 minutes and washed with autoclaved distilled water for 20 minutes. Further, the seeds were cultured on Hoagland nutrient medium (Hoagland & Arnon , 1950) with and without different concentrations of irons (0, 50 mM, 100 mM and 200 mM). In all the treatments iron was given in the form of Ferrous Sulfate. The pH of the nutrient solution was adjusted to 6.8 by the use of 0.1M HCl or 0.1 M KOH and the solution has changed in 2-day intervals to facilitate aeration of the roots, maintain the desired level of nutrients and the pH. The polysterol pots were kept in the growth chamber to study the seed germination, root and shoot growth. Percentage of germination was calculated after 4 days of culture.

% of germination = Total number of germinated seeds/ total number of seeds inoculated X 100.

The rate of shoot and root elongation in each genotype was determined by subtracting the length of the root / shoot recorded on the day of germination from that noted on the 10^{th} day. Tolerant index (TI) for the tested plants was calculated by using the formula:

Tolerance Index (TI) = Mean root or shoot elongation in solution with Fe/ mean root or shoot elongation in solution without Fe X 100.

2.2 Leaf scoring

The iron toxicity responses were scored by subjective visual assessment of symptoms on fully expanded leaves (bronzing symptoms) for the entire plant and expressed as percentage leaf area affected after 21 days of exposure to iron treatment. As scoring system, the standard evaluation system for scoring for leaf blast (*Pyricularia oryzae*) lesions provided by the International Network for the Genetic Evaluation of Rice (International Rice Research Institute, 1996) was adopted for iron toxicity; the scale measuring toxicity is as follows.

Score was determined according to the percentage leaf area affected

1 0– No symptoms,
2 1.0 - 9.0% = 1;
3 10 – 29% = 3;
4 30 – 49 % = 5;
5 50 -69% = 7;
6 70 -89% = 9;
7 90 -100% = 10 (Dead leaf).

Table 1 Screening of 51 rice varieties at different concentrations of Iron with regard to 50 % germination (LC_{50}).

S. No.	Cultivars	Av. Percentage seed Germination (%)				LC 50 Concentration after 10 days*	Remark (On the basis of germination)
		Control (Without iron)	50 mM iron	100mM iron	200mM iron		
1	Ghanteswari	100	100	80	50	>200mM	Tolerant
2	Subhadra	100	100	70	30	< 200mM	Moderate tolerant
3	Mandakini	100	50	0	0	50mM	Susceptible
4	Mrunalini	100	83	38	0	< 100mM	Susceptible
5	Mahanadi	100	100	100	60	>200mM	Tolerant
6	Lalitagiri	90	70	0	0	<100mM	Susceptible
7	Kharvela	100	80	53	0	100mM	Susceptible
8	Hema	100	85	40	0	100mM	Susceptible
9	Meher	100	50	30	0	50mM	Susceptible
10	Jagabandhu	100	90	90	0	<100mM	Susceptible
11	Surendra	100	93	80	80	>200mM	Tolerant
12	Sidhanta	100	90	60	20	<100mM	Moderate tolerant
13	Kanchan	100	100	60	0	<100mM	Susceptible
14	Pratap	100	100	80	30	<200mM	Moderate tolerant
15	Pratikshya	100	100	70	40	<200mM	Moderate tolerant
16	Jogesh	100	80	50	30	<100	Moderate tolerant
17	Bhanza	100	100	100	60	200mM	Tolerant
18	Urbashi	100	100	80	0	<100	Susceptible
19	Lalat	90	90	90	70	>200	Tolerant
20	Daya	100	100	90	50	>200mM	Tolerant
21	Keshan	100	100	80	60	>200mM	Tolerant
22	Gajapati	100	100	20	0	<100mM	Susceptible
23	Rajeswari	100	100	90	66	>200mM	Tolerant
24	Rambha	100	90	90	0	<200mM	Susceptible
25	Bhoi	100	100	80	0	<100mM	Susceptible
26	Samanta	100	81	54	0	<100mM	Susceptible
27	Indravati	100	90	50	0	<100 mM	Susceptible
28	Manaswini	100	100	66	0	100 mM	Susceptible
29	Konark	100	90	60	30	<200mM	Moderate tolerant
30	Sarathi	100	100	100	20	<200mM	Moderate tolerant
31	Parijata	100	100	50	30	<100mM	Moderate tolerant
32	Badani	100	100	80	0	<200mM	Susceptible
33	Sebati	100	80	50	0	100mM	Susceptible
34	Nilagiri	100	90	83	0	<200mM	Susceptible
35	Tejaswini	100	100	90	58	>200mM	Tolerant
36	Jajati	100	84	58	0	100mM	Susceptible
37	Sankar	100	90	81	63	>200mM	Tolerant
38	Jagannath	100	100	66	0	<200mM	Susceptible
39	Bhuban	100	92	83	83	>200mM	Tolerant
40	Birupa	100	100	87	0	<200mM	Susceptible
41	Uphar	100	100	90	60	>200mM	Tolerant
42	Mahalaxmi	100	85	84	18	<200mM	Moderate Tolerant
43	Ramachandi	91	84	83	0	<200mM	Susceptible
44	Prachi	100	90	90	0	<200mM	Susceptible
45	Khandagiri	100	92	85	69	>200mM	Tolerant
46	Udayagiri	100	91	91	80	>200mM	Tolerant
47	Manika	100	90	90	54	>200mM	Tolerant
48	Rudra	100	100	76	76	>200mM	Tolerant
49	Pathara	100	93	86	23	<200mM	Moderate tolerant
50	Suphala	100	80	60	40	<200mM	Moderate tolerant
51	Gouri	100	100	90	0	<200mM	Susceptible

*100 seeds/treatment; each treatment has 3 replicates

Table 2 Tolerance index of shoot and root after 28days of germination in 16 tolerant rice genotypes grown at 200 mM iron level.

Tolerance Variety	Iron Concentration (200 mM)	
	Shoot Tolerance Index (%)	Root Tolerance Index (%)
Bhuban	108.22 ± 7.5	110.34± 8.2
Surendra	116.34 ± 4.3	120.45± 5.2
Udayagiri	110.41± 8.6	115.42± 3.6
Rudra	115.12± 10.3	121.22±8.7
Lalat	118.3± 8.5	116.56± 7.3
Khandagiri	122.67±6.3	118.23±5.8
Rajeswari	98.21± 5.4	111.12± 6.3
Sankar	100.23± 6.8	108.23± 7.3
Uphar	97.11± 5.5	103.62±6.4
Keshan	96.12±7.2	110.54±5.8
Bhanza	98.23±2.8	107.23±8.6
Mahanadi	97.34±4.2	106.43±3.6
Tejaswini	96.44±4.3	105.11±4.6
Manika	98.11±3.5	102.23±4.8
Ghanteswari	87.12±7.2	98.23±6.8
Daya	84.23±2.3	97.34±4.2

Values are mean of 50 samples; value followed by ± represents Standard Error

2.3 Plant growth and toxicity level determination

Further, due to high toxicity and death of the shoot occurs in high concentration, the experiment was modified with low concentration of iron solution (0, 10, 20, & 40 mM) to study the physiological and biochemical trend of the tolerant, medium tolerant and susceptible (non-tolerant) genotypes. For this study, seeds were cultured hydroponically with nutrient solution in different concentrations of iron (0, 10, 20 and 40 mM). The observation was taken in 0, 5, 10, 21 and 28 days interval with regard to root & shoot length, shoot/root ratio and morphological toxicity up to 28 days.

2.4 Biochemical analysis

2.4.1 Chlorophyll Estimation

500 mg fresh leaves sample were collected from plant grown in control nutrient solution without and with 10, 20 and 40 mM iron for estimation of chlorophyll. The tissues were homogenized with 80% acetone in the dark. The amount of chlorophyll was estimated according to method described by Vernon (1960). Pigment content was expressed as mg/g fresh weight of sample.

