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Assessment of genotypic variability for grain zinc and iron content in traditional and improved rice genotypes using energy dispersive X-ray fluorescence spectrophotometer (ED-XRF)

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Abstract

Micronutrients are essential elements for plant and human development. The deficiency of these micronutrients hampering the crop productivity as well as deteriorating the quality of the produce. In the countries where staple foods consist of mainly cereals, the nutrient deficient foods causing human health hazard. The micronutrient content of grain can be elevated either by fortification or by agricultural strategies. The strategy involves enhance the micronutrients level through conventional plant breeding and biotechnology methods. The primary step in conventional breeding is to screen out the micronutrient-dense cultivars within natural existing germplasm. In rice natural variability exist for micronutrients (Fe, Zn, Vitamin A, etc.) content and bioavailability. Accordingly, the objective of the present study was to evaluate a panel of 192 diverse rice germplasm lines for iron and zinc content in brown and polished rice grain through energy dispersive X-ray fluorescence spectrophotometer (ED-XRF). Substantial variation was observed among screened genotypes. In brown rice iron and zinc content was ranged between 6.3 µg/g -24.5 µg/g and 15.4 µg/g -39.40 µg/g, respectively, whereas, polished rice showed iron and zinc content range from 0.1 µg/g -6.7 µg/g and 13.1 µg/g -32.6 µg/g, respectively indicating the nutritive richness of brown rice over the polished rice. The wild accessions showed the highest Fe and Zn content in grains before and after polishing. Thus, these micronutrient-rich wild species open up the possibilities for the exploitation as a donor in biofortification breeding programme and also in identification of genomic positions associated with iron and zinc contents in grains.

Keywords: germplasm, rice, variability, zinc content, iron content, correlation

Introduction

There is a growing demand for agricultural products of higher nutritional quality, in order to minimize the occurrence of nutritional deficiency. This nutritional deficiency in micronutrients such as iron (Fe) and zinc (Zn) have particularly affected, mainly in developing countries (Khush *et al*., 2012) [16]. It is estimated that more than 60% of the world population present Fe deficiency, and 30% or more present deficiency of Zn (Souza *et al*., 2013) [27] accounting for decreased work productivity, reduced mental capacity, stunting, blindness etc. (Baishya *et al*., 2015) [5]. To remedy this situation, it has been targeted the production of bio fortified foods, which is the increase in concentration of nutrients in the edible parts of plants, through breeding, in order to meet the human needs.

Rice is one of the global staple foods being cultivated for 10,000 years and provides 70-80% or more daily calorie intake for 3 billion people, which is almost half of the world's population (Ravindra Babu, 2013) [20]. The grain has large genetic variability in micronutrient concentration. Hence, rice was included in biofortification program (Graham *et al.*, 1999) [12]. The biofortification programme has been identified as an efficient means to develop as well as transfer the genetically improved high micronutrient containing rice grains to the poor people who depend on rice for both energy and nutrients. The first pre-requisite for initiating a breeding programme to develop micronutrient rich genotypes, is to screen the available germplasm and to identify the source of the genetic variation for the target trait which can be used in crosses, genetic variation, molecular marker development and to understand the basic enhancement of micronutrient. Thus, micronutrient rich lines can be selected from the existing variation in germplasm of rice.

During pre-green revolution period the poverty was the major issue, whereas, in green revolution era (1965-70) the introduction of high yielding varieties (HYV's) which are highly fertilizer responsive solved the problem of food grain insufficiency but today, most of us do get enough to eat, in terms of calories, but we still may not be getting our essential micronutrients, such as iron and zinc. In other words, our focus has shifted from quantity to quality.

Rice is consuming as the major calorie supplement for two thirds of the Indian population with a consumption of ~220 g per day. However polished rice is a poor source of micronutrients (Eric *et al.,* 2012) [10]. It is observed that in polished rice, Zn and protein content can be enhanced through conventional breeding, whereas, for increasing Fe, transgenics appears to be the only viable solution. Recent approaches for biofortification include identification of genomic regions or mapping of quantitative trait loci (QTL) followed by their introgression into popular varieties. Since bio fortified crops are developed through conventional breeding, regulatory constraints are not applicable for their release. Till now more than 5600 varieties of different crops have been released of which number of bio fortified varieties is negligible. These bio fortified varieties assume great significance to achieve nutritional security of the country.

The objective of this study was to evaluate the genotypic variation in a collection of 192 genotypes of diverse origin to assess the variability in iron and zinc content in dehusked rice grains for its utilization in micro-nutrient biofortification program. Laboratory bench top Energy Dispersive X-ray Florescence Spectrophotometer (ED-XRF) is the most commonly used technique because of its precision and rapid and cost effective screening for the estimation of large number of samples. Hence this study was proposed to (i) estimate rice germplasm for iron and zinc concentration in brown rice (ii) identifying lines with less loss of iron and zinc after polishing and (ii) analyse the correlation between Fe and Zn concentration in a population panel of 192 lines using ED-XRF method.

