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RESEARCH ARTICLE

Unveiling the genetic potential and diversity of rice landraces for grain Fe content

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Abstract

Addressing micronutrient deficiency is increasingly recognized as a critical aspect of food and nutrition security in developing nations. Leveraging diverse genetic resources offers a promising avenue for identifying and enhancing micronutrient-rich genotypes through breeding strategies, thus providing sustainable solutions to this pressing challenge. The study aimed to identify rice genotypes with high Fe content and to study the extent of genetic divergence based on morphological and grain quality traits in a set of 50 native rice landraces over 2 different locations. A wide range of variation for grain Fe content was observed among the studied genotypes, which varied from 9.28–14.45 mg kg^{-1} and 1.88–4.87 mg kg^{-1} in brown and polished rice respectively. Results showed that the genotypes Jaya, *Kalanamak, Kottara Samba*, *Gandakasala* and *Gopalbhog* recorded high grain Fe content before polishing whereas *Kottara Samba, Kalapathi Black, Jyothi, Chinnar* and *Kalanamak* were found to have high Fe content after polishing. Interestingly, landraces possessing red seed coat color and medium slender grain group were identified to possess high grain Fe content. This was further substantiated by the correlation study where kernel breadth recorded a negative association with Fe content after polishing. Clustering resulted in 5 groups where the high Fe content possessing genotypes were grouped into clusters 2 and 4. Thus, these genotypes could be utilized as donors in further bio-fortification breeding programs.

Keywords

grain iron content; grain type; rice (*Oryza sativa* L.); seed coat color

Introduction

Essential micronutrients in human diet include iron (Fe), zinc (Zn), copper (Cu), boron (B), manganese (Mn) and molybdenum (Mo). One of the most crucial micronutrients is Fe, as it plays a vital role in growth, development, blood clotting, metabolic activity of enzymes (co-factors), wound healing, bone mineralization and proper functioning of thyroid and immune system (1). Nearly half of the world's population consumes rice, making it a staple food crop. Rice provides for more than half of the daily calories of the global population and thus is targeted for bio-fortification schemes to increase the grain Fe content (2). Furthermore, the brown rice production reached approximately 520.56 million MT as per FAO and USAD and in Tamil Nadu alone, it accounted for about 7.85 million MT (3). Phytic acid (PA) is the most abundant storage form of phosphorus (P) in seeds. PA acts as a strong chelator of metal cations to form phytate and is considered an anti-nutrient as it reduces the bioavailability of important micronutrients (4). PA content is high in rice grain and is responsible to reduce the concentrations of most of the essential micronutrients. Studies with dry bean genotypes have suggested that sufficient amounts of P absorbed during the early growth stages can be easily redistributed to developing organs with subsequent translocation to seeds, where it is readily converted to PA (5). This observation indicates that there is a correlation between the PA content and the bio-availability of iron (Fe) content in grains. Additionally, the P content in different plant organs is also important in determining the grain PA content.

Most of the inhabitants of Asian countries prefer rice widely, especially well-milled white rice, which is relatively low in micronutrients (6). Brown rice, on the other hand, is a rich source of vitamins, minerals, proteins and phenolic compounds; mostly concentrated in the germ and outer layer of the starch endosperm. However, intensive milling reduces the nutritional quality of rice grains by removing the husk from the paddy and the outer bran layers from brown rice leading to a significant loss of the nutrients like protein, fat, fiber, minerals, oryzanol, thiamine and phenolic compounds (7). Therefore, brown rice is considered nutritionally superior to milled white rice and recommended as a staple food for human health benefits (9). However, edible brown rice is rarely consumed as most human populations prefer white polished rice for various reasons related to appearance, taste, palatability, ease of cooking, tradition, safety and shorter shelf of brown rice (8). Rice polishing is responsible for the most significant loss of Fe content in the rice grain, reducing the nutritional quality of the white rice grains (9).

Micronutrient deficiency, also known as ''hidden hunger'', emerged as a severe global problem in the late 1970s and early 1980 and has since increased. It refers to a condition where individuals may consume enough calories but lack essential vitamins and minerals necessary for proper growth, development and overall health. Iron deficiency is a common form of hidden hunger and has significant health consequences (10). The most severe consequence of iron deficiency is anemia, a condition arising due to insufficiency of Fe resulting in decreased hemoglobin production and thus, inadequate oxygen transport through bloodstream. Anemic individuals may experience fatigue, weakness, and an increased risk of infections (9, 10). Enhancing the total iron content in rice is of immense importance for nutritional security. The best way to solve this problem is through the bio-fortification of rice and other staple crops. These crops are inexpensive and easily available to local populations, making them suitable for breeding programs (11). The primary step in conventional breeding is to screen for micronutrient-dense cultivars within the existing germplasm (12). Local native landraces harbor great genetic potential and are endowed with tremendous genetic variability scattered across wide ecological niches. The high diversity of landraces indicates better adaptation to changing climatic conditions. The impact of selection, domestication and genetic drift on the nutrient composition of indigenous rice landraces and their potential benefits to rice cultivation, in general, remain mostly unknown. While elaborating on the natural biodiversity of rice germplasms, it is important to quantify the nutritional and biochemical composition of the rice varieties (13). Since nutrient variability among rice landraces reflects their inherent genetic diversity, these rice landraces function as a genetic pool for developing nutrient-rich varieties. Selecting suitable genotypes for high micronutrient content is also crucial for developing nutrient-rich rice varieties (12). Therefore, the aim of the current study is the nutritional profiling of 50 traditional native rice landraces for iron content in brown and polished rice across different locations. The outcome of this experiment will be useful for creating a genetic database for a specific breeding program to improve the micronutrient concentrations in rice varieties.

Materials and Methods

Plant collection

A panel of 50 rice landraces (*Oryza sativa* L.) was collected from different agroecological regions of Tamil Nadu, India (Table 1). The experiment was carried out using a randomized complete block design in 2 locations, namely Agricultural Research Station, Paramakudi and Rice Research Station, Tirur. Details of the experimental sites are provided in Table 2. In both locations, direct seeding was done in pulverized dry soil. Each entry was sown in 3 rows with a row length of 3 m and a row-to-row spacing of 20 cm. Plant-to-plant spacing was maintained at 20 cm by thinning the excess plants in the rows 14 days after germination. The germplasm accessions were evaluated to study important morphological parameters (Table 1, Fig. 1) along with grain iron (Fe) content in brown and polished rice. Observations on post-harvest grain traits, such as hulling percentage (HP), milling percentage (MP), head rice recovery percentage (HRR), kernel length (KL), kernel breadth (KB), length to breadth ratio (LBR) and quality traits Fe content in brown (FEB) and polished rice (FEP) were recorded following standard procedures.