2.4.2 Estimation of total phenol content

Estimation of total phenolic content in leaf samples collected from control as well as treated plants were evaluated by Folin-Ciocalteu method (Singleton & Rossi, 1965). To prepare a standard curve, 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml of the gallic acid stock solution was transferred to 100 ml flasks and then added equal volume of sodium carbonate solution in each flask and volume was adjusted to 20 ml with distilled water. The data was taken after 1 hr at 765 nm by UV Spectrophotometer against Folin-Ciocalteu reagent blank. The calibration curve of absorbance vs concentration was plotted. 1.0 ml of leave extracts was transferred in 25 ml flask; similar procedure was adopted as described above in preparation of calibration curve. With the help of calibration curve, the phenolic concentration of extracts was determined. The standard curve was prepared the total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass).

2.4.3 Estimation of Proline

Proline was quantified according to the method described by Bates et al. (1973). Fresh leaf samples (500 mg) were collected from control as well as treated plant and homogenized with 3% sulphosalicylic acid using a clean dry mortar pestle and sterile sand. The homogenate was filtered through Whattman no. 2 filter paper. From the filtrate, 2 ml was taken and added in 2 ml of freshly prepared ninhydrin reagent (1.25 g ninhydrin + 30 ml glacial acetic acid to make up the volume to 6 ml and the mixture was incubated at 100 ^{0}C for 1 hour. The reaction was stopped by putting in ice, then 4 ml of toluene was added and mixture was shaken vigorously for 15 to 20 second. The aqueous toluene layer was separated and warmed to room temperature and the absorbance of red color aliquot was measured at 520 nm against blank prepared along with sample using 3% sulphosalicylic acid. The proline content was expressed on fresh weigh basis (micromoles of proline per gm of tissue).

Table 3 Shoot & Root growth rate of tolerant varieties of rice grown on nutrient medium supplemented with and without and (40 mM) iron

Name of the Variety	Average Shoot/ Root length (cm)	Control				40 mM Fe			
		5 day	10 day	21 day	28 day	5 day	10 day	21 day	28 day
Bhuban	SL	3.2±0.7	21±0.8	21.5±0.9	21.6±0.3	3.1±0.9	10.2±0.9	10.4±2	10.5±0.9
	RL	2.1±0.6	3.5±0.7	3.5±2	3.5±0.4	2±0.7	2.5±0.7	2.5±0.9	2.5±0.7
	Shoot : Root	1.6	6.00	6.14	6.14	1.6	4.0	4.0	4.0
Surendra	SL	2.5±0.9	11.5±0.3	12.5±0.5	18.2±0.9	2.5±0.5	5.5±0.9	5.5±2	5.5±0.8
	RL	4.5 ±0.7	5.5±0.5	5.5±0.3	5.5±0.1	4±0.9	4.5±0.7	4.5±1	4.5±0.5
	Shoot : Root	0.62	2.09	2.27	3.27	0.62	1.22	1.22	1.22
Udayagiri	SL	3.5±0.9	11±0.7	18.5±0.7	21±0.5	3.5±0.7	5±0.8	5±0.7	5±0.7
	RL	3.0±0.1	5.5±0.8	5.5±0.6	5.5±0.9	3±0.9	5.5±0.9	5.5±0.5	5.5±0.5
	Shoot : Root	1.16	2.00	3.36	3.81	1.16	1.1	0.9	0.9
Rudra	SL	1.5±0.8	16.5±0.3	22±0.7	23±1.4	1.5±0.9	4.5±0.2	10±0.3	10±0.9
	RL	3.5±0.5	5.5±0.1	5.5±0.4	5.5±0.8	3.5±0.5	5±0.9	5±0.9	5±0.5
	Shoot : Root	0.42	3.00	4	4.18	0.42	0.9	2.0	2 .0
Lalat	SL	4.5±0.1	15.5±0.8	19±0.5	20±0.3	4.5±0.1	7.5±0.9	7.5±0.7	7.5±0.8
	RL	4±0.3	5±0.7	5±0.3	5±0.2	4±0.3	5±0.5	5±0.1	5±0.9
	Shoot : Root	1.12	3.10	3.8	4	1.12	1.5	1.5	1.5
Khandagiri	SL	3±1.5	9.5±0.4	21±0.5	21.2±1.8	3±0.8	4.5±0.2	5±0.9	5±3
	RL	4.1±0.8	4.5±0.2	4.5±0.9	4.5±0.3	4±0.9	5±0.3	5±0.3	5±3.5
	Shoot : Root	0.75	2.11	4.66	4.71	0.75	0.9	1	1
Rajeswari	SL	4±0.7	10.5±0.5	14±0.3	16±0.5	3.5±0.4	6±0.9	6.5±0.2	6.5±0.2
	RL	5.5±0.1	5.5±0.8	5.5±0.9	5.5±0.7	4.5±0.6	4.5±0.8	4.5±0.3	4.5±0.3
	Shoot : Root	0.72	1.90	2.54	3.55	0.77	1.33	1.44	1.44
Sankar	SL	3.5±2	2.5±0.9	26.5±0.5	27.5±0.4	3.5±0.3	8±0.8	11±0.1	11±0.1
	RL	3±1	4±0.7	4.5±0.3	4.5±0.5	3±0.3	3.5±0.3	4.5±0.7	4.5±0.3
	Shoot : Root	1.16	6.00	5.88	6.11	1.16	2.28	2.44	2.44
Uphar	SL	3.5±0.7	14±0.9	20±1.5	21±0.5	3.5±0.1	8.5±0.9	12.5±1	12.5±0.9
	RL	4±0.5	5.5±0.1	5.5±1.3	5.5±0.4	4±0.9	5.5±1	5.5±0.8	5.5±0.7
	Shoot : Root	0.87	2.54	3.63	3.81	0.87	1.54	2.27	2.27
Keshan	SL	4±0.1	11±0.3	16±0.9	16.5±1	4±0.5	6.5±0.9	6.5±0.4	6.5±0.4
	RL	5.5±0.9	5.5±0.4	5.5±1	5.5±1	5.5±0.7	5.5±0.8	5.5±0.3	5.5±0.3
	Shoot : Root	0.72	2.00	2.9	3	0.72	1.18	1.18	1.18
Bhanza	SL	3.5±0.8	17.5±1	24±2.1	26±0.9	3.5±0.8	9±1	12±0.3	12±0.3
	RL	4.5±0.9	5±0.9	5±1	5±0.7	4.5±0.1	5±3.5	5±0.1	5±0.1
	Shoot : Root	0.77	3.50	4.8	5.2	0.77	1.8	2.4	2.4
Mahanadi	SL	3±o.1	13.5±2.7	15±1	16.5±0.9	3±0.1	4.5±0.5	6±0.3	6±0.2
	RL	4±0.9	5±1	5±1.5	5±0.2	4±0.7	4±0.3	4±0.9	4±0.8

	Shoot : Root	0.75	2.70	3	3.3	0.75	1.12	1.5	1.5
Tejaswini	SL	3.5±0.8	8±0.9	17±0.1	19±0.7	3.5±0.9	4±2	5.5±0.9	5.5±0.3
	RL	4±0.4	5.5±0.5	5.5±0.4	5.5±0.4	4±0.1	4±1.5	5.5±0.3	5.5±0.4
	Shoot : Root	0.87	1.45	3.09	3.45	0.87	1	1	1
Manika	SL	2.5±1.5	7±0.9	17±0.8	19±0.9	2.5±0.7	5±1	5.5±0.5	5.5±0.3
	RL	4.5±0.3	5±0.8	5±0.6	5.5±0.5	4.5±0.3	5±0.9	5±0.3	5±0.2
	Shoot : Root	0.55	1.40	3.4	3.45	0.55	1	1.1	1.1
Ghanteswari	SL	4±0.5	15±0.7	20±2.3	22±0.1	4±0.09	5±0.3	5±0.7	5±0.4
	RL	4.5±0.7	4.5±0.3	4.5±0.8	4.5±0.5	4.5±0.08	4.5±0.8	4.5±0.3	4.5±0.3
	Shoot : Root	0.88	3.33	4.44	4.88	0.88	1.11	1.11	1.11
Daya	SL	4±0.8	12.5±0.3	14.5±0.1	16.5±0.9	4±0.9	7.5±0.9	8.5±0.5	8.5±0.5
	RL	5±0.7	5±0.9	5±0.8	5.3±0.8	5±0.7	5±0.8	5±0.1	5±0.1
	Shoot : Root	0.8	2.50	2.9	3.11	0.8	1.5	1.7	1.7

50 plants /treatment; Data collected from 10 plants; the experiments were repeated twice; value given after ± represent Standard Error.

