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Screening of rice genotypes for grain micronutrient concentrations to identify potential candidates for biofortification

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Abstract
Health related issues due to malnutrition are one of the serious concerns of present world. It is also a major limiting factor in achievement of the Millennium Development Goals. Currently around 872.9 million people of the world are undernourished and more than 2 billion people suffer from micronutrient based hidden hunger. Intervention by providing more nutritious staple food like nutri-rich rice offers a potential solution to such key issues. Considering serious consequences due to micronutrient malnutrition a study was performed to examine the variability among rice genotypes. A set of rice panel of 192 genotypes was evaluated for polished grain micronutrient (Zinc and Iron) concentrations. The work categorizes rice material into high, medium and low subsets for the grain zinc (Zn) and iron (Fe) levels. Grain Zn content ranged from 13.0-33.4 mg/kg whereas Fe from 2.0-16.6 mg/kg among polished grains of all the genotypes. Total 43 and 27 potential rice genotypes showing high zinc and iron levels respectively have been identify, of which 12 were high for both nutrients. The concentration level of the Zn/Fe among the high lines is within the target range specified by the HarvestPlus that makes these genotypes a vital candidate for biofortification programmes.

Keywords: Rice, Malnutrition, Micronutrients, Iron, Zinc

Introduction

Malnutrition and diet-related chronic diseases remain major constraints to human health and sustainable development, particularly in developing countries (Dipti *et al.*, 2012) [1]. Micronutrient malnutrition, often described as "hidden hunger", results from an inadequate intake of essential vitamins and minerals through the daily diet. It is especially prevalent among populations whose diets are predominantly based on staple crops that provide energy but contain insufficient quantities of essential micronutrients. Prolonged deficiencies can adversely affect immunity, growth, cognitive development and physical capacity, and may increase susceptibility to disease and mortality (Banerjee *et al.*, 2010; Banerjee *et al.*, 2011; Gupta *et al.*, 2015; Muthusamy *et al.*, 2015) [2, 3, 4, 5]. In several rice-dependent regions, including India, deficiencies of iron (Fe), zinc (Zn) and vitamin A have been recognized as important nutritional concerns (Dipti *et al.*, 2012; Babu *et al.*, 2013) [1, 6]. Iron deficiency is a major contributor to nutritional anaemia, while inadequate zinc intake is associated with impaired growth, immune function and child development. These concerns underline the importance of improving the nutritional quality of commonly consumed staple foods (Bailey *et al.*, 2015; Trijatmiko *et al.*, 2016) [7, 8].

Rice cultivation is one of the major agricultural activities and an important source of income for millions of households in Asia and Africa. More than two billion people in Asia obtain a substantial proportion of their dietary energy from rice-based foods. The expression 'Rice is Life' aptly describes the importance of this crop, particularly in India, where rice plays a vital role in national food security and provides livelihoods for millions of rural people (Umadevi *et al.*, 2012) [9]. The literature says that India holds 1st position among largest producers of rice cultivation and produced about 217.9 million tonnes (FAO, 2024) [10], with rice contributing approximately 31% of the calories in the Indian diet (Babu, 2013) [11]. Rice is an important source of dietary energy because of its high carbohydrate content; however, polished rice is comparatively poor in protein and essential micronutrients, particularly Fe and Zn (Potrykus, 2003; Sperotto *et al.*, 2010; Nagesh *et al.*, 2013) [13, 14, 12].

