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Biofortification of iron in rice (*Oryza sativa*) grown in acid soil of Assam, India

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In rice (*Oryza sativa* L.), iron (Fe) concentration drastically reduced due to post-harvest processing. Paddy contains 38 mg/kg of Fe that is reduced to 8.8 mg/kg in brown rice after processing and finally 4.1 mg/kg in milled rice (Majumder *et al.* 2019). The recommended daily dietary intake of Fe for human is 10–15 mg. A slight increase in its nutritive value would be highly beneficial for alleviation of Fe malnutrition. There are two billion anaemic people worldwide and 50% of all the anaemic cases can be attributed to Fe deficiency (Krupa *et al.* 2017). Fe biofortification can be achieved *via* agronomic practices, conventional breeding and genetic engineering. Combined application of both NPK fertilizer and Fe fertilizer could be a potential approach to increase Fe bioavailability in rice through root development, shoot transport and re-localization, which improved the translocation of Fe into rice grain (Kok *et al.* 2018). Earlier (Borah *et al.* 2000) studies were conducted to know the effect of different levels of soil Fe content on Fe content of rice leaves. However, there is hardly any report on studies conducted in northeast region of India to know the effect of different levels of soil Fe content on Fe content of brown rice. Therefore, the present study was carried out to know the effect of soil Fe content on grain Fe content of rice.

Rice varieties Ranjit, Mahsuri and Kajoli Chakua were collected from Regional Agricultural Research Station, Titabar, Assam. A pot experiment was conducted during 2019 as winter rice under rain protected condition. Bulk surface soil sample (0–20 cm depth), classified as clay was collected from rice growing areas of of Assam Agricultural University, Jorhat, Assam. Plastic pots of 15 kg capacity (24 cm diameter and 30 cm depth) were used for growing the rice plants with recommended doses of NPK fertilizer. A 6 kg of well prepared soil was put into each of the pots. The soil of the pots of the control set contained no added iron, instead 1 litre distilled water, added 2 days before transplanting. The 1 litre solution of 50 ppm iron in the form of Ferrous sulphate ($FeSO_4.7H_2O$) was added to the soils of each of the treated pots, two days before transplanting. The soil of the pots was puddled after addition of water and Fe solution. Representative soil samples from both control and treated pots were collected at this stage for analysis of soil characteristics before plant growth. Three rice seedlings (30 days old) were transplanted in each pot. Each variety was replicated thrice. The soil was submerged up to 5 cm with distilled water from transplanting till the grain filling stage to ensure anaerobic condition. The characteristics of the soil of experimental pots in terms of *p*H, organic carbon, cation exchange capacity and Diethylene triamine pentaacetate (DTPA) extractable Fe before planting were 5.10 and 4.80, 9.57 g/kg and 11.26 g/kg, 13.13 [cmol (p^+)/kg] and 12.66 [cmol (p^+)/kg]) and 159.40±0.51 mg/kg and 182.35±0.57 mg/kg for control and treated, respectively.

The leaves were analysed at three stages; maximum tillering, grain filling and harvesting. Brown rice and respective husk were analysed at grain filling and harvesting stage. The brown rice was analysed at harvesting stage for different positions of grain within the panicle, viz. top primary rachis (TPR), top secondary rachis (TSR), middle primary rachis (MPR), middle secondary rachis (MSR), bottom primary rachis (BPR) and bottom secondary rachis (BSR). The Fe content and phytic acid content were determined according to Wong (1928) and Wheeler and Ferrel (1971), respectively. The mean data of three varieties were analysed statistically using paired t-test in MS-Excel for comparison between control and treated, at 5% significant level.