Materials and Methods

Experimental Site: A set of 192 genotypes were grown in Research Cum Instructional Farm, College of Agriculture, Raipur (C.G.). India during *Kharif* 2017. The experiment was laid out in augmented design with a spacing of 15×20cm. Normal cultural practices were followed as per standard recommendation.

Plant Materials: The experimental material consists of a set of 192 rice genotypes including advanced breeding lines (24), landraces (113), farmer's varieties (15), cultivated varieties (16) and wild rice (14) accessions belonging to IGKV gene pool and different states of India as well as exotic landraces (10) from Indonesia, Philippines, Thailand, Iran, China, Vietnam and Myanmar (Table No. 1).

Iron and Zinc Content Estimation: Micronutrient content can be estimated by both destructive and non-destructive methods. Iron and zinc were estimated using non-destructive ED-XRF machine at Indian institute of Rice Research, Hyderabad. ED-XRF is an Oxford Instruments X-supreme 8000 which has 10 place auto-samplers. 10g paddy sample having similar moisture content from each genotype was de-husked through non-metallic de-husker (Krishi international 810 de-husker) having roller made up of polymer to avoid iron and zinc contamination. De-husked rice was cleaned for broken and debris and 5g of each sample was weighed and transferred to sample cups. The sample cups were gently shaken for uniform distribution of samples and kept for analysis. Scans were conducted in sample cups assembled from 21 mm diameter and the cup combined with polypropylene inner cups was sealed at one end with 4 µm Poly-4 XRF sample film. Concentration was expressed in microgram per gram (µg/g).

Results and Discussion

In the present study the micronutrient content (Zn and Fe) of 192 diversely rice accessions including cultivated varieties, advanced breeding lines, germplasm accessions and wild rice accessions were analysed in brown rice. The accessions having more than 23 µg/g Zinc were selected and milled for estimation of Fe and Zn content in polished rice. Iron concentration ranged from 6.3 µg/g to 24.5 µg/g and zinc concentration from 15.4 µg/g to 39.4 µg/g (Figure 1) (Table No. 1). The mean value of iron in the germplasm lines is 10.38 µg/g and Zinc, 25.04 µg/g. The lowest concentration of iron was recorded in Goindi (farmer's variety), which is a Chhattisgarh landrace and the lowest zinc content in red rice landrace, Mokdo.

Table 1: Iron and Zinc content of diverse rice germplasm used in the study

S. No		Name of Genotypes	Origin	Iron (µg/g) unpolished (polished)	Zinc (µg/g) unpolished (polished)
1	RG1	Longkulabat	Indonesia/DRR	12.1 (1.6)	24.9 (16.1)
2	RG2	Hasan Serai	Iran/DRR	9.2	20.6
3	RG3	NiiawHawm	Pant nagar/DRR	10.7	21.0
4	RG4	Lua Nhe Den	Thailand/DRR	9.6	19.3
5	RG5	Hawm Jan	Indonesia/DRR	11.7 (1.4)	23.7 (18.0)
6	RG6	Hung-mi-hsiang-ma-Tsan	Vietnam/DRR	9.4	15.8
7	RG7	Guinata	Thailand/DRR	11.1	20.7
8	RG8	Dawleuang	China/DRR	11.2 (2.7)	23.3 (15.9)
9	RG9	Bongcay	Vietnam/DRR	8.2	20.3
10	RG10	Binirhen	Phillii pines/DRR	9.8 (0.7)	32.8 (25.7)
11	RG11	Lalbasmati	J & K/DRR	10.7 (0.9)	24.9 (17.1)
12	RG12	Kalikhasa	AS/DRR	11.2 (2.8)	24.8 (17.8)
13	RG13	Kamini Joha	AS/DRR	9.9	20.0
14	RG14	BAS 837	US Patented line/DRR	11.0 (2.0)	28.0 (18.5)
15	RG15	Ayepyaung	Myanmar/DRR	10.7 (0.9)	24.5 (16.0)
16	RG16	Dubraj (D: 1251)	IGKV, CG, India	8.6	22.0
17	RG17	Luchai (L: 246)	IGKV, CG, India	7.5	17.8
18	RG18	Mancha (M: 1028)	IGKV, CG, India	9.1 (1.4)	25.2 (18.8)