Although rice is a major dietary staple, polished rice generally provides limited amounts of essential micronutrients such as Fe and Zn. However, considerable genetic variability for the concentrations of these elements has been reported among rice genotypes. Thus, the mineral content of rice grain is not uniform but varies with genetic background and environmental conditions. Dietary diversification, conventional food fortification and micronutrient supplementation are important strategies for addressing nutritional deficien

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cies; nevertheless, their reach and sustained implementation may be challenging mainly in resource-limited areas. Biofortification offers a complementary, food-based approach in which the nutritional quality of the edible portion of a crop is improved through conventional breeding, agronomic management or biotechnological interventions (Welch and Graham, 2002 [15]; Anuradha *et al.*, 2012 [16]; Nagesh *et al.*, 2012 [17]; Masuda *et al.*, 2013 [18]; Nachimuthu *et al.*, 2014 [19]; Verma and Srivastav, 2017) [20]. Recent literature study have highlighted the potential of genetic resources, molecular breeding, functional genomics approaches and genome editing to enhance Fe and Zn accumulation in rice grains (Senguttuvel *et al.*, 2023; Dhanyalakshmi *et al.*, 2024; Zubair *et al.*, 2024) [22, 21, 23].

As rice is a regularly diet for a large proportion of the world population, even a moderate improvement in its micronutrient concentration could contribute to better dietary mineral intake. Nevertheless, the nutritional value of rice depends not only on the total concentration of minerals in the grain but also on their distribution within the grain, retention during milling and bioavailability after consumption. Recent evidence indicates that polishing can substantially reduce the Fe and Zn concentrations of rice, emphasizing the need to evaluate nutrient levels in the form actually consumed (Senguttuvel *et al.*, 2023; Senthilkumar *et al.*, 2026) [22, 24]. Although several studies have assessed rice germplasm for nutritional quality, many accessions remain uncharacterized for grain micronutrient traits. Systematic screening is therefore necessary to identify promising genetic resources for biofortification and to generate phenotypic information useful for subsequent molecular studies. Accordingly, the present study was undertaken to determine Fe and Zn concentrations in the polished grains of 192 rice genotypes, with the objective of identifying potential candidates for rice biofortification and generating phenotypic data for molecular research.

Material and Methods

Experimental material

A total of 192 rice (*Oryza sativa* L.) genotypes (Table 1) were used to determine grain Zn and Fe concentrations. All genotypes were grown at the farm of Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India. Micronutrient concentrations were determined in the polished (white) grains of all the rice accessions.

Processing of rice grains

Seeds were carefully harvested, dried under clean, shaded conditions until the post-harvest grain moisture content was reduced to approximately 12%. Seeds of each genotype were dehusked using a polyurethane-coated dehusker. The dehusked grains from each variety were polished for one minute then grain whiteness and moisture content were recorded using a reflectance meter and a moisture meter, respectively.

Determination of grain micronutrients (Zn/Fe)

Polished rice grain samples were wet-digested using a microwave digestion system and analysed by atomic absorption spectrophotometry (AAS) to quantify Fe and Zn concentrations. The procedures for sample preparation, digestion and AAS-based quantification were based on the HarvestPlus guidelines (Stangoulis *et al.*, 2008) [25], the analytical procedures described by PerkinElmer (1996) [26], and AOAC Official Method 999.10 (AOAC, 2002) [27].

Analysis and presentation of data

Data on grain whiteness, moisture content, Zn concentration and Fe concentration were recorded for all genotypes. The data were analysed using Microsoft Excel and presented graphically using GraphPad Prism software (version 7.02) for Windows (GraphPad Software, 2016) [28].