Table 4 Shoot & Root growth rate of medium tolerant varieties of rice grown on nutrient medium supplemented with and without (40 mM) iron

Name of the Variety	Average Shoot/ Root length (cm)	Control				40mM Fe			
		5 day	**10 day**	**21 day**	**28day**	**5 day**	**10 day**	**21 day**	**28day**
Suphala	SL	2±0.5	8.5±1	15.5±0.9	17±0.5	2±0.1	5.5±0.9	5.8±0.7	5.8±0.7
	RL	4.5±0.3	5±0.7	5.5±0.2	5.5±0.2	4.5±0.2	4.5±1	4.5±0.7	4.5±0.7
	Shoot : Root	0.44	1.70	2.81	3.09	0.44	1.22	1.28	1.28
Pratikshya	SL	3±0.8	16.5±1	22±0.9	23.5±0.7	3±0.9	6±0.7	6.5±0.9	6.5±0.3
	RL	4.5±1	5±0.9	5.5±0.7	5.5±0.8	4.5±0.1	4.5±0.3	4.5±1	4.5±0.9
	Shoot : Root	0.66	3.30	4	4.27	0.77	1.33	1.44	1.44
Subhadra	SL	4±0.03	15.5±0.3	20.2±0.23	22±0.9	4±0.1	5±0.9	6±0.25	6±0.5
	RL	3.5±0.1	4.5±0.7	4.8±0.14	4.8±0.3	3.5±0.5	4.5±0.6	4.5±0.23	4.5±0.2
	Shoot : Root	1.14	3.44	4.2	4.58	1.14	1.11	1.33	1.33
Parijata	SL	3±2	10±0.9	17±0.7	18±0.9	3±0.9	4.5±0.3	9±0.9	9±0.8
	RL	4.5±0.2	5±0.5	5.5±0.9	5.5±0.3	4.5±0.9	4.5±0.5	4.5±0.8	4.5±0.3
	Shoot : Root	0.66	2.00	3.09	3.27	0.66	1	2	2
Konark	SL	3.5±0.6	11.5±0.8	20±0.3	22±0.8	3.5±0.7	6±0.7	8.5±0.3	8.5±0.3
	RL	5±0.8	5.5±0.5	5.5±0.8	5.5±0.8	5±0.6	5±0.1	5±0.7	5±0.7
	Shoot : Root	0.7	2.09	3.63	4	0.7	1.2	1.7	1.7
Pratap	SL	3±0.9	13±0.3	19±0.9	20±0.5	3±0.9	6.5±0.8	7±1	0
	RL	3.5±o.7	5±0.25	5±0.2	5.5±0.3	3.5±0.8	4.5±0.7	4.5±0.7	0
	Shoot : Root	0.85	2.60	3.8	3.63	0.85	1.44	1.55	0
jogesh	SL	3±0.9	17.5±0.3	23±0.2	24±0.4	3±0.5	6.5±0.8	7.5±0.7	7.5±0.3
	RL	4±0.l	4.5±0.7	4.5±0.4	4.5±0.6	4±0.6	3.5±0.7	3.5±0.5	3.5±0.4
	Shoot : Root	1.33	3.88	5.11	5.33	1.33	1.85	2.14	2.14
Pathra	SL	2.5±0.8	8±0.9	19±0.7	20±0.1	2.5±0.9	8±0.7	8±0.1	8±0.1
	RL	4±0.7	4.5±0.1	5±0.3	5±0.4	4±0.7	4.5±0.5	4.5±0.3	4.5±0.4
	Shoot : Root	0.62	1.77	3.8	4	0.62	1.77	1.77	1.77

Sarathi	SL	2.5±0.7	7.5±0.8	12.5±0.5	14.5±0.9	2.5±0.1	3±1.3	6.5±1	6.5±1
	RL	4±0.9	4.5±0.7	5±0.3	5±0.3	4±0.5	4±1	4.5±0.9	4.5±0.9
	Shoot : Root	0.62	1.66	2.5	2.9	0.62	0.75	1.44	1.44
Sidhanta	SL	4.5±0.1	19.5±0.7	27±0.3	28.5±0.9	4.5±0.3	9±0.5	9±0.5	0
	RL	4±0.8	5.5±0.8	5.5±0.2	5.7±0.3	4±0.2	5±0.4	5±0.4	0
	Shoot : Root	1.12	3.54	4.9	5	1.12	1.8	1.8	0
Mahalaxmi	SL	4.5±0.9	16±0.3	18.5±0.2	21±0.3	4.5±0.9	8±0.5	8±0.4	8±0.4
	RL	4.5±0.1	5±0.9	5.5±0.1	5.5±0.9	4.5±0.4	5±0.6	5±0.6	5±0.5
	Shoot : Root	1	3.20	3.36	3.81	1	1.6	1.6	1.6

50 plants /treatment; Data collected from 10 plants; the experiments were repeated twice; value given after ± represent Standard Error

Table 5 Shoot & Root growth rate of susceptible varieties of rice grown on nutrient medium supplemented with and without (40 mM) iron. (50 plants /treatment)
Data collected from 10 plants; repeated twice)

