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RESEARCH ARTICLE

Exploring the Hidden Potential of Traditional Rice Landraces and Mutants for Iron and Zinc Concentration in Brown Rice to Develop Bio-fortified Rice Varieties

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Abstract

In the present study, 58 rice genotypes were evaluated for three consecutive seasons to enucleate the elite lines with high Fe and Zn contents. ANOVA revealed significant differences among the genotypes for Fe and Zn contents during all three seasons. Furthermore, PCV and GCV were moderate to high for Fe and Zn content during all three seasons. Similarly, high level of heritability and genetic advance were also recorded for both the traits. Iron content of Safri mutant 17-2-48 was consistently high for all the seasons whereas zinc content of Loktimachi was high for two seasons. Interestingly, rice genotypes Chhindmauri, Badsahbhog, Rudra, Danighoda and Suapankhi have been identified to have moderate to high Fe and Zn contents which could be used as donors for developing bio-fortified rice varieties. Stability analysis revealed that the genotypes Jhimiprass, Loktimachi, and CG Zinc Rice 2 for iron content and Rela Dhan, Chhindmauri, and CG Zinc Rice-1 for zinc content have had an average response to environment, making them appropriate for all seasons

Keywords: Rice landraces, Bio-fortification, Fe & Zn content, Variability, Stability analysis.

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Introduction

Rice is a major staple food for more than half of the world's population and contributes to solve the problem of hunger among poor people, but they are rarely accessible to nutrient rich food sources. The prevalence of micronutrient deficiency, especially for crucial elements like Fe content and Zn content among the rice-consuming population, prompted in initiation bio-fortification programme in rice which has been identified as a sustainable source to develop nutrient-rich rice grains to poor people who depend solely on rice for the energy and nutrients (Rao *et al*., 2014). Traditional rice landraces have tremendous genetic variability and possess valuable genes for nutritional traits. Several biotic and abiotic stresses have adaptability in wide agroecological niches, unmatched qualitative traits and therapeutic properties, and thus possesses great genetic potential for rice improvement. Along with the rice landraces, mutants developed from them are a valuable source of micronutrients that need to be quantified and utilized to develop bio-fortified rice varieties. Production of varieties containing high amounts of bioavailable Fe would improve Fe nutrition in regions with prevalent iron deficiency (Aggett, 2020; Maret and Sandstead, 2006; Shahzad *et al*., 2014). Studies by Harvest Plus and others have shown considerable iron and zinc

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losses during rice polishing. Therefore, an increase in the concentration of Fe and Zn in brown rice is a high-priority research area and later on that could be quantified in white rice for varietal development (Ryu and Aydemir, 2020). For this concern, we need to focus on micronutrient-rich sources which can be easily reachable and acceptable to people.

Biofortification is one of the sustainable approaches for improving the iron and zinc content and their bioavailability in rice grains. Nevertheless, before the implementation of thebiofortification process, the primary step in conventional breeding is to screen out the micronutrient-dense cultivars within the natural existing germplasm because the presence of awide range of genetic variations in the population helps in selecting the desired genotypes. Furthermore, selecting stable genotypes for high micronutrient content is also crucial for developing nutri-rich rice varieties. Therefore, the current study utilized 44 rice landraces and 8 mutants along with the 6 check to explore the genetic variability, stability and hidden potential of traditional rice landraces and mutants for iron and zinc concentration in brown rice over different seasons. Moreover, the outcome of this study will be further helpful to create the genetic database for breeding program specific for improving the micronutrient in the rice varieties.

Materials and Methods

Experimental Site

The field experiments were conducted at Instructional Farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492012 (C.G.) India, during three seasons *viz.*, *Kharif* season-2020, *Rabi* season -2020-21, and *Kharif* season -2021. Geographically, Chhattisgarh state of India is located between 17°14′ N and 24°45′ N latitude and longitudeof 79°16′ E. The experiment was laid out by following a randomized complete block design with two replications during all three seasons. All the standard agronomic practices were adopted during the crop seasons.

Plant Materials

A total of 58 accessions, including 44 landraces, 08 mutants, and 06 checks, were used in the experiment. All the genotypes were accessed from the Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492012 (C.G.). The list of experimental materials is presented in Table 1. Dehusked brown rice was used to estimate Zinc and Iron content during all three seasons.

Estimation of Iron and Zinc Contents

Iron and zinc content were estimated using the S2 RANGER ED-XRF Method at International Rice Research Institute, South Asia Hub, ICRISAT Campus, Hyderabad, India center. The seed samples were dehusked by the help of Zaccaria testing rice mill having rubber roller. Before dehusking, the grains were washed with 0.1 N HCl followed by rinsing with double distilled water to make the sample free from contamination. Total 5 g of properly cleaned brown rice in triplicates were used for the estimation of Fe and Zn contents. The published protocol of ED-XRF was used to estimate both the micro-nutrients (Rao *et al.,* 2014). The data from the application was exported in MS Excel sheet for further analysis.