19	RG19	Luchai (L: 1099)	IGKV, CG, India	8.2	20.1
20	RG20	Mahipal (M: 27A)	IGKV, CG, India	9.2	18.5
21	RG21	Umari (CGR: 17451)	IGKV, CG, India	8.0	19.2
22	RG22	Kekai (K: 927 II)	IGKV, CG, India	11.1 (1.8)	27.9 (23.9)
23	RG23	Bantha Luchai (B: 2733)	IGKV, CG, India	8.5 (1.0)	26.9 24.2)
24	RG24	Mokdo (M: 550)	IGKV, CG, India	6.6	15.4
25	RG25	Shri kamal (S: 660 I)	IGKV, CG, India	10.2 (3.1)	23.1 (17.4)
26	RG26	Kankadiya (K: 18 II)	IGKV, CG, India	8.1	21.9
27	RG27	Khuddi (K: 1128 IV)	IGKV, CG, India	8.4	22.5
28	RG28	Jira Dhan (J: 53)	Tilda/RPR	13.3 (2.5)	23.9 (14.5)
29	RG29	Javaphool (J: 333)	Lailunga/RPR	11.3	19.6
30	RG30	Maharaji (M: 504)	Ghughari/Mandla	10.0	20.7
31	RG31	Fundri (F: 28)	Antagarh/Bastar	11.9	19.1
32	RG32	Banspatri (B: 728)	Gariaband/RPR	10.6	19.4
33	RG33	Anterved (A: 217)	Debra/Damob	8.0 (2.0)	27.0 (19.2)
34	RG34	Jaigundi (J: 248)	Saraipali	8.5	17.3
35	RG35	Chinnor (C: 151)	Tilda/RPR	8.4	22.7
36	RG36	Keraghul (K: 2034)	Gharghoda/Raigarh	9.2	16.3
37	RG37	R 2053-202-2-145-1	IGKV, CG, India	9.3	21.6
38	RG38	R 1607-321-1-34-1	IGKV, CG, India	9.2	22.0
39	RG39	R 1661-1372-1-601-1	IGKV, CG, India	8.4	20.6
40	RG40	R 1700-2247-1-2313-1	IGKV, CG, India	8.8	21.6
41	RG41	Improved Chepti Gurmatiya	IGKV, CG, India	12.3	18.7
42	RG42	Improved Dokra-Dokri	IGKV, CG, India	10.9 (0.9)	24.2 (18.4)
43	RG43	R 1779-321-1-112-1	IGKV, CG, India	11.6	21.5
44	RG44	R 2054-685-1-205-1	IGKV, CG, India	11.0	19.4
45	RG45	R 1882-306-4-243-1	IGKV, CG, India	11.5 (1.1)	23.1 (16.0)
46	RG46	R 1973-206-2-86-1	IGKV, CG, India	10.2	17.7
47	RG47	Sonagathi	CG, India	11.0	21.0
48	RG48	KodhaPhool	CG, India	8.4	21.3
49	RG49	Balamsar	CG, India	8.8	20.6
50	RG50	HarikhutaDhan	CG, India	8.1 (0.1)	23.3 (17.9)
51	RG51	Barabali	CG, India	8.9	22.6
52	RG52	Lahunchi	CG, India	9.1 (1.8)	24.5 (18.4)
53	RG53	Bagdisona	CG, India	9.7 (2.3)	27.3 (21.5)
54	RG54	Netakalani	CG, India	10.4	20.7
55	RG55	Damrubaba-3	CG, India	9.0	22.0
56	RG56	Nardha	CG, India	8.3	24.2
57	RG57	Mehar Dhan	CG, India	8.0	20.5
58	RG58	Kata Mehar	CG, India	8.4 (1.7)	24.3 (20.7)
59	RG59	Goindi	CG, India	6.3	17.2
60	RG60	Luchai	CG, India	7.5 (1.1)	26.6 (22.2)
61	RG61	Tetirpakhi	CG, India	9.2 (1.2)	24.7 (17.0)
62	RG62	Hadrasal	CG, India	8.6	19.3
63	RG63	Dubraj	IGKV, CG, India	12.1 (0.3)	23.6 (13.2)
64	RG64	Baihaguda	IGKV, CG, India	7.2	20.8
65	RG65	Bhathaguda	IGKV, CG, India	7.9	17.7
66	RG66	IC: 41843	IGKV, CG, India	10.0 (0.5)	27.3 (18.4)
67	RG67	IC: 466901	IGKV, CG, India	8.0	21.9
68	RG68	R-RKM-1	IGKV, CG, India	8.8	21.3
69	RG69	Ateya (CGR: 2)	Bastar, CG, India	12.9 (3.0)	34.8 (23.7)
70	RG70	Dubraj 11 (CGR: 12230)	Bastar, CG, India	8.4 (1.2)	24.3 (15.0)
71	RG71	Newari (CGR: 4056)	Shahdol, MP, India	10.5 (0.5)	23.5 (16.2)
72	RG72	Badshahbhog (CGR: 10919)	Bastar, CG, India	10.9	22.2
73	RG73	SathaDhan (CGR: 394)	Raigarh, CG, India	12.8 (1.3)	33.2 (25.7)
74	RG74	Piso (CGR: 16109)	Bastar, CG, India	9.7 (2.0)	28.1 (19.9)
75	RG75	Newari (CGR: 4053)	Panna, MP, India	10.6 (1.0)	25.1 (17.7)
76	RG76	Lonkti Monchhi (CGR: 16804)	Bastar, CG, India	10.4	19.9
77	RG77	Kanji Local (CGR: 174)	Panna, MP, India	12.1 (3.0)	29.7 (28.0)
78	RG78	Selection of Badshahbhog (CGR: 17760)	Bastar, CG, India	13.3	22.2
79	RG79	Fara (CGR: 113)	Bastar, CG, India	13.1 (4.6)	30.7 (24.6)
80	RG80	Dondagi (CGR: 12135)	Raigarh CG, India	7.6 (1.1)	23.0 (14.6)
81	RG81	Kadam Phool (CGR: 943)	Bastar, CG, India	11.8 (3.3)	26.4 (21.1)
82	RG82	Khatiya Pati (CGR: 14230)	Raigarh, CG, India	11.1 (0.4)	26.4 (25.9)
83	RG83	Mekara Ghol (CGR: 270)	Raipur, CG, India	11.3 (2.3)	27.0 (19.7)
84	RG84	Dokra Dokri (CGR: 12126)	Raipur, CG, India	10.0	22.2
85	RG85	Chhatri (CGR: 669)	Jabalpur, MP, India	9.1 (2.3)	30.4 (23.12)
86	RG86	Sugarkand (CGR: 17213)	Damoh, MP, India	10.8 (1.5)	24.8 (18.0)
87	RG87	Angur Guchcha (CGR: 1803)	Durg, CG, India	10.3 (3.2)	29.3 (23.7)