Estimation of grain Fe content

In each genotype, 3 plants in the middle row were harvested separately and pooled for grain Fe content analysis. One hundred grams of seeds were de-husked and milled using non-metallic de-husker (Krishi International 810 de-husker) at the Grain Quality Laboratory, Tamil Nadu Rice Research Institute, Aduthurai, Tamil Nadu, India. Samples were polished for 30–45 sec and cleaned using tissue paper. Only full, undamaged grains free of debris were analyzed. The iron content in brown and polished rice samples was estimated using a non-destructive, energy-dispersive X-ray fluorescence

Table 1. Pooled mean of traits associated with grain Fe content of native rice landraces

Sl.No.	Genotypes	HP	MP	HRR	KL	KB	LBR	FEB	FEP	GT	SCC
1	*AathurKichadi Samba*	81.66	75.50	55.00	5.40	1.80	3.00	11.80	4.60	Short-slender	White
2	*Aanai Komban*	81.00	71.33	56.33	7.33	2.03	3.61	10.03	3.77	Long-slender	White
3	*Adukkan*	79.50	67.83	51.17	6.00	2.13	2.81	11.80	3.00	Medium-slender	Red
4	*Altera*	81.50	72.67	52.83	6.28	2.18	2.88	11.15	2.43	Medium-slender	Brown
5	*Athira*	80.16	71.67	56.50	5.38	1.80	2.98	10.25	3.62	Short-slender	White
6	*Athur Kichadi*	79.50	70.17	55.17	5.30	1.80	2.94	11.12	2.95	Short-slender	White
7	*Bavani*	75.66	66.83	47.67	5.67	2.05	2.76	11.78	3.70	Medium-slender	White
8	*Chenellu*	78.66	70.17	55.83	6.4	2.25	2.90	11.28	2.73	Long-slender	White
9	*Chinna Adukku Nel*	76.50	67.67	55.00	5.48	1.82	3.01	11.23	2.20	Medium-slender	Brown
10	*Chinkinikar*	78.83	68.17	56.17	5.53	2.70	2.04	11.87	2.47	Short-bold	Red
11	*Chinnar*	81.50	73.33	57.17	7.85	2.10	3.73	12.33	4.77	Long-slender	Red
12	*Chinthamani*	79.66	70.33	59.00	5.63	1.50	3.76	11.68	2.87	Medium-slender	White
13	*Chithirai Kar*	76.33	64.50	56.50	5.92	2.00	2.96	9.45	2.12	Medium-slender	Brown
14	*Edakkal*	75.67	65.83	60.50	6.51	3.10	2.06	11.02	2.10	Long-bold	Brown
15	*Gandakasala*	82.33	74.33	61.17	6.05	2.02	3.00	12.73	3.12	Medium-slender	White
16	*Geb-24*	79.00	67.00	55.50	5.57	1.89	2.95	12.03	3.78	Medium-slender	White
17	*Gedumani*	79.17	68.50	56.00	7.00	2.37	3.04	9.82	2.15	Long-slender	White
18	*Gopal Bhog*	79.67	69.00	58.33	5.62	2.03	2.76	12.65	2.95	Medium-slender	White
19	*IllupaiPooSamba*	81.17	72.17	54.17	5.63	1.90	2.96	10.98	4.28	Medium-slender	White
20	*Indian Samba*	75.50	66.83	48.50	6.77	2.35	2.89	9.28	2.83	Long-slender	White
21	*Irunaazhi*	77.50	68.67	58.17	6.87	2.15	3.19	10.42	2.02	Long-slender	Red
22	*Jai Sri Ram*	78.17	69.33	50.50	5.38	1.50	3.59	11.78	3.45	Short-slender	White
23	Jaya	77.00	65.67	49.17	6.10	2.03	3.00	14.45	2.85	Medium-slender	Red
24	*Jyothi*	74.67	64.17	54.50	5.38	2.22	2.43	11.82	4.82	Short-slender	Red
25	*Kaan*	80.83	70.83	59.00	5.72	2.85	2.00	11.35	2.07	Short-bold	Red
26	*Kaatu Ponni*	79.17	70.17	56.33	5.85	2.00	2.95	11.65	4.68	Medium-slender	Brown
27	*KaatuSamba*	77.67	68.33	52.67	5.87	2.00	2.94	12.60	3.52	Medium-slender	Brown
28	*KaivaraSamba*	80.33	56.67	49.00	5.45	1.90	2.87	11.98	3.47	Short-slender	Red
29	*Kalanamak*	80.00	72.17	59.17	6.68	2.15	3.10	13.43	4.75	Long-slender	White
30	*KalapathiBlack*	82.17	71.50	60.67	6.73	2.37	2.83	10.98	4.85	Long-slender	Black
31	*Kalavai*	76.00	68.83	51.67	6.68	2.97	2.10	10.13	3.17	Long-bold	White
32	*Kallimadaiyan*	80.67	70.83	59.17	5.50	2.00	2.75	10.53	2.17	Short-bold	White
33	*Kallrandaikar*	78.33	68.67	53.00	6.37	2.07	3.08	9.88	3.68	Medium-slender	White
34	*Kallundai*	81.17	70.83	54.67	6.63	3.20	2.05	11.33	1.88	Long-bold	Light brown
35	*Kalyani*	74.00	66.50	53.67	6.13	2.07	2.96	11.05	3.17	Medium-slender	Red
36	*Kanchana*	77.00	68.33	50.67	6.30	2.10	3.00	10.72	3.50	Medium-slender	Red
37	*Kandhasali*	80.17	71.33	57.17	5.47	2.02	2.71	9.32	3.80	Short-slender	White
38	*Karikajanavalli*	81.00	71.50	55.17	5.28	2.33	2.26	12.20	3.45	Short-bold	Red
39	*Karikardiderum*	76.83	69.50	53.00	6.15	1.93	3.18	9.75	3.58	Medium-slender	Brown
40	*Karimbalan*	79.17	68.83	54.33	5.93	2.00	2.96	10.57	2.28	Medium-slender	Red
41	*Karnel*	78.17	71.17	56.00	5.51	2.63	2.09	12.02	3.88	Short-bold	Red
42	*Karuppu Nel*	80.17	72.00	58.67	6.18	2.10	2.94	11.52	2.28	Medium-slender	Brown
43	*Katta Samba*	78.00	70.17	54.17	6.80	3.00	2.00	11.50	2.87	Long-bold	Brown
44	*Kavuni*	74.50	68.33	54.33	5.52	2.25	1.85	10.18	3.53	Short-bold	Black
45	*KichiliSamba*	74.50	66.17	53.50	6.45	2.16	2.98	10.82	3.22	Medium-slender	White
46	*Kochin Samba*	78.33	68.33	54.67	5.60	1.89	2.96	9.47	2.43	Medium-slender	White
47	*KoomVazhai*	78.83	69.67	51.83	6.68	2.28	2.92	10.78	2.75	Long-slender	Red
48	*KothamalliSamba*	80.00	71.33	56.17	4.87	2.40	2.03	10.82	4.28	Short-bold	Brown
49	*KottaraSamba*	80.17	65.33	55.33	6.33	2.00	3.15	13.41	4.87	Medium-slender	Red
50	*Kudavaragai*	79.33	71.83	56.67	7.28	3.40	2.14	12.37	2.58	Long-bold	Red

