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FULL-LENGTH RESEARCH ARTICLE

Variation in Iron and Zinc Content in Traditional Rice Genotypes

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Abstract Micronutrient deficiency is considered as one of the emerging challenges to food and nutrition security particularly in developing countries and there is a growing realization of a food based approach for addressing this. The wide diversity of plant genetic resources provides opportunity for identifying micronutrient-rich genotypes for direct use or for genetic enhancement of staple crops using breeding strategies. In the present study, we have collected 159 rice germplasm from different agroclimatic regions and analysed their iron and zinc content growing them in a single location for both brown and polished rice and checked consistency of micronutrient density over two seasons. Grain micronutrient content analysis was done through the non-destructive method, energy-dispersive X-ray fluorescence spectrophotometry. Considerable variation was observed in the micronutrient density among the germplasm assessed. Iron concentration varied from 6.9 to 22.3 mg/kg, whereas zinc concentration ranged from 14.5 to 35.3 mg/kg in unpolished, brown rice. There was substantial loss of iron than zinc, upon polishing. The loss of iron ranged from 16 to 97.4%, whereas that of zinc from 1 to 45%. Time series analysis indicates that the micronutrient concentration in a given genotype remains more or less constant when cultivated under the similar environmental conditions. Moreover, there is a moderate positive correlation between iron and zinc content of brown (r = 0.5) and polished rice (r = 0.3) indicating the probability of simultaneous effectual selection for both the micronutrients.

Keywords Rice · Brown · Polishing · X-ray florescence · Germplasm · Micronutients · Iron · Zinc

Abbreviations

Fe	Iron
Zn	Zinc
Cu	Copper
Mn	Manganese
ED-XRF	Energy-dispersive X-ray florescence spectrophotometer
ICP-OES	Inductively coupled plasma optical emission spectrometry
AAS	Atomic absorption spectrometry

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Introduction

Optimal health is ensured by a diverse, well-balanced diet containing both macronutrients and micronutrients. Macronutrients—carbohydrates, lipids and proteins constitute the bulk of daily food basket and the primary source of energy. Micronutrients are vitamins or minerals present in trace amounts and are not the energy source, but are nevertheless vital for good health [8]. Cassava, wheat, rice and corn constitute the primary staple foods across the world but are poor sources of many essential micronutrients.

Approximately 3.5 billion people depend on rice for nearly 20% of their daily calories, and Asia accounts for 90% of global rice consumption. Rice is usually preferred in polished form (white rice). Rice polishing, however, affects the nutritional quality of rice grains through the removal of husk from paddy and removal of outer bran layers of brown rice. Hence, fewer nutrients are present in rice that is consumed. In fact, rice polishing accounts for the largest loss of iron in the rice grain. Micronutrient deficiency or "hidden hunger" surfaced as a serious world problem during the late 1970s and early 1980s and has only worsened [3]. Hidden hunger is inherently difficult to apprehend and devastating. Hidden hunger stunts physical and intellectual growth, weakens the immune system and can lead to death. It inflicts economic mayhem as well, trapping countries into cycles of poor nutrition, poverty and lost productivity that hinders the economic growth. Micronutrient deficiencies account for approximately seven percentage of the global disease burden annually [22].

Iron and zinc are important micronutrients and are crucial for tissue growth and maintenance, wound healing, prostaglandin production, cognitive functions, bone mineralization, sperm production, proper function of thyroid and immune system, blood clotting, foetal growth and metabolic activity of enzymes (as cofactor). An extensive National Family Health Survey (NFHS-4)[1] for 2015–2016 covering 13 states and two union territories of India found a staggering increase in anaemia across all ages even though other nutrition parameters seemed to have improved over the last decade. According to the World Health Organization (WHO), there are two billion people with anaemia in the world and half of the anaemia is due to iron deficiency [40]. Anaemia is a late indicator of iron deficiency, so it is estimated that the prevalence of iron deficiency is 2.5 times that of anaemia [40, 42]. Anaemia is a major health problem in India.

Hidden hunger has been targeted for intervention, given the immensity of the issue posed by these deficiencies. Micronutrient content of the staple diets among the economically underprivileged (rice, wheat, maize, beans, cassava and sweet potatoes) is a major research strategy, mainly through breeding and biotechnological approaches [17]. Making dietary changes using local foods is usually arduous when one is economically underprivileged. Breeding for micronutrient-dense cereals (biofortification) is considered most economical and effective for tackling micronutrient deficiencies. This is an economical and sustainable approach that neither demands a change in eating habits nor imposes recurring costs that co-occur with supplement and fortification strategies. Breeding for a micronutrient concentration with a biological impact, but without compromising agronomic traits, has been evinced for crops including pearl millet and sweet potato [18, 38].

Rice, being the primary staple food for about half of the world's population, is an appropriate crop to be enhanced for its micronutrient content. Other reasons favouring biofortification of rice are its wide availability and genetic variability for micronutrients that make it suitable for breeding programs. Also, it is important to identify genetically superior germplasm for conservation purposes. Bangladesh rice research institute (BRRI) has released a new zinc-enriched rice variety named BRRI Dhan 62, which is an early maturing and high-yielding variety by employing breeding strategies [41].

Estimation of mineral content by energy-dispersive X-ray florescence spectrophotometer (ED-XRF) is more advantageous than colorimetric method, or atomic absorption spectrometry (AAS), and inductively coupled plasma optical emission spectrometry (ICP-OES). Colorimetric method is a qualitative method, whereas, among the quantitative methods, ICP-OES and AAS are destructive methods. Laboratory bench top ED-XRF is the most suitable high throughput screening method for application in quantity evaluation of large sample size owing to the advantages of high precision, non-destructive, high efficiency, rapidity, low cost and easy operation [23, 25, 27, 28]. Hence, considering the advantages of grain micronutrient content analysis through non-destructive method, grain zinc and iron content were analysed with ED-XRF in this study. The objectives of this study were to quantify the micronutrients (Fe, Zn) in brown rice of 159 genotypes in two seasons by ED-XRF grown in an uniform field, to eliminate micronutrient variability in soil, assess the extent of rice grain micronutrient density variations in two successive *Rabi* seasons and assess and quantify the impact of polishing on the loss of rice grain micronutrients.

Secondary centres of rice diversity in India include the Western Ghats and the Jeypore tract [24, 30, 35, 37] from where the accessions have been collected for the present investigation. The overall goal of the research study is to determine the extent of germplasm variability for complex quantitative traits such as micronutrient density in the rice grain. ED-XRF studies are accurate and can therefore further the understanding towards developing nutrient-rich rice varieties.

Materials and Methods

Experimental Site

A total of 159 genotypes consisting indigenous land races, wild rice and high-yielding varieties collected from four different states of India were used in this study (Table 1). These genotypes were grown in MSSRF field-site at Kalpakkam, on the East coast of Indian peninsula, during November–January (*Rabi* season) as few genotypes are photosensitive. The coordinates for this site are 12.5576°N and longitude of 80.1754°E. Kalpakkam has a tropical

[1] http://rchiips.org/NFHS/factsheet_NFHS-4.shtml. Accessed 14 September 2017.

climate and is considered to be *As*[2] according to the Köppen–Geiger climate classification. The average annual temperature in Kalpakkam is 28.4 °C. Precipitation here averages 1202 mm. The soil in the area is essentially sand with sandy clay and soft disintegrated rock with a pH of 5.6. The experiment was laid out in augmented block design with a spacing of 20 × 20 cm. Regular agricultural practices were followed as per standard recommendations by the Krishi Vigyan Kendra, Government of India.

Plant Materials

After harvest, all samples were processed using a husker and polisher devoid of iron or zinc, at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. All samples were polished for 45 s. The samples were cleaned, and whole, intact grains free from any debris were used for analyses.

Iron and Zinc Content Estimation

Iron and zinc were estimated using non-destructive ED-XRF (OXFORD Instruments X-Supreme 8000) as per HarvestPlus guidelines [32]. Paltridge et al. [25] reported that a minimum of 4 g sample mass was required for both rice and pearl millet. Rao et al. [28] found 5 g to be optimum for XRF screening. In this study, clean sample weighing 5 g was transferred to clean sample cups. For uniform distribution of sample, the sample cups were gently shaken before analyses. Analysis time per sample was approximately 3.1 min which incorporated 60 s acquisition time for each Fe and Zn conditions and a 66 s "dead time" during which the XRF establishes the measurement parameters. All samples were measured in triplicates.

Statistical Analysis

All statistical analyses were done using the softwares PAST [14] and Microsoft EXCEL.