88	RG88	Parmal (CGR: 15971)	Raipur, CG, India	8.8 (0.9)	27.2 (20.0)
89	RG89	Lakhouwal (CGR: 1128)	Damoh, MP, India	11.9 (1.9)	27.8 (17.8)
90	RG90	Anjan (CGR: 1814)	Raipur, CG, India	9.4 (1.0)	26.1 (19.3)
91	RG91	Anjan (CGR: 1816)	Raigarh, CG, India	14.1 (4.1)	27.0 (18.2)
92	RG92	Mani Gurmatia (CGR: 2730)	Raipur, CG, India	11.0 (1.3)	25.9 (17.9)
93	RG93	Makado (CGR: 3831)	Bastar, CG, India	11.5 (0.7)	26.2 (16.2)
94	RG94	Moti Basmati (CGR: 5817)	Seoni, MP, India	12.3 (0.5)	24.7 (16.0)
95	RG95	Bauwara (CGR: 5854)	Raipur CG, India	12.8 (0.4)	29.2 (17.7)
96	RG96	Cross 116 (CGR: 6366)	Raigarh, CG, India	8.2	19.7
97	RG97	Kotari (CGR: 7805)	Raipur, CG, India	8.0 (1.1)	25.1 (16.9)
98	RG98	Krishna Koliyari (CGR: 7812)	Rajnandgaon, CG, India	11.3 (2.2)	25.7 (15.1)
99	RG99	Malpa (CGR: 8409)	Raipur, CG, India	11.1 (2.1)	31.2 (19.3)
100	RG100	Niwari (CGR: 8748	Jabalpur, MP, India	13.2 (2.4)	30.2 (16.5)
101	RG101	IC: 116076 (CGR: 10007)	CG, India	10.9 (3.3)	26.4 (18.3)
102	RG102	Nagbel (CGR: 4003)	Raipur, CG, India	13.9 (3.3)	25.2 (14.8)
103	RG103	Surmatia (CGR: 4909)	Bastar, CG, India	10.1	20.2
104	RG104	IC: 459172	NBPGR, New Delhi, India	12.6 (1.6)	27.3 (15.9)
105	RG105	IC: 460160	NBPGR, New Delhi, India	15.0 (1.6)	25.0 (13.1)
106	RG106	IC: 74637 A1	NBPGR, New Delhi, India	9.5	21.9
107	RG107	IC: 277830	NBPGR, New Delhi, India	12.9	22.9
108	RG108	IC: 323957	NBPGR, New Delhi, India	12.9	22.0
109	RG109	IC: 453927	NBPGR, New Delhi, India	8.6 (1.2)	29.5 (21.8)
110	RG110	IC: 457989	NBPGR, New Delhi, India	11.2 (0.9)	30.2 (20.6)
111	RG111	IC: 459147	NBPGR, New Delhi, India	9.4	21.9
112	RG112	IC: 449793	NBPGR, New Delhi, India	11.2 (1.2)	32.9 (23.8)
113	RG113	IC: 124346	NBPGR, New Delhi, India	9.3 (0.3)	26.6 (18.4)
114	RG114	IC: 124366	NBPGR, New Delhi, India	10.7	19.1
115	RG115	IC: 125267	NBPGR, New Delhi, India	10.2 (1.4)	26.9 (17.4)
116	RG116	IC: 133146	NBPGR, New Delhi, India	8.9	22.9
117	RG117	IC: 135827	NBPGR, New Delhi, India	8.6 (2.0)	28.3 (21.2)
118	RG118	IC: 135877	NBPGR, New Delhi, India	15.8 (2.2)	30.9 (26.4)
119	RG119	IC: 123505	NBPGR, New Delhi, India	10.0 (1.0)	28.5 (19.9)
120	RG120	IC: 124525	NBPGR, New Delhi, India	11.7 (2.7)	33.4 (24.8)
121	RG121	IC: 206322	NBPGR, New Delhi, India	10.4	22.9
122	RG122	IC: 206693	NBPGR, New Delhi, India	11.6 (2.0)	25.0 (20.0)
123	RG123	IC: 206754	NBPGR, New Delhi, India	10.4 (1.9)	25.7 (22.3)
124	RG124	IC: 206866	NBPGR, New Delhi, India	13.1 (1.0)	31.0 (22.7)
125	RG125	IC: 206615	NBPGR, New Delhi, India	9.8	21.3
126	RG126	IC: 331668	NBPGR, New Delhi, India	10.1	21.3
127	RG127	IC: 379109	NBPGR, New Delhi, India	10.4 (2.1)	29.2 (19.1)
128	RG128	IC: 379122	NBPGR, New Delhi, India	10.5 (1.9)	26.6 (13.9)
129	RG129	IC: 296890	NBPGR, New Delhi, India	9.9 (0.9)	25.1 (13.7)
130	RG130	IC: 388204	NBPGR, New Delhi, India	12.9 (2.0)	24.5 (13.3)
131	RG131	IC: 388737	NBPGR, New Delhi, India	11.4 (0.9)	38.4 (21.4)
132	RG132	IC: 389351	NBPGR, New Delhi, India	15.9 (1.7)	28.8 (16.1)
133	RG133	IC: 389509	NBPGR, New Delhi, India	9.5 (1.0)	28.6 (21.3)
134	RG134	IC: 389838	NBPGR, New Delhi, India	12.4	22.9
135	RG135	IC: 390299	NBPGR, New Delhi, India	12.6 (2.2)	31.4 (22.6)
136	RG136	IC: 435091	NBPGR, New Delhi, India	11.2 (1.6)	27.6 (18.9)
137	RG137	IC: 435541	NBPGR, New Delhi, India	10.5 (1.3)	24.4 (13.2)
138	RG138	IC: 435559	NBPGR, New Delhi, India	13.2 (2.7)	38.0 (27.6)
139	RG139	CR 3969-24-1-2-1-1 (IR 73907-753-2-3/Pratiksya)	IGKV/DRR, India	10.7 (1.6)	24.0 (13.5)
140	RG140	OR 2487-13 (OR 2076-2/Ashoka228)	IGKV/DRR	10.5 (1.1)	25.1 (14.2)
141	RG141	NPT 14-10 (NPT 29/R 296)	IGKV/DRR	9.9 (1.4)	26.3 (14.9)
142	RG142	JAYA	IGKV/DRR	11.3	22.0
143	RG143	CR 3561-3-2-1-1-1-1 (Surendra/Annapurna)	IGKV/DRR	8.5	19.7
144	RG144	Swarna	IGKV/DRR	8.7	19.7
145	RG145	CR 3856-44-22-2-1-10-1-5 (IR 73963-86-1-5-2-2/CR2324-1)	IGKV/DRR	9.0	21.0
146	RG146	OR 2573-11 (Birupa/IR 76561-AC-8-8)	IGKV/DRR	12.5 (0.7)	28.6 (16.1)
147	RG147	YNP 7060 (NPG 6/NPG 15)	IGKV/DRR	10.5	21.7
148	RG148	CR 3856-29-14-2-1-1-7-1 (IR 73963-86-1-5-2-2/CR 2324-1)	IGKV/DRR	6.4	16.3
149	RG149	PA 6444	IGKV/DRR	8.0	20.1
150	RG150	CR 3504-12-2-1-1-1-1 (IR 36/Birupa)	IGKV/DRR	9.6 (2.1)	23.8 (13.4)
151	RG151	NDR 359	IGKV/DRR	9.6 (2.2)	29.0 (21.8)
152	RG152	Loktimachhi	IGKV, CG, India	9.2	17.7
153	RG153	Swarna	IGKV, CG, India	11.7	17.8
154	RG154	Madhuraj-55	IGKV, CG, India	9.2 (1.3)	23.6 (16.1)