HP-hulling percentage; **MP**-milling percentage; **HRR**-head read rice recovery; **KL**-kernel length; **KB**-kernel breadth; **LBR**-length-to-breadth ratio; **FEB**-Fe content in brown rice; **FEP-**Fe content in polished rice; **GT-**grain type; **SCC-**seed coat color

Table 2. Details of meteorological data of 3 locations

Parameters	Paramakudi	Tirur
Duration	October–February	November–March
Latitude (°N)	9.48	13.07
Longitude (°E)	78.36	57.21
Annual temperature (°C)	26.5	27.9
Average rainfall (mm)	840	1152
Type of soil	Clay loam	Sandy clay
pH	8.4	6.7

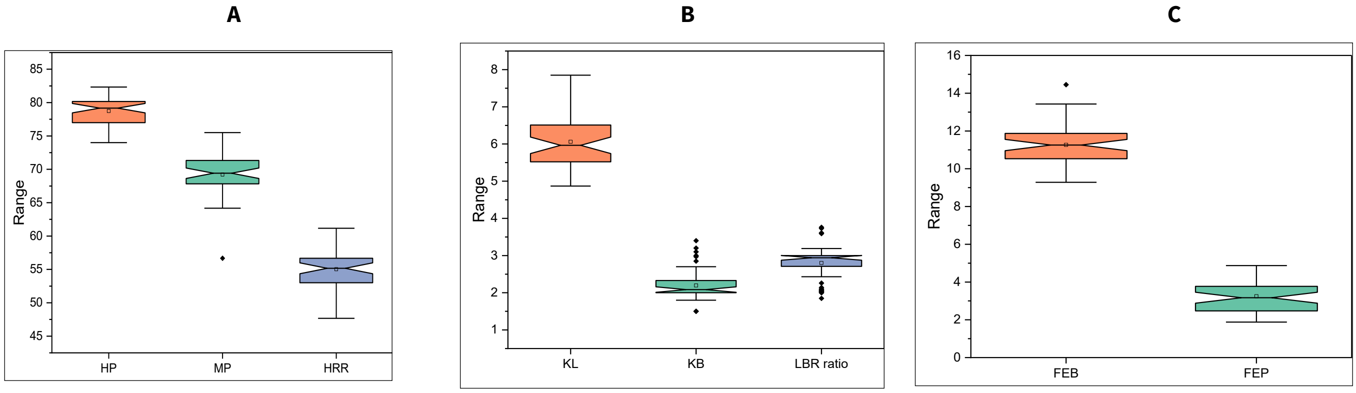

Fig. 1. Box plots showing the mean performance for **(A)** HP, MP and HRR **(B)** KL, KB and LBR and **(C)** FEB and FEP

spectrometry (ED-XRF) instrument (Hitachi X-Supreme following Harvest Plus guidelines (14). Each sample of brown and polished rice (5 g) was weighed and transferred to sample cups. The sample cups were gently shaken for uniform distribution of samples and then set aside for analyses. The concentration of iron was expressed in milligrams per kilogram (mg kg^{-1}) or parts per million (ppm) of grains. The samples were analyzed in triplicate (15).

Statistical analysis

Data on morphological and quality traits were analyzed using the 'R' statistical software with the 'agricolae' package to examine the pooled mean and analysis of variation (mean sum of squares) (16,17). Correlation studies among the traits were estimated using the 'corrplot' package and principal component analysis (PCA) (18). The genetic diversity of clustering was assessed using the Ward. D2 method with the assistance of the 'Euclidean' distance matrix to identify the best genotypes.

Results

ANOVA and *per se* performance of the genotypes

The pooled analysis of variance (Table 3) was found to be highly significant for season and environment (mean sum of squares) for all traits studied and it was also significant for environment X genotype interaction except for HP and HRR over the 2 locations. A wide range of genetic variation was observed across the germplasm for the studied traits. The analyzed data on 6 morphological traits along with their mean differences including Fe content in brown and polished rice were presented (Table 1). The genotypes, *Gandakasala* (82.33%, 74.33% and 61.7%), *Kalapathi Black* (82.17%, 71.50% and 60.67%), *Aathur Kichidi Samba* (81.6%, 75.50% and 55%), *Chinnar* (81.50%, 73.33% and 57.17%) and *Illupoi Poo Samba* (81.17%, 72.17% and 54.17%), exhibited high mean for HP, MP and HRR values. These genotypes could be utilized as donors in future breeding programs.

A higher LBR was observed for *Chinnar* (3.73), which had a 'long slender' shaped grain with the highest length (7.85 mm) and also possessed Fe content of 12.33 mg kg^{-1} and 4.77 mg kg^{-1} in brown and polished rice respectively. Whereas the lowest LBR ratio was noticed in *Kavuni* (1.85 mm) which had a 'short bold' shaped grain with a length of (5.52 mm) and recorded 10.18 mg kg^{-1} of Fe content in brown rice and 3.53 mg kg^{-1} in polished rice.

Grain Fe content in brown and polished rice

Fe content is a major nutritional trait used in bio-fortification programs by donors. Among the 50 landraces studied, the Fe content in brown rice ranged from 14.45 to 9.28 mg kg^{-1} (Jaya and *Indian Samba*) with an average of 11.38 mg kg^{-1}. The top 5 performing genotypes for Fe content in brown rice were Jaya, *Kalanamak*, *Kottara Samba*, *Gandakasala* and *Gopalbhog*. Conversely, the Fe content in polished rice ranged from 4.87 to 1.88 mg kg^{-1} (*Kottara Samba* and *Kallundai*) with an average of 3.25 mg kg^{-1} (Table 4). The top performing genotypes for Fe content in polished rice were *Kottara Samba*, *Kalapathi Black*, *Jyothi*, *Chinnar* and *Kalanamak.* The polishing process resulted in significant variation in Fe content, with studied genotypes experiencing a 2 fold reduction in Fe content, after 30-45 sec of polishing, After de-husking and polishing, the Fe content of the 50 genotypes was classified into 3 categories: low, moderate and high (Table 3, Fig. 2) based on previous research (9).