Results

The Zn and Fe concentrations, together with grain whiteness and moisture content, recorded in the polished grains of the rice genotypes are presented in Figure 1 and Table 2. Significant differences were observed among the rice accessions for grain micronutrient concentrations and the other quality traits. Variation in grain Zn and Fe concentrations among the genotypes is illustrated in Figure 2. Analysis of polished grains from the 192 rice genotypes showed that Zn concentration ranged from 13.0 mg/kg to 33.4 mg/kg, whereas Fe concentration ranged from 2.0 mg/kg to 16.6 mg/kg. The mean Zn and Fe concentrations were 20.8 mg/kg and 7.2 mg/kg, respectively. Genotype R172 recorded the lowest Fe concentration (2.0 mg/kg), while R21 recorded the highest (16.6 mg/kg). Based on the obtained results, 39 genotypes contained up to 5 mg/kg Fe, 126 contained more than 5 but less than 10 mg/kg, and 27 contained at least 10 mg/kg grain Fe concentration were categorized as low, medium and high iron genotypes. For Zn, genotype R76 recorded the lowest concentration (13.0 mg/kg), while R17 recorded the highest (33.4 mg/kg) (Fig. 3). Based on the result in this study zinc classes adopted, 32 genotypes were classified as low (< 17 mg/kg), 117 as medium (17 to < 24 mg/kg), and 43 as high (≥ 24 mg/kg) in grain Zn concentration (Figure 3). Twelve genotypes were identified as potential candidates with high concentrations of both Zn and Fe in polished grains (Table 3). A positive correlation ($r = 0.348$) was also observed between grain Zn and Fe concentrations among the rice genotypes.

Discussion

Iron and zinc are essential mineral nutrients for human health, and inadequate dietary intake can result in serious nutritional and health consequences. Biofortification of staple crops such as rice with higher concentrations of Fe and Zn is therefore considered a promising complementary strategy for addressing micronutrient malnutrition (Anuradha *et al.*, 2012; Lu *et al.*, 2013; Bailey *et al.*, 2015; Verma *et al.*, 2017) [16, 29, 7, 20]. Screening available germplasm is an important first step in identifying useful genetic variation and selecting promising donors for breeding programmes. The present study revealed a wide range of Fe and Zn concentrations in the polished grains of the rice genotypes evaluated, indicating the presence of useful variability within the experimental panel.

Grain whiteness is an important quality parameter from a consumer and market perspective because polished white rice is preferred in many rice-consuming regions. Dehusking and polishing remove the bran layers, aleurone layer and embryo to varying degrees, leaving a larger proportion of the starchy endosperm. These outer grain tissues contain relatively high concentrations of minerals; consequently, prolonged or intensive polishing can reduce the micronutrient content of rice (Lu *et al.*, 2013; Masuda *et al.*, 2013) [29, 18]. In the present study, all rice samples were polished for a fixed duration of one minute, and grain whiteness was recorded. This provided a standardized processing condition; however, it should be noted that equal polishing time does not necessarily remove an identical proportion of bran from every genotype because grain size, shape, hardness and surface characteristics may differ. Grain whiteness measurements therefore provide useful information on polishing response and differences in grain pigmentation. The variation in whiteness observed after polishing for the same duration may partly reflect the diversity of the material, including red and other pigmented rice types. Grain moisture is another important factor influencing rice quality and analytical measurements. In this study, polished grains were stored under appropriate conditions to obtain relatively uniform moisture levels. Moisture content ranged from 10.4% to 11.7%, with a mean of 11.12 ± 0.23% (SD), thereby limiting variation associated with sample moisture. Genotypic differences in grain mineral concentrations have also been documented in earlier studies (Pearson *et al.*, 1994; Grusak *et al.*, 1999; Anuradha *et al.*, 2012) [30, 31, 16]. Recent synthesis further confirms that milling and polishing can cause substantial losses of Fe and Zn, particularly because these minerals are concentrated in

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the outer grain tissues (Senthilkumar *et al.*, 2026) [24]. A global *meta*-analysis published in 2026 estimated average reductions of approximately 21% in Zn and 70% in Fe following conversion of brown rice to polished white rice, although the magnitude of loss varied among studies and processing conditions (Senthilkumar *et al.*, 2026) [24].