Results

Grain Iron and Zinc Content in Brown Rice

A total of 159 genotypes consisting indigenous land races, wild rice and high-yielding varieties were analysed for iron and zinc concentration in brown and polished rice (Table 1). Iron concentration in the brown rice ranged from 8.4 to 22.6 mg/kg in season 1 and from 6.9 to 22.3 mg/kg in season 2, whereas zinc concentration ranged from 14.6 to 39.2 mg/kg in season 1 and from 14.5 to 35.5 mg/kg in season 2 (Fig. 1). The mean concentration of iron in brown rice was 12.7 mg/kg in season 1 and 13.2 mg/kg in season 2. The mean values of zinc concentration in two seasons were recorded as 24.1 mg/kg and 23.8 mg/kg. The coefficient of variation for iron concentration in brown rice in season 1 was 16.3% and 18.8% in season 2. The coefficient of variation of zinc concentration in brown rice was 15.5% and 17.7%, respectively, in seasons 1 and 2. The genotypes T4 (Kuliveelichan) and T3 (Karuppukavuni) contained the lowest levels of iron, respectively, in seasons 1 and 2. The highest concentration of iron in season 1 was found in genotype O96 (Patrali) while in genotype KL8 (Kaima) in season 2. In zinc estimation, the genotype Jaya (H10) had the highest concentration in season 1 and the landrace Matidhan (O83) in season 2. The genotype H5 (Chaitanya) had the least concentration of zinc in both the seasons.

To ascertain the randomness of the data sets obtained from two seasons, autocorrelations were computed. The autocorrelations are significantly nonzero signifying that the correlation is non-random (Fig. 2).

Effect of Polishing

After polishing, large variation in iron content was observed, than in zinc, among the varieties analysed (Table 1). Compared with zinc (∼ 1 to 45%), loss of iron (∼ 16 to 97.4%) was more than twice, after 45 s of polishing (Fig. 3). The association between iron concentration in brown and polished rice is much weaker ($r = 0.45$) than that of zinc content ($r = 0.78$). The concentrations of zinc in brown and polished rice are in tandem.

Classification of Genotypes Based on Micronutrient Density

The 159 genotypes could be categorized based on the iron and zinc content after dehusking and polishing as low, moderate and high (Fig. 4). Brown rice genotypes with iron content less than 12 mg/kg were categorized as low (41 genotypes), iron content between 12.1 and 15 mg/kg was grouped under moderate (99 genotypes), and more than 15.1 mg/kg (19 genotypes) were considered high. Similarly for zinc, less than 20 mg/kg (18 genotypes) was considered low, between 20.1 and 29 mg/kg as moderate (126 genotypes), and more than 29 mg/kg was grouped as high.

Polished rice genotypes with iron content less than 4 mg/kg were placed in low category (72 genotypes), 4.1–8 mg/kg were considered moderate (69 genotypes) and more than 8.1 mg/kg as high (18 genotypes). With respect to zinc in polished rice, less than 16 mg/kg (33 genotypes) were grouped as low, between 16.1 mg/kg and 25 mg/kg as

[2] https://www.pmfias.com/climatic-regions-of-india-stamps-koeppens-classification/. Accessed 14 September 2017.

Table 1 Genotypes used in the study and their iron and zinc content

Acc. no.	Genotype	State	Br Fe S1[a]	Br Fe S2[a]	Pol Fe[a]	%Loss Fe	Br Zn S1[a]	Br Zn S2[a]	Pol Zn[a]	%Loss Zn
O1	ALASIKIBA	OD	10 ± 0.03	16.2 ± 0.02	3.4 ± 0	79	20.5 ± 0.03	20.9 ± 0.04	14.5 ± 0.08	30.7
O2	ASAMCHUDI	OD	12.8 ± 0.01	11.5 ± 0	5.2 ± 0.07	54.8	23.1 ± 0.06	21.3 ± 0	16.6 ± 0.01	22.3
O3	BADADHAN	OD	13.8 ± 0.02	14.5 ± 0	7.4 ± 0.02	49	23.4 ± 0.07	26.9 ± 0.15	20.4 ± 0.01	24.2
O4	BADALI	OD	14.3 ± 0.18	14.9 ± 0.01	2.5 ± 0.01	83.6	30.2 ± 0.11	33.7 ± 0.09	26.8 ± 0	20.5
O5	BADALOCHI	OD	12.3 ± 0.03	12.9 ± 0.02	7.1 ± 0.01	44.7	25.3 ± 0.17	23.9 ± 0.01	20.3 ± 0.06	15.3
O7	BANKEI	OD	16.9 ± 0.05	14.2 ± 0.02	6.5 ± 0	54.1	23.6 ± 0.01	23.6 ± 0.01	20.4 ± 0.06	13.8
O8	BANSKANTHIA	OD	13.4 ± 0.07	15 ± 0.01	8 ± 0.02	46.8	27.9 ± 0.07	25.6 ± 0.04	22.9 ± 0.06	10.4
O9	BARAMASI	OD	11.8 ± 0.11	16 ± 0.03	3.4 ± 0.01	78.8	25.2 ± 0.26	24.1 ± 0.06	18.5 ± 0.08	23.2
O10	BASNAMUNDI	OD	11.7 ± 0.11	15.2 ± 0.03	3.2 ± 0	78.9	26.2 ± 0.04	26.6 ± 0	24.9 ± 0.04	6.4
O11	BASTUBHOGA	OD	11.8 ± 0.07	12.6 ± 0.02	3.7 ± 0.01	70.5	24.7 ± 0.09	24.2 ± 0.02	18.5 ± 0.03	23.8
O12	BASUBHOGA	OD	13 ± 0.09	14.6 ± 0	9.6 ± 0.01	34.6	20.2 ± 0.07	21.7 ± 0.01	20.2 ± 0.08	6.9
O13	BAUNSADUBRAJ	OD	13.2 ± 0.02	13.1 ± 0.01	9.9 ± 0	24.4	27.5 ± 0.05	27.8 ± 0.03	26.3 ± 0.03	5.2
O14	BAYAHUNDAR	OD	13.5 ± 0.02	15.7 ± 0.01	8.2 ± 0.02	47.8	27.3 ± 0.09	31.7 ± 0.04	25.6 ± 0.03	19.2
O15	BEDAGURUMUKHI	OD	12.3 ± 0.02	12.5 ± 0.01	2.6 ± 0.01	79.2	22.6 ± 0.1	20.5 ± 0.08	17.7 ± 0.02	13.7
O16	BEDAMANKADA	OD	12.5 ± 0.08	17 ± 0.04	3.1 ± 0.03	81.8	29.7 ± 0.21	29.8 ± 0.03	20.8 ± 0.11	30.1
O17	BHANDAMASHURI	OD	11 ± 0.08	9.7 ± 0.02	2.5 ± 0.03	74.7	18.1 ± 0.01	19.2 ± 0.07	13.2 ± 0.02	31.3
O18	BHATACHUDI	OD	11.4 ± 0.04	11.1 ± 0.02	4.1 ± 0.01	63.5	21.2 ± 0.13	20.8 ± 0.02	13.4 ± 0.01	35.6
O19	BHATAGUNDA	OD	13.1 ± 0.01	12.8 ± 0.01	4.1 ± 0.01	68.4	25.7 ± 0.09	22.8 ± 0.02	18.4 ± 0.04	19.3
O20	BHATAGURUMUKHI	OD	12.4 ± 0.06	13 ± 0.02	6.6 ± 0.01	49.2	21.3 ± 0.15	26.1 ± 0.08	20 ± 0.07	23.4
O21	BHATAMALLI	OD	14.6 ± 0.12	14.7 ± 0.03	3.6 ± 0.02	75.4	30.2 ± 0.12	32.2 ± 0.12	27.2 ± 0.03	15.7
O22	BHATASAPURI	OD	8.5 ± 0.02	8.5 ± 0.03	1.4 ± 0.02	83.5	19.9 ± 0.01	20.2 ± 0.03	13.8 ± 0.04	31.9
O23	BHUDEI	OD	11.4 ± 0.03	14.1 ± 0.02	3.7 ± 0	73.7	27.1 ± 0.13	20.2 ± 0.06	16.3 ± 0.02	19.4
O24	BIDIDHAN	OD	12.8 ± 0.03	13.2 ± 0.01	5.1 ± 0.03	61.4	25.9 ± 0.33	25.6 ± 0.01	22.1 ± 0.01	13.9
O26	BODIKABURI	OD	10.5 ± 0.18	15.3 ± 0.07	4.5 ± 0	70.5	19.4 ± 0.09	23.4 ± 0.02	22.7 ± 0.11	3
O27	BUTKI	OD	12.9 ± 0.06	10.2 ± 0.04	7.4 ± 0.01	27.6	25.4 ± 0.1	21.7 ± 0.04	19.2 ± 0.01	11.8
O28	CHATIACHUDI	OD	12.3 ± 0.04	14.2 ± 0.01	4.4 ± 0.01	69.4	26.4 ± 0.15	30.4 ± 0.08	20.8 ± 0.02	31.6
O29	CHEPTIMASURI	OD	13.5 ± 0.03	13.8 ± 0.16	6.4 ± 0.01	53.8	19.5 ± 0.15	18.1 ± 0.08	17.5 ± 0.04	3.3
O30	CHETEK	OD	12 ± 0.08	13 ± 0.13	3.8 ± 0	70.8	27.2 ± 0.09	26.5 ± 0.26	21.9 ± 0.02	17.2
O31	CHIKLAKOLI	OD	12 ± 0.02	13.1 ± 0.08	3.5 ± 0.04	73.6	20.1 ± 0.03	21.6 ± 0.01	14.4 ± 0.09	33.3
O32	CHUDIDHAN	OD	12.6 ± 0.01	12.3 ± 0.01	3.4 ± 0.01	72.4	25 ± 0.11	21.4 ± 0.02	17.8 ± 0	16.6
O33	DANGARBASUMATI	OD	15.5 ± 0.02	14.3 ± 0.01	3.1 ± 0.02	78.6	30.3 ± 0.15	26.4 ± 0.06	17.6 ± 0.06	33.4
O34	DHOBLAKUNDA	OD	10.6 ± 0.16	12.6 ± 0.02	3.4 ± 0.02	72.9	21.3 ± 0.3	28.5 ± 0.11	20.7 ± 0.06	27.2
O35	DOBLAKALIKUJI	OD	13.9 ± 0.01	13.8 ± 0.01	3 ± 0.02	78.6	25.5 ± 0.06	26.9 ± 0.12	21.9 ± 0.03	18.8
O36	DOKRAKUJI	OD	13.8 ± 0.08	13.4 ± 0.01	4.1 ± 0.01	69.8	25.7 ± 0.01	28.4 ± 0.04	23.1 ± 0.11	18.7
O37	DOLOBHOGA	OD	13.8 ± 0.09	15 ± 0.03	4.9 ± 0.04	67.7	25.6 ± 0.05	27 ± 0.07	19.8 ± 0.04	26.7
O38	DUBRAJ	OD	12.1 ± 0	13.4 ± 0	6.6 ± 0.01	51.1	26.6 ± 0.04	28.8 ± 0.01	26.3 ± 0.06	8.5
O39	DUDHAMANI	OD	14.4 ± 0.04	15 ± 0.02	3.2 ± 0.02	78.9	24.1 ± 0.01	27 ± 0.02	20 ± 0.01	25.9
O40	FARMCHUDI	OD	11.5 ± 0.04	14.6 ± 0.02	3.7 ± 0.02	74.9	22.5 ± 0.13	20.4 ± 0.09	14.5 ± 0.02	29.2
O41	GADAKUTA	OD	11.9 ± 0.01	13.3 ± 0.03	5.6 ± 0.04	58.1	22.1 ± 0.1	24.9 ± 0.08	21.7 ± 0.08	12.7
O42	GANGABALI	OD	13.9 ± 0.01	14.3 ± 0.05	10.6 ± 0.04	26	21.2 ± 0.02	20.8 ± 0.01	20.6 ± 0.03	1
O44	GURUJI	OD	15 ± 0.02	14.2 ± 0.07	5.5 ± 0.02	61.1	31.7 ± 0.19	29.2 ± 0.03	21.9 ± 0.06	24.9
O45	GURUMUKHI	OD	14.2 ± 0.01	18.2 ± 0.02	3.8 ± 0.02	79.4	19.8 ± 0.04	25.2 ± 0.02	19.7 ± 0.04	22
O46	HALADI	OD	10.4 ± 0.06	12.7 ± 0.08	3.3 ± 0	74	23.6 ± 0.03	24.1 ± 0.04	21.4 ± 0.04	11.2
O47	HALADISARI	OD	10.9 ± 0.06	10.7 ± 0.03	2.7 ± 0.05	75.2	22 ± 0.07	23.1 ± 0.05	19.1 ± 0.03	17.1
O48	HALDICHUDI	OD	12.5 ± 0.05	16.2 ± 0.02	6.1 ± 0.01	62.3	25.2 ± 0.02	33.9 ± 0.07	29.3 ± 0.11	13.4
O49	HIRAKHANDI	OD	13.2 ± 0.03	16.4 ± 0.02	6.4 ± 0.04	61.3	28.4 ± 0.12	24.8 ± 0.45	22.7 ± 0.01	8.3
O50	IRPISONA	OD	11.7 ± 0.03	13.7 ± 0.01	4.6 ± 0.02	66.8	22.7 ± 0.06	24.3 ± 0.06	20.4 ± 0.04	16.1

