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Research Note

Genetic diversity studies for morphological characters and micronutrient content in red rice germplasm

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Abstract

Rice is the most staple crop of the world consumed by poorest to richest person in this world. However, rice is a poor source of essential micronutrients such as Iron (Fe) and Zinc (Zn). In rice, evaluating genetic diversity is an essential step during the process of development of trait-specific varieties. Genetic diversity studies among red rice germplasm lines was conducted to evaluate 66 red rice lines for morphological traits and zinc, iron and protein content. Three lines, namely Kalavathi, Misebhatta and Karikagga exhibited unique characters for most of the morphological traits. Correlation studies revealed significant positive association of grain yield with grain zinc content. Cluster analysis classified the 66 red rice germplasm lines into nine distinct clusters. Cluster I was the largest and Cluster VI had germplasm with high zinc content and grain yield per plant. Iron content was highest in cluster VIII. The first five components in the principal component analysis with Eigen values >1 contributed to 66.97% of the total variability. Among the germplasm, Shahara, Hallaga and Karthika recorded high zinc, iron and protein content respectively and could serve as potential source for grain nutritional content in red rice.

Keywords: Red rice, micronutrient content, genetic diversity, cluster analysis

A major and escalating issue in the underdeveloped and developing countries is malnutrition. Zinc (Zn) and iron (Fe) are the most commonly deficient mineral elements in the human diet with iron ranking the fifth and the zinc ranking the sixth among the top ten risk factors contributing to burden of disease (WHO, 2017). Nutrient deficiency in humans, especially in children and lactating women, is a major concern. Zinc malnutrition is a major problem in developing countries where polished rice is a staple food. Zinc is an essential component of many enzyme systems and is necessary for the body to function normally. Zinc has a key role in immunity, cell division, gene expression, and reproduction (Brown *et al.,* 2004). Zn deficiency poses health risks for both mothers and infants during child birth and more than 1,16,000 children die each year due to Zn deficiency (Ryu and Aydemir, 2020). The main component of haemoglobin and a vital part of human blood is iron. Additionally, it plays a significant function in the immune system and controls enzyme activity (Abbaspour *et al.,* 2014). Its deficiency includes anaemia, increased risk of maternal mortality and premature birth. Young children and pregnant women are more vulnerable for zinc and iron deficiencies (Frossard *et al.,* 2000). Protein is an essential component of the human diet; each individual must consume a recommended amount of protein depending on age group and body weight. Protein deficiency and amino acids imbalance are known to cause specific health disorders and also affect growth and brain development (Upadhyaya *et al.,* 2011 and Hanamaratti *et al.,* 2015).

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Red rice has been in cultivation in India since ancient times and was domesticated in India or India – China. Hence, India is accomplished with a great diversity of germplasm and still have largest collection of diverse red rice germplasm lines and varieties of landraces. The genetic diversity analysis is an essential component in plant breeding for the improvement and transfer of desirable traits (Tripathy, 2020 and Varshney *et al.,*2008). The red pigment in rice grains is due to the presence of pro-anthocyanidin pigment (Oki *et al.,* 2002) and shows deterrent effects to pathogens and predators (Scalbert, 1991). Also red and black husked rice are comparatively more resistant to storage insect pests. It also possesses several medicinal properties which promotes blood circulation and digestion. Iron and zinc content of red rice is 2–3 times higher than that of white rice (Ramaiah and Rao,1953) and manganese content of red rice bran contributes to anti-oxidative properties to a great extent (Kaneda *et al.*, 2007). It also has more Vitamin B1, B2, C and elements N, P, K, S, Mg, Ca and edible cellulose than achromatic rice (Jing *et al*., 2000).

Seed protein content in rice is one of the important nutritional parameters. In rice grain protein content varies from 4.5 to 19.3 per cent in *Oryza sativa* varieties and 10.2 to 15.9 per cent in *O.glaberrima* varieties (Wang *et al.,* 2008). Landraces often have more protein content than high yielding varieties, which were developed mainly to optimize yield and the nutritional value (Sindhumole, 2012). Polished rice is generally preferred by most people for daily eating, it is deficient in iron and zinc with respect to the daily requirements (Bouis and Welch, 2010). The systematic use of rice germplasm in rice biofortification attempts requires an understanding of the genetic process underlying high grain iron and zinc in rice (Swamy *et al.,* 2016; Neeraja *et al.,* 2017). It is a viable, long-lasting, and economical approach to malnutrition and nutrition security, particularly in developing nations (Babu, 2013). A number of scientists have examined the morphological and physical/chemical quality parameters, such as iron and zinc, in rice germplasm, including landraces, cultivars, and advanced breeding lines (Roy and Sharma 2014; Wadbok *et al.,* 2019; Haritha *et al.,* 2020).