Name of the variety	Average Shoot/ Root length (cm)	Control				40mM Fe			
		5 day	**10 day**	**21 day**	**28 day**	**5 day**	**10 day**	**21 day**	**28 day**
Mandakini	SL	2±0.8	9.5±0.7	15±0.6	16±0.5	2±0.9	4.5±0.1	7±0.3	0
	RL	4.5±0.7	4.5±0.8	5±0.3	5.5±0.4	4.5±0.6	4.5±0.9	4.5±0.2	0
	Shoot : Root	0.44	2.11	3	2.55	0.44	1	1.55	0
Mrunalini	SL	1.5±0.9	14±0.3	17.5±0.9	18±0.9	1.5±0.8	4±0.7	6±0.5	0
	RL	3±0.8	4±0.8	4.5±0.6	4.5±0.5	3±0.7	3±0.6	3±0.4	0
	Shoot : Root	0.5	3.50	3.88	4	0.5	1.33	2	0
lalitagiri	SL	3±0.8	17±0.2	22±1	22±0.9	3±0.9	6.5±1	6.5±0.9	0
	RL	4.5±0.9	5±0.1	5.5±1.2	5.7±0.8	4.5±0.5	4±0.9	4±0.5	0
	Shoot : Root	0.66	3.40	4	3.85	0.66	1.62	1.62	0
Kharvela	SL	3.5±2.1	15±0.5	15.5±0.3	19.5±0.3	3.5±0.5	6±0.9	6±0.3	6±0.3
	RL	4.5±1.5	5.5±0.1	5.5±0.1	5.5±0.7	4.5±1	4±0.8	4±0.3	4±0.5
	Shoot : Root	0.77	2.72	2.81	3.54	0.77	1.5	1.5	1.5
Hema	SL	3±0.8	11±1	16±1	19.5±3.1	3±0.9	8±0.7	9±0.9	0
	RL	3.5±0.7	4±0.3	4.5±0.2	4.5±0.3	3.5±0.5	3.5±0.5	4±0.5	0
	Shoot : Root	0.85	2.75	3.55	4.33	0.85	2.28	2.25	0
Meher	SL	3.5±1.4	14.5±0.9	14.5±0.9	16.5±0.3	3.5±0.8	10±0.8	10±0.5	0
	RL	4±1	5±0.3	5±0.5	5±0.8	4±0.9	3.5±0.5	3.5±0.9	0
	Shoot : Root	0.87	2.90	2.9	3.3	0.87	2.85	2.85	0
Jagabandhu	SL	3±0.9	15±0.1	19±0.7	21±0.1	3±0.3	7.5±0.1	7.5±0.1	0
	RL	4±0.1	5.5±0.3	5.5±0.8	5.5±0.3	4±0.4	3.5±0.9	3.5±0.9	0
	Shoot : Root	0.75	2.72	3.45	3.2	0.75	2.14	2.14	0
Kanchan	SL	2±0.3	15.5±0.7	19.5±0.8	20±0.7	2±0.4	7±0.9	7±0.2	0
	RL	3.5±0.5	5±0.9	5±0.7	5±0.3	3.5±0.6	4.5±0.7	4.5±0.7	0
	Shoot : Root	0.57	3.10	3.9	4	0.57	1.55	1.55	0
Urbashi	SL	2.5±0.23	11±0.7	23±0.7	23±0.6	2.5±0.7	4.5±0.6	4.5±0.2	0
	RL	4±0.6	4.5±0.3	5.5±0.3	5.5±0.4	4±0.1	5±0.3	5±0.4	0
	Shoot : Root	0.62	2.44	4.18	4.18	0.62	1	1	0
Gajapati	SL	3.5±0.7	14±0.1	14.5±0,7	19±0.9	3.5±0.8	6.5±1	7.5±0.9	0
	RL	4.5±0.9	5±0.5	5±0.6	5.5±1.2	4.5±0.4	4.5±0.7	4.5±1	0
	Shoot : Root	0.77	2.80	2.9	3.45	0.77	1.44	1.66	0
Rambha	SL	4±0.7	11±0.5	20±0.5	20±0.1	4±1	6±0.7	10.5±0.5	0

	RL	3.5±0.8	4.5±0.3	5±0.3	5.5±0.4	3.5±1.5	4.5±0.3	4.5±0.3	0
	Shoot : Root	1.14	2.44	4	3.63	1.14	1.33	2.33	0
Bhoi	SL	3.5±0.9	14.5±2.5	15.5±0.7	16.5±1	3.5±0.2	7.5±0.3	7.5±0.3	0
	RL	5.5±1	4.5±1.5	5.5±0.9	5.5±0.1	5.5±0.3	5±0.8	5±0.3	0
	Shoot : Root	0.63	3.22	2.81	2.81	0.63	1.87	1.87	0
Samanta	SL	2.5±0.1	12±0.5	12±0.3	14.5±0.8	5.5±0.3	5.5±0.2	5.5±0.3	0
	RL	4±0.8	4.5±0.6	4.5±0.2	4.5±0.9	4±0.4	4±0.2	4±0.5	0
	Shoot : Root	0.62	2.66	2.66	3.22	1.37	1.37	1.37	0
Indravati	SL	3±1.7	10±0.9	14.5±0.7	16.5±0.8	3±1	5.5±0.4	5.5±0.8	0
	RL	3.5±0.7	4.5±0.9	5.5±0.3	5.5±0.9	3.5±0.3	4±0.3	4.5±0.7	0
	Shoot : Root	0.85	2.22	2.63	3	0.85	1.37	1.22	0
Manaswini	SL	2.5±0.5	14.5±0.4	16.5±0.3	17±0.7	2.5±0.4	3.5±0.9	5±0.7	0
	RL	3±0.1	5.5±0.3	5.5±0.4	5.5±0.4	3±0.8	3.5±1	3.5±0.8	0
	Shoot : Root	0.87	2.63	3	3.09	0.87	1	1.42	0
Badani	SL	4.5±0.3	10±0.6	17.5±0.7	19±0.5	4.5±0.2	6.5±0.8	9±0.4	0
	RL	4±0.6	5.5±0.8	5.5±0.9	5.5±0.3	4±0.5	5.5±0.3	5.5±0.5	0
	Shoot : Root	1.12	1.81	3.18	3.45	1.12	1.18	1.63	0
Sebati	SL	3±0.2	10±0.7	15.5±0.1	15.5±0.8	3±0.1	3.5±0.3	6.5±0.9	0
	RL	5±0.3	5.5±0.1	5.5±0.3	5.5±0.3	4.5±0.3	4.5±0.4	4.5±1	0
	Shoot : Root	0.6	1.53	2.81	2.81	0.6	0.77	1.44	0
Nilagiri	SL	4.5±0.9	12±0.7	19±0.9	21±0.8	4.5±1	5.5±1	9±1.5	0
	RL	3±0.5	4.5±0.5	4.5±0.7	4.5±0.7	3±0.7	4.5±0.2	5±0.3	0
	Shoot : Root	1.5	2.66	4.22	4.66	1.5	1.22	1.8	0
Jajati	SL	2.5±0.9	8.5±1	17±0.9	17.5±0.1	2.5±0.3	3±0.3	8±0.1	0
	RL	4±0.4	5±0.9	5±0.5	5.5±0.3	4±0.4	4.5±0.9	4.5±0.9	0
	Shoot : Root	0.62	1.70	3.4	3.18	0.62	0.66	1.77	0
Jagannath	SL	3±0.5	17±0.9	18.5±1	19.5±0.7	3±1.2	8±0.2	8±0.1	0
	RL	3.5±0.7	4.5±1.2	4.5±1.2	5±0.9	3.5±0.8	4.5±0.9	4.5±0.2	0
	Shoot : Root	0.85	3.77	4.11	3.9	0.85	1.77	1.77	0
Birupa	SL	3±0.3	21.5±0.9	21.5±0.9	22±0.4	3±0.3	11±0.7	11±0.3	0
	RL	3.5±0.2	5.5±0.8	5.5±0.8	5.5±0.7	3.5±0.4	4.5±0.9	4.5±0.5	0
	Shoot : Root	0.85	3.90	3.9	4	0.85	2.44	2.44	0
Ramachandi	SL	3.5±0.6	17.5±0.9	20.5±0.5	22±0.9	3.5±0.1	9.5±0.5	10±0.1	0
	RL	4.5±0.3	5.5±0.8	5.5±0.7	5.7±0.1	4.5±0.3	5±0.3	5±0.4	0
	Shoot : Root	0.77	3.18	3.72	3.85	0.77	1.9	2	0
Prachi	SL	3±0.3	14.5±0.8	18.5±0.3	20±0.7	3±0.4	6.5±0.3	8.5±0.8	0
	RL	4±0.9	5.5±0.1	5.5±0.2	5.5±0.4	4±0.9	5±0.1	5±0.5	0
	Shoot : Root	0.75	2.63	3.36	3.63	0.75	1.3	1.7	0
Gouri	SL	3±0.9	11±0.3	13.5±0.5	16.5±0.3	3±0.8	7.5±0.1	7.5±0.3	0
	RL	4±0.8	5.5±0.8	5.5±0.8	5.5±0.9	4±0.1	5±0.4	5±0.4	0
	Shoot : Root	0.75	2.00	2.45	3	0.75	1.5	1.5	0

50 plants /treatment; Data collected from 10 plants; the experiments were repeated twice; value given after ± represent Standard Error