Table 1 continued

Acc. no.	Genotype	State	Br Fe S1[a]	Br Fe S2[a]	Pol Fe[a]	%Loss Fe	Br Zn S1[a]	Br Zn S2[a]	Pol Zn[a]	%Loss Zn
O51	JAKASURA	OD	14.5 ± 0.02	14.8 ± 0.05	2.4 ± 0.01	83.7	26.5 ± 0.31	28.3 ± 0.11	21.4 ± 0.07	24.4
O52	JHILLI	OD	13.4 ± 0.06	12.2 ± 0.03	2.4 ± 0.03	80.7	23.8 ± 0.01	24 ± 0.02	20.3 ± 0.03	15.2
O53	JUMPACHUDI	OD	14.4 ± 0.01	11.4 ± 0.01	8.3 ± 0.03	27.2	20.3 ± 0.02	19.7 ± 0.01	19.3 ± 0.01	2.3
O54	KABURI	OD	10.4 ± 0.04	12.5 ± 0.03	1.1 ± 0.02	91.2	23.7 ± 0.03	26.3 ± 0.04	22.7 ± 0.05	13.9
O55	KAKAMARANGA	OD	12.8 ± 0.1	15.5 ± 0.07	3.3 ± 0.05	79	25.7 ± 0.16	24.8 ± 0.03	20.1 ± 0.06	19
O56	KALAMOHRA	OD	15.6 ± 0.06	15 ± 0.09	8.8 ± 0.02	41.3	24.9 ± 0.02	22.7 ± 0.01	20.2 ± 0.01	11
O57	KALACHUDI	OD	13.2 ± 0.01	12.7 ± 0.02	1.8 ± 0.03	86.2	28.7 ± 0.06	23.4 ± 0.12	15.9 ± 0.01	32.1
O58	KALAMA	OD	13.6 ± 0.02	11.4 ± 0.03	2.9 ± 0	74.4	25.5 ± 0.16	21.9 ± 0.01	16.9 ± 0.04	22.9
O59	KALAMATIA	OD	14.5 ± 0.01	17.6 ± 0.06	1.8 ± 0.02	89.8	28 ± 0.07	32.3 ± 0.07	19.2 ± 0.03	40.5
O60	KALAZIRA	OD	15.3 ± 0.1	14 ± 0	8 ± 0.01	42.9	25.6 ± 0.05	28.8 ± 0.07	26.8 ± 0.08	6.8
O61	KAMUMTANA	OD	11.2 ± 0.02	13.7 ± 0.03	2.4 ± 0.01	82.8	23.1 ± 0.01	23.8 ± 0.13	19.4 ± 0.12	18.5
O62	KANAKCHUDI	OD	12.7 ± 0.01	13.6 ± 0.03	2 ± 0.02	85.3	21 ± 0.04	27.3 ± 0.08	19.1 ± 0.06	29.9
O63	KANDULAKATHI	OD	12.6 ± 0.03	15.8 ± 0.06	1.1 ± 0.01	93	23.1 ± 0.01	21 ± 0.01	19.1 ± 0.02	9.1
O64	KANEI	OD	11.5 ± 0.06	11.9 ± 0.02	3.5 ± 0.01	70.5	23 ± 0	25.8 ± 0.03	20.7 ± 0.02	19.6
O65	KATIABANDHA	OD	13 ± 0.01	13.4 ± 0.01	0.4 ± 0.01	97.4	24.2 ± 0.11	22.8 ± 0.02	15.7 ± 0.03	31.2
O66	KERANDI	OD	13.4 ± 0.04	12.8 ± 0.02	1.2 ± 0.01	90.6	24.1 ± 0.01	25.5 ± 0.04	19.4 ± 0.04	24
O67	KHARADHAN	OD	12.8 ± 0.01	13 ± 0.03	3.2 ± 0.02	75.3	24 ± 0.11	19.6 ± 0.12	17 ± 0.01	13.3
O68	KHUJI	OD	11.4 ± 0.01	12.5 ± 0.06	2 ± 0.02	84.4	25.9 ± 0.1	18.8 ± 0.01	13 ± 0.07	30.7
O69	KOSIKAMAN	OD	13.8 ± 0.09	17.3 ± 0.05	2.7 ± 0.03	84.6	26.9 ± 0.1	30.8 ± 0.1	20.8 ± 0.06	32.6
O70	KUDESIRE	OD	11.8 ± 0.03	14.3 ± 0.01	3.4 ± 0.02	76.1	23 ± 0.11	28.9 ± 0.06	23.4 ± 0.02	19.1
O71	KUYERKULING	OD	14.4 ± 0.06	12.9 ± 0.02	6.2 ± 0.01	52.3	19.2 ± 0.04	18 ± 0.07	17.4 ± 0.06	3.3
O72	LACTIMASI	OD	15.7 ± 0.01	16.5 ± 0.02	7.4 ± 0.01	55	24.4 ± 0.01	28.8 ± 0.01	22.3 ± 0.08	22.6
O73	LAKUDIKUJI	OD	10.3 ± 0.07	12 ± 0.02	1.5 ± 0.01	87.5	26.2 ± 0.07	23.4 ± 0.05	17.3 ± 0.02	25.9
O74	LAXMIPATI	OD	10.6 ± 0.04	9.8 ± 0.06	0.5 ± 0.01	95.4	22.7 ± 0.11	21.4 ± 0.04	11.8 ± 0.04	45
O75	LEDIARI	OD	11.4 ± 0.02	17.4 ± 0.11	2.9 ± 0.01	83.6	24.4 ± 0.17	28.9 ± 0.01	21.8 ± 0.02	24.6
O76	LOCHI	OD	9.8 ± 0.02	10.2 ± 0.01	2.3 ± 0.02	77.8	128.6 ± 11.78	21.4 ± 0.06	17.7 ± 0.06	17.1
O77	LULUBAYA	OD	9.3 ± 0.03	8.7 ± 0.03	4 ± 0.02	54	22.7 ± 0.13	18.6 ± 0.03	17.2 ± 0.01	7.5
O78	MACHAKANTA	OD	13.3 ± 0.01	14.6 ± 0.01	6.1 ± 0.02	58.4	27.5 ± 0.03	31.4 ± 0.01	27.3 ± 0.04	13.1
O79	MAGURA	OD	12.6 ± 0.01	13.7 ± 0.02	3.6 ± 0.01	74.1	26.9 ± 0.1	23.8 ± 0.01	20.3 ± 0.03	14.5
O80	MALAGOINDI	OD	12 ± 0.04	13.9 ± 0.02	1.8 ± 0.02	87.1	24 ± 0.07	23.7 ± 0	18.1 ± 0.01	23.8
O82	MANDHAGANDHEI	OD	10.1 ± 0.01	11.5 ± 0.08	2.8 ± 0.04	75.7	18.9 ± 0.01	16.9 ± 0.01	14.9 ± 0.01	11.6
O83	MATIDHAN	OD	15.7 ± 0.02	20.3 ± 0.02	4.9 ± 0.03	75.8	31 ± 0.07	35.3 ± 0.05	26.2 ± 0.02	25.7
O84	MERLO	OD	14.3 ± 0.03	11.8 ± 0.03	3.8 ± 0.04	67.7	28.9 ± 0.1	22.6 ± 0.02	17 ± 0.07	24.8
O85	METRO	OD	13.1 ± 0.08	17 ± 0.03	5.5 ± 0.01	67.6	25.3 ± 0.13	24.6 ± 0.07	19.8 ± 0.02	19.3
O86	MILO	OD	10.1 ± 0.01	10.7 ± 0.01	2.4 ± 0.02	77.6	22 ± 0.06	24.7 ± 0.01	18.1 ± 0.02	26.7
O87	MUGUDI	OD	13.3 ± 0	12.9 ± 0.01	3.5 ± 0.01	73.2	26.1 ± 0.005	31 ± 0.01	25.6 ± 0.02	17.6
O89	NADIARASA	OD	13.9 ± 0.01	16 ± 0.02	10.6 ± 0.02	33.9	20.8 ± 0.02	19.8 ± 0.05	18.8 ± 0.02	5.1
O90	OSAGANTHULU	OD	12.7 ± 0.02	12.4 ± 0.02	7.5 ± 0.04	39.9	17.3 ± 0.04	17 ± 0.01	15.4 ± 0.04	9.4
O91	OZAN	OD	11.5 ± 0.02	13 ± 0.01	1.9 ± 0.04	85.4	22.1 ± 0.04	25.7 ± 0.05	16.8 ± 0.04	34.5
O92	PANDKAGUDA	OD	11.2 ± 0.04	13.1 ± 0.01	3.2 ± 0.03	76	28.2 ± 0.02	23.5 ± 0.02	19.2 ± 0.01	18.3
O93	PATADHAN	OD	12.4 ± 0.04	11 ± 0.01	0.5 ± 0.01	95.9	23.7 ± 0.07	20 ± 0.12	12 ± 0.03	40.1
O94	PATHANGADA	OD	15.6 ± 0.12	12.5 ± 0.02	2.1 ± 0.04	83.6	27.2 ± 0.44	24.8 ± 0.06	22.2 ± 0.02	10.5
O95	PATRACHUDI	OD	15.3 ± 0.02	15.2 ± 0.06	4.9 ± 0.06	67.7	19.8 ± 0.01	22.9 ± 0.04	19.2 ± 0.02	16
O96	PATRALI	OD	19 ± 0.02	15.5 ± 0.01	4.4 ± 0.02	71.8	25.4 ± 0.16	24.1 ± 0.04	22.3 ± 0.02	7.3
O97	KAKHIA	OD	12.9 ± 0.04	11.8 ± 0.03	6.3 ± 0.03	47	29.2 ± 0.13	25.4 ± 0.01	24.9 ± 0.01	2
O98	RANGAKHANDA	OD	13.4 ± 0.06	11.8 ± 0.02	5.3 ± 0.02	55.3	28.3 ± 0.19	26.8 ± 0.02	22.4 ± 0.01	16.4
O99	RANICHETEK	OD	10.4 ± 0.1	10.5 ± 0.02	2.2 ± 0.01	79	23.1 ± 0.01	19.2 ± 0.06	15.9 ± 0.02	17.2