Grain nutrient composition is influenced by both genetic and environmental factors (Verma *et al.*, 2017) [20]. The elemental analysis in the present study revealed significant genotypic variation in Fe and Zn concentrations. The ranges recorded were broadly comparable with those reported in earlier investigations. Traditional varieties and landraces represented an important source of variability in the present panel and were frequently represented among the genotypes with higher Fe and Zn concentrations. Genetic variation for grain Fe and Zn has been reported in rice, with concentrations ranging from 2.5–24.4 mg/kg for Fe and 4.4–33 mg/kg for Zn among cultivated rice and wild rice materials (Graham *et al.*, 1999 [32]; Gregorio *et al.*, 2000 [33]; Chandel *et al.*, 2005 [34]; Virk *et al.*, 2007 [35]; Anuradha *et al.*, 2012 [16]; Verma and Srivastav, 2017) [20]. Banerjee *et al.* (2011) [2] reported Fe and Zn concentrations of 8.50–20.51 mg/kg and 13.954–39.32 mg/kg, respectively, in brown rice. Anuradha *et al.* (2012) [16], while evaluating 126 rice genotypes, reported Fe concentrations of 6.2–71.6 mg/kg and Zn concentrations of 26.2–67.3 mg/kg in brown rice; wild accessions showed particularly high mineral concentrations. Similarly, Nachimuthu *et al.* (2014) [19] reported Fe and Zn concentrations of 6.6–16.7 mg/kg and 7.1–32.4 mg/kg, respectively, in brown rice. Verma and Srivastav (2017) also reported substantial variation in Zn and Fe concentrations among Indian rice accessions. In a recent work Parte *et al.* (2023) [36] identified landraces and mutants with useful Fe and Zn concentrations and reported genotype and seasonal effects, supporting the continued value of local germplasm in biofortification programmes. Recent reviews have further emphasized the importance of combining conventional selection with molecular breeding, genomic tools and functional characterization of nutrient-transport pathways to improve mineral accumulation in rice grains (Senguttuvel *et al.*, 2023; Dhanyalakshmi *et al.*, 2024) [22, 21].

A positive correlation ($r = 0.348$) was observed between grain Zn and Fe concentrations among the rice genotypes evaluated, indicating a modest positive association between the two traits in this experimental panel. Nachimuthu *et al.* (2014) [19] similarly reported a positive correlation ($r = 0.364$) between grain Zn and Fe concentrations in 192 rice genotypes, while Verma *et al.* (2017) [20] reported a stronger positive relationship ($r = 0.795$) in a set of eight rice genotypes. These findings suggest that simultaneous improvement of grain Zn and Fe may be possible through appropriate selection; however, the strength of the association may vary with genetic material and growing environment. The observed variability in grain Zn and Fe concentrations highlights the potential of rice germplasm as a source of useful alleles for nutritional improvement. HarvestPlus has used a target concentration of approximately 28 mg/kg Zn in polished rice, while the Fe target has been discussed at around 13 mg/kg in recent rice biofortification literature (Senguttuvel *et al.*, 2023; HarvestPlus, n.d.) [22]. These targets are breeding objectives and should not be interpreted as universal thresholds for nutritional adequacy, because the contribution of rice to human mineral intake also depends on daily consumption, mineral bioavailability and the presence of absorption inhibitors such as phytate. Recent reviews highlight that Fe and Zn accumulation is controlled by multiple uptake, transport, chelation and grain-partitioning mechanisms, and that genomic and molecular breeding approaches may help improve these traits (Senguttuvel *et al.*, 2023; Dhanyalakshmi *et al.*, 2024; Zubair *et al.*, 2024) [22, 21, 23]. Moreover, a recent *meta*-analysis of 245 studies reported that genetic and agronomic interventions can jointly influence grain Zn and Fe concentrations, while milling and polishing may markedly reduce mineral retention (Senthilkumar *et al.*, 2026) [24]. Therefore, the high-Fe and high-Zn genotypes identified in the present study can be considered preliminary candidates for further evaluation, including multi-environment testing, assessment of yield and grain quality, and estimation of mineral bioavailability before their direct use in breeding or deployment. An analysis of Indian multi-environment breeding trials has also highlighted the need to evaluate micronutrient concentration together with grain yield and cooking or processing quality, since nutritional improvement must be achieved without compromising farmer and consumer preferences (Prasad *et al.*, 2025) [37].