Table 1: List of rice genotypes used as experimental materials under the study

S. No.	*Name of genotypes*	*Parentage*
1	Red Barhasal	landrace
2	Rudra	landrace
3	Tulsibhog	landrace
4	Loktimachi	landrace
5	Odha Dhan	landrace
6	Gathuwan Dhan	landrace
7	Raja Bangla	landrace
8	Brown Rice-1	landrace
9	Munibhog	landrace
10	Danighoda	landrace
11	Saraitolia	landrace
12	Pangudi Goindi	landrace
13	Safri-17	landrace
14	Dubraj	landrace
15	Byalo	landrace
16	Badshahbhog	landrace
17	Jonyaphool	landrace
18	Mohlyanbanko	landrace
19	Kanakbhog	landrace
20	Shwet Ganga	landrace
21	Matko Dhan	landrace
22	Suapankhi	landrace
23	Jhimiprass	landrace
24	Jauphool	landrace
25	Kalajeera	landrace
26	Chhindmauri	landrace
27	Motipeera	landrace
28	Pedgadi	landrace
29	Sonagathi	landrace
30	Jana Dhan	landrace
31	Rela Dhan	landrace
32	Ram Shree	landrace
33	Kari Alcha	landrace
34	Jhilli Safri	landrace
35	Siyar	landrace
36	Karhani	landrace

37	Nandel	landrace
38	Safri	landrace
39	Dhaura Mundariya	landrace
40	Karhani-2	landrace
41	Sanchuriya	landrace
42	Alsakar	landrace
43	Luchai	landrace
44	Mai Dubraj	landrace
45	TCDM-1	Mai Dubraj
46	Vikram-TCR	Safri-17
47	C.G. Jawaphool Trombay	Jawaphool
48	Alsakar Mutant-3	Alsakar
49	Sanchuriya Mutant-6	Sanchuriya
50	Safri-17-5 Mutant	Safri-17
51	Safri-17-2-48 Mutant	Safri-17
52	Luchai Mutant -1	Luchai
53	Dubraj Sel-1	Dubraj
54	CG Zinc Rice 1	Poornima x Annada
55	CG Zinc Rice 2	IR681444-B-18-2-1-1 x HMT
56	Zinco Rice MS	Lalmati x IR681444
57	Rajeshwari	R320-300 x ChaptiGurmatiya
58	Protazin	Ciherang x R-7586-106-28-3-B-B-B

Statistical Analysis

Statistical analysis for analysis of variance (ANOVA), genetic variability parameters like mean, range, phenotypic coefficients of variation (PCV), genotypic coefficients of variation (GCV), heritability in broad sense (Hb), and genetic advance as percent of the mean parameters were calculated through the standard software (Windostat version 9.2 from Indostat services, by following the standard methodology. Stability analysis over the three seasons was performed following the Eberhart and Russell (1966) Model through Windostat version 9.2 from Indostat services.

Results

Total 58 accessions were analyzed and the result of pooled variance analysis (Table 2) revealed high variability for the iron and zinc as represented by the highly significant mean sum of squares over three seasons. However, the results of different genetic variability parameters revealed that the phenotypic coefficients of variation (PCV) were slightly higher than the corresponding genotypic coefficients of variation (GCV) for both traits among all three seasons (Table 3). Heritability in broad sense (Hb) were found to be medium for iron (36.30%) and zinc (41.78%) in season I while in season II it was estimated high for Iron (83.54%) and Zinc (80.21%). In season III heritability was high for Zn (73.17%) but found medium for Fe (64.68%). Similarly, high estimate of genetic advance as a percent of mean was recorded for Fe in season II (39.92) and season III (27.3), while it was estimated as medium in season I (16.66). However, genetic advance as a percent of mean for Zn was estimated to be medium in all three seasons (Table 3). Total 58 accessions were analyzed and variation in iron and zinc contents in brown rice was studied in different seasons.

Table 2: Pooled analysis of variance (ANOVA) for Fe and Zn contents over the three seasons (*Kharif season* 2020, Rabi season 2020-21, and *Kharif season* 2021.)

Source of variation	*Degree of freedom*	*Mean sum of square*	
		Fe	*Zn*
Replications	1	5.945	4.661
Genotypes	57	28.017**	36.577**
Environment	2	5.413	6.977
Genotype x environment	2	12.916**	23.267**
Errors	285	2.273	2.765

** Significant at 1% level of significance, * Significant at 5% level of significance

In season I, range for the concentration of iron varied from 8.13 ± 0.46 to 20.83 ± 0.71 ppm with an average of 11.41 ppm iron, while zinc content among the 58 genotypes ranged from 14.41 ± 1.29 to 27.45 ± 1.45 ppm with an average of 21.41 ppm zinc. Among all genotypes, Safri mutant 17-2-48 had the highest iron content (20.83 ppm), while Red Barhasal recorded the lowest iron content during *Kharif*-2020.

Meanwhile, Munibhog recorded the highest zinc content and Odha Dhan recorded the least (Table 4). Among the genotype tested, 78% were found high for iron content while 22% of genotypes had low iron content, 74% contained high zinc, and 26% had low zinc content (Figure 1) in season I. The top five elite genotypes with high iron during season I are Safri-17-2-48 Mutant, Badshahbhog, Rajeshwari, Chhindmauri, and Rudra (Table 5). The top five elite

Table 3: Genetic variability parameters for Fe and Zn contents over the three seasons

Micronutrients	*Kharif* season 2020					*Rabi* season 2020-21					*Kharif* season 2021				
	Mean	PCV %	GCV %	Hb	GA % mean	Mean	PCV %	GCV %	Hb	GA % mean	Mean	PCV %	GCV %	Hb	GA % mean
Fe	11.42	22.29	13.43	36.30	16.66	11.85	23.2	21.21	83.54	39.92	11.63	20.47	16.46	64.68	27.3
Zn	21.42	15.69	10.14	41.78	13.51	20.93	12.47	11.17	80.21	20.61	21.18	12.58	10.76	73.17	19

genotypes with high zinc are Munibhog, Byalo, Jhimiprass, Jonyaphool, and Loktimachi (Table 6). Iron concentration in season II ranged from 8.31 ± 0.01 ppm to 22.19 ± 2.63 ppm and zinc concentration from 16.33 ± 1.03 to 25.80 ± 0.01 ppm with the mean value of 11.85 and 20.93 ppm for iron and zinc respectively (Table 4). Meanwhile, out of tested genotypes, 83% of total genotypes had high iron content while 17% of genotypes have low iron content, whereas for a concentration of zinc 67% of genotypes contained high zinc while 33% has low zinc in brown rice (Figure 2).