Table 1 continued

Acc. no.	Genotype	State	Br Fe S1[a]	Br Fe S2[a]	Pol Fe[a]	%Loss Fe	Br Zn S1[a]	Br Zn S2[a]	Pol Zn[a]	%Loss Zn
O100	RASKADAM	OD	12.4 ± 0.04	11.4 ± 0.06	1.7 ± 0.02	85	27.4 ± 0.04	24.7 ± 0.03	18.1 ± 0.08	26.9
O101	BAGADICHUDI	OD	12.8 ± 0.12	11.5 ± 0	3.9 ± 0.01	66.5	28.9 ± 0.1	29.7 ± 0.1	23.5 ± 0.02	20.9
O102	BHUDEI	OD	11.5 ± 0.11	12.7 ± 0.02	4.5 ± 0.04	64.6	30.9 ± 0.15	29.3 ± 0.02	20.9 ± 0	28.7
O107	RETDHAR	OD	11.9 ± 0.08	12.3 ± 0.01	4.7 ± 0.03	61.6	25.5 ± 0.16	23.8 ± 0.01	16.9 ± 0.01	29.2
O108	SAMUDRABALI	OD	14.5 ± 0.05	13.7 ± 0.02	5.8 ± 0.02	57.9	28.3 ± 0.15	20.2 ± 0.04	19 ± 0.01	5.7
O109	SANKARZIRA	OD	15.6 ± 0.06	13.3 ± 0.06	8 ± 0.01	39.6	27.8 ± 0.08	26.9 ± 0.02	23.8 ± 0.08	11.5
O110	SAPURI	OD	11.9 ± 0	12.6 ± 0.03	4.2 ± 0.02	66.5	23.2 ± 0.07	20.3 ± 0.06	15.4 ± 0.02	24.4
O111	SONIACARD	OD	14.1 ± 0	13.2 ± 0	4.6 ± 0.01	65.5	26.9 ± 0.06	24.4 ± 0.02	20.4 ± 0.02	16.6
O112	SUNAKHADIKA	OD	13.1 ± 0.04	11.2 ± 0.03	3.3 ± 0.01	70.4	24.3 ± 0.08	20.5 ± 0.06	15 ± 0.04	26.8
O113	SUNASERI	OD	11.5 ± 0.03	9.9 ± 0.06	0.9 ± 0.02	90.9	23.8 ± 0.06	19.4 ± 0.02	14.5 ± 0.08	25.3
O114	SURUDAKA	OD	13 ± 0.01	13.1 ± 0.05	4.6 ± 0.01	65.1	27.4 ± 0.01	23.1 ± 0.02	20.5 ± 0.03	11.3
O115	TARMANDHAN	OD	12.2 ± 0.01	13.5 ± 0.05	7.1 ± 0.04	47.6	20.4 ± 0.03	20 ± 0.02	19.6 ± 0.06	1.8
O116	TIKICHUDI	OD	11.8 ± 0.02	16.9 ± 0.03	5.4 ± 0.02	68.3	23.7 ± 0.06	26.7 ± 0.02	18.2 ± 0.02	31.7
O117	TIKRAMUNDI	OD	13.8 ± 0.03	12.7 ± 0.04	4.1 ± 0	67.7	28.9 ± 0.1	24.6 ± 0.01	20.3 ± 0.06	17.5
O118	TUDIAKUJI	OD	13.2 ± 0.0	12.5 ± 0.04	2.2 ± 0.04	82.7	29.4 ± 0.04	23.4 ± 0.04	15.9 ± 0.1	32.3
O119	TULSI	OD	12.4 ± 0.05	13.4 ± 0.03	4.9 ± 0.02	63.8	24.2 ± 0.07	23.4 ± 0.02	21.9 ± 0.04	6.6
O120	TULSIGANTHI	OD	12 ± 0.04	9.6 ± 0.01	5.5 ± 0.07	42.4	23.6 ± 0.01	17.5 ± 0.15	14.7 ± 0.03	16
O121	UMARAJACHUDI	OD	9.8 ± 0.05	9.9 ± 0.03	3.3 ± 0.05	67	22 ± 0.02	21 ± 0.06	18.9 ± 0.1	10
O122	BODHEBARING	OD	14 ± 0.01	14 ± 0.01	7.2 ± 0.04	48.9	23.7 ± 0.05	23.7 ± 0.05	17.3 ± 0.01	27.1
O125	MALLIMANKADA	OD	13.1 ± 0	14.5 ± 0.04	4.9 ± 0.05	66.4	22.8 ± 0.03	18.9 ± 0.08	14.1 ± 0.06	25.5
O127	ZAMUKOLI	OD	12.8 ± 0.01	19.1 ± 0.03	13.7 ± 0.06	28.3	21.9 ± 0.12	23.8 ± 0.03	22.2 ± 0.09	6.5
A1	BONDALU	AP	10.7 ± 0.07	10.7 ± 0.02	5.8 ± 0.03	46.3	16.2 ± 0.03	14.7 ± 0.08	11.3 ± 0.02	22.9
A3	ISSUKARAVVALU	AP	12 ± 0.08	15.1 ± 0.01	9.7 ± 0.01	35.5	22.8 ± 0.1	22.1 ± 0.01	20.4 ± 0.05	7.9
T1	JEERAKASAMBA	TN	16.7 ± 0.01	11.8 ± 0.01	8.7 ± 0.01	26	23.5 ± 0	23.5 ± 0.01	15.1 ± 0.01	35.7
T2	KAARNEL	TN	12.1 ± 0	12 ± 0.06	4 ± 0.01	67.1	27.3 ± 0	28 ± 0.06	19.5 ± 0.04	30.5
T3	KARUPPUKAVUNI	TN	13.6 ± 0	6.9 ± 0.62	5.7 ± 0.02	17.5	21.8 ± 0	24.3 ± 0.06	19 ± 0.13	21.6
T4	KUZHIVEELICHAN	TN	8.4 ± 0	9.7 ± 0.03	3.4 ± 0.01	64.9	18.1 ± 0	19.2 ± 0.1	15.6 ± 0.02	18.5
T5	PACHAMALAI PUZHUTHINEL	TN	12.8 ± 0.08	15.3 ± 0.03	7 ± 0.01	54.1	18.5 ± 0.25	22.7 ± 0.04	19.6 ± 0.03	13.5
T6	PUZHUTHIKARNEL	TN	11 ± 0.04	11.7 ± 0.01	1.1 ± 0.05	91	18.4 ± 0.1	17.1 ± 0.01	11.4 ± 0.06	33.4
T7	PUZHUTHINEL	TN	10.2 ± 0.02	11.4 ± 0.06	5.4 ± 0.01	53.1	21.5 ± 0.21	18.8 ± 0.11	18.1 ± 0.05	3.7
T8	SOORAKURUVAI	TN	9.1 ± 0	10 ± 0.01	3.1 ± 0.02	68.8	21.1 ± 0	20.4 ± 0	17.5 ± 0.08	14.2
H4	BPT	HY	13.3 ± 0	11.4 ± 0.01	7.5 ± 0.02	34.2	19.4 ± 0	19.9 ± 0.06	17.5 ± 0.08	12.1