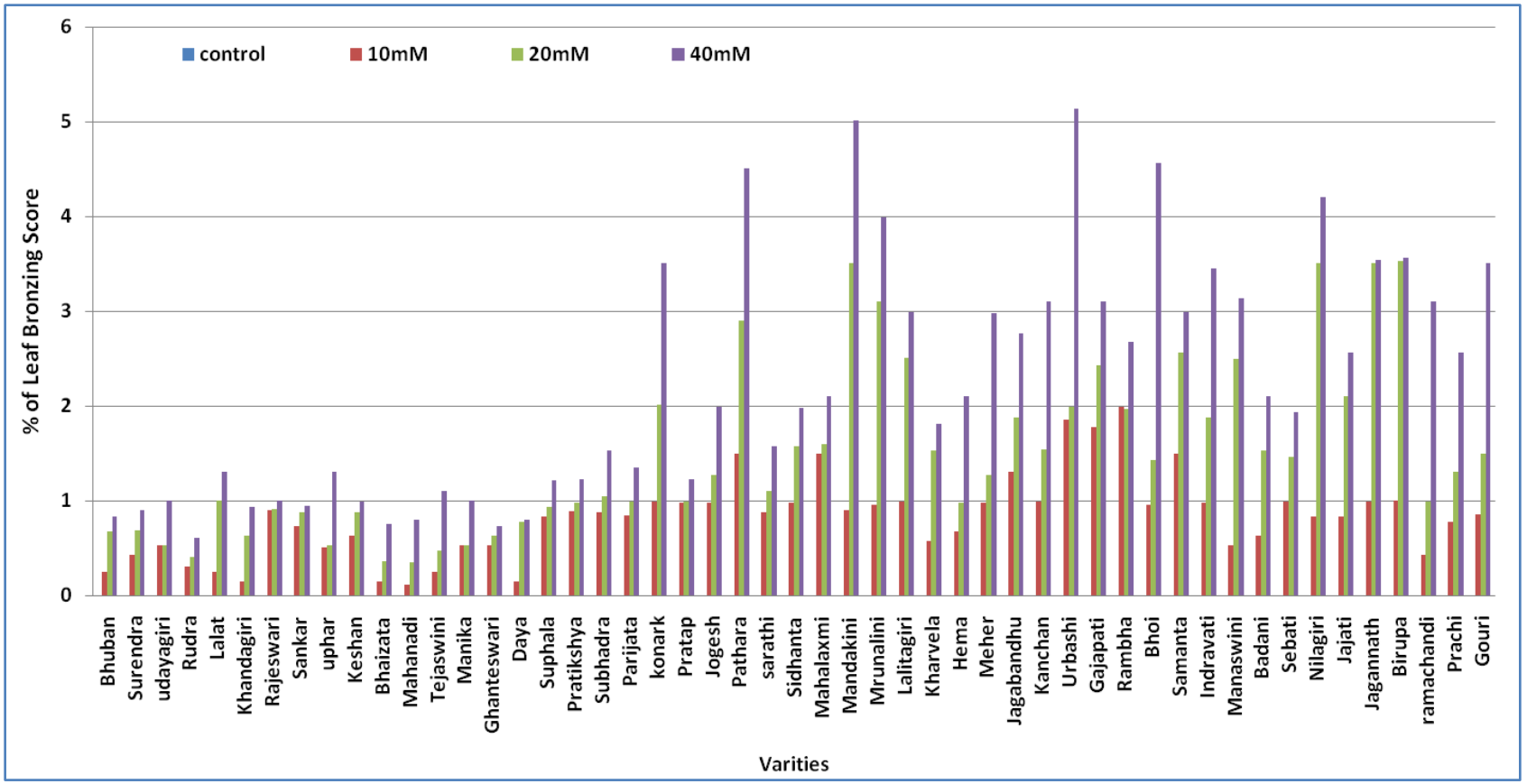

Figure 1 Leaf Bronzing score of different varieties of rice after 5 days of growth in different concentration of Iron.

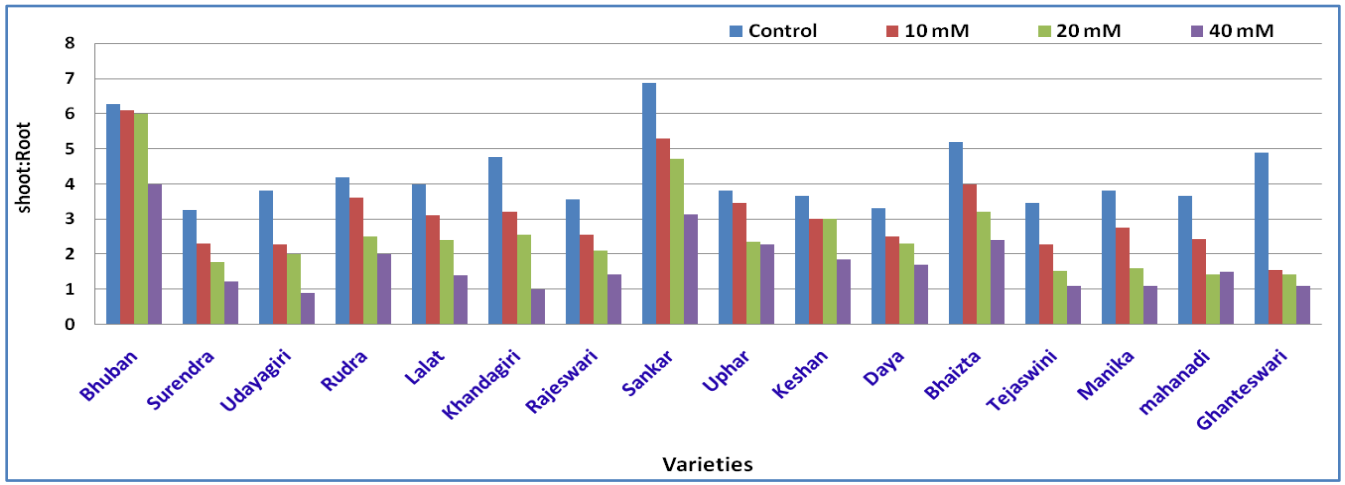

Figure 2 Effect of Different concentrations of Feon shoot: root ration of tolerant rice varieties after 28 days of growth.

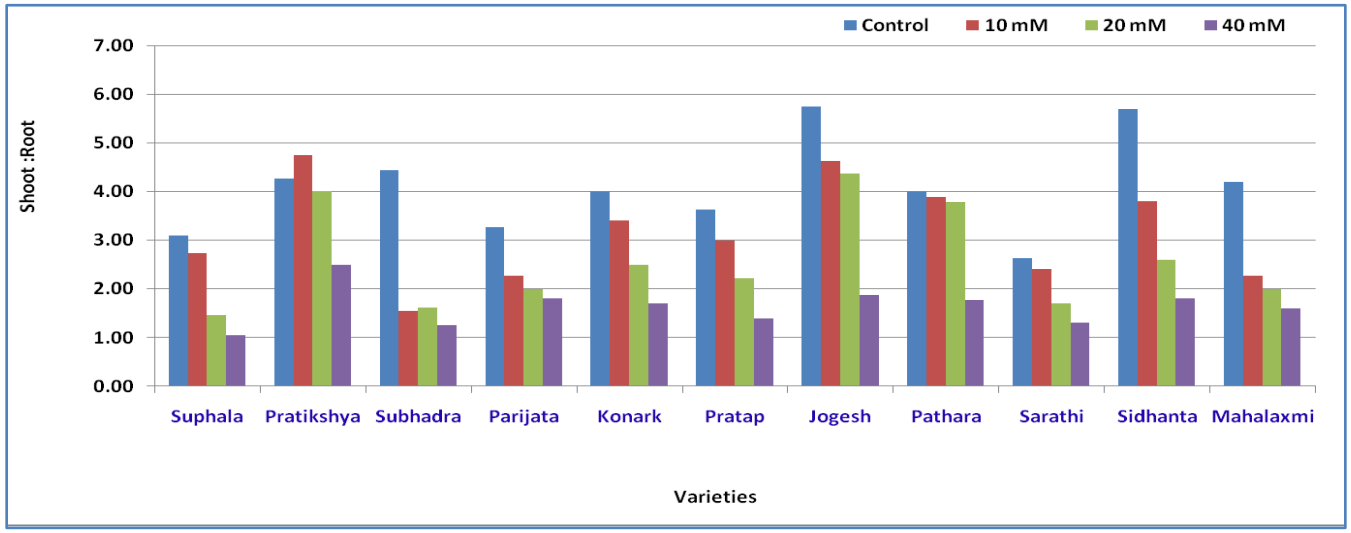

Figure 3 Effect of different concentration of Feon shoot: root ratio of medium tolerant varieties of rice after 28 days growth.