Correlation

The degree of association among the traits was determined by their correlation coefficients. The

Table 3. Pooled analysis of variance (ANOVA) for morpho-quality traits in rice

Source of variation	df	MSS							
		HP	MP	HRR	KL	KB	L/B	FEB	FEP
Replications	4	147.3	28.00	12.03	2.2	0.87	0.6	26.48	14.15
Genotypes	49	28.51**	59.21**	63.63**	1.57**	0.51**	3.79**	7.72**	4.52**
Environment	1	21.87**	253.92**	74**	0.16*	0.09*	1.37**	104.19**	6.9**
E X G	49	3.39	28.34*	22.92	0.09**	0.06**	0.28**	12.18**	0.58*
Error	196	3.79	26.67	29.23	0.05	0.03	0.05	1.93	0.27
Mean		78.74	69.37	55.11	5.94	2.27	3.15	11.38	3.25
CV(%)		2.47	7.44	9.81	3.61	7.68	7.19	12.22	15.87
CD(P=01)		2.90	7.69	8.05	0.32	0.26	0.34	2.07	0.77
SED		1.12	2.98	3.12	0.12	0.10	0.13	0.80	0.42

****, *** Significance at 1% and 5% level respectively. **HP**-hulling percentage; **MP**-milling percentage; **HRR**-head read rice recovery; **KL**-kernel length; **KB**-kernel breadth; **LBR**-length-to-breadth ratio; **FEB**-Fe content in brown rice; **FEP**-Fe content in polished rice

Table 4. Classification of genotypes based on iron content in both brown and polished rice

Brown rice				Polished rice			
Sl.No.	**Class**	**Range**	**No. of genotypes**	**Sl.No.**	**Class**	**Range**	**No. of genotypes**
1	Low	<12 mg kg^{-1}	39	**1**	Low	<4 mg kg^{-1}	41
2	Moderate	12.1-15 mg kg^{-1}	11	**2**	Moderate	4.1-8 mg kg^{-1}	9
3	High	>15.1 mg kg^{-1}	0	**3**	High	>8 mg kg^{-1}	0

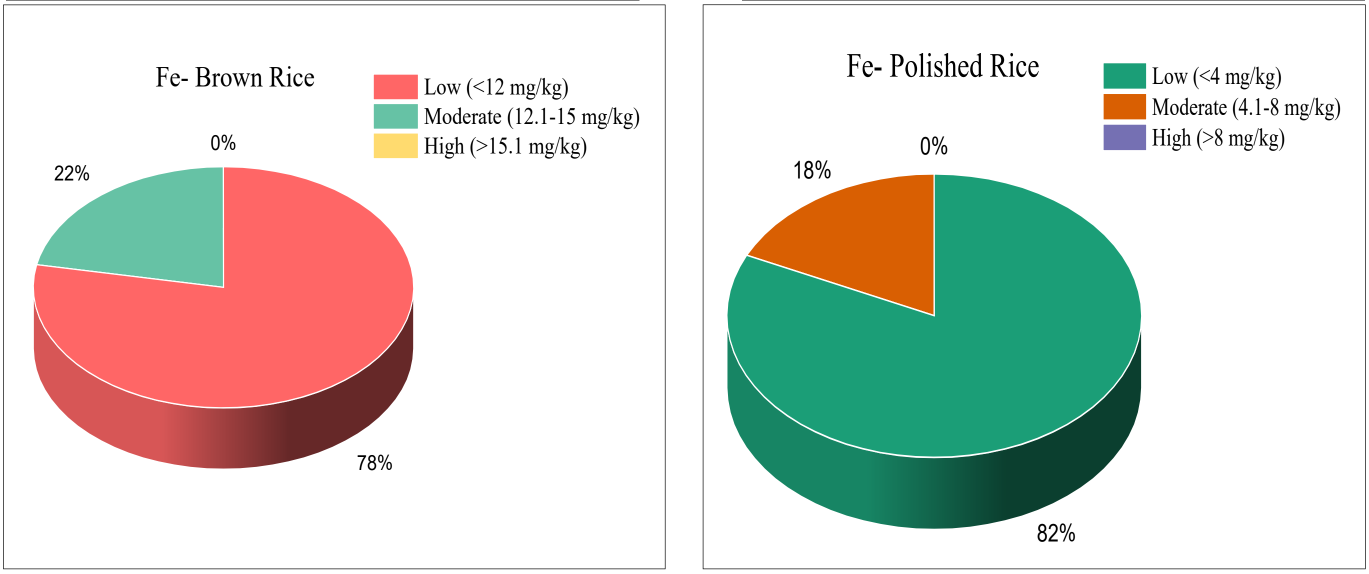

Fig. 2. Percentage of genotypes having low and moderate Fe content from t 2 locations

association analysis aids in examining the possibility of increasing Fe content through the indirect selection of its highly correlated component traits. The HP had a strong and positive relationship with MP (r = 0.57****, p<0.0001), HRR (r = 0.44**, p<0.01) and FEB (r = 0.29*, p<0.05). The milling percentage recorded a strong positive correlation with HRR (r = 0.48***, p<.001). KL showed a positive and significant association with KB (r =0.31* p<0.05). Additionally, KB exhibited a negative association with FEP (r = -0.31*, p<0.05) and LBR (r = -0.31*, p<0.05). Highly significant positive correlations were observed between HP and FEB. The associations between these traits were strong and significant (Fig. 3).

Principal component analysis (PCA)

PCA is a multivariate statistical analysis used to examine and simplify complex and large datasets. It studies the

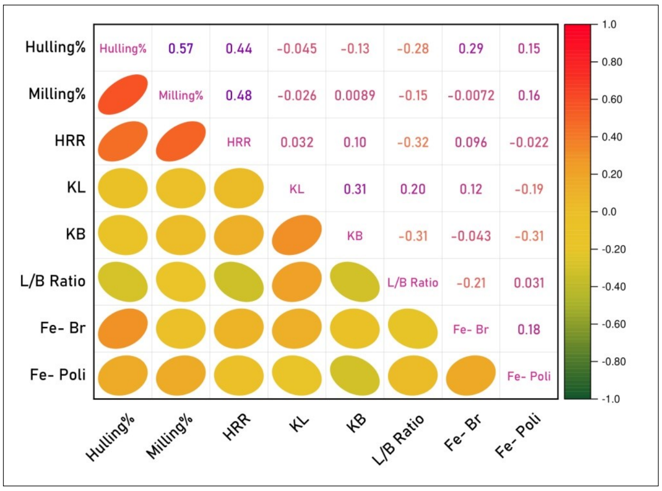

Fig. 3. Correlogram showing an association between grain quality traits. **HP**-hulling percentage; **MP**-milling percentage; **HRR**-head read rice recovery; **KL**-kernel length; **KB**-kernel breadth; **LBR**-length-to-breadth ratio; **FEB**-Fe content in brown rice; **FEP**-Fe content in polished rice