155	RG155	Barhasaal	IGKV, CG, India	8.1	20.6
156	RG156	Bisni	IGKV, CG, India	12.6 (2.6)	31.5 (21.8)
157	RG157	Tarunbhog	IGKV, CG, India	11.0 (3.7)	27.7 (15.6)
158	RG158	Chhattisgarh Zinc Rice-1	IGKV, CG, India	10.8 (1.7)	28.4 (20.9)
159	RG159	Lalloo 14	IGKV, CG, India	10.5 (2.8)	31.3 (22.0)
160	RG160	Lalmati	IGKV, CG, India	10.4 (1.8)	31.5 (26.9)
161	RG161	Kanak Jira	IGKV, CG, India	11.8 (3.4)	26.2 (17.1)
162	RG162	*O. Officinalis*	IGKV, CG, India	24.5 (4.4)	36.1 (16.1)
163	RG163	*O. Latifolia*	IGKV, CG, India	22.5 (6.7)	33.8 (20.7)
164	RG164	*O. Sativa var. fatua*	IGKV, CG, India	9.9 (0.7)	28.5 (21.3)
165	RG165	*O. Sativa var. fatua*	IGKV, CG, India	12.7 (3.0)	38.7 (28.2)
166	RG166	*O. nivara*	IGKV, CG, India	11.2 (2.1)	39.4 (32.6)
167	RG167	*O. nivara*	IGKV, CG, India	11.2 (2.1)	30.0 (24.4)
168	RG168	*O. nivara*	IGKV, CG, India	10.6 (1.9)	29.8 (21.8)
169	RG169	*O. nivara*	IGKV, CG, India	9.9 (2.7)	31.8 (23.4)
170	RG170	*O. nivara*	IGKV, CG, India	11.6 (2.1)	30.1 (22.5)
171	RG171	*O. nivara*	IGKV, CG, India	10.4 (1.7)	27.1 (22.0)
172	RG172	*O. nivara*	IGKV, CG, India	11.9 (2.7)	38.0 (29.0)
173	RG173	*O. nivara*	IGKV, CG, India	9.5 (1.8)	34.4 (20.9)
174	RG174	*O. nivara*	IGKV, CG, India	7.7 (1.3)	31.9 (23.7)
175	RG175	*O. nivara*	IGKV, CG, India	8.8 (0.9)	30.9 (19.1)
176	RG176	Chanda (C: 287)	IGKV, CG, India	10.6 (0.9)	35.3 (25.4)
177	RG177	Bhantha Luchai (B: 2733)	IGKV, CG, India	10.5 (1.5)	27.8 (21.9)
178	RG178	Karhani (K: 1276)	IGKV, CG, India	10.0 (1.6)	27.1 (22.4)
179	RG179	Adhan Chilpa (A: 701)	IGKV, CG, India	9.3	21.5
180	RG180	Katarni Bhog (K: 415 II)	IGKV, CG, India	9.9 (1.2)	28.5 (19.0)
181	RG181	Laxmibhog (L: 708)	IGKV, CG, India	8.9 (1.9)	23.2 (15.7)
182	RG182	Barounda Offtype (BOT: 61 II)	IGKV, CG, India	9.9 (1.3)	28.2 (18.3)
183	RG183	Chhatri (C: 54 I)	IGKV, CG, India	8.7 (4.1)	28.7 (18.4)
184	RG184	Umariya Chudi (U: 229)	IGKV, CG, India	7.5 (0.3)	25.6 (19.5)
185	RG185	Dubraj (D: 1289)	IGKV, CG, India	8.1	21.8
186	RG186	Muchchhan Moti (IC: 387442)	IGKV, CG, India	8.1 (1.5)	26.4 (20.9)
187	RG187	Aruna	Kerala, India	9.1 (0.9)	24.8 (16.0)
188	RG188	Krishnanjana	Kerala, India	8.6	22.3
189	RG189	Dubraj Selection-1	IGKV, CG, India	8.2 (2.4)	25.0 (17.2)
190	RG190	Badshahbhog Selection-1	IGKV, CG, India	14.2	21.8
191	RG191	Jaldubi	IGKV, CG, India	12.6 (2.5)	26.2 (17.6)
192	RG192	IGKV R1 (Rajeshwari)	IGKV, CG, India	10.0 (0.2)	23.0 (16.1)