H5	CHAITANYA	HY	9.1 ± 0	8.4 ± 0.02	4.1 ± 0.02	51.5	14.6 ± 0	14.5 ± 0.09	12.1 ± 0.05	16.9
H8	IR20	HY	10.7 ± 0.19	9.6 ± 0.02	3.9 ± 0.03	59.9	15.4 ± 0.16	14.5 ± 0.07	12.6 ± 0.03	13.4
H9	IR64	HY	13.2 ± 0.02	14.2 ± 0.17	7.5 ± 0.06	47.3	22 ± 0.06	20.9 ± 0.03	19.3 ± 0.06	7.7
H10	JAYA	HY	15.3 ± 0.16	14.6 ± 0.02	4.6 ± 0.01	68.8	39.2 ± 0.18	33.6 ± 0.01	28 ± 0.14	16.7
H14	MTU1010	HY	11.1 ± 0	12.5 ± 0.08	4.7 ± 0	62.4	18.8 ± 0	16.4 ± 0.01	13.8 ± 0.01	15.9
H15	NLR	HY	12.6 ± 0.31	11.9 ± 0.02	8.6 ± 0.01	28.2	18 ± 0.02	15.7 ± 0.06	14.9 ± 0.06	4.8
H17	ORYZAJAPONICA	HY	12 ± 0.04	12 ± 0.04	4.2 ± 0.03	65	20.1 ± 0.02	20.1 ± 0.02	15.2 ± 0.01	24.4
H18	PUSA	HY	10 ± 0	10 ± 0.04	8.1 ± 0.03	18.6	16.1 ± 0	17.5 ± 0.02	13.8 ± 0.07	21.4
H19	RASI	HY	8.4 ± 0	8.1 ± 0.02	3.8 ± 0.01	52.8	17.5 ± 0	16.9 ± 0.02	16 ± 0.01	5.3
H23	SWARNA	HY	9.2 ± 0	12.2 ± 0.01	10.3 ± 0.01	16	18.2 ± 0.03	17.5 ± 0.08	16 ± 0.1	8.6
H24	WHITEPONNI	HY	8.6 ± 0	10 ± 0.02	5.8 ± 0.02	42.5	15 ± 0	16.4 ± 0.02	14.5 ± 0.03	11.6
KL1	ADUKKAN	KL	11.6 ± 0.11	12.5 ± 0.01	2.5 ± 0.01	80.4	24.1 ± 0.13	25.5 ± 0.03	17.8 ± 0.01	30.1
KL2	CHENNELLU	KL	10.7 ± 0.07	11.4 ± 0.01	2.5 ± 0.01	78.5	24.9 ± 0.1	23.8 ± 0.02	18.9 ± 0.03	20.8
KL3	CHENTHADI	KL	11.1 ± 0.1	9.4 ± 0.02	3.3 ± 0.03	64.9	25.3 ± 0.11	27.5 ± 0.1	20.7 ± 0.1	24.7

Table 1 continued

Acc. no.	Genotype	State	Br Fe S1[a]	Br Fe S2[a]	Pol Fe[a]	%Loss Fe	Br Zn S1[a]	Br Zn S2[a]	Pol Zn[a]	%Loss Zn
KL4	CHOMALA	KL	11.3 ± 0.03	11.4 ± 0.01	3 ± 0.02	74.1	27 ± 0.36	25.9 ± 0.07	18.6 ± 0.05	28.4
KL5	GANDHAKASALA	KL	16.4 ± 0.19	15 ± 0.06	9.2 ± 0	38.7	25.2 ± 0.01	26.5 ± 0	22.4 ± 0.04	15.5
KL6	JEERAKASALA	KL	15.5 ± 0.5	13.9 ± 0.01	8 ± 0.01	42.2	24.8 ± 0.17	26.2 ± 0.04	20.5 ± 0.17	21.8
KL7	KALLADIYARAN	KL	10.3 ± 0.03	12.4 ± 0.02	6.1 ± 0.01	51.2	23.1 ± 0.21	24.3 ± 0.08	22.4 ± 0.02	7.8
KL8	KAYAMA	KL	14.7 ± 0	21 ± 0.01	11.2 ± 0.01	46.9	20.5 ± 0.02	22 ± 0.01	18.1 ± 0.01	17.5
KL9	KUNJUTTIMATTAN	KL	14.6 ± 0	16.1 ± 0.01	5.1 ± 0.01	68.2	22.6 ± 0.01	22 ± 0.12	17.6 ± 0.05	20.2
KL10	KURUMATTAN	KL	15.9 ± 0	11.9 ± 0.05	4.1 ± 0.01	65.4	24.8 ± 0	22.3 ± 0.06	20.4 ± 0.01	8.5
KL11	KURUVA	KL	12.2 ± 0	12.6 ± 0.01	6.8 ± 0.01	46	24.1 ± 0	21.6 ± 0.1	18.9 ± 0.02	12.5
KL12	MULLANKAYAMA	KL	12.9 ± 0	14 ± 0.02	7.1 ± 0.02	49.6	21.5 ± 0.01	21.5 ± 0.06	18.7 ± 0.08	13
KL13	MULLANPUNJA	KL	13.7 ± 0	13.6 ± 0.08	5.2 ± 0.03	62	25.7 ± 0.01	26.9 ± 0.04	19.3 ± 0.01	28.3
KL14	THONDI	KL	16.7 ± 0.01	8.8 ± 0.04	6.8 ± 0.01	23.3	25.7 ± 0.01	22.9 ± 0.02	20.9 ± 0.03	8.5
KL15	THONNURAMTHONDI	KL	15 ± 0	17.6 ± 0.01	4.4 ± 0.03	75.3	25.2 ± 0.01	25.4 ± 0.03	20.3 ± 0.05	20.1
KL16	VALICHURI	KL	17.7 ± 0	18.2 ± 0.02	5.2 ± 0.03	71.3	25.6 ± 0.01	25.1 ± 0.05	21.3 ± 0.04	15
KL17	VELYAN	KL	12 ± 0.13	10.9 ± 0.01	3.4 ± 0.02	68.8	22.3 ± 0.14	21.2 ± 0.02	18.4 ± 0.03	13.4
W1	WILD RICE	OD	15.6 ± 0.03	17.1 ± 0.07	9.9 ± 0.07	42.2	29.7 ± 0.04	29.2 ± 0.04	26.1 ± 0.01	10.5
W2	O. *barthii*	WR	13.1 ± 0.02	13.1 ± 0.02	7.4 ± 0.04	43.7	29.5 ± 0.02	29.5 ± 0.02	24.9 ± 0.01	15.4
W3	O. *glaberrima*	WR	14.3 ± 0.12	14.3 ± 0.12	5 ± 0.06	65.4	31.6 ± 0.02	31.6 ± 0.02	27 ± 0.01	14.4
W6	O. *nivara*	WR	17.5 ± 0.07	17.5 ± 0.07	8.9 ± 0.08	49.3	26.5 ± 0.06	26.5 ± 0.06	24.4 ± 0.02	7.8
W7	O. *officinalis*	WR	22.6 ± 0.01	22.4 ± 0.01	17 ± 0.12	24.6	25.9 ± 0.06	25.9 ± 0.06	24.9 ± 0.01	3.9