correlation among traits and clusters, to determine the most significant characteristics that contribute to the diversity among native landraces (14). A total of 8 principal components (PCs) were extracted, with the 4 most informative PCs having Eigen-values of 2.13, 1.9, 1.30 and 1.02, which are responsible for 79.62% of the total variation (Table 5). PC1 accounted for 26.66 % of the total variability and was mainly contributed to by MP and LBR. PC2 contributed 23.90% of the total variance, which was explained mainly by FEP and LBR. PC3 contributed about 16.29 % and was linked with FEB, KL and FEP. The PC4 accounted for a 12.78 % variance and was contributed to by LBR, KL and MP. The PCA-variables plot (Fig. 4a) and PCA bi-plots (Fig. 4b) analysis showed that quality traits such as FEB, LBR, KL and MP were critical traits contributing to the total variability of the landraces, while the remaining traits contributed a less to the phenotypic variability.

A PCA biplot analysis can help select traits that can be categorized into main groups and subgroups based on homogeneity and dissimilarity. In this data set, 4 groups were identified in the PCA biplot considering both PC1 and PC2 (Fig. 4c). HP, MP and HRR were clustered in group I, while FEB, FEP and LBR were in group II and KL clustered as group III; KB belonged to group IV. Group I traits, main contributors to PC2, were strongly correlated with the genotypes of *Gandakasala*, *Kalapathi Black* and *Aathur Kichadi Samba* (top left) according to the PCA biplot. In contrast, group II traits, significant contributors to PC1, were correlated with the genotypes of *Chintamani*, *Kalanamak, Jai-Sri-Ram* and *Kottara Samba* (bottom left). The genotypes *Aathur Kichadi Samba*, *Chinkinikar*, *Karimbalan*, *Gedumani* and *Kaatu Ponni* showed the strongest correlations with the features of groups III and IV, leading to PC2 (Table 5).

Cluster analysis

The grouping of landraces into different clusters was done using the Ward 2.0 method and the 'Euclidean' genetic distance matrix between all possible pairs of genotypes. A total of 50 native landraces were grouped into 5 distinct genetic clusters (Fig. 5, Table 6). The number of landraces in each cluster varied, with 5 in clusters II and IV and nineteen in cluster III. Genotypes like *Bavani*, Jaya, *Jyothi*, *Kaivara Samba* and *Kottara Samba*, which had high mean values for FEB (12.68 mg kg^{-1}), belonged to cluster IV. *Aanai Komban*, *Chinnar*, *Jai-Sri-Ram*, *Chinthamani* and *Kalanamak*, with high mean values for HRR (57.73 %), LBR (3.55 mm) and the second highest mean value for FEB

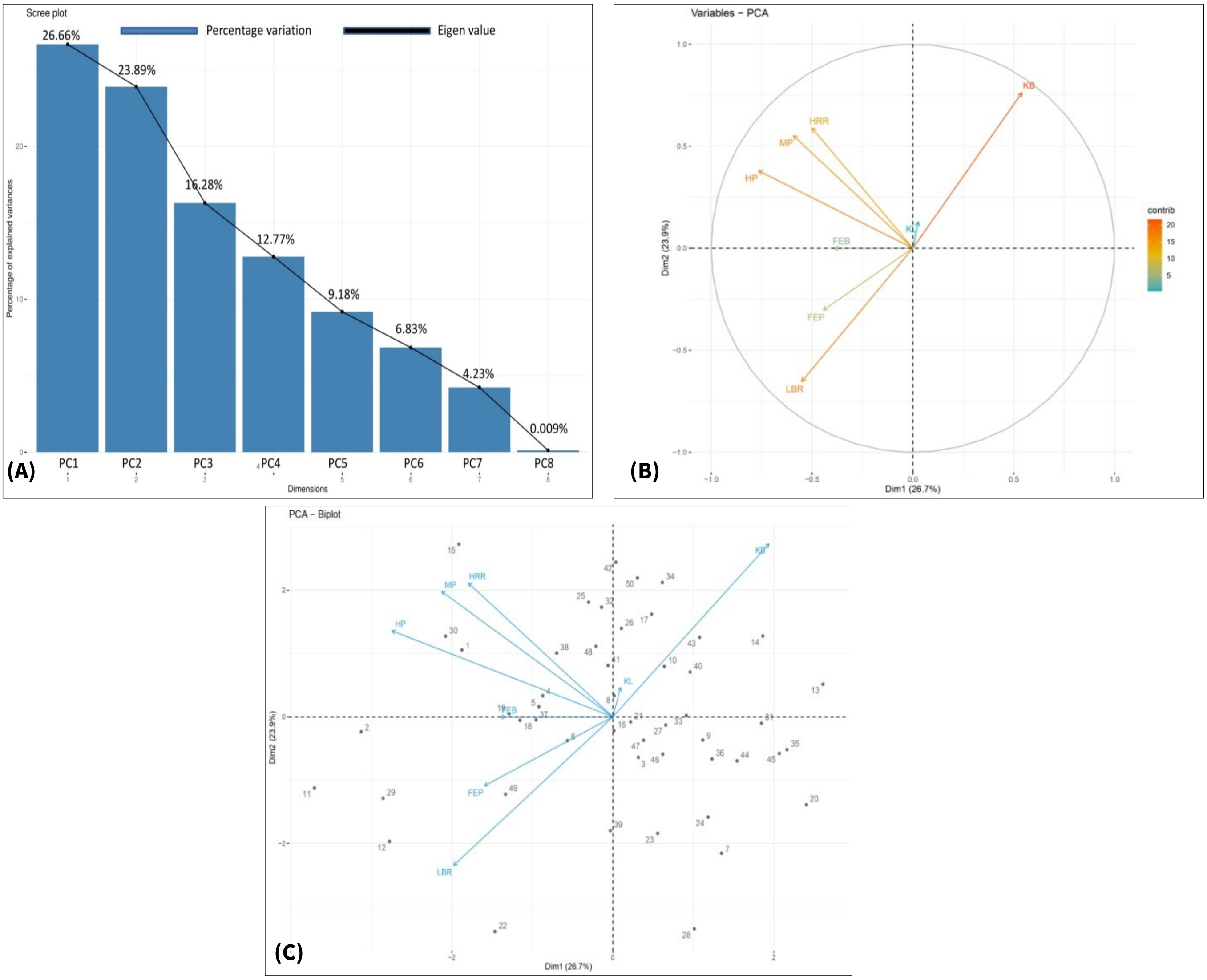

Fig. 4. (A) Scree plot PCA depiction of the 50 landraces and their eigen values contribution to total variability. **(B)** Clustering of variables towards PCs. **(C)** PCA biplot of 50 local landraces and 8 quality traits plotted by PC1 *vs* PC2**. HP-**hulling percentage; **MP-**milling percentage; **HRR-**head read rice recovery; **KL-**kernel length; **KB-**kernel breadth; **LBR-**length-to-breadth ratio; **FEB-**Fe content in brown rice; **FEP-**Fe content in polished rice