In the current state of nutritional security, it is critical to find potential red rice germplasm lines with high iron, zinc, and protein content. The current work sought to analyze red rice germplasm lines based on their morphological traits as well as grain zinc, iron, and protein content in order to assess genetic diversity and identify promising lines with high protein, zinc, and iron contents.

Plant material and Field evaluation: The experimental material comprised of 66 indigenous red rice germplasm lines maintained at All India Coordinated *Rice* Improvement project, Brahmavar, Udupi District, Karnataka. The present experiment was conducted at Seed farm of Zonal Agricultural and Horticultural Research Station, Brahmavar, Udupi District, Karnataka during *Kharif,*2023. All the 66 germplasm lines were evaluated under normal rice growing conditions in a Randomized Complete Block Design (RCBD) with two replications. Twenty one-days-old, healthy seedlings and three checks were transplanted to the main field with well-puddled land from raised nursery bed with a distance of 15 cm between each plant and 20 cm between rows.

Morphological observations: The red rice germplasm lines were characterized for important agro-morphological parameters along with grain micronutrient contents (zinc, iron and protein in rice). The data on 12 agro-morphological traits *viz.,* days to maturity, plant height (cm), panicle length (cm), number of productive tillers plant^{-1}, number of grains panicle^{-1}, test weight (g), grain yieldplant^{-1} (g), grain length (cm), grain width (cm), protein content (g/100g), Zinc content (ppm) and Iron content (ppm) was recorded. DUS characters were also recorded from ten randomly selected representative plants as per the DUS guidelines given by Subba Rao *et al.*(2013).

Protein estimation: Harvested seeds from each red rice germplasm were collected, cleaned thoroughly using winnower to remove the chaff and other foreign matters then sundried, dehusked using palm dehusker. One gram of the powered sample was used to estimate the protein content by Micro Kjeldhal method as proposed by Piper (1966) .

Assessment of grain zinc and iron: Seeds of red rice germplasm were sent to the Indian Institute of Rice Research (IIRR), Hyderabad to analyze the grain zinc and iron content. Five grams sample from each genotype was analyzed to estimate the grain zinc and iron content using an energy dispersive X-ray fluorescence spectrophotometer (ED-XRF) (OXFORD Instruments X-Supreme 8000).

Data analysis: RStudio Version 4.3.1a statistical software was used to analyze the data for principal component analysis, correlation and diversity studies.

For all 12 quantitative parameters, including grain zinc, iron, and protein, a broad spectrum of genetic variability was detected among the red rice germplasm lines (Ratnam *et al.,* 2023) (**Table 1**). This extensive variability observed in red rice genotypes indicated scope for identification and selection of potential genotypes with desirable characteristics, thereby facilitating the development of improved red rice varieties (Arunkumar *et al.,* 2025; Jasmine *et al.,* 2022; Singh *et al.,* 2020 and Paramanik *et al.,* 2023).

Morphological characterization is a critical prerequisite for crop improvement activities since it provides vital information for plant breeding activities (Chakravorty *et al.,* 2013). Sixty-six red rice germplasm were morphologically

Table 1. Genetic variability for yield parameters and grain zinc, iron and protein content in red rice germplasm lines

S. No.	Characters	Mean	Range		GCV (%)	PCV (%)	Heritability (%)	GAM (%)
			Min	Max				
1	Days to maturity	133.21	113.50	152.00	4.47	4.61	93.96	8.93
2	Plant height (cm)	105.63	52.00	149.90	18.79	18.83	99.55	38.61
3	Panicle length (cm)	23.70	13.95	34.15	13.76	15.67	77.05	24.88
4	Number of productive tillers per plant	10.04	5.50	19.00	28.34	30.32	87.36	54.57
5	Number of grains per panicle	103.03	56.00	184.50	26.47	28.00	89.36	51.55
6	Test weight (g)	24.97	18.67	31.57	13.89	14.08	97.42	28.25
7	Grain length (cm)	8.31	5.97	9.50	5.00	9.50	27.74	5.43
8	Grain width (cm)	2.88	1.66	3.90	16.26	16.99	91.56	32.05
9	Grain yield per plant (g)	24.15	11.17	45.74	26.02	28.29	84.61	49.30
10	Protein content (g/100g)	8.13	5.00	11.43	20.88	21.67	92.86	41.45
11	Zinc content (ppm)	22.31	11.40	44.70	40.20	40.46	98.71	82.27
12	Iron content (ppm)	19.60	13.11	37.12	31.42	31.60	98.87	64.35