OD Odisha, *AP* Andhra Pradesh, *TN* Tamilnadu, *HY* high yield, *KL* Kerala, *WR* wild rice. Br Fe S1—brown rice iron concentration in season 1, Br Fe S2—brown rice iron concentration in season 2, Pol Fe—iron in polished rice, Br Zn S1—brown rice zinc concentration in season 1, Br Zn S2—brown rice zinc concentration in season 2, Pol Zn—zinc in polished rice

[a]Mean ± standard error, concentration of iron and zinc in mg/kg

moderate (113 genotypes) and above 25.1 mg/kg as high (13 genotypes).

Correlation Between Iron and Zinc

In this study, a moderate positive correlation between iron and zinc content of brown ($r = 0.5$) and polished rice ($r = 0.3$) was observed, implying the likelihood of concurrent assemblage of both the micronutrients. Few genotypes O33, O44, O49, O59, H10 and W1 have high iron as well as zinc content in brown rice (Table 1). The genotypes O60, W1 and W6 retain both the micronutrients even after polishing (Table 1). The relationship between the two variables, namely iron and zinc content in both brown and polished rice grain, is evident from the scatter plots (Fig. 5). The confidence ellipse aids in the visualization of the spread (variance), mean and correlation between the variables since 95% of the data points are expected to lie within it. The group means accord with the centre of the ellipses. The measure of linear correlation between the variables is indicated by the eccentricity, such that highly correlated variables give a very narrow ellipse. It is evident from the scatter plot that there is a strong association between the zinc levels of brown and polished rice.

Discussion

Oryza is an agronomically pivotal genus consisting species with diversified morphology. Tremendous efforts are being made to understand the nutritional dynamics of rice. Screening the accessible germplasm and identifying the source of genetic variation for the desired trait in order to effectuate crosses, genetic studies, molecular marker development and understanding the process of micronutrient uptake is a prerequisite for micronutrient-dense crop breeding. Plant breeding programs in biofortification of staple food crops necessitate assessing of germplasm, elite lines and varieties with iron and zinc-dense grains to be employed as donor parents. Anti-nutritional factors (ANF) such as tannins and phytic acid, present in cereals, bind to Fe/Zn reducing their bioavailability [19]. The naturally functional alleles conferring phenotypes of low ANF and high grain Fe/Zn concentration found among diverse rice accessions could be used to fine-tune grain mineral density and bioavailability. Therefore, practicability of breeding for enhanced bio-available micronutrients in grains is higher when the potential genetic variation is exploited to the maximum.

Hitherto, genetic variability for iron and zinc contents has been researched in various crops with the aim to

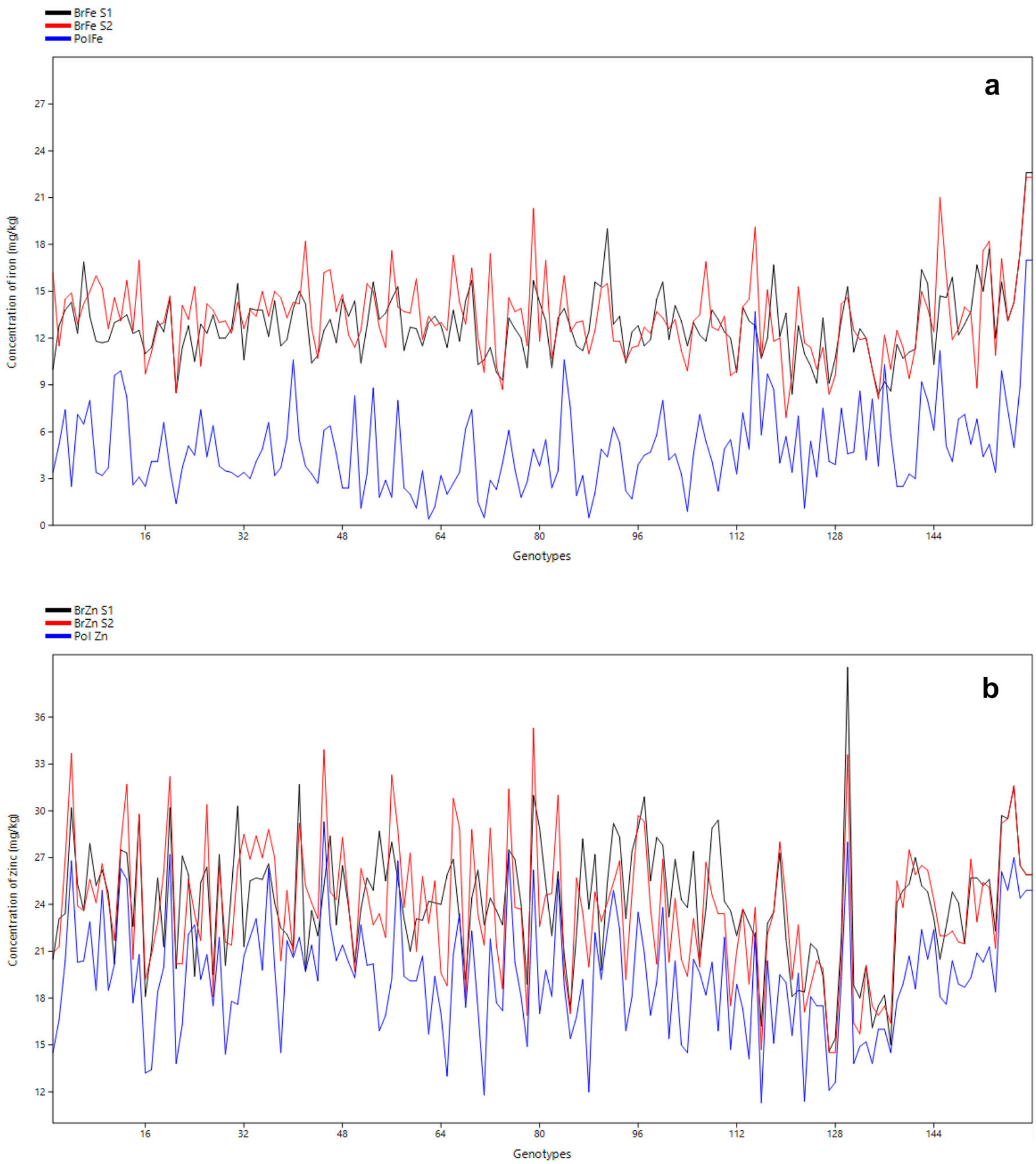


Fig. 1 Variation in micronutrient content in the population. Concentration of zinc (**a**) and iron (**b**) in brown rice in two seasons and polished rice one season

identify the donor genotypes that have micronutrient-dense grains. Approximately fourfold variation in rice grain iron content was identified in a research that evaluated 939 genotypes with iron content spanning between 7.5 and 24.4 mg/kg and zinc content between 15.9 and 58.4 mg/kg in brown rice [9]. Manifold variations in iron and zinc content in 192 varieties of brown rice were recently reported by Nachimuthu et al. [23]. A screening study among 84 landraces by Sharma [29] found that iron content ranged from 0.25 to 34.8 mg/kg and zinc content from 0.85 to 195.3 mg/kg. In a study where 1138 genotypes were screened by Gregorio [10], iron ranged from 6.3 to 24.4 mg/kg while zinc from 15.3 to 58.4 mg/kg. In the current study, the concentration of both micronutrients is well within the range as reported by Gregorio [10]. The wild genotypes had the highest iron as reported in many papers [5]. Wild species, therefore, have immense potential in biofortification of popular cultivars through acceptable, conventional and non-transgenic methods. Earlier studies have recorded a significant positive correlation between iron and zinc in rice, double haploid rice populations, wheat and beans indicating co-segregation of concerned