The iron content of leaves: The Fe content of the leaves (Table 1) was significantly higher in treated than in control plants, only at grain filling stage. The Fe content of leaves (*Mahsuri*) was found to be comparable with those reported by Borah *et al.* (2000). There was no Fe toxicity symptom on the leaves in the plants grown at two different soil Fe contents. This might be due to detection

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Table 1 Iron content (mg/100 g, dry weight basis) of rice leaves at 3 different growth stages as affected by differential soil iron content

Stage	Varieties	Iron (mg/100 g)	
		C	T
Maximum tillering stage	Ranjit	27.64±0.06	54.17±0.25
	Mahsuri	16.12±0.20	33.91±0.03
	Kajoli Chakua	27.29±0.36	36.01±0.04
Mean		23.68±6.55	41.36±11.14
P value		0.07 NS	
Grain filling stage	Ranjit	58.81±0.26	75.01±0.26
	Mahsuri	18.39±0.12	36.76±0.12
	Kajoli Chakua	47.05±0.03	62.26±0.07
Mean		41.42±20.79	58.01±19.47
P value		0.00*	
Harvesting stage	Ranjit	57.58±0.01	69.71±0.19
	Mahsuri	24.77±0.21	26.75±0.14
	Kajoli Chakua	35.12±0.06	39.62±0.20
Mean		39.16±16.77	45.36±22.04
P value		0.17 NS	

The data represented are the mean of 3 replications ± Standard deviation. *Significant at 5% level of probability. NS, Not significant; C, Control; T, Treated.

of sufficiently lower Fe content in the leaves than the toxic concentration (300–2000 mg/kg) reported earlier (Baruah and Bharali 2015).

The iron content of brown rice: At grain filling stage (Table 2), there were significant differences in Fe content of brown rice due to Fe fertilization, being the higher amount detected in treated than in control. The study (Table 3) also indicated variation in grain Fe content at harvesting stage, according to its position on the rachis; the highest being detected at TPR followed by TSR, MPR, MSR, BPR and BSR, respectively, which was comparable with the findings of Su *et al.* (2014). The highest Fe content of brown rice (Table 3) was observed in Ranjit followed by Kajoli Chakua and Mahsuri for all the panicle positions. The Fe content of brown rice of all the three varieties increased significantly (more than 100 % than that of control) due to added Fe content of soil. Considering maximum 80% loss of Fe due to polishing, it can be estimated that the polished form of Ranjit, Mahsuri and Kajoli Chakua may retain about 2 mg/100 g, 1.34 mg/100 g and 1.59 mg/100 g Fe (dry weight basis), respectively. Kok *et al.* (2018) reported that if 15 μg/g (15 mg/kg or 1.5 mg/100 g) Fe would be incorporated in polished rice through biofortification, the recommended target was met. Singh *et al.* (2018) also suggested that the split application of nitrogen at 160 kg/ha, in combination with soil and foliar application of Fe can be a good agricultural practice to enhance the Fe concentration in grain. Saini *et al.* (2017) reported that with increase of Fe in soil, the Fe content was consistently increased in all rice tissues, while the percent increase depended on genotypic variation.

The iron content of husk: The Fe content of rice husk at both grain filling and harvesting stages (Table 2) were found to be comparable with the findings of Meng *et al.* (2005). At both the stages, the Fe content of rice husk were also found to be higher than the same in brown rice. The higher Fe content of rice husk at harvesting stage than at grain filling stage indicated transport and accumulation of Fe till the maturity of the grain.

The phytic acid P content of brown rice: The phytic acid P content, (which can interfere with cation absorption) of brown rice (Table 2) were non-significant and found to be comparable with Su *et al.* (2014).

On average, biofortification of Fe in brown rice from 6.70±0.73 mg/100 g (Mahsuri) to 10.39±0.47 mg/100 g (Ranjit), due to single application of Fe fertilizer to soil was revealed by the present study. Position of grain in the panicle also affected accumulation of Fe, ranging from 11.89% (Kajoli Chakua) to 75.26% (Mahsuri) increase in the brown rice of TPR in comparison to BSR. The highest uptake of Fe was found in the rice variety Ranjit followed by Kajoli Chakua and Mahsuri. Therefore, application of Fe fertilization might be a good cultural practice to increase the brown rice Fe content for winter rice.