Table 5. Eigen-values, percentage of variation, cumulative percentage and eigen vectors values of the first 4 principal components

Parameters	PC1	PC2	PC3	PC4
Eigen Value	2.13	1.91	1.30	1.02
Variance %	26.66	23.90	16.29	12.78
Cumulative Variance %	26.66	50.56	66.85	79.63
HP	-0.55	0.10	0.07	0.14
MP	**0.50**	0.02	-0.24	**0.38**
HRR	-0.49	-0.19	-0.16	0.15
KL	0.07	-0.42	**0.50**	**0.54**
KB	-0.01	-0.66	0.00	-0.14
LBR	0.36	**0.25**	0.07	**0.66**
FEB	-0.23	0.05	**0.78**	-0.26
FEP	-0.15	**0.52**	**0.24**	-0.03

PC-principal component; **HP-**hulling percentage; **MP-**milling percentage; **HRR-**head read rice recovery; **KL**-kernel length; **KB-**kernel breadth; **LBR-**length-to-breadth ratio; **FEB-**Fe content in brown rice; **FEP-**Fe content in polished rice

Table 6. Distribution of 50 landraces of rice

Cluster	Number of landraces	Names of genotypes
I	12	*Aathur-Kichadi-Samba, Athira, Kandhasali, Karnel, Kaatu-Ponni, Kalapathi-Black, Karikajanavalli, Gandakasala, Illupai-Poo-Samba, Kattu-Samba,GEB-24, Kothamalli-Samba*
II	5	*Aanai-Komban, Chinnar, Jai-Sri-Ram, Chinthamani, Kalanamak*
III	19	*Chinna Adukku Nel, Gedumani, Irunaazhi, Kaan, Athur Kichadi, Chinkinikar, Gopalbhog, Kallundai, Karuppu Nel, Kochin Samba, Adukkan, Karikardiderum, Katta Samba, Koom Vazhai, Altera, Chenellu, Kallimadaiyan, Karimbalan, Kudavaragai*
IV	5	*Bavani,* Jaya*, Jyothi, Kaivara Samba, Kottara Samba*
V	9	*Chithiraikar, Kallrandaikar, Kichili Samba, Edakkal, Kalavai, Kalyani, Indian Samba, Kachana, Kavuni*

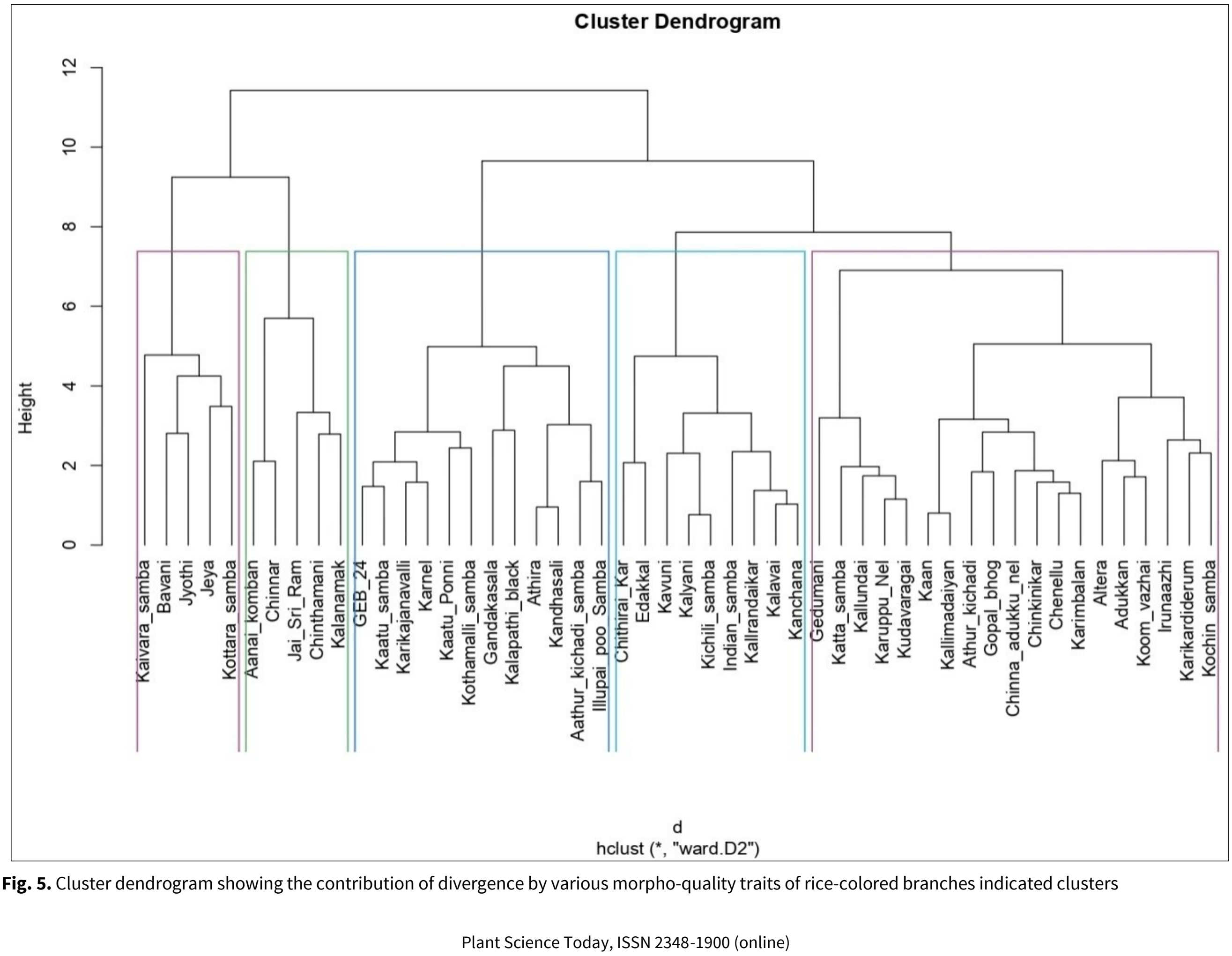

Fig. 5. Cluster dendrogram showing the contribution of divergence by various morpho-quality traits of rice-colored branches indicated clusters

(12.25 mg kg^{-1}), were in cluster II. These clusters were selected for crossing programs to develop bio-fortified varieties. Most genotypes with higher mean values for HP (80.22 %), MPB (71.33 %) and FEP (3.98 mgkg^{-1}) were placed in cluster I. Additionally, cluster V had 9 genotypes with high mean values for KL (6.22 mm) and KB (2.34 mm).