characterized by DUS guidelines for fifty-four traits (**Fig. 1**). Attributes, such as anthocyanin colouration of various plant parts, presence of awns, lemma and palea colour and decorticated grain colour can serve as morphological markers in breeding programs as these traits exhibited highest polymorphism. Additionally, traits like high leaf pubescence (e.g., Peetasale), strong thick stems, purple stigma (e.g., Kavalakannu, Misebhatta, Kempudadi and Kartha) and presence of awns (e.g., Misebhatta, Balesuli, Karikagga, Nagasampige and Nereguli) are associated with resistance to insect pests. Certain germplasm displayed distinctive traits such as purple lines on the basal leaf sheath (Misebhatta), anthocyanin colouration of leaf tips (Kanjaddu), a purple-coloured ligule (Kalanamak and Rakthashali) and pronounced anthocyanin colouration of stem nodes (Bilikannuhegga).Comparable findings were also reported by Rai *et al.* (2013), who investigated Indian landraces of aromatic and non-aromatic accessions and discovered that morphological diversity can be contributed to panicle density, leaf length, plant height and grain characteristics. Among the germplasm studied, Kalavathi is especially notable for exhibiting distinct traits *viz.,* anthocyanin colouration of the leaf, basal leaf sheath, auricle, keel of the lemma, apex of the lemma, stem internodes, lemma tip, lemma and palea colour and sterile lemma colour. Kalavathi's comprehensive range of traits makes it a unique genotype with potential applications in addressing both biotic and abiotic stresses (Bate-Smith, 1973 and Subba Rao *et al.,* 2013). Whereas, presence of leaf collar, leaf ligule, shapes of leaf ligule, male sterility, stem thickness displayed no significant variation (Manjunatha *et al*., 2018 and Gayathri *et al.,* 2023). Moderate variability was observed for rest of the traits. Frequency distributions also revealed wide variability which is in accordance with the previous studies on variability in traditional varieties using DUS descriptors (Lavanya *et al.,* 2021; Poudel *et al.,* 2020 ; Akshay *et al.,*2022). The germplasm examined in this study displayed significant distinctiveness, uniformity and stability for most of the morphological traits.

Correlation analysis (**Table 2**) revealed positive and significant association of grain yield per plant with panicle length, number of productive tillers per plant, number of grains per panicle, test weight and zinc content (Prasannakumari *et al.,* 2020). Interestingly, there was significant positive correlation between protein and zinc content. Days to maturity and days to flowering exhibited negative correlation with test weight, grain length, grain width, iron and zinc content andgrain yield per plant. Thuy *et al.* (2023) recorded significant negative association of grain yield with protein content, non-significant positive association with Zn content and non-significant negative association with Fe content and panicle length. The current study also showed a positive relationship between zinc concentration and grain production suggesting that germplasm lines with both high yield and high zinc content could be chosen. These findings are consistent with Singh *et al*. (2020).

Using the Mahalanobis' D^2 statistic, 66 red rice lines were grouped into nine clusters. Cluster I was the largest, containing 37 lines, followed by Cluster II with 12 lines, Cluster III with 4 lines, Cluster VIII with 3 and IV,V, VI, VII, IX clusters had 2 lines each (**Table 3**).The cluster mean values for all the studied traits varied across the different clusters. Cluster I exhibited the highest mean value for grain yield per plant. Cluster III had the highest mean values for days to maturity, number of productive tillers per plant, and number of grains per panicle. Cluster IV exhibited the highest mean values for panicle length, protein content and Zn content. Cluster VI recorded the