Table 2 Iron content (mg/100 g, dry weight basis) of brown rice and rice husk and phytic acid content (mg/100 g, dry weight basis) of brown rice as affected by differential soil iron content

Variety	Iron (mg/100 g) (Brown rice) (Grain filling stage)		Iron (mg/100 g) (Rice husk) (Grain filling stage)		(Harvesting stage)		Phytic acid (mg/100 g) (Brown rice) (Harvesting stage)	
	C	T	C	T	C	T	C	T
Ranjit	2.18±0.08	5.92±0.10	5.50±0.30	9.37±0.01	8.91±0.01	12.07±0.30	647.46±55.43	941.82±112.40
Mahsuri	1.17±0.04	4.18±0.03	3.11±0.02	6.96±0.10	6.95±0.18	11.17±0.32	875.85±68.41	986.90±32.20
Kajoli Chakua	1.87±0.14	5.13±0.08	3.68±0.01	8.91±0.10	7.96±0.58	11.46±0.22	844.38±73.41	997.11±43.26
Mean	1.74±5.17	5.08±8.70	4.09±1.24	8.41±1.27	7.94±0.98	11.56±0.45	789.23±123.78	957.27±29.42
P value		0.00*		0.01*		0.01*		0.07NS

The data represented are the mean of three replications ± Standard deviation. The results of iron content of rice husk at harvesting stage represent mean for the cumulative samples irrespective of the position on the panicle. *Significant at 5% level of probability. NS, Not significant; C, Control; T, Treated.

Table 3 Iron content (mg/100 g, dry weight basis) of brown rice at harvesting stage

Variety/Grain position	TPR		TSR		MPR		MSR		BPR		BSR		Average	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Ranjit	5.18 ± 0.15	11.09 ± 0.22	4.85 ± 0.08	10.62 ± 0.13	4.77 ± 0.15	10.54 ± 0.26	4.04 ± 0.08	10.26 ± 0.36	3.91 ± 0.11	10.21 ± 0.32	3.76 ± 0.11	9.67 ± 0.17	4.41 ± 0.55	10.39 ± 0.47
Mahsuri	3.26 ± 0.09	7.94 ± 0.01	3.04 ± 0.05	6.89 ± 0.07	2.71 ± 0.04	6.77 ± 0.11	2.28 ± 0.11	6.73 ± 0.06	1.97 ± 0.13	6.01 ± 0.07	1.86 ± 0.05	5.88 ± 0.06	2.52 ± 0.57	6.70 ± 0.73
Kajoli Chakua	3.86 ± 0.16	8.37 ± 0.17	3.60 ± 0.19	8.34 ± 0.12	3.53 ± 0.14	8.02 ± 0.24	3.36 ± 0.07	7.85 ± 0.14	3.25 ± 0.09	7.66 ± 0.15	3.21 ± 0.07	7.48 ± 0.01	3.46 ± 0.24	7.95 ± 0.36
Mean	4.10 ± 0.98	9.13 ± 1.70	3.83 ± 0.92	8.61 ± 1.87	3.67 ± 1.03	8.44 ± 1.92	3.23 ± 0.89	8.28 ± 1.80	3.04 ± 0.98	7.96 ± 2.11	2.94 ± 0.98	7.68 ± 1.89	3.46 ± 1.12	8.34 ± 1.87
P value		0.00*		0.01*		0.01*		0.01*		0.01*		0.01*		0.01*

The data represented are the mean of 3 replications ± Standard deviation. *Significant at 5% level of probability. C, Control; T, Treated.

SUMMARY

A pot experiment was conducted during 2019 under rain protected condition at Assam Agricultural University, Jorhat to know the effect of two different levels of soil iron (Fe) content on Fe content of rice tissues including grain (brown rice). Soils of initial DTPA extractable Fe content of 159.40±0.51 mg/kg was applied with 50 ppm Ferrous sulfate solution to increase the soil Fe content to 182.35±0.57 mg/kg. Two popular rice varieties, Ranjit and Mahsuri, and one traditional variety Kajoli Chakua were cultivated as winter rice under submerged condition in pots. There was no Fe toxicity symptom in the leaves for the plants grown at two different soil Fe content. At harvesting stage, the concentration of Fe in different tissues of rice were detected in the order: leaves>husk>grain. The Fe content of brown rice was significantly higher (>100% than that of control) in plants grown in soils of higher Fe content. Significant variation in the Fe content of brown rice was observed according to its position on the rachis, being the highest at the top and the lowest at the bottom. Considering initial soil Fe status, application of Fe fertilizer of suitable concentration to the soil was found to be successful in biofortification of Fe in brown rice, leading to increase of Fe content depending on varieties.

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