Table 1. Rice genotypes used in the study

S.N.	Genotype	S.N.	Genotype	S.N.	Genotype
1	R 1	42	R 42	83	R 83
2	R 2	43	R 43	84	R 84
3	R 3	44	R 44	85	R 85
4	R 4	45	R 45	86	R 86
5	R 5	46	R 46	87	R 87
6	R 6	47	R 47	88	R 88
7	R 7	48	R 48	89	R 89
8	R 8	49	R 49	90	R 90
9	R 9	50	R 50	91	R 91
10	R 10	51	R 51	92	R 92
11	R 11	52	R 52	93	R 93
12	R 12	53	R 53	94	R 94
13	R 13	54	R 54	95	R 95
14	R 14	55	R 55	96	R 96
15	R 15	56	R 56	97	R 97
16	R 16	57	R 57	98	R 98
17	R 17	58	R 58	99	R 99
18	R 18	59	R 59	100	R 100
19	R 19	60	R 60	101	R 101
20	R 20	61	R 61	102	R 102
21	R 21	62	R 62	103	R 103
22	R 22	63	R 63	104	R 104

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S.N.	Genotype	S.N.	Genotype	S.N.	Genotype
23	R 23	64	R 64	105	R 105
24	R 24	65	R 65	106	R 106
25	R 25	66	R 66	107	R 107
26	R 26	67	R 67	108	R 108
27	R 27	68	R 68	109	R 109
28	R 28	69	R 69	110	R 110
29	R 29	70	R 70	111	R 111
30	R 30	71	R 71	112	R 112
31	R 31	72	R 72	113	R 113
32	R 32	73	R 73	114	R 114
33	R 33	74	R 74	115	R 115
34	R 34	75	R 75	116	R 116
35	R 35	76	R 76	117	R 117
36	R 36	77	R 77	118	R 118
37	R 37	78	R 78	119	R 119
38	R 38	79	R 79	120	R 120
39	R 39	80	R 80	121	R 121
40	R 40	81	R 81	122	R 122
41	R 41	82	R 82	123	R 123
124	R 124	157	R 157	190	R 190
125	R 125	158	R 158	191	R 191
126	R 126	159	R 159	192	R 192
127	R 127	160	R 160		
128	R 128	161	R 161		
129	R 129	162	R 162		
130	R 130	163	R 163		
131	R 131	164	R 164		
132	R 132	165	R 165		
133	R 133	166	R 166		
134	R 134	167	R 167		
135	R 135	168	R 168		
136	R 136	169	R 169		
137	R 137	170	R 170		
138	R 138	171	R 171		
139	R 139	172	R 172		
140	R 140	173	R 173		
141	R 141	174	R 174		
142	R 142	175	R 175		
143	R 143	176	R 176		
144	R 144	177	R 177		
145	R 145	178	R 178		
146	R 146	179	R 179		
147	R 147	180	R 180		
148	R 148	181	R 181		
149	R 149	182	R 182		
150	R 150	183	R 183		
151	R 151	184	R 184		
152	R 152	185	R 185		
153	R 153	186	R 186		
154	R 154	187	R 187		
155	R 155	188	R 188		
156	R 156	189	R 189		

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Table 2. Summary of statistical parameters used for evaluating different traits

Parameter	Zinc (mg/kg)	Iron (mg/kg)	Grain Whitness (%)	Grain Moisture (%)
Mean	20.8	7.2	40.1	11.12
Standard Error	0.3	0.2	0.9	0.02
Standard Deviation	4.1	2.7	12.8	0.23
Sample Variance	16.8	7.5	164.0	0.05
Minimum	13.0	2.0	10.5	10.40
Maximum	33.4	16.6	79.3	11.70
Corellation (between Zn & Fe)	0.348**			