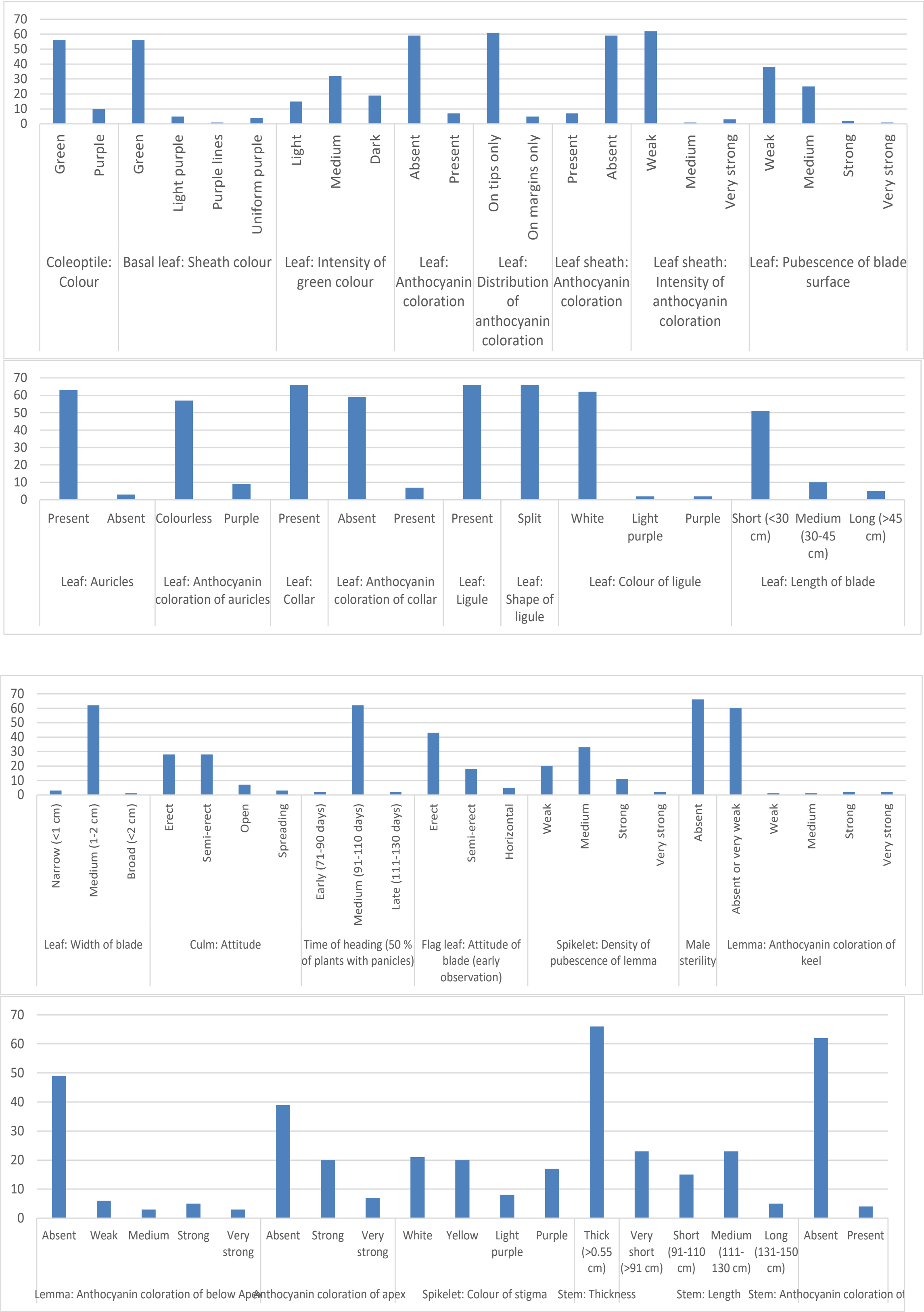

Fig. 1. Frequency distribution, number of genotypes of red rice characterized according to DUS test guidelines

Fig. 1. Frequency distribution, number of genotypes of red rice characterized according to DUS test guidelines

Table 2. Correlation coefficients for yield, its attributing traits, grain zinc, iron and protein in red rice germplasm lines

	DM	PH	PL	PT	NG	TW	GL	GW	P	Zn	Fe	GY
DM	1	0.0647	0.0241	0.0733	0.0054	-0.0996	-0.2996 **	-0.1827 *	0.0327	-0.0168	-0.0514	-0.0299
PH		1	0.4769 **	-0.1758 *	0.1195	0.2267 **	-0.005	0.0227	-0.0189	0.2048 *	-0.1128	0.0665
PL			1	-0.1444	0.2337 **	0.1461	-0.0559	0.1072	-0.1483	0.1315	-0.1402	0.2438 **
PT				1	-0.1736 *	-0.1826 *	0.1002	-0.0011	0.1131	0.1476	-0.058	0.4218 **
NG					1	0.1579	-0.1792 *	0.0649	-0.2411 **	0.0715	0.0391	0.5551 **
TW						1	0.1083	0.0187	-0.1782 *	0.0633	-0.132	0.4328 **
GL							1	0.2581 **	-0.0404	-0.0648	0.0158	0.023
GW								1	-0.0982	-0.0232	0.071	0.0883
P									1	0.2421 **	0.056	-0.1906 *
Zn										1	-0.1306	0.2762 **
Fe											1	-0.1027
GY												1