Each trait showed significant variations in mean values for each cluster (Table 7). The phenotypic variation within cluster distance was highest in cluster III followed by clusters II, IV and V, while, cluster I had the lowest mean distance. The highest distance was observed between clusters I and IV followed by II and IV and II and V, with the lowest distance seen in clusters III and V (Fig. 6).

Discussion

Oryza is an agronomically important genus with a wide range of morphologies. Significant efforts are being made to understand the nutritional dynamics of rice. Modern high-yielding rice varieties are deficient in Fe content. However, some landraces such as basmati varieties namely Basmati KS 282 and Super Basmati and wild rice (*Japonica*) still have high levels of Fe (19–22). Screening available germplasm helps identify genotypic sources and potential donors for targeted traits in improvement programs. This process also aids breeders in conducting genetic investigations and developing molecular markers which can accelerate breeding program (9).

The PA content of seeds is primarily influenced by genetic and environmental factors like climatic conditions and soil characteristics (23). Effects of PA on mineral bioavailability also depend on the PA/micronutrient ratio and can be a determinant for understanding mineral availability in food types (24). The molar ratio of PA/Fe is recommended to be 1 or lower for better Fe absorption from cereals (25). Brown rice is superior to milled rice in terms of bioactive compounds and minerals but also has PA that may reduce the bioavailability of minerals. The effect of PA on Fe and Zn bioavailability from milled rice grain was investigated. Rice with contrasting PA was analyzed for Fe and Zn bioavailability in milled and cooked rice. The genotype, Khira, with the lowest PA (2.0 g kg^{-1}), exhibited high Fe and Zn bioavailability, while Phalguni, with the highest PA (11.2 g kg^{-1}), showed low Fe and Zn bioavailability. The PA had a significant negative correlation with Fe and Zn bioavailability (26). The earlier report suggested that the dietary fibre and PA present in higher concentrations in brown rice might inhibit mineral absorption in the human digestive system (27).

In the current study, 50 rice genotypes were evaluated at the Agricultural Research Station, Paramakudi and the Rice Research Station, Tirur, Tamil Nadu, India, to identify Fe-rich genotypes for the development of bio-fortified, high-yielding rice varieties. The results indicated a significant variation in Fe content among the studied rice genotypes. The statistical analysis (pooled ANOVA) of this investigation demonstrated a considerable and wide range of variance across all the traits (genotypes and environments) associated with Fe content in both brown and polished rice. On the other hand, the mean sum squares of environment × genotype showed significant association except for HP and HRR. Previous researchers had also observed a significant level of variation in rice landraces for grain nutrients (28, 29). The coefficient of variation for morpho-quality traits such as HP, MP, HRR, KL, KB, LBR, FEB and FEP ranged from 2.47 to 15.87 %. The coefficient of variation is a statistical tool used to quantify the degree of relationship or variation between 2 variables. It provides information about the direction and intensity of a linear relationship between 2 variables, leading to improvements in yield in the offspring and predicting the performance of certain traits based on other traits. Correlation analysis helps validate the effectiveness of markers by comparing phenotypic correlations (9, 30).

Genotypes in the study were divided into 5 categories based on grain size: long-slender (9), long-bold (5), medium-slender (22), short-slender (7) and short-bold (7). The seed coat colors of the studied landraces (de-hulled grains) were classified as white, light brown, brown, black and red according to the rice descriptors provided by IRRI, 1980 (31). Out of the 50 genotypes, 21 had white kernels, 10 were brown, 2 were black (*Kavuni* and *Kalabathi Black*) and 1 (*Kallundai)* had a light brown color.

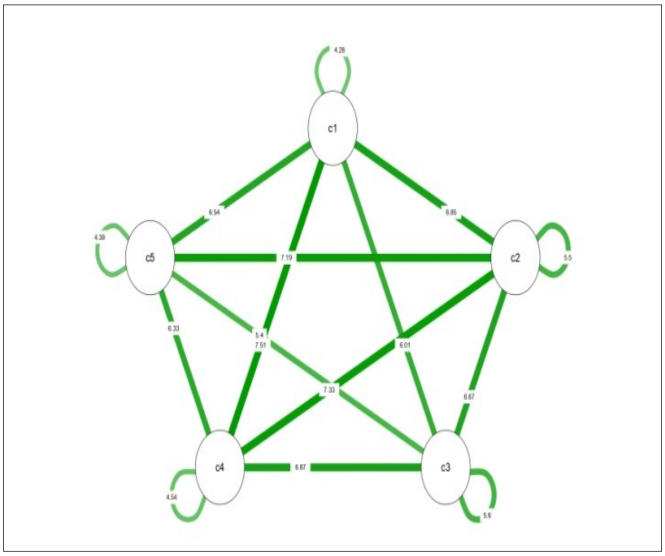

Fig. 6. Inter and intra-cluster distance matrix of 50 local landraces of rice

Table 7. Cluster means and per contribution of 8 quality traits in rice

Clusters	HP	MP	HRR	KL	KB	LBR	FEB	FEP
I	**80.22**	**71.33**	56.37	5.56	1.93	2.72	11.44	**3.98**
II	80.06	71.29	**57.73**	6.16	1.85	**3.55**	12.25	3.92
III	79.16	69.77	55.51	6.02	2.32	2.60	11.29	2.51
IV	77.56	63.73	51.13	5.78	2.20	2.84	**12.69**	3.94
V	75.75	67.11	53.59	**6.22**	**2.34**	2.69	10.28	3.03

HP-hulling percentage; **MP-**milling percentage; **HRR-**head read rice recovery; **KL**-kernel length; **KB-**kernel breadth; **LBR-**length-to-breadth ratio; **FEB-**Fe content in brown rice; **FEP-**Fe content in polished rice

16 landraces were classified as red rice. In domestication and advanced breeding programs, long-grained rice is typically chosen based on consumer demand. Grain dimensions, including length, width and shape are considered crucial during the domestication of rice (32, 33).