** significant at 0.05 and 0.01 level

Table 3. List of genotypes showing high grain micronutrient levels

High Zinc Rice Genotypes	High Iron Rice Genotypes
R2, R4, R5, R6, R16, R17, R20, R21, R24, R27, R29, R37, R39, R40, R41, R42, R52, R53, R54, R 57, R59, R68, R70, R71, R77, R84, R90, R91, R98, R104, R105, R111, R118, R119, R123, R131, R137, R142, R146, R152, R161, R184, R191	R12, R13, R16, R17, R21, R22, R23, R24, R29, R30, R54, R69, R74, R89, R90, R91, R92, R120, R122, R123, R126, R131, R137, R152, R153, R177, R184

Bold number indicates genotypes high for both Zinc and Iron content

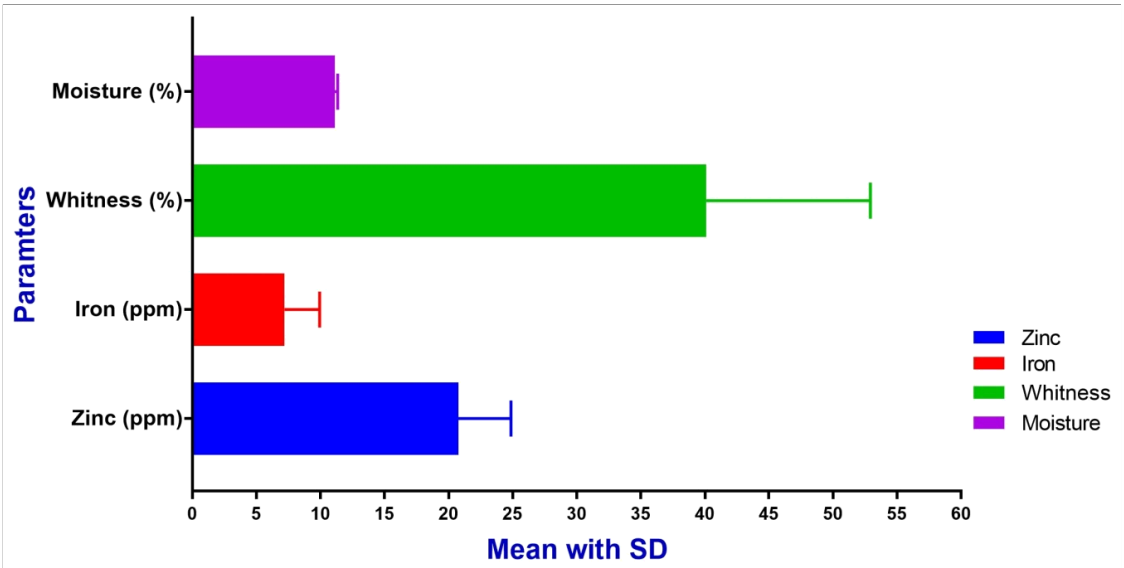


Fig. 1. Levels of Grain Quality Parameters for 192 Rice Genotypes

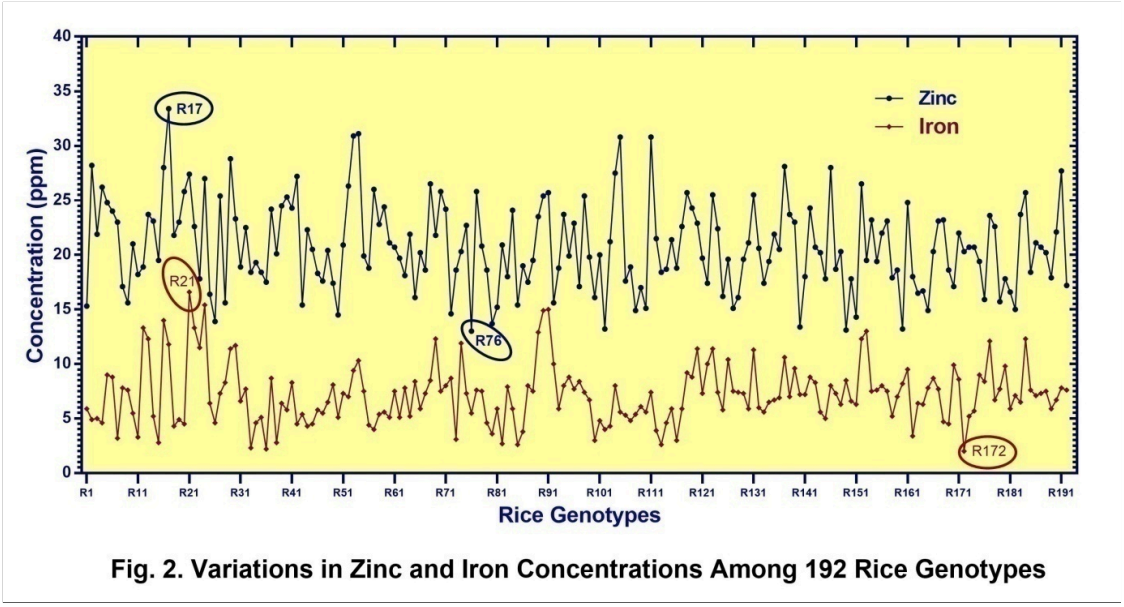


Fig. 2. Variations in Zinc and Iron Concentrations Among 192 Rice Genotypes

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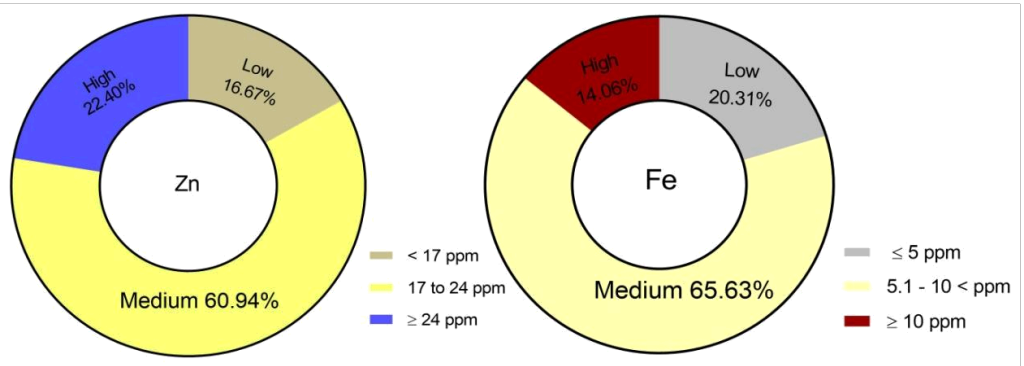


Fig. 3. Categorization of Rice Genotypes Based on Zinc and Iron Levels

Conclusions

Considerable variation in grain Zn and Fe concentrations was observed among the polished grains of the rice genotypes evaluated. Based on the variability detected, the genotypes were classified into low, medium and high micronutrient groups. Grain Zn and Fe concentrations ranged from 13.0 to 33.4 mg/kg and 2.0 to 16.6 mg/kg, respectively. Forty-three genotypes were classified as high in grain Zn and 27 as high in grain Fe; 12 genotypes were reported as common to both groups. The results demonstrate the value of rice germplasm evaluation for identifying sources of grain micronutrient variation. The identified Zn and Fe rich genotypes may be useful directly as donor materials or indirectly in crop breeding programmes aimed at improving the nutritional quality trait of rice. Further evaluation across seasons and locations, together with assessment of grain yield, processing quality and mineral bioavailability, will be necessary before recommending these genotypes for wider cultivation or nutritional interventions.

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