Table 3. Clustering pattern of red rice germplasm lines

Clusters	No. of entries	Germplasm lines
I	37	Hallinga, Kavalakannu, Moradda, Neerambadi, Karikagga, Kajejaya, Latha, Maskath, Pokkali, Mallige, Narunga, Rakthashali, Atire, Kalabatha, Navaraa, Chakavoporiyat, Nyaremind, Kabbaga, Kaavadari, Halagabatta, Z.Gulvadisannakki, Varadababanyasale, Balesuli, Arundathi, Mundoni, Akkalu, Karimeesebhatta, Doodaalur, Dambarsali, Bilikannuhegga, Bara ratnachoodi, Karidadi, Karavaikalanji, Karana, Kanjaddu, Kempurajesh, Kartha
II	12	Nagasampige, Nishmitha, Lavanya, Jyosthana, Halaga, Aishwarya, Kasargodu, Athikaraya, Mysore sanna, Kandadivaalya, Bolumallige, MO-4
III	4	Nereguli, Peetasale, Kuttimanja, Chittimutyalu
IV	2	Kempusanna, Karikandaka
V	2	Kempudadi, Karthika
VI	2	Shahara, Karinesaru
VII	2	Misebhatta, Kiruvanna
VIII	3	Hallaga, Muttu, Kalanamak
IX	2	Kalavathi, Karikantaka

highest mean values for plant height, test weight and Fe content (**Table 4**). Lines with high grain yield per plant, test weight, grain zinc, iron and protein content were present in III,V,VI,VII,VIII,VI and IX clusters. Hence, lines of these clusters can be utilized in future hybridization programmes for development of red rice varieties with high grain yield coupled with grain zinc, iron and protein content. Similar findings were observed by Manohara and Singh (2013) for plant height.

The intra and inter cluster D^2 values of yield, its related traits, grain zinc, iron and protein content are given in **Table 5**. The intra cluster distance ranged from 234.54 to 790.68 and Cluster IX was observed with maximum intra cluster distance *i.e.* 790.68 followed by cluster VIII (740.22). These results indicated the presence of significant genetic variation among the red rice genotypes in these clusters (IX and VIII) and better scope for selection of diverse lines with desirable traits for hybridization. These results are in alignment with the reports of Vennela *et al.* (2017) and Chaudhary *et al.* (2023) in rice.

Maximum inter cluster distance was found between cluster VI and IX (6388.33) followed by cluster V and VI (6143.30). High genetic distance observed among these red rice genotypes belonging to these clusters suggests a broad genetic diversity, which can be used to exploit maximum variability. Whereas, the lowest inter cluster distance was found between cluster I and III (711.047) followed by cluster II and IV (737.061) which indicated that the red rice genotypes falling into these clusters are genetically close. Similar results were recorded by Kamera *et al.* (2015) and Gnaneswari, *et al.* (2023). Plant height contributed maximum towards total divergence and percentage contribution of different traits towards total divergence is given in **Fig. 2**. The germplasm lines evaluated in this

Table 4. Cluster means for yield, its attributing traits, grain zinc, iron and protein content in red rice germplasm lines

Clusters	DM	PH	PL	PT	NG	TW	GL	GW	P	Zn	Fe	GY
I	133.68	113.63	24.49	9.92	105.43	25.34	8.31	2.96	8.01	26.06	16.96	24.89
II	130.75	80.81	21.91	11.38	97.42	25.57	8.35	2.96	8.23	16.00	20.12	25.92
III	140.38	104.95	23.48	8.88	121.50	21.87	8.14	2.94	7.52	23.46	26.40	22.64
IV	134.00	82.10	25.85	7.25	72.25	20.58	8.54	2.24	9.51	26.08	16.11	10.55
V	132.50	145.93	27.05	8.00	100.00	26.28	8.13	2.36	11.27	34.00	15.95	20.60
VI	135.00	58.13	16.50	14.25	103.75	24.48	8.34	2.45	7.93	43.33	17.28	26.93
VII	123.25	113.10	22.58	8.50	85.75	23.93	8.35	3.10	8.77	41.58	29.70	20.37
VIII	134.00	101.80	22.93	10.83	89.00	23.63	8.45	2.86	8.62	20.00	36.12	20.07
IX	132.25	136.90	24.30	7.50	126.75	27.28	7.91	2.31	5.11	12.63	20.49	24.41