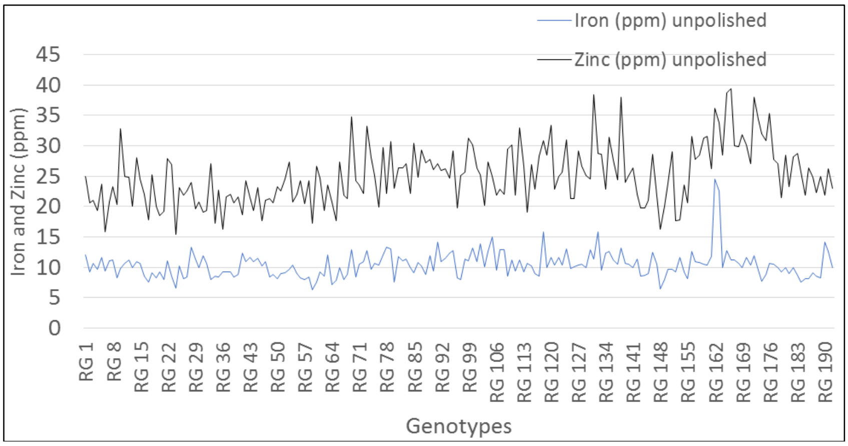

Fig 1: Iron and Zinc content in diverse rice germplasm

The wild rice accessions *Oryza officinalis* and *Oryza latifolia* were found to have both higher iron and zinc content i.e. 24.5 µg/g &36.1 µg/g and 22.5 µg/g and 33.87 µg/g, respectively. Other than wild rice accessions there were 30 more genotypes having higher iron as well as zinc content. Banerjee *et al.* (2010) [6] and Anuradha *et al.* (2012) [4] showed that wild rice accessions had high iron and zinc content in rice grain. These results are in consistent with the present study. Anandan *et al.* (2011) [3], Sanjeeva Rao *et al.* (2014) [9] and Roy and Sharma (2014) [21] showed that landraces have high Fe and Zn content than the improved cultivars. Previous studies and present study have indicated that though the variation in the micronutrient content depends on several factors, the germplasm stock can be exploit to further enhance the micronutrient content through conventional breeding for developing stable lines.