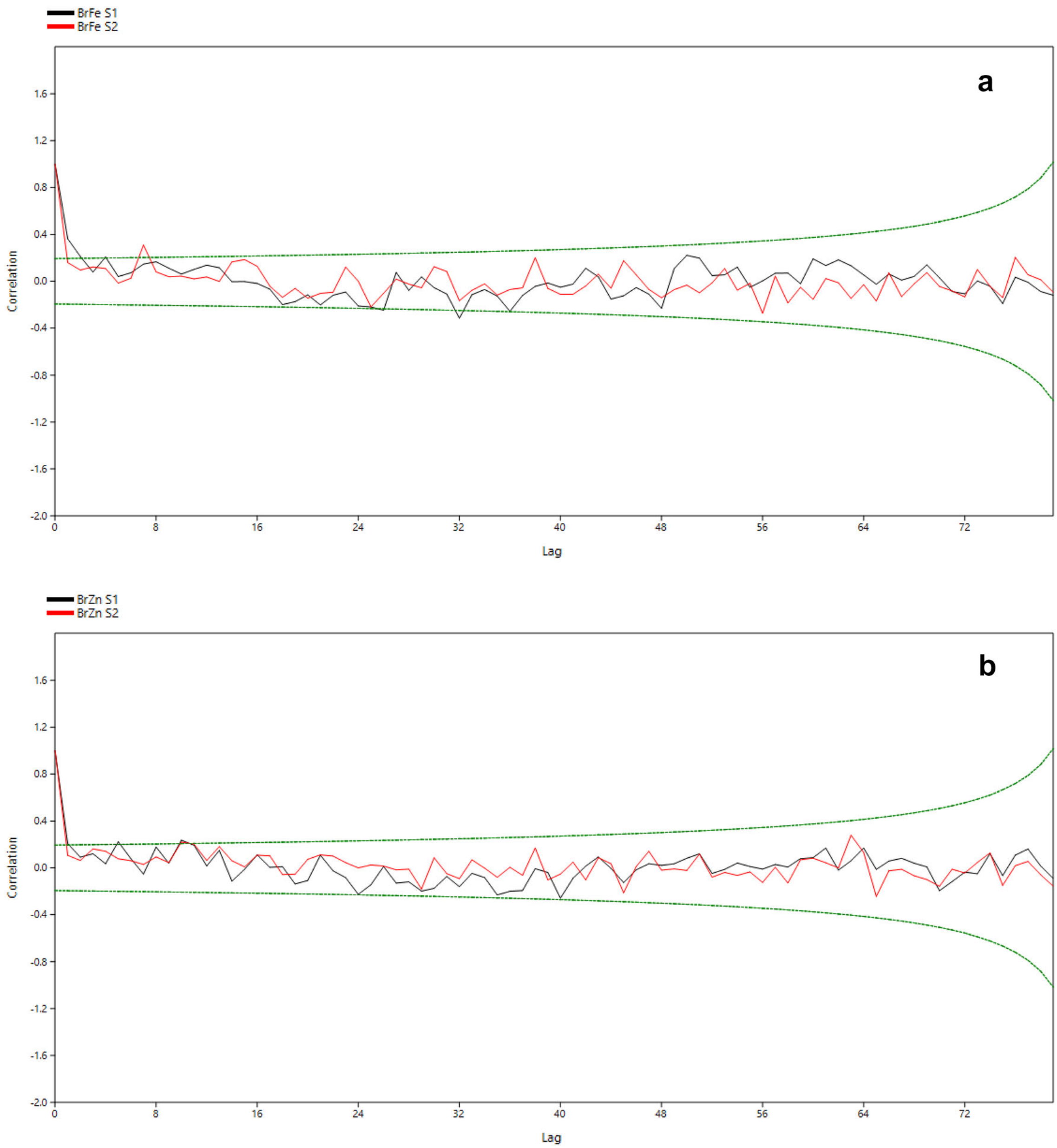


Fig. 2 Lag plot showing variation in micronutrient concentration in two seasons iron (**a**) and zinc (**b**)

factors [9, 33]. This study finds a moderate positive correlation implying the possibility of concurrent selection of both the micronutrients.

Iron concentration in rice grains is significantly affected by environment, genotype and genotype × environment interaction [34]. Several multi-environment trial investigations conducted in India, Vietnam, Philippines, Bangladesh and Korea have revealed a conspicuous effect of environmental factors, such as wet and dry season, inherent soil properties like salinity, pH and period of water logging during crop growth, on traits related to grain nutritive value in rice [2, 7, 11]. This study found that the performance of genotypes is more or less stable when cultivated in the same environment. Comparative analysis of grain iron and zinc of the 159 genotypes grown during two successive *Rabi* showed slight variation, although the overall trend for grain micronutrient concentration remained largely unchanged over both seasons (Fig. 2). There is minimal temporal effect on the trend of micronutrient density in the grain. Also, the degree of variation is much higher for iron content than for zinc. As reported earlier by various research groups, this study also found that zinc content in the grain seemed to be more constant than iron content [2, 7].

This study showed higher accumulation of micronutrients in five genotypes, viz. Raskadam, Jeerakasamba,

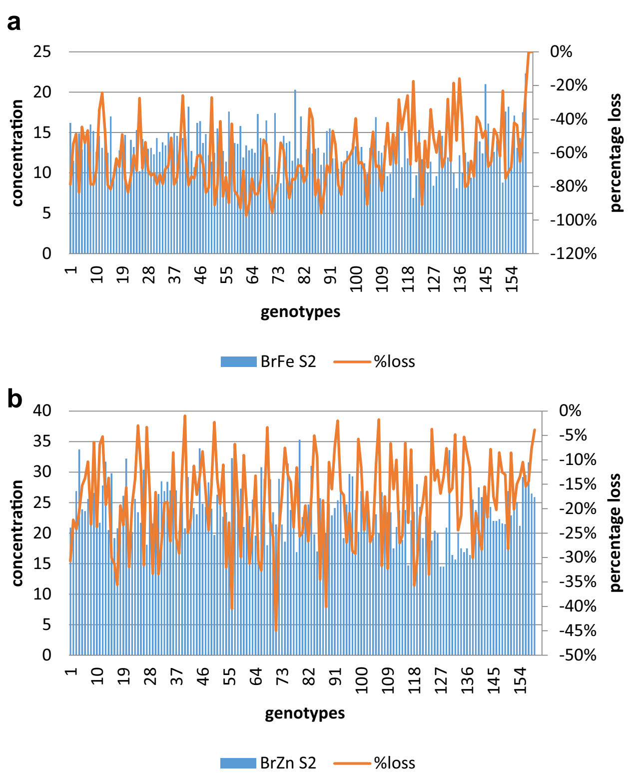
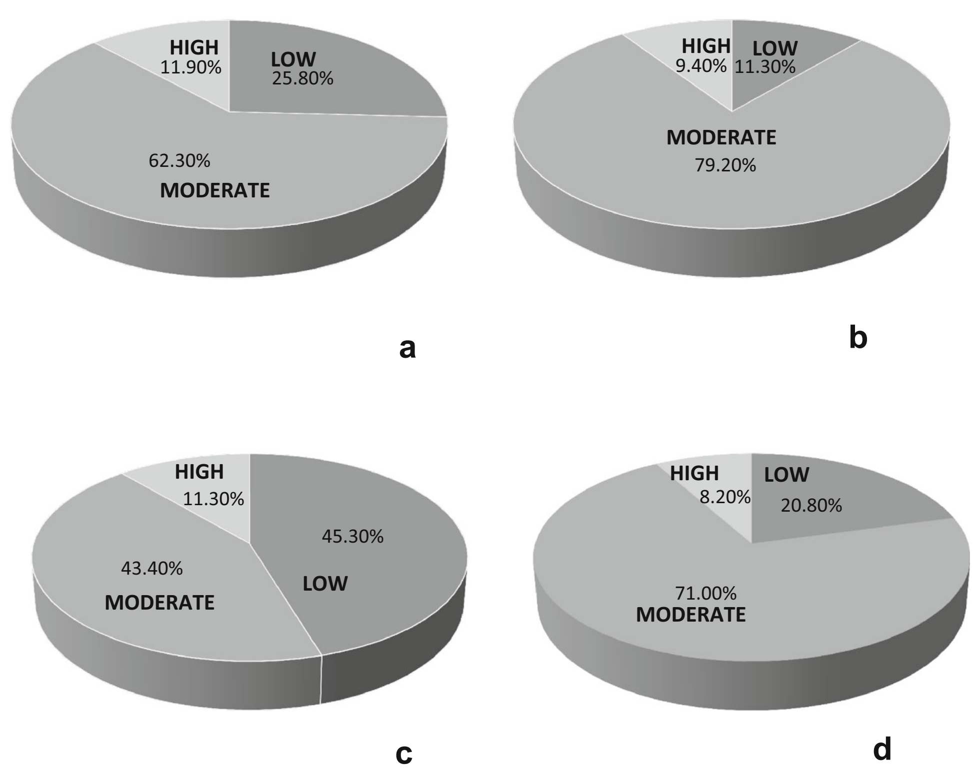