The top elite genotypes with high iron during season II are Safri-17-2-48 Mutant, TCDM-1, Rudra, Chhindmauri, and Suapankhi, respectively (Table 5). The top elite genotypes with high zinc are Loktimachi, TCDM-1, Jhimiprass, Badshahbhog, and Chhindmauri (Table 6). Chhindmauri and TCDM-1 can be used as the donor parent for both iron and zinc.

However, variation in iron during the third season ranged from 8.68 ± 0.35 (Raja Bangla) to 21.51 ± 0.96 ppm (Safri-17-2-48 Mutant) and zinc concentration from 15.38 ± 1.37 (Odha Dhan) to 25.80 ± 0.01 ppm (Loktimachi) with the mean value of 11.63 ppm for iron and 21.18 ppm for zinc (Table 4). Out of 58 genotypes, 84% found high iron while 16% have low iron content, 67% contain high zinc while 33% have low zinc, as shown in Figure 3. The top five genotypes with high iron during season III are Safri 17-2-48 Mutant, Rudra, Chhindmauri, TCDM-1, and Badshahbhog (Table 5). While the top five genotypes which having high zinc are Loktimachi, Byalo, Munibhog, Jhimiprass, and Chhindmauri (Table 6) these genotypes can be utilized to develop biofortified varieties.

Among all the seasons, the concentration of Iron was found high for Safri mutant 17–2-48, where Munibhog recorded for highest zinc concentration during season I, whereas Loktimachi found the highest during the second and third seasons. Thus, variations in rice grains' iron and zinc content were observed during different seasons in the present study. These variations can be attributed to different factors such as environment, soil properties, nature of grain, genotype, and interaction of genotype X environment, micronutrient homeostasis, method of analysis, *etc*. Furthermore, Chhindmauri, Badshahbhog, Rudra, Danighoda, Suapankhi, TCDM-1 and Luchai Mutant-1 exhibited best for both Zn and Iron. Thus, these genotypes can be used as donor parents for developing Iron and Zinc rich varieties.

To test the significance among the genotypes, mean sum of square due to genotypes has been tested against mean sum of square due to pooled deviation. The results indicated highly significant mean sum of square due to genotypes for both iron and zinc contents in brown rice, indicating the availability of ample variability among the genotypes (Table 7). Stability analysis revealed that all the genotypes included in the study deviated non-significantly from zero

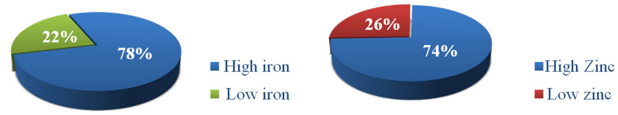

Figure 1: Percentage of genotypes having high and low Iron and Zinc content during *Kharif* season 2020 (season I)

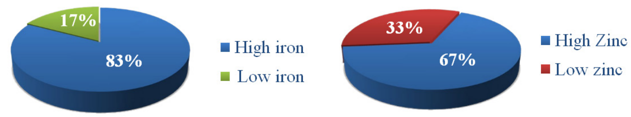

Figure 2: Percentage of genotypes having high and low Iron and Zinc content during *Rabi* season 2020-21 (season II)

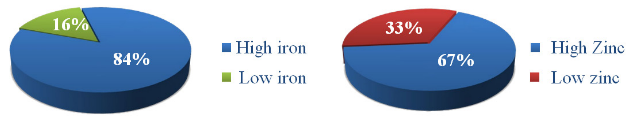

Figure 3: Percentage of genotypes having high and low Iron and Zinc content during *Kharif* season 2021 (season III)

($\sigma^2 d$=0). Still, only genotypes Jhimiprass, Loktimachi and CG zinc rice two deviated non-significantly from unity of regression for iron content. The 3 genotypes *viz*., Rela Dhan, Chhindmauri and CG Zinc Rice 1 were deviated non-significantly from unity of regression for zinc content (Table 8). Hence these genotypes have an average response to environments and are recommended for all seasons (environments), while the regression coefficient of 32 genotypes for iron and 27 genotypes for zinc recorded significant deviation from unity and less than unity ($b<1$). Hence these genotypes have below average response to environments and are recommended for poor unfavorable environments. Furthermore, the regression coefficient of 23 genotypes for iron and 25 genotypes for zinc recorded significant deviation from unity and more than unity ($b>1$). Therefore, it has an above-average response to environments and is recommended for favorable environments.