DM- Days to maturity, PH- Plant height, PL- Panicle length, PT- Number of productive tillers per plant, NG-Number of grains per panicle, TW- Test weight, GY-Grain yield perplant, P-Protein, Zn-Zinc and Fe- Iron

Table 5. Inter and intra cluster distances for yield, its attributing traits, grain zinc, iron and protein content in red rice germplasm lines

Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	517.56	1302.02	711.047	1071.12	1214.942	3059.81	1069.28	1599.07	1259.41
II		437.307	1060.37	737.061	3600.721	1597.74	1737.52	1413.67	2854.61
III			378.550	1004.93	1866.616	2830.49	833.63	786.54	1463.11
IV				234.543	2937.737	1180.79	1421.35	1912.04	3039.13
V					397.2822	6143.30	1754.14	2999.20	1056.93
VI						518.794	2871.12	3567.57	6388.33
VII							583.234	1132.18	2107.57
VIII								740.22	2113.85
IX									790.68

*Diagonal values indicate intra cluster distances*Above diagonal values indicate inter cluster distances

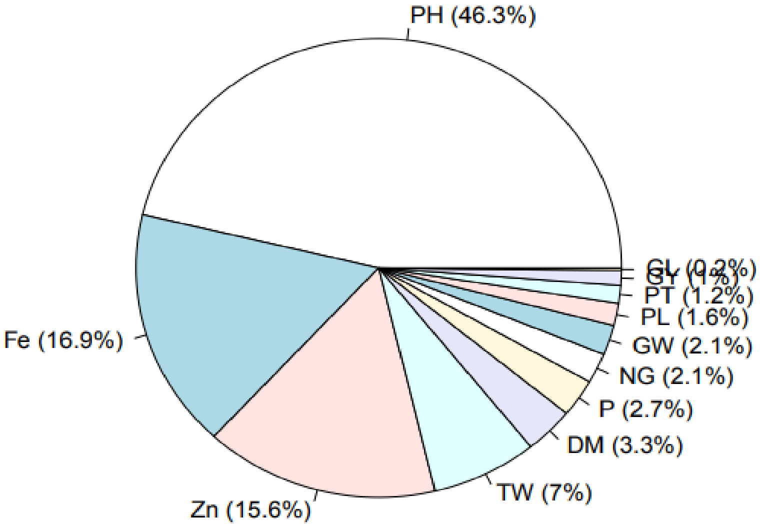

Fig. 2. Percent contribution of different traits towards total divergence

study showed significant genetic diversity, suggesting that this material could be used to select a plant for rice biofortification efforts.

Principal component Analysis: Principal Component Analysis (PCA), is a reduction technique for multivariate data and is used more frequently to assess the significance and contribution of each factor to the over-all variance in addition to providing details on the influence of a specific trait to the total variance, (Bhargavi *et al.,* 2023). The first five components in the PCA analysis with Eigen values > 1 contributed 66.97 percent variability existing in the rice germplasm. Remaining components with Eigen values < 1 contributed 33.02 percent variability. Principal component 1 (PC 1) with Eigen value 2.34 accounted 19.56% of total overall variability. PC 2 with Eigen value of 1.66 and PC 3 with Eigen value 1.57 contributed 13.93% and 13.10% to the total variability, respectively (Salem *et al.,* 2021). Hence, the maximum variation was observed in first PC (**Table 6**) indicating the wider scope for selection of genotypes.

According to PCA analysis, PC 1 had a high discriminating power of principle components (2.34), whereas PC 5 had a low one (1.08). PC 1 explained 19.56 per cent of variation and had positive significant association with Plant height, Panicle length, number of grains per panicle, test weight and grain yield per plant. However, negative loadings for Days to maturity were observed for PC 1. The second component (PC 2) accounted for 13.90% of the variation and had a negative correlation with days to maturity, test weight, protein and iron content and a positive correlation with the number of productive tillers per plant and grain production per plant. The PC 3 explained 13.10% variation and correlated positively with grain protein and zinc content. PC 4 explained 11.38% variation and correlated positively with Days to maturity and negatively with pant height, panicle length, grain yield per plant. PC 5 explained 9.01% variation and significantly positive association with Fe and negatively association with Panicle length, number of productive tillers per plant, grain yield per plant, while component 6 explained only 8.13% variation (**Fig. 3, Fig. 4, Fig. 5**).