Fig. 3 Effect of polishing on the concentration of iron (a) and zinc (b) in rice grain

Machakanta, Haladichudi and IR 64, compared to an earlier study [23]. Agarwal et al. [1] investigated 126 rice genotypes for iron and zinc by atomic absorption spectroscopy, of which nine genotypes are common with the present study. While our study is consistent with the iron concentrations in five genotypes—Pusa, Rasi, Tulasi, BPT 5204 and IR64, the levels of zinc in all the nine were much lower than the earlier report. The remaining genotypes had lower concentration of both micronutrients in our study. Micronutrient density in rice grains depends on a plethora of interrelated metabolic pathways that are involved in uptake from soil, transportation to source tissues and mobilization and/or remobilization to developing grains which probably explains the differences in content with earlier reports [7, 12]. Each of these processes is regulated by an eclectic mix of genes and environmental factors like soil type, fertilizer application, drought, genotype × environment interaction, etc. [7, 10, 16]. Factors such as annual rainfall, pH, soil organic matter content, inherent trace element levels and fertilizer application affect the concentration as well as availability of mineral ions in soil solution [6]. Intensive agriculture leads to the depletion of inherent microelements in soils, while continuous fertilizer application in fields leads to increased accumulation of inorganic salts resulting in alkaline pH which, in turn, reduces the availability of micronutrients for plants [7].

Fig. 4 Classification of genotypes based on iron and zinc content. a iron in brown rice, b zinc in brown rice, c iron in polished rice and d zinc in polished rice



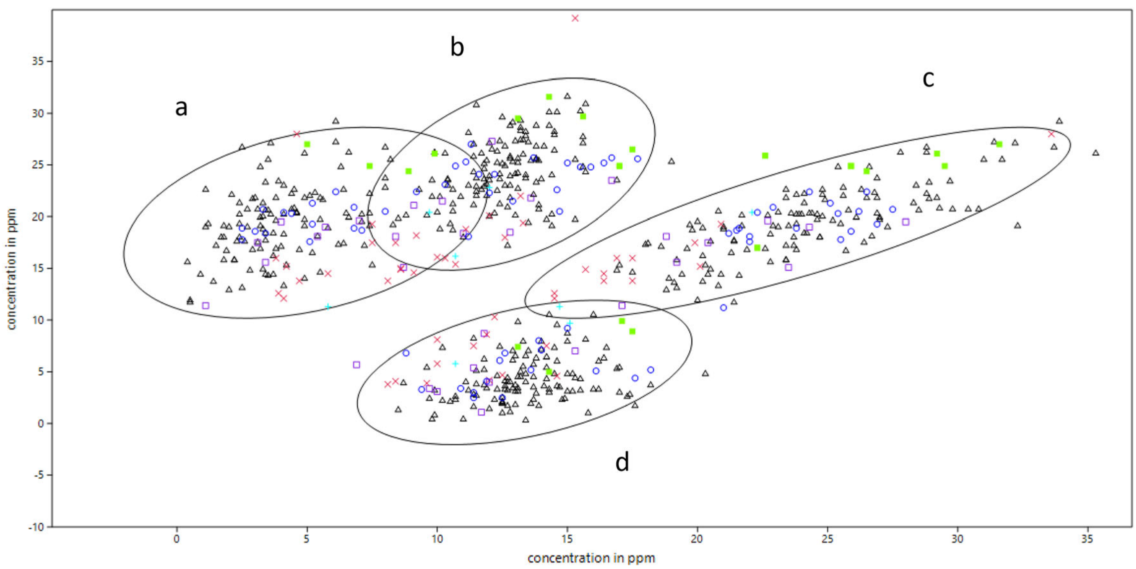


Fig. 5 Scatter plot between iron (*X* axis) and zinc (*Y* axis) content in polished rice (**a**), iron (*X* axis) and zinc (*Y* axis) content in brown rice (**b**), zinc content in brown (*X* axis) and polished (*Y* axis) rice (**c**), iron content in brown (*X* axis) and polished (*Y* axis) rice (**d**)

Hao et al. [15] have adumbrated the linear correlation between irrigation and fertilizer management to that of accumulation of Zn, Mn, Cu and Fe in grains of rice and wheat. A recent study conducted by ICMR [20] estimated the Fe concentration in commercially available polished rice collected from 107 districts of India to be 6.5 mg/kg which is at variance with several other studies that suggest the general baseline of Fe in popular polished indica varieties to be about 2–3 mg/kg [36, 39]. In this study, the variability of environmental factors is lowered by cultivating in the same field in contrast to collection from different places [20]. The minerals Fe and Zn are abundant in the environment, and hence, the potential for contamination during estimation is much higher [13]. Moreover, environmental contamination very likely impacts the Fe results [26]. Hence, reducing the possible contamination from external sources (insects/soil/dust) is essential to reduce inaccuracy of results. Analytical techniques such as ICP-OES and AAS require pre-analysis sample preparation. Many sample preparation processes (grinding, polishing and others) make use of plastics (i.e. with equipment), which can contain Zn. It is also important that polishing and grinding devices used for micronutrient analysis are non-contaminating. Modified equipment with a suitable non-Zn-containing alternative has been reported by [32].

Zinc in rice grains is distributed all through the endosperm. Hence, estimates of zinc in brown rice are effective indicators of zinc in polished rice; this does not hold good for iron since most of it is present in outer aleurone layer that is lost during polishing. Worldwide, the polished form of rice, obtained after removal of the bran, is preferred for consumption. Significant reduction in iron content in polished rice was observed by Martinez et al. [21] who investigated Fe/Zn concentration in 11,400 samples of brown and polished rice and found that brown rice contained 10–11 mg/kg Fe and 20–25 mg/kg Zn while polished rice contained 2–3 mg/kg Fe and 16–17 mg/kg Zn. Similar Fe (3.64–5.66 mg/kg) and Zn (18.62–25.46 mg/kg) pattern was reported [19]. Hence, it is pivotal to ascertain what fraction of iron is lost during polishing. The findings in this paper are similar to [10, 21] who also observed more loss of iron, than zinc during polishing. Iron content also decreases drastically as polishing time increased [11, 31]. Besides the loss of iron during polishing, another 10 percentage is lost during washing before cooking, whereas loss of zinc during washing before cooking is almost negligible [28]. Considering this, losses during polishing as well as washing and international threshold values of 7 mg/kg for iron and 24 mg/kg for zinc, and varieties having $\geq$ 30.0 mg/kg zinc/iron in brown rice can be considered as potential donors for breeding programme for enhancing zinc/iron. Nonetheless, genotypic variations do prevail with respect to the distribution of micronutrients across the layers of rice grain. Gregorio et al. [11] have reported a notable retention of iron content of IR68144-3B-2-2 and Xua Bue Nuo, a traditional high-iron rice from China, upon polishing as compared with other varieties. These underlying genetic differences can manifest as variation in the thickness of the aleurone layer or embryo size or both, etc. Scope for further enhancement of zinc through conventional breeding is higher, whereas transgenic approach appears inevitable for iron.

Bioavailability is a complex phenomenon governed by various dietary components. Anti-nutrients like phytic acid bind to these ions and make them unavailable for absorption and contrarily, citric acid being a pro-nutrient promotes iron absorption. The composition and availability of these components vary among the genotypes, and thus, only a part of the available iron and zinc in the cooked food enters the blood stream. Emphatically biofortification calls for interconnecting agricultural research with the human health and nutrition sectors [4].

Conclusions

Despite many leads from diversity screens, iron biofortification of rice based on classical breeding has only progressed from infantile to novice and is yet to learn the intricacies to advance further. The width of variation demonstrated by this study indicates that these secondary centres of diversity are good repositories of divergent germplasm that could be exploited in breeding programs to mitigate micronutrient malnutrition. Breeders and scientists can respond better to future challenges when the available genetic diversity is well documented for grain mineral density for developing robust and nutrient-rich lines. Most polished rice grains especially of the popular cultivated mega-varieties have about 2 mg/kg of iron. The degree of iron enrichment attained to date is still very low, and the improvement in nutritional quality of plants is still a daunting task due to uncertainties around G x E interactions, bioavailability along with auxiliary concerns such as grain polishing, method of cooking which underscore the inherent intricacies and difficulties of the problem. This study is useful in chronicling the variability of micronutrient content in rice germplasm in India, and further studies through inclusion of other genotypes will enable development of an appropriate strategy for molecular analyses and possibly identify markers associated with iron and or zinc uptake into grains.

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Author Contributions SM prepared the samples, carried out data analysis and drafted the manuscript. RS designed the experiment and revised the manuscript. AP participated in study design and revised the manuscript. All authors have read and approve the final manuscript.

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