Discussion

Iron and zinc deficiency is notably the most widespread micronutrient deficiency among the human population. As a staple food, a lot of efforts are being made to enrich the nutritional status of rice to prevent malnutrition. However, the effect of these efforts is still limited because of the low consumer acceptability of fortified food and the low bioavailability of iron and zinc in our resources (Pyo *et al.,* 2022). For this concern, knowledge of our germplasm resources and their preferentially elemental constitutions of the different grain tissues and their remobilization patterns during germination makes the possibility of designing target products with a nutritionally optimal constitution more feasible (Arora *et al*., 2010; Omary *et al*., 2012). While a better understanding of the physiological basis of nutrient

Table 4: Mean performance of rice landraces and mutant for Fe and Zn contents over the three seasons

Treatments (Genotypes)	*Season 1*		*Season 2*		*Season 3*	
	Fe (ppm)	*Zn (ppm)*	*Fe (ppm)*	*Zn (ppm)*	*Fe (ppm)*	*Zn (ppm)*
Red Barhasal	8.13 ± 0.46	16.45 ± 2.76	10.95 ± 1.48	21.03 ± 3.08	9.54 ± 2.00	18.74 ± 3.24
Rudra	14.55 ± 3.61	23.85 ± 2.33	18.45 ± 3.04	20.03 ± 1.03	16.50 ± 2.76	21.94 ± 2.70
Tulsibhog	13.00 ± 2.12	22.33 ± 0.04	12.00 ± 0.71	22.30 ± 0.01	12.50 ± 0.71	22.31 ± 0.02
Loktimachi	11.80 ± 0.57	25.80 ± 0.02	12.20 ± 0.01	25.80 ± 0.01	12.00 ± 0.28	25.80 ± 0.00
Odha Dhan	9.78 ± 2.65	14.41 ± 1.29	13.00 ± 2.40	16.35 ± 1.34	11.39 ± 2.28	15.38 ± 1.37
Gathuwan Dhan	11.30 ± 5.52	22.13 ± 3.57	10.85 ± 1.63	23.13 ± 1.66	11.08 ± 0.32	22.63 ± 0.71
Raja Bangla	8.93 ± 2.30	24.03 ± 5.83	8.43 ± 1.60	19.90 ± 0.00	8.68 ± 0.35	21.96 ± 2.92
Brown Rice-1	11.58 ± 0.81	21.63 ± 1.59	11.90 ± 0.42	22.15 ± 0.78	11.74 ± 0.23	21.89 ± 0.37
Munibhog	11.20 ± 1.56	27.45 ± 1.45	10.10 ± 0.00	23.70 ± 0.01	10.65 ± 0.78	25.58 ± 2.65
Danighoda	11.23 ± 0.46	23.53 ± 0.92	13.00 ± 0.99	22.48 ± 0.04	12.11 ± 1.26	23.00 ± 0.74
Saraitolia	9.53 ± 0.67	22.68 ± 1.45	10.75 ± 0.35	22.35 ± 0.64	10.14 ± 0.87	22.51 ± 0.23
Pangudi Goindi	9.10 ± 19.8	22.95 ± 0.92	10.50 ± 0.01	22.30 ± 0.00	9.80 ± 0.99	22.63 ± 0.46
Safri-17	10.00 ± 2.12	21.65 ± 5.3	11.50 ± 0.00	17.90 ± 0.01	10.75 ± 1.06	19.78 ± 2.65
Dubraj	10.08 ± 0.88	22.53 ± 1.66	11.15 ± 0.21	20.18 ± 1.66	10.61 ± 0.76	21.35 ± 1.66
Byalo	12.23 ± 1.73	27.15 ± 4.31	11.00 ± 0.00	24.10 ± 0.00	11.61 ± 0.87	25.63 ± 2.16
Badshahbhog	16.55 ± 2.76	23.28 ± 1.94	15.43 ± 0.39	24.68 ± 1.31	15.99 ± 0.80	23.98 ± 0.99
Jonyaphool	10.63 ± 1.66	25.98 ± 1.94	10.23 ± 0.74	21.45 ± 1.48	10.43 ± 0.28	23.71 ± 3.20
Mohlyanbanko	11.45 ± 0.64	18.42 ± 2.57	12.43 ± 0.25	20.58 ± 1.87	11.94 ± 0.69	19.50 ± 1.53
Kanakbhog	9.40 ± 1.13	16.77 ± 0.90	8.76 ± 0.52	18.50 ± 0.19	9.08 ± 0.45	17.63 ± 1.22
Shwet Ganga	10.83 ± 0.75	22.66 ± 2.04	10.38 ± 0.04	21.03 ± 1.45	10.60 ± 0.32	21.84 ± 1.16
Matko Dhan	12.55 ± 1.06	23.88 ± 2.3	11.13 ± 1.03	18.53 ± 3.29	11.84 ± 1.01	21.20 ± 3.78
Suapankhi	9.28 ± 1.24	21.48 ± 2.93	16.50 ± 3.82	20.30 ± 0.42	12.89 ± 5.11	20.89 ± 0.83
Jhimiprass	11.03 ± 0.67	26.23 ± 1.87	11.50 ± 0.00	24.90 ± 0.01	11.26 ± 0.34	25.56 ± 0.94
Jauphool	11.30 ± 0.14	20.80 ± 5.09	11.20 ± 0.00	17.20 ± 0.01	11.25 ± 0.07	19.00 ± 2.55
Kalajeera	11.92 ± 0.59	22.92 ± 1.11	11.50 ± 0.01	23.70 ± 0.00	11.71 ± 0.29	23.31 ± 0.56
Chhindmauri	14.83 ± 4.35	25.10 ± 0.71	17.90 ± 0.00	24.60 ± 0.00	16.36 ± 2.17	24.85 ± 0.35
Motipeera	10.40 ± 0.85	22.12 ± 1.11	10.10 ± 0.14	22.83 ± 0.04	10.25 ± 0.21	22.47 ± 0.50
Pedgadi	11.72 ± 1.01	24.16 ± 3.05	11.00 ± 0.00	23.00 ± 1.41	11.36 ± 0.51	23.58 ± 0.82
Sonagathi	9.80 ± 0.01	19.30 ± 3.25	10.85 ± 0.49	17.83 ± 0.39	10.33 ± 0.74	18.56 ± 1.04
Jana Dhan	11.68 ± 4.35	21.23 ± 10.2	10.18 ± 0.74	17.08 ± 1.45	10.93 ± 1.06	19.15 ± 2.93
Rela Dhan	10.20 ± 2.97	21.56 ± 5.32	9.72 ± 0.69	20.98 ± 3.32	9.96 ± 0.34	21.27 ± 0.41
Ram Shree	10.11 ± 2.84	17.41 ± 1.54	11.85 ± 1.77	16.33 ± 1.03	10.98 ± 1.23	16.87 ± 0.77
Kari Alcha	9.27 ± 1.36	21.68 ± 0.45	9.88 ± 0.74	23.50 ± 0.71	9.57 ± 0.43	22.59 ± 1.29
Jhilli Safri	11.55 ± 0.35	22.63 ± 2.86	11.58 ± 0.11	20.60 ± 0.00	11.56 ± 0.02	21.61 ± 1.43
Siyar	12.77 ± 0.65	22.28 ± 2.75	14.95 ± 0.54	21.18 ± 2.75	13.86 ± 1.54	21.73 ± 0.78
Karhani	12.92 ± 0.83	22.11 ± 2.81	9.90 ± 0.97	22.50 ± 0.71	11.41 ± 2.14	22.31 ± 0.28
Nandel	10.60 ± 2.97	21.75 ± 1.2	12.25 ± 1.77	22.03 ± 0.53	11.43 ± 1.17	21.89 ± 0.19
Safri	11.18 ± 1.45	22.60 ± 1.27	10.28 ± 1.73	21.73 ± 0.56	10.73 ± 0.63	22.16 ± 0.62
Dhaura Mundariya	9.64 ± 1.61	15.00 ± 1.41	9.25 ± 0.35	16.55 ± 1.2	9.45 ± 0.28	15.78 ± 1.10
Karhani-2	10.26 ± 0.04	20.69 ± 0.53	9.87 ± 0.90	21.47 ± 0.19	10.06 ± 0.28	21.0 ± 0.55
Sanchuriya	9.01 ± 1.40	18.00 ± 1.98	10.75 ± 0.35	18.05 ± 0.64	9.88 ± 1.23	18.03 ± 0.04
Alsakar	10.74 ± 1.90	20.71 ± 0.69	12.63 ± 1.52	21.95 ± 0.35	11.68 ± 1.33	21.33 ± 0.88
Luchai	11.30 ± 1.27	20.83 ± 1.38	11.06 ± 0.23	21.16 ± 1.34	11.18 ± 0.17	20.99 ± 0.23