Table 6 Chlorophyll content (mg/g fresh weight basis) in both tolerant (T) and non- tolerant (NT) varieties of rice.

Varieties	Fe Concentration	Chl a	Chl b	Total Chlorophyll (a+b)	Carotenoid
Surendra (T)	Control	240.5 ± 3.3	120.8 ± 2.1	361.3 ±2.7	143.0±1.1
	10mM	785.8 ± 6.5	344.6 ± 1.5	1130.4±4.0	283.9±1.5
	20mM	790.6 ± 8.6	432.6 ± 1.3	1223.2±4.9	345.9±1.6
	40mM	693.1 ± 6.8	263.8 ±1.4	956.9 ± 4.1	260.6 ±1.7
Lalat (T)	Control	226.7± 7.2	271.9 ±2.5	498.6± 4.8	104.0 ±1.8
	10mM	673.8 ± 7.7	365.0 ±2.6	1038.8±5.1	257.0 ±1.3
	20mM	752.9 ± 7.4	288.2 ± 1.1	1041.1±4.2	288.9 ±1.9
	40mM	479.1 ± 8.4	469.5 ± 3.2	948.6±5.8	235.9±1.7
Khandagiri	Control	141.30 ±4.3	146.5 ± 2.6	287.8±3.4	85.2±1.3
	10mM	676.5 ± 5.3	205.5 ± 2.3	882.0±3.8	247.3±1.4
(T)	20mM	649.4 ± 3.9	352.3 ±2.4	1001.7±3.1	252.3±1.5
	40mM	617.9 ± 3.6	279.6 ±2.8	897.5±3.2	244.5±1.7
Lalitgiri (NT)	Control	180.4 ± 2.5	282.6 ±2.5	463.0±2.5	26.9 ±1.9
	10mM	515.6 ± 3.8	488.9 ±3.4	1004.5±3.6	200.8±1.3
	20mM	722.7 ± 4.9	178.0 ±1.4	900.7 ±3.1	223.6±1.4
	40mM	573.3 ± 2.6	188.1 ±1.3	761.4±1.9	224.5±1.7
Urbashi (NT)	Control	416.4 ± 3.9	151.4 ±2.7	567.8 ±3.3	45.8±1.7
	10mM	491.0 ± 2.7	198.2 ±1.5	689.2 ±2.1	174.2±1.3
	20mM	657.3 ± 4.7	266.6 ± 1.6	923.9± 3.1	235.57±1.6
	40mM	618.0 ± 2.9	191.6 ± 1.8	809.6±2.3	220.75±1.5
Jagannath	Control	130.0 ± 1.4	289.8 ± 2.3	419.8±1.8	62.43±1.9
	10mM	389.5 ± 3.9	335.0 ± 1.6	724.5±2.7	145.13±1.9
(NT)	20mM	713.6 ± 5.8	237.6 ± 2.7	951.2±4.2	164.89±1.8
	40mM	547.1 ±4.1	245.9 ± 2.5	793.0±3.3	175.69±1.6

*Three replicates/treatment; repeated twice.

2.4.4 Total protein content

500 mg fresh leaf samples were collected from control as well as treated plant for total nitrogen estimation by micro kjeldahl method. Soluble nitrogen was determined by this method after precipitating the protein in the extract of the fresh material with trichloroacetic acid (Anonymous, 1970).

2.4.5 Total Carbohydrate content

Fresh leaf samples (500 mg) were collected from control as well as treated plant for carbohydrate estimation. 500 mg of leaf sample were taken in a test tube with 5 ml of 2.5 N HCl and kept in a boiling water bath for three hours. Subsequently, it neutralize with solid sodium carbonate until the effervescence ceases.

Table 7 Total carbohydrate content (mg/g fresh weight basis) after 28 days of growth in tolerant (T) and non- tolerant (NT) varieties of rice grown at different concentrations of iron.

Varieties	Different Concentration of Fe			
	Control	10mM	20mM	40mM
Khandagiri (T)	162.52 ± 14.4^{a}	120.6± 13.3^{a}	164.52 ± 7.2^{a}	225.49 ± 12.5^{a}
Surendra (T)	376.94 ± 11.7^{b}	392.15± 12.7^{b}	485.45 ± 8.3^{b}	495.05 ± 13.1^{b}
Lalat (T)	567.57 ± 13.4^{d}	682.26± 13.2^{c}	900.96 ± 10.3^{c}	841.70 ± 12.5^{c}
Lalitgiri (NT)	500.16 ± 12.18^{c}	872.91± 12.7^{d}	971.43 ± 12.1^{d}	1022.2±1 5.5^{d}
Urbashi (NT)	528.05± 15.71^{c}	866.97± 11.8^{d}	1097.89 ± 13.2^{e}	1368.26 ±13.3^{e}
Jagannath(NT)	512.57± 14.78^{c}	945.78± 21.1^{e}	983.24 ± 12.4^{d}	986.00 ± 13.7^{d}

Five replicates/treatment; each treatments were repeated twice; Data were analyzed as the mean values followed by the same letter are not significantly different at the 5% significance level with Duncan's multiple range test.

Table 8 Total phenolic content (µg/g fresh weight basis) after 28 days of growth in tolerant (T) and non-tolerant (NT) varieties of rice grown at different concentrations of iron

Varieties	Different Concentration of iron			
	Control	10mM	20mM	40mM
Lalat (T)	777.93 ±8.37^{c}	1934.57 ±22.27^{e}	2922.42±14.46^{c}	3531.6±28.40^{a}
Khandagiri (T)	1436.63 ±8.48^{e}	2860.63 ±14.67^{f}	11057.9±22.50^{e}	12167.38±35.87^{f}
Surendra (T)	612.43 ±16.97^{a}	1129.94 ±8.44^{b}	3494.92±33.67^{d}	9924.90±28.96^{e}
Lalitgiri (NT)	842.73 ±8.48^{d}	1474.16 ±8.44^{d}	1631.56±8.44^{b}	1043.67± 24.58^{b}
Urbashi (NT)	700.63 ±16.98^{b}	1202.92 ± 9.57^{c}	1650.48±17.17^{b}	1175.93± 29.2^{d}
Jagannath (NT)	756.78 ±13.87^{c}	1098.98 ±7.52^{a}	1545.58±13.46^{a}	1109.87±28.98^{c}

Five replicates/treatment; each treatments were repeated twice; Data were analyzed as the mean values followed by the same letter are not significantly different at the 5% significance level with Duncan's multiple range test.

Table 9 Total proline content (µg/g fresh weight basis) after 28 days of growth in tolerant (T) and non-tolerant (NT) varieties of rice grown at different concentrations of iron.