The most significant characteristics that contributed to the classification of the variation were determined to be days to maturity, test weight, grain yield, number of productive tillers per plant, number of grains per panicle, panicle length, grain length, plant height and zinc content (Sanni *et al.,* 2012; Radhamani *et al.,* 2015 and Lakshmi *et al.* (2019). Madhubabu *et al.* (2020) observed the number of effective tillers, panicle length, thousand grain yields, grain length, and zinc content, all significantly contributed to the variation. Bollinedi *et al.* (2020) found prospective donors for biofortification and rice grain quality improvement after studying a sizable collection of rice germplasm.

Micronutrients are crucial for human growth and development. In regions where staple foods primarily consist of cereals, deficiencies in the mentioned nutrients can pose significant health risks. Improvement of micronutrient content in staple food grains like rice through fortification or any other biotechnological tools is best method to enhance nutritional security. Enhancement of the micronutrient levels through conventional plant breeding is the most widely followed that involves screening germplasm with high micronutrient content (Parikh *et al.,*2019 and Agustin *et al.,* 2021.). In the present study, the red rice genotypes exhibited considerable variability for grain Zn content with the highest value recorded for Shahara (44.70 ppm) followed by Misebhatta (43.75 ppm), Karinesaru (41.95 ppm) and Maskath (40.75 ppm). Grain Fe content also varied widely, the germplasm line Bilihallaga had highest Fe content i.e. 37.13 ppm followed by Muttu (36.08 ppm), Peetasale

Table 6. Eigen values, % variance and cumulative percentage of variance of different components

S.No	Components	Eigen value	% of variance	cumulative percentage of variance
1	PCA 1	2.34811653	19.56	19.56
2	PCA2	1.66872679	13.90	33.47
3	PCA 3	1.57283301	13.10	46.58
4	PCA 4	1.36639724	11.38	57.96
5	PCA 5	1.08133809	9.01	66.97
6	PCA 6	0.97604639	8.13	75.11
7	PCA 7	0.78371857	6.53	81.64
8	PCA 8	0.69374998	5.78125	87.42439
9	PCA 9	0.55060901	4.588408	92.0128
10	PCA 10	0.48729699	4.060808	96.0736
11	PCA 11	0.40959149	3.413262	99.48687
12	PCA 12	0.06157592	0.513133	100