Mai Dubraj	9.48 ± 0.11	21.65 ± 0.21	12.03 ± 1.24	22.18 ± 0.18	10.75 ± 1.80	21.91 ± 0.37
TCDM-1	13.00 ± 2.83	22.50 ± 0.71	19.48 ± 1.20	24.99 ± 0.48	16.24 ± 4.58	24.62 ± 0.52
Vikram-TCR	11.50 ± 0.71	18.35 ± 0.49	11.30 ± 0.14	18.33 ± 0.18	11.40 ± 0.14	18.34 ± 0.02
CG Jawaphool Trombay	10.96 ± 1.05	20.45 ± 0.64	9.68 ± 0.95	18.95 ± 0.92	10.32 ± 0.91	19.70 ± 1.06
Alsakar Mutant-3	11.07 ± 0.23	17.06 ± 1.20	12.18 ± 0.60	16.55 ± 0.64	11.62 ± 0.78	16.80 ± 0.36
Sanchuriya Mutant-6	11.32 ± 2.71	17.77 ± 2.03	13.53 ± 1.94	16.95 ± 1.06	12.42 ± 1.56	17.36 ± 0.58
Safri-17-5 Mutant	11.20 ± 1.13	17.86 ± 0.48	10.40 ± 0.00	18.95 ± 0.35	10.80 ± 0.57	18.41 ± 0.77
Safri-17-2-48 Mutant	20.83 ± 0.71	17.82 ± 0.26	22.19 ± 2.63	19.25 ± 0.10	21.51 ± 0.96	18.53 ± 1.01
Luchai Mutant-1	13.87 ± 0.52	21.82 ± 0.69	11.55 ± 0.92	21.85 ± 0.21	12.71 ± 1.64	21.83 ± 0.02
Dubraj Sel-1	10.67 ± 2.35	21.02 ± 0.45	10.50 ± 0.71	23.25 ± 1.20	10.58 ± 0.12	22.13 ± 1.58
CG Zinc Rice-1	9.70 ± 0.71	22.34 ± 1.33	8.31 ± 0.01	21.84 ± 1.19	9.01 ± 0.98	22.09 ± 0.35
CG Zinc Rice-2	12.50 ± 0.71	22.50 ± 0.71	13.00 ± 1.41	23.50 ± 0.71	12.50 ± 0.71	22.50 ± 0.71
Zinco Rice MS	12.07 ± 0.37	19.93 ± 1.80	9.93 ± 0.88	18.80 ± 1.13	11.00 ± 1.51	19.36 ± 0.80
Rajeshwari	16.03 ± 7.46	22.20 ± 0.57	11.49 ± 1.19	20.82 ± 0.73	13.76 ± 3.21	21.51 ± 0.98
Protazin	12.65 ± 0.35	18.81 ± 0.69	12.81 ± 0.57	19.61 ± 0.40	12.73 ± 0.11	19.21 ± 0.57