Varieties	Different concentration of iron			
	Control	10mM	20mM	40mM
Lalat (T)	3.98±0.92^{c}	7.95±1.7^{d}	8.89±1.91^{c}	17.67±1.8^{c}
Sudendra (T)	5.18±1.25^{d}	6.62±1.19^{c}	8.97±1.05^{c}	18.24±1.5^{d}
Khandagiri (T)	5.48± 1.56^{d}	8.88±0.78^{e}	9.47±1.18^{d}	18.45± 1.^{d}
Lalitgiri (NT)	3.41±0.91^{c}	4.45±0.91^{a}	4.95±1.13^{a}	7.87±1.3^{b}
Urbashi (NT)	1.82±0.72^{a}	4.87±0.82^{a}	4.76±1.36^{a}	5.05±0.8^{a}
Jagannath (NT)	2.46± 1.0^{b}	5.52±0.89^{b}	5.15±1.08^{b}	5.37±1.2^{a}

Five replicates/treatment; each treatments were repeated twice; Data were analyzed as the mean values followed by the same letter are not significantly different at the 5% significance level with Duncan's multiple range test.

Table 10 Total protein content (µg/g fresh weight basis) after 28 days of growth in tolerant (T) and non-tolerant (NT) varieties of rice grown at different concentrations of iron.

Varieties	Different concentrations of iron			
	Control	**40mM**	**80mM**	**200mM**
Lalat (T)	9.83±1.3^{c}	4.99±1.2^{d}	3.46±0.82^{b}	0.43±0.07^{b}
Surendra (T)	10.37±1.2^{d}	4.28±1.7^{d}	3.28±0.76^{b}	0.82±0.02^{c}
Khandagiri (T)	9.45±1.4^{e}	5.41±1.1^{e}	3.29±0.72^{b}	0.33±0.09^{b}
Lalitgiri (NT)	7.18±1.5^{a}	3.52±0.85^{c}	1.09±0.51^{a}	0.10±0.03^{a}
Urbashi (NT)	7.06±1.5^{a}	2.82±0.93^{b}	1.30±0.21^{a}	0.13±0.04^{a}
Jagannath (NT)	8.89±1.2^{b}	1.84±0.65^{a}	1.83±0.72^{a}	0.17±0.04^{a}

Five replicates/treatment; each treatments were repeated twice; Data were analyzed as the mean values followed by the same letter are not significantly different at the 5% significance level with Duncan's multiple range test.

Table 11 Superoxide dismutase activity (unit/mg protein) after 28 days of growth in tolerant (T) and non-tolerant (NT) varieties of rice grown on different concentrations of iron.

Varieties	Different concentration of iron SOD activity (unit/mg.protein)			
	Control	**10mM**	**20mM**	**40mM**
Llalat (T)	0.276±0.13^{c}	3.421±0.86^{e}	5.909±1.52^{e}	5.923±1.27^{e}
Sudendra (T)	0.261±0.12^{c}	3.252±0.25^{d}	4.062±1.35^{c}	5.024±1.23^{d}
Khandagiri (T)	0.232± 0.13^{b}	3.823±1.02^{f}	4.642±1.22^{d}	5.018±1.16^{d}
Lalitgiri (NT)	0.205±0.05^{b}	0.305±0.15^{a}	0.550±0.08^{a}	0.619±0.83^{b}
Urbashi (NT)	0.312±0.09^{d}	0.570±0.22^{b}	0.585±0.26^{b}	0.691±0.08^{c}
Jagannath (NT)	0.167±0.08^{a}	0.698±0.17^{c}	0.546±0.37^{a}	0.537±0.06^{a}

Five replicates/treatment; each treatments were repeated twice; Data were analyzed as the mean values followed by the same letter are not significantly different at the 5% significance level with Duncan's multiple range test.

Total 100 ml volume was made up and centrifuged at 8000 rpm for 15 minutes. From the supernatant 1 ml was exacted and mixed with 1 ml of distilled water and 4 ml of anthrone reagent. The prepared mixture was boiled at water bath for eight minutes and cools rapidly for taking observation at 630 nm. The carbohydrate content was expressed on mg of glucose per gram of fresh weight (Hedge & Hofreiter, 1962).

2.4.6 Enzyme extraction and assay:

2.4.6.1 Peroxidase activity:

Fresh leaf samples (500 mg) were collected from control and treated plants, homogenized with mortar and pestle in cold 0.1M phosphate buffer (pH 6.1) containing 30 mg of insoluble polyvinylpyrrolidone and 15 mg sodium ascorbate. The homogenate was filtered through four layers of muslin cloth and centrifuged at 12,000g for 10 min at 4^0 C. The supernatant was used for the peroxidase assay. The assay mixture contained 0.1M phosphate buffer (pH 6.1), 4 mM Guaiacol, 3 mM H_2O_2 and 0.4 ml of crude enzyme extract. The total reaction volume was 1.2 ml. The rate of change in absorbance at 420 nm was measured using a UV spectrophotometer. The levels of enzyme activity were expressed as µmol H_2O_2 destroyed/min/mg protein (Bergmeyer et al. 1974).

2.4.6.2 Catalase activity:

Fresh leaf samples (500 mg) were collected from control and treated plants, homogenized with mortar and pestle in 0.1M sodium phosphase buffer (pH 7.0) and centrifuged at 1000g for 10 min at 4^0 C. One milliliter of supernatant was added to the reaction mixture containing 1 ml 0.1M H_2O_2 and 3 ml 0.1M sodium phosphate buffer (pH 7.0). The reaction was stopped by adding 10 ml 2% H_2SO_4 after 1 min incubation at 20^0 C. The acidified reaction mixture with or without the supernatant was titrated against 0.01M $KMnO_4$ to determine the quantity of H_2O_2 utilized by the enzyme. The catalase activity was expressed as µmol H_2O_2 destroyed/min/mg protein (Bergmeyer et al., 1974). Soluble proteins in the supernatant were determined according to Bradford (1976) using bovine serum albumin as standard.

2.4.6.3 Superoxide dismutase activity

Fresh leaf samples (500 mg) were grinded with 10 ml chilled 50 mM potassium phosphate buffer pH 7.8 in a mortar and pestle. Sample was centrifuged at 10,000g for 10 min on 4^0C in a refrigerated centrifuge. The supernatant was collected and kept in the deep frezer for further experiment. 50 µl enzyme extract mixed with 3 ml of reaction mixture (50 mM Potassium Phosphate buffer pH 7.8 + 13 mM methionine + 2 uM riboflavin + 0.1 mM EDTA + 75 mM NBT) and make up the volume with distilled water up to 2 ml. The reaction table exposed to light with 400 W for 15 minute and take absorbance at 560 nm. The enzyme activity is expressed as units/mg of protein. The 50% inhibition of the reaction between riboflavin and NBT in the presence of methionine is taken as 1 unit of SOD activity

2.7 Statistics

In order to ascertain the significant differences of growth among various cultivars of rice, an ANOVA test was performed (Sokal & Rohlf, 1973). Regression analysis was performed to assess the response of root length of different varieties of rice to iron over the time of exposure. Effects of iron on growth variables at each level were noted with the separation of mean using the Waller-Duncan multiple range test (Harter, 1960).

3 Results and Discussion

Iron toxicity is a wide spread nutrient disorder affecting the rice cultivation in the tropical regions of Asia and Africa. It is a major stress in lowland and medium land rice varieties. The present study is to early screening of iron tolerant genotypes and its physiological and biochemical mechanism for iron improvement program. Fifty one upland and lowland rice varieties were treated with three levels (0, 50, 100 and 200 mM) of iron showed significant variations in respect of seed germination, root and shoot elongation and leaf bronzing symptoms. The results of the study showed that the seed germination was not affected at lower concentration of iron (data not shown). Whereas, at higher concentration (50 – 200 mM) germination rate were declined and various genotypes respond variously against selected iron concentration. A good degree of variation in germination (LC_{50}) and growth response was observed between 50 - 200 mM iron; therefore, this concentration was chosen to compare the performance of different genotypes. On the basis of $LC_{50,}$ 16 varieties were tolerant to iron at the level of 200 mM while eleven varieties showed medium tolerance against the 100 mM irons. The rest of 24 varieties are susceptible against the lowest level of irons viz 50 mM (Table 1).