Genotypes *Gandakasala*, *Kalapathi Black*, *Aathur Kichidi Samba*, *Chinnar* and *Illupoi Poo Samba* exhibited high mean values for HP, MP and HRR compared to other genotypes. These genotypes displayed medium slender, white; long slender, black; short slender, white; long slender, red and medium slender, white grain types and seed coat colors, respectively. The Fe content of these genotypes was recorded as 12.73/3.12, 10.98/4.85, 11.80/4.60, 12.33/4.77 and 10.98/4.28 mg kg^{-1} in brown rice and polished rice respectively. Grain type and seed coat color demonstrated differences among the genotypes in terms of Fe content in brown rice and polished rice. The majority of landraces with high FEB had medium slender grain type and red seed coat color compared to others. In general, red rice types have higher iron content than white rice variants. The iron content of rice landraces tends to vary significantly geographically, not only within India but also between countries due to genetic causes. Numerous studies have found that the traditional genotypes exhibit significantly high Fe content levels (6, 34, 35). A screening study among 50 landraces for Fe content in brown rice ranged from 9.28 to 14.45 mg kg^{-1}. The results also partially agree with an earlier study where the Fe content varied from 6.9 to 22.3 mg kg^{-1} among 33 landraces of Arunachal Pradesh (9). Panels of 939 genotypes were evaluated for the variation in rice grain Fe content, which ranges from 15.9 to 58.4 mg kg^{-1} in brown rice (36). Variation in Fe and Zn concentration was also documented using 192 rice genotypes (37, 38).

The Fe content of 50 different rice genotypes in polished rice ranged from 1.88 to 4.87 mg kg^{-1}. Genotypes such as *Kottara Samba, Kalapathi Black*, *Jyothi*, *Chinnar* and *Kalanamak* showed high Fe content, ranging from 4.68 to 4.87 mg kg^{-1}. The study also found a weak correlation between Fe content in brown and polished rice, attributable to the intensive milling process that reduces nutrients. Previous studies have reported a direct relationship between Fe content and MP (9, 20, 39 & 40). Therefore, brown rice is considered nutritionally superior to milled white rice and is recommended as a staple food for human health benefits.

Correlation studies are useful in determining the relationship between various qualities, allowing plant breeders to select accessions with desirable traits. The highest associations were observed between HP and MP followed by FEB. Kernel breadth had a negative association with FEP. Previous studies reported a negative correlation between grain dimensions and Fe content in polished rice (30, 32, 41). The contribution of each component to the total variance is calculated using PCA. In the current study, 4 PCs explained 79.62% of the variation. A similar percentage of cumulative variance was observed in earlier studies (41). Selecting features that contribute to the highest morphological variation via 2 primary PCs would be desirable. The 4 genotypes namely *Chintamani*, *Kalanamak*, *Jai-Sri-Ram* and *Kottara Samba* in PC1 had medium to long slender grain types and a combination of red-white seed coat color. The 3 genotypes (*Gandakasala*, *Kalapathi Black* and *Aathur Kichadi Samba*) in PC2 observed different grain dimensions (long, medium and short slender) and white to black seed coat color, contributing to the maximum genetic diversity. Therefore, it would be beneficial to use these accessions as a donor parent in rice breeding programs targeting nutritional improvements in rice.

Cluster analysis grouped 50 rice genotypes into 5 different clusters. Cluster III comprised 19 accessions accounting for medium slender to long slender grain types. The Fe content within this group ranged from 9.47 to 12.6 mg kg^{-1} and 1.88 to 3.58 mg kg^{-1} in brown and polished rice, respectively. The red rice genotypes exhibited a higher amount of Fe content than the brown rice genotypes. The genotype *Kallundai* (1.88 mg kg^{-1}), exhibited a light brown seed coat color and recorded the lowest Fe content in polished rice. The genotypes with the highest FEB, *viz*., Jaya, *Kalanamak* and *Kottara Samba*, belong to clusters IV and II and had medium to long slender grain type and red-white seed coat colors. Clusters (II and IV) represented the lowest number of genotypes thus they may be directly used as parents in further hybridization programs to combine desirable characters. Similar results were reported by an earlier study (42).

This study found a positive correlation that indicates the selection of the most critical micronutrient (Fe), simultaneously. The Fe content in rice grains is significantly affected by environments, genotypes and genotype × environment interactions (9, 43). A comparison of the grain Fe content of 50 genotypes evaluated in 2 different locations revealed significant variance. Despite this, the overall trend for grain micronutrient content changed significantly between the locations (Fig. 7). The genotypes, *viz*., Jaya (14.45 mg kg^{-1}), *Kalanamak* (13.43 mg kg^{-1}), *Kottara Samba* (13.41 mg kg^{-1}), *Gandakasala* (12.73 mg kg^{-1}) and *Gopal bhog* (12.65 mg kg^{-1}), were top ranking

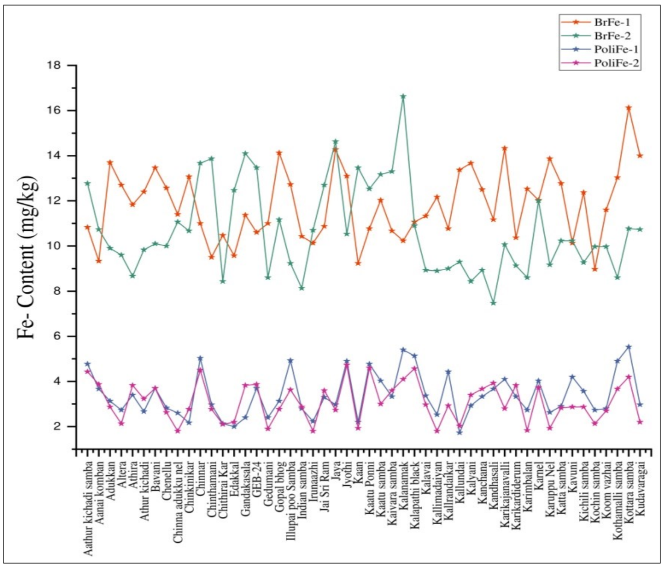

Fig. 7. Representation of Fe content variation over 2 locations

with the high pooled mean value for grain Fe content in brown rice. Interestingly, among these 5 genotypes, '*Kalanamak'* also had a high pooled mean value (4.75 mg kg^{-1}) for Fe content in polished rice. Micronutrient density in rice grains is determined by several interconnected metabolic pathways involved in nutrient uptake, transportation to source tissues and mobilization as well as remobilization to developing grains, which likely explains the differences in content with previous reports (26, 40).

Conclusion

Findings from current research suggest that the genetic diversity present in native landraces provides an opportunity to develop nutrient-rich variants. Significantly, there was considerable variation in the Fe content, indicating the potential genetic capacity to increase the Fe content of rice grains. The study identified the genotypes like Jaya, *Kalanamak* and *Gandakasala* as rich in iron. Therefore, these genotypes are being utilized in the current breeding program at the Tamil Nadu Rice Research Institute, Aduthurai aiming to develop nutrient-rich bio-fortified rice varieties.

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Authors' contributions

All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.

Compliance with ethical standards

Conflict of interest: The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical issues: None

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