Table 5: Top performing genotypes for Fe contents in rice genotypes

	Season 1 (*Kharif* season 2020)		Season 2 (*Rabi* season 2020-21)		Season 3 (*Kharif* season 2021)	
	Genotypes	Fe (ppm) content in brown rice	Genotypes	Fe (ppm) content in brown rice	Genotypes	Fe (ppm) content in brown rice
1	Safri-17-2-48 Mutant	20.83 ± 0.71	Safri-17-2-48 Mutant	22.19 ± 2.63	Safri-17-2-48 Mutant	21.51 ± 0.96
2	Badshahbhog	16.55 ± 2.76	TCDM-1	19.48 ± 1.20	Rudra	16.50 ± 2.76
3	Rajeshwari	16.03 ± 7.46	Rudra	18.45 ± 3.04	Chhindmauri	16.36 ± 2.17
4	Chhindmauri	14.83 ± 4.35	Chhindmauri	17.90 ± 0.00	TCDM-1	16.24 ± 4.58
5	Rudra	14.55 ± 3.61	Suapankhi	16.50 ± 3.82	Badshahbhog	15.99 ± 0.80

Table 6: Top performing genotypes for Zn contents in rice genotypes

	Season 1		Season 2		Season 3	
	Genotypes	Zn (ppm)content in brown rice	Genotypes	Zn (ppm)content in brown rice	Genotypes	Zn (ppm)content in brown rice
1	Munibhog	27.45 ± 1.45	Loktimachi	25.80 ± 0.01	Loktimachi	25.80 ± 0.01
2	Byalo	27.15 ± 4.31	TCDM-1	24.99 ± 0.48	Byalo	25.63 ± 2.16
3	Jhimiprass	26.23 ± 1.87	Jhimiprass	24.90 ± 0.01	Munibhog	25.58 ± 2.65
4	Jonyaphool	25.98 ± 1.94	Badshahbhog	24.68 ± 1.31	Jhimiprass	25.56 ± 0.94
5	Loktimachi	25.80 ± 0.02	Chhindmauri	24.60 ± 0.00	Chhindmauri	24.85 ± 0.35

uptake (Kong *et al*., 2022), their translocation, and the maintenance of homeostasis in grain is essential for the genetic biofortification of rice, complete knowledge of these processes in rice is still lacking (Olsen and Palmgren, 2014; Kobayashi *et al.,* 2021).

Ultimately, the concentration of micronutrients in rice grain depends on the genotype's efficiency to uptake and translocate the nutrients from root to the grain, which involves many complex physiological processes at different levels. Therefore, rice genotypes to be developed should have the genetic potential and physiological efficiency to utilize the available micronutrients from the soil (Olsen and Palmgren, 2014). Knowledge of mineral localization within rice grains is important for understanding the role of different elements in seed development and facilitating the biofortification of seed micronutrients to enhance seeds' values in human diets (Lu *et al.*, 2013).

To initiate a breeding program to improve or develop any variety depends on the availability of variation in germplasm for the targeted trait, which can be further used in genetic studies, to make crosses, molecular marker development, and to understand the basis of the uptake process for micronutrients. A large genetic variation for grain iron and zinc has been observed in different germplasm of rice and it was exploited in breeding programs. In our study Iron content in season I, ranged between 8.13 ± 0.46 to

Table 7: Analysis of variance for stability of iron and zinc content among rice genotypes

Source of variation	*Mean sum of squares*		
	Degree of Freedom	*Fe (ppm)*	*Zn (ppm)*
Replications within Environment	3	5.297 **	8.525 **
Genotypes	57	14.009 **	18.289 **
Env.+ (Gen.* Env.)	116	1.029 **	1.043 **
Environments	2	2.706 **	3.488 **
Gen.* Env.	114	0.999 **	1.000 **
Environments (Lin.)	1	5.412 **	6.977 **
Gen.* Env. (Lin.)	57	1.997 **	1.987 **
Pooled Deviation	58	0.001	0.013
Pooled Error	171	1.228	1.638
Total	173	5.305	6.725

20.83 ± 0.71 ppm and zinc content between 14.41 ± 1.29 to 27.45 ± 1.45 ppm in brown rice. Similarly, in season II, Iron content ranged from 8.31 ± 0.01 to 22.19 ± 2.63 ppm and zinc from 16.33 ± 1.03 to 25.80 ± 0.01 ppm. Interestingly, the range of micronutrients were slightly higher in season III, *viz*., 8.68 ± 0.35 to 21.51 ± 0.96 ppm for iron content and 15.38 ± 1.37 to 25.80 ± 0.01 ppm for zinc content. Zhang *et al.,* (2018) experimented on a set of 698 rice germplasms and found that the iron concentration ranged between 0.9 to 9.1 µg/g while zinc concentration ranged from 5.8 to 29.6 µg/g in the polished rice. Similarly, Roy and Sharma (2014) found ample variation for Fe and Zn content in 84 rice cultivars and reported that Iron content varied between 0.25 to 34.8 µg/g and zinc content from 0.85 to 195.3 µg/g where local cultivars had the highest iron content. The grain iron content was found to be relatively higher in the second season with the maximum values of 22.19 ± 2.63

Table 8: Mean performance and stability parameters for Iron content (ppm) and Zinc content (ppm) of rice genotypes