There was a significant variation between in root tolerant index (RTI) and shoot tolerant Index (STI) in different rice varieties with respect to 200 mM of Fe concentration (Table 2). According to the Wilkins (1978) to quantify the inhibitory effect of metal ions, root growth was widely used in ecological studies. Similarly, Toyler & Foy (1985) suggested that the root tolerance index (RTI) is one of the most important markers to screen genotypes and varieties for metal tolerance. Tolerance index (TI) derived from ratios between the data of different treatments and the control solutions have been useful to characterize individual populations for metal tolerance. Observations of this study therefore provide further evidences that 16 varieties of rice were tolerant to 200 mM iron concentration having RTI ranged from 97.34 to 121.22 (Table 2). Leaf-browning scores were determining in both susceptible and tolerant genotypes grown on different concentration of iron (Figure 1).

Table 12 Effect of different iron concentrations on the catalase activity (µmol H_2O_2 $min^{-1}g^{-1}$ fresh weight) after 28 days of growth in tolerant (T) and non-tolerant (NT) varieties of rice .

Varieties	Different concentration of iron Catalase activity (µmol H_2O_2 $min^{-1}g^{-1}$ fresh weight)			
	Control	10mM	20mM	40mM
Llalat (T)	1.8 ± 0.7^{b}	1.85 ± 0.52^{d}	2.46 ± 0.78^{c}	4.54 ± 1.1^{c}
Sudendra (T)	1.6 ± 0.8^{b}	2.14 ± 0.62^{e}	2.82 ± 0.88^{d}	4.82 ± 0.9^{c}
Khandagiri (T)	2.2 ± 0.7^{c}	3.72 ± 1.02^{f}	4.55 ± 1.21^{e}	5.43 ± 1.3^{d}
Lalitgiri (NT)	1.2 ± 0.9^{a}	1.22 ± 0.84^{a}	0.98 ± 0.32^{a}	0.67 ± 0.7^{a}
Urbashi (NT)	1.5 ± 0.8^{b}	1.45 ± 0.43^{c}	1.22 ± 0.81^{b}	0.73 ± 0.5^{a}
Jagannath (NT)	1.6 ± 0.7^{b}	1.34 ± 0.66^{b}	1.16 ± 0.65^{b}	0.89 ± 0.6^{b}

Five replicates/treatment; each treatments were repeated twice; Data were analyzed as the mean values followed by the same letter are not significantly different at the 5% significance level with Duncan's multiple range test.

Some of the varieties showed some disorders such as chlorosis, dark brown speckles, leaf yellowish green and inner venial spots on the leaves due to Fe toxicity. Similar observations were made in different plant species at higher concentration of metal either in solution or in soil (Tiffin, 1972; Moore & Patrick, 1991; Rout & Das, 2002; Wu et al. (2014). High concentration of iron causes nutrient imbalance through antagonistic effects on the uptake of nutrients, including K and Zn. The presence or absence of other nutrients can also affect the plants ability to decrease uptake of iron in the shoots through physiological functions by roots such as iron oxidation, iron exclusion, and iron retention (Yoshida, 1981, Sahrawat, 2005).

Furthermore, these varieties were treated with three different concentrations of iron (0, 10, 20, 40 mM) up to 28 days in nutrient culture. The result showed that the growth rate, shoot & root ratio of tolerant and medium tolerant genotypes varied in different concentrations of iron (Figures 2 & 3). The result showed that the tolerant varieties have higher growth at 40 mM iron compare with non-tolerant one. The growth data of tolerant, medium tolerant and susceptible (non-tolerant) genotypes grown with or without iron showed significant variation (Tables. 3 -5). Growth reduction under iron toxicity has been reported in rice plants by many researchers (Becker & Asch, 2005; Dorlodot et al., 2005). Biochemical analysis was conducted between tolerant and non-tolerant genotypes to compare the biochemical activity under iron stress. The total chlorophyll and carotenoid content varied differently between tolerant and non-tolerant one (Table 6). The protein, carbohydrates, polyphenols and proline content were differing between tolerant and non-tolerant varieties (Tables. 7 -10). Leaf soluble protein and carbohydrates were decreased with increase of Fe concentration. Phenolic content also increased at high concentration of iron probably due to the production of H_2O_2 (Michalak, 2006).

Acceleration in the oxidative enzymes activities such as catalse, peroxidase and superoxide dismutase (SOD) are believed to play a metabolic role under metal stress (Van Assche & Clijsters, 1990) and therefore may have a subtle role in metal tolerance. The enzyme activity varied in both tolerant and non-tolerant varieties. The catalase activity was higher in case of tolerant than the non-tolerant varieties. The activity increased up to 50% in case of peroxidase, 65% in case of SOD and 56% in case of catalase respectively, as compared to control (Tables 11 -13). The enzyme activity increased with increasing the concentration of iron in nutrient solution. Greater activity of oxidative enzymes in tolerant varieties indicates that the tolerant plants were under stress, a feature often associated with tolerance (DeVos & Schat, 1991). The enzyme activity was indicators of heavy metal toxicity and subsequent stress situation in plants (Nashikhar & Chakrabarti, 1994).

Table e13 Efect of different concentrations of iron on peroxidase activity ((µmol Guaiacol $min^{-1}g^{-1}$ fresh weight) after 28 days of growth in tolerant (T) and non-tolerant (NT) varieties of rice.

Varieties	Different concentration of iron Peroxidase activity (µmol Guaiacol $min^{-1}g^{-1}$ fresh weight)			
	Control	10mM	20mM	40mM
Llalat (T)	80.23 ± 3.7^{c}	88.23 ± 4.2^{c}	92.45 ± 5.6^{c}	51.43 ± 1.4^{d}
Sudendra (T)	78.56 ± 5.2^{c}	92.68 ± 3.8^{c}	98.44 ± 3.9^{d}	64.82 ± 1.2^{e}
Khandagiri (T)	110.2 ± 4.5^{d}	118.4 ± 4.6^{d}	123.67 ± 5.6^{e}	75.43 ± 2.3^{f}
Lalitgiri (NT)	51.2 ± 3.2^{b}	46.53 ± 2.5^{b}	36.81 ± 2.6^{b}	30.81 ± 1.2^{c}
Urbashi (NT)	42.3 ± 2.9^{a}	32.45 ± 4.3^{a}	28.62 ± 3.1^{a}	26.42 ± 1.8^{b}
Jagannath (NT)	56.1 ± 3.8^{b}	42.34 ± 2.2^{b}	34.96 ± 2.5^{b}	20.81 ± 1.4^{a}

Five replicates/treatment; each treatments were repeated twice; Data were analyzed as the mean values followed by the same letter are not significantly different at the 5% significance level with Duncan's multiple range test.

The plants exposure to excess of iron shift the balance of free radical metabolism toward production of hydrogen peroxide due to deceased activity of reaction oxygen scavenging enzymes (Mehraban et al., 2008). The peroxidase activity increased during iron toxicity due to the production of intracellular metal binding compounds, alterations in metal compartmentation patterns, alteration of cellular metabolism and membrane structure as reported by many researchers (Verkleij & Schat, 1990; Van Assche & Clijsters, 1990; Van Gronsfeld & Clijsters, 1994; Harahap et al., 2014). Furthermore, Qureshi et al (2007) reported that the catalase activity increased with increase of iron concentration in nutrient solution due to metabolizing peroxide decomposition in peroxisome after converting glycolate through photorespiration. On the basis of the growth parameters, shoot and root ratio and growth pattern in different concentrations of iron exposure, the varieties were categorized with regard to iron tolerance as tolerant, medium tolerant and non-tolerant. Iron toxicity can be minimized by using iron tolerant varieties and by applying other nutrients whose presence is negatively affected by a high concentration of iron in soil. An integrated use of tolerant genotypes and improved soil and nutrient management is more practical for sustainable increases in rice productivity. Further experiments are necessary to know the hidden facts on the mechanisms of Fe tolerance in starch rich plant.

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