Based on the iron and zinc content, these 192 diverse rice genotypes can be classified into three categories, low, moderate and high (Nachimuthu *et al.*, 2014) [17]. For iron content, 52 genotypes with the iron content of 0-9 µg/g was considered in low category, whereas,108 genotypes having iron content from 9.1 to 12 µg/g were grouped in moderate and 32 genotypes were placed in high category having more than 12 µg/g iron content (Figure 2a). No genotype was observed with the low zinc content (0-12 µg/g), 26 genotypes with the zinc content from 12.1 to 20 µg/g was grouped in moderate category and the genotypes (166 genotypes) with more than 20 µg/g to 32.4 µg/g was placed in high category (Figure 2b).

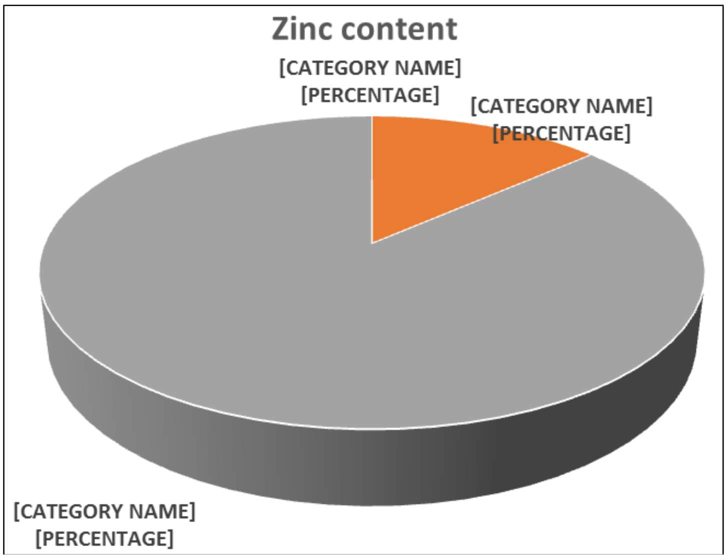

Fig 2a: Genotypes classification based on Zinc content

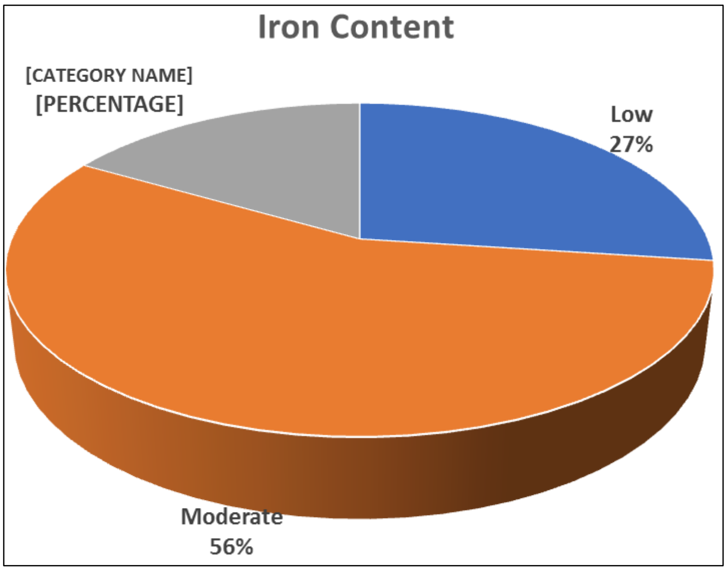

Fig 2b: Genotypes classification based on Iron content

Out of these 192 diverse rice genotypes, 118 genotypes having more than 23 µg/g zinc content were further analysed for iron and zinc content after polishing the grain. In polished rice Fe content was ranged from 0.1 µg/g (Harikhuta) to 6.7 µg/g (*Oryza latifolia*) and Zn content was ranged from 13.1 µg/g (IC: 460160) to 32.6 µg/g (*Oryza nivara*) through XRF method, with a mean value of Fe 1.79 µg/g and Zn 19.53 µg/g (Table 1).