Genotypes	Fe (ppm)			Zn (ppm)		
	Mean (µ)	βi	σ^2di	Mean (µ)	βi	σ^2di
Red Barhasal	9.54	6.54**	-1.30 NS	18.74	-9.33**	-1.75 NS
Rudra	16.50	9.03**	-1.30 NS	21.94	7.80*	-1.76 NS
Tulsibhog	12.50	-2.31**	-1.30 NS	22.31	0.05**	-1.76 NS
Lokti musi	12.00	0.93 NS	-1.30 NS	25.80	0.00**	-1.76 NS
Odha dhan	11.39	7.46**	-1.30 NS	15.38	-3.95**	-1.76 NS
Gathuan dhan	11.08	-1.04**	-1.30 NS	22.63	-2.04**	-1.76 NS
Raja bagla	8.68	-1.15**	-1.30 NS	21.96	8.41*	-1.75 NS
Brown Rice-1	11.74	0.75*	-1.30 NS	21.89	-1.07**	-1.76 NS
Munibhog	10.65	-2.55**	-1.30 NS	25.58	7.64*	-1.76 NS
Danighoda	12.11	4.11**	-1.30 NS	23.00	2.14*	-1.76 NS
Saraitolia	10.14	2.84**	-1.30 NS	22.51	0.66*	-1.76 NS
Pangudi Goindi	9.80	3.24**	-1.30 NS	22.63	1.33*	-1.76 NS
Safri-17	10.75	3.47**	-1.30 NS	19.78	7.64*	-1.76 NS
Dubraj	10.61	2.49*	-1.30 NS	21.35	4.79*	-1.76 NS
Byalo	11.61	-2.84**	-1.30 NS	25.63	6.22*	-1.76 NS
Badshahbhog	15.99	-2.60**	-1.30 NS	23.98	-2.85**	-1.76 NS
Jonyaphool	10.43	-0.93**	-1.30 NS	23.71	9.22*	-1.75 NS
Mohlyanbanko	11.94	2.26*	-1.30 NS	19.50	-4.40**	-1.76 NS
Kanakbhog	9.08	-1.48**	-1.30 NS	17.63	-3.53**	-1.76 NS
Shwet Ganga	10.60	-1.05**	-1.30 NS	21.84	3.33*	-1.76 NS
Matko Dhan	11.84	-3.30**	-1.30 NS	21.20	10.91*	-1.75 NS
Suapankhi	12.89	16.72**	-1.29 NS	20.89	2.40*	-1.76 NS
Jhimiprass	11.26	1.10 NS	-1.30 NS	25.56	2.70*	-1.76 NS
Jauphool	11.25	-0.23**	-1.30 NS	19.00	7.34*	-1.76 NS
Kalajeera	11.71	-0.96**	-1.30 NS	23.31	-1.60**	-1.76 NS
Chhindmauri	16.36	7.12**	-1.30 NS	24.85	1.02 NS	-1.76 NS
Motipeera	10.25	-0.69**	-1.30 NS	22.47	-1.45**	-1.76 NS

Pedgadi	11.36	-1.65**	-1.30^{NS}	23.58	2.36*	-1.76^{NS}
Sonagathi	10.33	2.43*	-1.30^{NS}	18.56	3.01*	-1.76^{NS}
Jana Dhan	10.93	-3.47**	-1.30^{NS}	19.15	8.46*	-1.75^{NS}
Rela Dhan	9.96	-1.11**	-1.30^{NS}	21.27	1.18^{NS}	-1.76^{NS}
Ram Shree	10.98	4.04**	-1.30^{NS}	16.87	2.21*	-1.76^{NS}
Kari Alcha	9.57	1.41*	-1.30^{NS}	22.59	-3.71**	-1.76^{NS}
Jhilli Safri	11.56	0.06*	-1.30^{NS}	21.61	4.13*	-1.76^{NS}
Siyar	13.86	5.03**	-1.30^{NS}	21.73	2.24*	-1.76^{NS}
Karhani	11.41	-6.99**	-1.30^{NS}	22.31	-0.80**	-1.76^{NS}
Nandel	11.43	3.82**	-1.30^{NS}	21.89	-0.56**	-1.76^{NS}
Safri	10.73	-2.07**	-1.30^{NS}	22.16	1.78*	-1.76^{NS}
Dhaura Mundariya	9.45	-0.90**	-1.30^{NS}	15.78	-3.16*	-1.76^{NS}
Karhani-2	10.06	-0.91**	-1.30^{NS}	21.08	-1.59**	-1.76^{NS}
Sanchuriya	9.88	4.03**	-1.30^{NS}	18.03	-0.10**	-1.76^{NS}
Alsakar	11.68	4.36**	-1.30^{NS}	21.33	-2.53**	-1.76^{NS}
Luchai	11.18	-0.56**	-1.30^{NS}	20.99	-0.67**	-1.76^{NS}
Mai Dubraj	10.75	5.90**	-1.30^{NS}	21.91	-1.07**	-1.76^{NS}
TCDM-1	16.24	15.0**	-1.30^{NS}	24.04	-5.04^{NS}	-1.21^{NS}
Vikram-TCR	11.40	-0.46**	-1.30^{NS}	18.34	0.05**	-1.76^{NS}
CG Jawaphool Trombay	10.32	-2.96**	-1.30^{NS}	19.70	3.06*	-1.76^{NS}
Alsakar Mutant-3	11.62	2.57*	-1.30^{NS}	16.80	1.03^{NS}	-1.76^{NS}
Sanchuriya Mutant-6	12.42	5.12**	-1.30^{NS}	17.36	1.66*	-1.76^{NS}
Safri-17-5 Mutant	10.80	-1.85**	-1.30^{NS}	18.41	-2.22***	-1.76^{NS}
Safri-17-2-48 Mutant	21.51	3.15*	-1.30^{NS}	18.53	-2.93**	-1.76^{NS}
Luchai Mutant-1	12.71	-5.36**	-1.30^{NS}	21.83	-0.07**	-1.76^{NS}
Dubraj Sel-1	10.58	-0.38**	-1.30^{NS}	22.13	-4.56**	-1.76^{NS}
CG Zinc Rice-1	9.01	-3.22**	-1.30^{NS}	22.09	1.02^{NS}	-1.76^{NS}
CG Zinc Rice-2	12.67	1.16^{NS}	-1.26^{NS}	22.83	-2.06^{NS}	-1.60^{NS}
Zinco Rice MS	11.00	-4.95**	-1.30^{NS}	19.36	2.29*	-1.76^{NS}
Rajeshwari	13.76	-10.50**	-1.30^{NS}	21.51	2.82*	-1.76^{NS}
Protazin	12.73	0.36**	-1.30^{NS}	19.21	-1.63**	-1.76^{NS}
Grand mean	11.63			21.17		