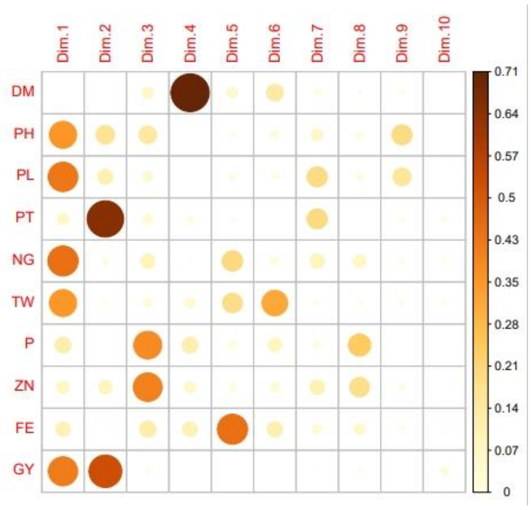

Fig. 3. Correlation with PCA

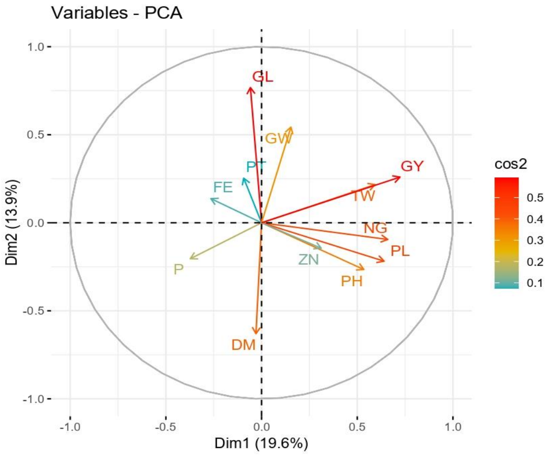

Fig. 4. Correlation of variables

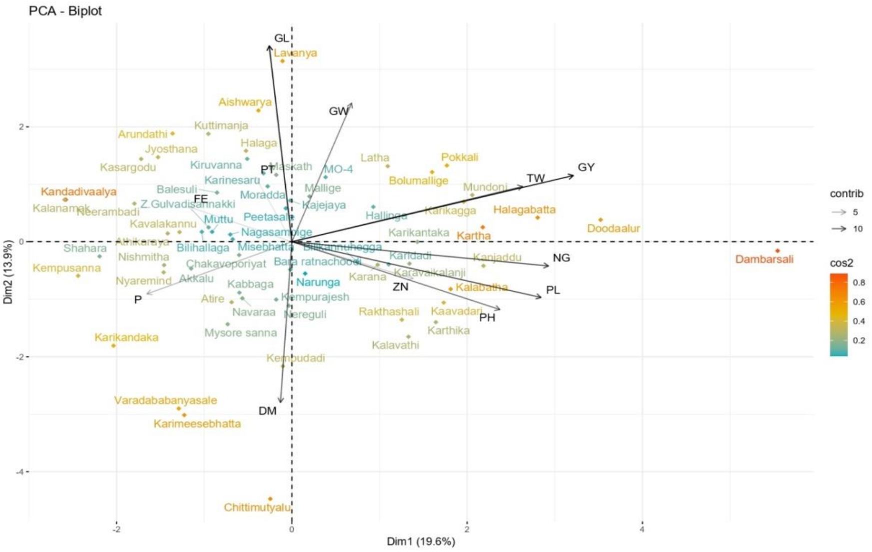

Fig. 5. Distribution of germplasm lines across two components

Table 7. Best performing red rice genotypes with high zinc, iron, protein and yield

S. No.	Red rice germplasm lines	Zinc (ppm)	Red rice germplasm lines	Iron (ppm)	Red rice germplasm lines	Protein (g/100g)	Red rice germplasm lines	Grain yield per hill (g)
1	Shahara	44.70	Hallaga	37.13	Karthika	11.44	Doddaalur	40.60
2	Misebhatta	43.75	Muttu	36.08	Kempudadi	11.11	Dambarsali	40.06
3	Karinesaru	41.95	Peetasale	35.50	Karimeesebhatta	11.11	Mundoni	39.72
4	Rakthashali	41.20	Kiruvanna	35.45	Moradda	10.94	Kanjaddu	34.14
5	Karthika	41.00	Nereguli	35.25	Kempusanna	10.81	Kartha	32.90
6	Maskath	40.75	Jyosthana	27.79	Bilikannuhegga	10.47	Karikagga	31.45
7	Karavaikalanji	40.15	Bilikannuhegga	26.7	Neerambadi	10.44	Halagabatta	30.73
8	Kiruvanna	39.40	Misebhatta	24.3	Hallinga	10.01	Latha	29.60
9	Dambarsali	37.85	Mysore sanna	22.55	Shahara	10.37	Karavaikalanji	29.45
10	Moradda	37.85	Kuttimanja	21.5	Nagasampige	10.29	Shahara	28.08

(35.50 ppm) and Kiruvanna (35.45 ppm). Protein content ranged from 5.01 g/100g in the genotype Karikantaka to 11.44 g/100g in Karthika and other notable entries include Karimesebhatta (11.11 g/100g), and Moradda (10.94 g/100g).This variability indicated difference in capabilities for the uptake of different micronutrients and similar findings were also reported by Gogoi *et al.* (2024). Ten lines with high grain yield, high zinc, high iron and high protein content were selected from a total of 66 lines (**Table 7**). This wide range of variation suggests notable diversity in the grain protein, zinc and iron content among the genotypes.These germplasm lines with high protein content can be utilized in breeding program to develop protein and micro nutrient rich red rice varieties.

All the red rice germplasm lines in the study were distinct, uniform and stable for important morphological traits and demonstrated a broad range of variability with higher estimates of heritability and genetic advance as *per cent* mean (Zn, Fe, Protein content) indicating that these traits are controlled by additive gene action offering high potential for selection of trait specific genotypes. Germplasm, such as Kalavathi, Karikagga, Misebhatta, Balesuli and Rakthashali, displayed unique characteristics and these traits can be used as morphological markers. Maximum inter cluster distance was observed between clusters VI and IX, therefore, genotypes belonging to these clusters are more diverse.PCA revealed high level of genetic variation (66.97 %) among the red rice germplasm. Highest grain Zn content was recorded in Shahara (44.70ppm), Misebhatta (43.75ppm), Karinesaru (41.95ppm) and Maskath (40.75ppm).High grain Fe content was recorded for Bilihallaga(37.13ppm),Muttu (36.08ppm), Peetasale (35.50ppm) and Kiruvanna (35.45 ppm). Similarly, Protein content was high in Karthika (11.44 g/100g), Karimeesebhatta (11.11 g/100g), and Moradda (10.94 g/100g). Hence, screening of red rice gemrplasm for morphological traits and micronutrient content with special reference to grain protein, Zn and Fe content can help in selection of potential genotypes for hybridization and development of improved varieties enriched with high protein and micronutrients.

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