During polishing there is loss of Fe and Zn content through bran layer. In the present study large variation in iron and zinc levels was observed among the rice genotypes after polishing. Polished rice of landrace CGR: 14230 showed minimum loss of Zn content (0.5 µg/g) followed by CGR: 174 (1.7 µg/g), Bantha Luchai (2.7 µg/g) and Kata Mehar (3.6 µg/g). Highest loss of Zn content was seen in CGR: 8748 (13.7 µg/g) followed by *O. nivara* (13.5 µg/g) and *O. latifolia* (13.1 µg/g). In polished rice, loss of Fe content in grain was lowest in Chhatri (4.6 µg/g) followed by Harad Guhidahi (5.8 µg/g) and Anterved (6.0 µg/g). Highest loss of Fe was recorded in *O. officinalis* (20.1 µg/g) followed by *O. latifolia* (15.8 µg/g). Neha *et al.* (2015), Sanjeeva Rao *et al.* (2014) [9], Chandel *et al.* (2010) [7] and Sellappan *et al.* (2009) [25] reported the similar findings in diverse rice germplasm and found that brown rice contain higher concentration of minerals and vitamins than polished rice, due to removal of nutritive aleurone layer, can be a better supplement of these minerals in daily diet.

A highly positive significant correlation (0.438**) was observed between iron and zinc contents of 192 rice genotypes (Figure 3) which are in accordance with Ajmera *et al.* (2017) [2], Baishali *et al.* (2016), Kavita *et al.* (2015) and Sanjeeva Rao *et al.* (2014) [9]. The result suggesting that selection of one can be automatically yield a higher estimation of the other and thus implying the chance of simultaneous effective selection or concurrent selection for both the micronutrients.

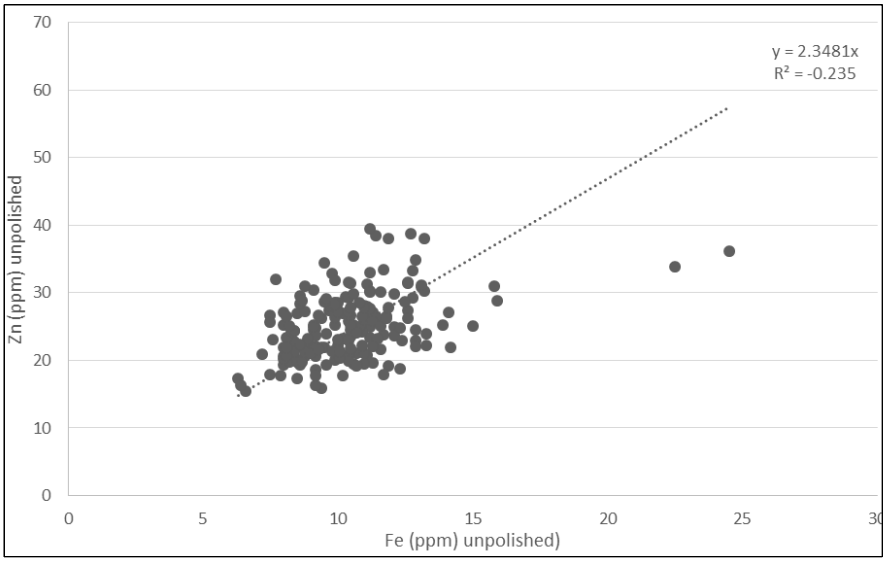

Fig 3: Relationship between grain iron content and grain zinc content

However, Nagesh *et al.*, (2018) [19] reported that the grain iron content had significant negative correlation with grain yield while grain zinc had negative and non-significant correlation with grain yield. Several reports indicate a significant negative association between grain Zn concentration and yield in rice but a positive relationship between grain yield and grain Zn concentration was observed under Zn-deficient soil (Gangashetty *et al.* 2013; Sathisha 2013) [11, 24]. Thus, it can be concluded that it is possible to develop high yielding varieties with high levels of Zn. The release of high Zn rice lines with high yield potential in Bangladesh provide positive evidence for the possibility of combining high Zn and high yield potential in rice (Harvest Plus 2014) [14]. Sala and Geetha, 2015, showed that iron content had a negative correlation with yield but zinc showed positive correlation with yield in a cross combination, this result is in contradicting with Nagesh *et al*., 2012 [18] where he observed that there is no correlation between grain iron and zinc content with grain yield. These results are in contradicting with Kalmeshwer Goud Patil (2008) [15] where iron and zinc were reported positive non-significant correlation with grain yield both genotypic and phenotypic level.

Conclusion

There is a wide genetic variability existing in rice germplasm which suggests the existence of genetic potential to accelerating the development of more nutritious rice varieties by increasing the concentration of Fe and Zn content in rice grain. The wild accessions showed the highest Fe and Zn content in grains before and after polishing. These genotypes identified after screening of national and international rice germplasm for rich and poor micronutrient content may be deployed in breeding programme to develop high-yielding and mineral rich rice genotypes. It also helps to identify genomic location for micronutrients content. Thus, the modern conventional breeding techniques including molecular MAS may be useful in the development of rice varieties by combining yield and high grain nutritional value without and genetic manipulation.

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