in the currentstudy. However, the seasonal variations for these elements were observed as reported earlier (Dixit *et al.,* 2019; Suman *et al.,* 2021). The variation in values may arise due to properties of grain such us relative position on the panicle (Su *et al.*, 2014), moisture content (Rao *et al*., 2014), number of aleurone layer (Sellappan *et al*., 2009), etc.; soil properties such as pH, organic carbon content and inherent availability of iron and zinc in the soil (Chandel *et al*., 2010). Furthermore, high significant interaction effect of genotype x environment on the content of these micronutrients has been reported by Chandel *et al.* (2010); and Suwarto (2011). In the current study, iron and zinc contents were found high in landraces and similar results were observed and reported earlier (Anandan *et al.,* 2011; Nachimuthu *et al.,* 2014; Parikh *et al*., 2019). Sanjeeva *et al.,* (2020) experimented on various sets of germplasm and reported that 99 genotypes from germplasm and 344 lines from mapping populations exhibited ≥ 28 mg/kg zinc content in polished rice, meeting the target zinc content set by HarvestPlus.

In this study, Safri mutant 17-2-48 has the highest iron content, a newly developed mutant from Safri 17 local variety. This mutant can be directly released as a new variety with high iron content. Munibhog and Loktimachi were identified for the highest zinc content. Thus, these genotypes open the possibilities for the exploitation as a donor in biofortification breeding programs and the identification of genomic positions associated with iron and zinc contents in grains. Though the variation in micronutrient content depends on several factors, the germplasm stock in rice has sufficient variation to exploit for developing stable lines. As micronutrient malnutrition poses a significant global challenge, the development of micronutrient enriched genotypes serves as the need of the hour. The top-performing genotypes identified in this research will be useful for selecting and breeding lines with enriched micronutrient status.

A stable variety for grain nutrition quality is an important consideration for biofortification. The genotype, environment, linear genotype X environment and non-linear genotype X environment interaction when tested against pooled deviation, showed significance for both iron and zinc content in the brown rice. Similar significance for zinc and iron content have earlier been reported by Oikeh *et al*., (2004), Prasanna *et al*., (2011), Suwarto (2011), Velu *et al*., (2012) and Ajmera *et al*., (2017). For iron content the genotypes Jhimiprass, Loktimachi and CG Zinc Rice 2 did not deviate from unity of regression, showing their stable and predictable performance. Therefore, they can be recommended for growing in all environments ranging from optimum to marginal environments. Similarly, for Zinc content the genotypes Rela Dhan, Chhindmauri and CG Zinc Rice 1 did not deviate significantly from the unity of regression. Therefore, these genotypes can be grown in any environment to get more or less stable performance in terms of the Zinc content in brown rice. Genotypes 32 and 27 recorded significant deviation from unity and less than unity ($b < 1$) for Iron and Zinc content, respectively, showing their below-average performance. These genotypes cannot take full advantage of the favorable conditions when grown in the optimum environment. Therefore, they are suitable for cultivation in marginal or suboptimal environments such as *Rabi* season. However, 23 and 25 genotypes for iron and zinc content have recorded significant deviation from unity and more than unity ($b > 1$). When grown in the optimum environment with the most suitable condition, such genotypes will result in above-average performance in terms of the micronutrient content in brown rice. Therefore, to take the best advantage, these varieties can be grown in *Kharif* season at regions having good soil fertility.

Conclusion

Existing genetic variation in rice germplasm offers scope for developing nutrient-rich varieties. Notably, there was an ample difference in Fe and Zn content suggesting the existence of genetic potential to increase the concentration of these micronutrients in rice grain. Micronutrient-rich rice genotype *viz*., Safri-17-2-48 Mutant, Loktimachi, Chhindmauri, Badsahbhog, Rudra, Danighoda, Suapankhi, TCDM-1, and Luchai Mutant-1 identified through this study may be further utilized in the breeding program as a donor for development of nutrient-rich or biofortified rice varieties. Additionally, genotypes Jhimiprass, Loktimachi, and CG zinc Rice 2 for iron content and Rela Dhan, Chhindmauri, and CG zinc rice 1 for zinc content exhibited an average response to the environment, making them suitable for all seasons.

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