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Diversity Analysis of Red Rice (*Oryza sativa L.*) Genotypes for Yield and Grain Zinc, Iron and Protein Content

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Abstract

Rice (*Oryza sativa* L.) is the most important staple food and primary nutrient source in the many countries of the world. In the present study, 108 diverse red rice genotypes were assessed to decipher genetic diversity in a Randomized Complete Block Design (RCBD) with two replications. Mahalanobis' D^2 statistics was performed for grain yield and yield contributing features along with grain zinc, iron and protein content to calculate the genetic divergence between the genotypes. Cluster VI (1843.40) recorded maximum intra cluster distance followed by cluster V (1462.46), indicating significant genetic variation among the red rice genotypes within these clusters and maximum inter cluster distance was found between cluster V and VI (4178.22) followed by cluster IV and V (2691.43). Cluster IV exhibited the highest mean values for panicle length, protein content and Zn content. Cluster VI recorded the highest mean values for plant height, test weight and iron content. Hence, the genotypes from cluster IV and VI could be used for hybridization with the genotypes of other clusters to develop high yielding varieties with high grain zinc, iron and protein content. The lowest inter cluster distance was found between cluster II and III (872.56) followed by cluster I and III (998.76) which indicates that the red rice genotypes falling into these clusters are genetically close. The maximum contribution towards divergence was made by plant height, followed by zinc content, iron content and test weight.

Key words: Red rice, iron, zinc, protein, genetic diversity

Introduction

Rice (*Oryza sativa* L.) is the predominant and most important staple food crop for more than half of the world's population and is playing a crucial role in providing human body nutrition, energy supply and food security (Billa *et al*., 2024). However, polishing removes the bran layer, aleurone, embryo and part of the endosperm, significantly reducing its nutritional content (Pillai *et al*., 2011; Modgil and Rani, 2016) and most of the iron and zinc is found in rice bran-aleuronic layer and 80 % of it gets removed in polishing (Tukaram *et al*., 2024). For proper growth and development, humans need mineral elements especially iron, zinc and also protein. Nutrient deficiencies are a significant issue, particularly for children and lactating women. zinc deficiency can lead to health risks for mothers and infants during child birth (Ryu and Aydemir, 2020). Young children and pregnant women are especially susceptible to severe effects of iron deficiency and according to World Health Organization (WHO, 2023) 40 % of children 6–59 months of age, 37 % of pregnant women and

30 % of women 15–49 years of age worldwide are anemic. There are two main syndromes associated with protein deficiencies, Kwashiorkor and Marasmus. Kwashiorkor symptoms include edema of legs and feet, light-colored, thinning hair, anemia, a potbelly and shiny skin. The symptoms of Marasmus are acute fatigue, poor skin health, diarrhea, hunger and growth retardation.

Modern rice varieties have limited genetic base which is becoming a major bottleneck for crop improvement programmes. There is a need to increase the rice production to meet the demand of ever growing population with nutritional security. Development of new high yielding and quality rice varieties, superior to the existing varieties, mainly depends on the amount of genetic variability present in the population. The rice germplasm is a rich reservoir of valuable genes that plant breeders can harness for crop improvement and efficient utilization of crop wild relatives is a most promising approach to increase genetic diversity in cultivated crops for yield and yield characteristics (Chandra *et al*., 2024). Genetic divergence study is therefore important for choice of parents in hybridization programme. Information on genetic diversity in terms of nature and degree of divergence for grain yield, yield components and quality characters would help the plant breeder in selection of right type of parents for hybridization programmes and designing effective breeding strategies aimed at development of high yielding varieties with good grain quality. The more diverse the parents, the greater are the chances of obtaining higher amount of heterotic expression in F1s and broad spectrum of variability in segregating generations (Vivekananda and Subramaniam, 1993). Red rice contains 2-3 times more iron and zinc content compared to white rice (Ramaiah and Rao, 1953), and its bran is rich in manganese, enhancing its antioxidant properties (Kaneda *et al*., 2007). Additionally, red rice has higher levels of protein, vitamins and edible cellulose compared to white rice (Jing *et al*., 2000). Rice also has good quality protein compared to other cereals and is rich in branched chain amino acids such as leucine, isoleucine and valine (Krishna Veni *et al*., 2024). Diversity analysis is used to assess the genetic divergence existing in the germplasm collections and to identify parental genotypes with greater divergence for hybridization to develop high yielding varieties (Bhatt, 1970). Mahalanobis' D^2 statistics has been widely recognized as a powerful tool for plant breeders to select suitable parental genotypes with a broader range of variability for various traits (Sinha *et al.*, 2020). The clustering can reveal the pattern of divergence among the genotypes and help in selecting diverse parental lines for breeding (Kamera, 2015). Hence, the present investigation was undertaken to estimate the magnitude of genetic divergence among 108 red rice genotypes for yield attributes and also its grain zinc, iron and protein content.

Materials and Methods

Plant material

Total of 108 red rice genotypes were sown and seedlings were raised in wet nursery method and twenty one days old healthy seedlings from each treatment along with checks were transplanted to the well prepared puddled field in a Randomized Complete Block Design (RCBD) with two replications. A spacing of 15 cm within a row and row-to-row spacing of 20 cm was maintained in a 5 square meter area during *Kharif,* 2023 at Zonal Agricultural and Horticultural Research Station (ZAHRS), Brahmavar, Udupi district, Karnataka. The required cultural operations and plant protection measures as per the package of practices were taken up to ensure uniform and healthy crop.

Morphological observation

All the genotypes were evaluated for genetic variability and genetic diversity along with grain zinc, iron and protein content in brown rice. Observations were recorded from ten randomly selected plants in each genotype from each replication for agromorphological traits such as days to maturity, plant height (cm), panicle length (cm), number of productive tillers per plant, number of grains per panicle, test weight (g), grain length, grain yield per plant (g), grain width (mm), protein content, zinc content and iron content. Mean values were used for statistical analysis.

Zinc, Iron and Protein estimation

The seed samples of all the red rice germplasm were harvested separately from the pooled panicles of three middle row plants and divided into two parts to be analyzed as two replicates. Fifty grams of the powered sample was used to estimate the protein content by Kjeldhal method as given by Piper (1966) and grain iron and zinc content were estimated with the help of Atomic Absorption Spectrophotometer (AAS) as suggested by Lindsay and Norvell (1978).

Data analysis

Mahalanobis' (1963) D^2 statistics was used for assessing the genetic divergence and statistical analysis was carried out using 'R' statistical software (R Studio *Version 4.3.1.)* for variance and diversity.

Results and Discussion

Analysis of variance revealed highly significant differences among all 108 red rice genotypes for all the evaluated traits. The significant mean sum of squares for all the studied traits indicates that the genotypes displayed considerable variability in their mean performance (**Table 1**).

Table 1: Analysis of variance for yield, its attributing traits, grain zinc, iron and protein content in red rice genotypes

Source of variation	Degrees of freedom	Days to maturity	Plant height (cm)	Panicle length (cm)	Number of productive tillers per plant	Number of grains per panicle
Replication	1	0.66	0.39	5.77	1.04	18.37
Genotype	107	80.60**	832.62**	24.45**	16.97**	1425.93**
Error	107	2.70	1.81	3.12	1.12	78.30
CD (5 %)		3.25	2.67	3.50	2.10	17.54
CD (1 %)		4.31	3.53	4.63	2.78	23.20
CV		1.22	1.38	7.76	10.39	8.34

Source of variation	Degrees of freedom	Test weight(g)	Grain yield per plant (g)	Protein (g/100g)	Zinc (ppm)	Iron (ppm)
Replication	1	1.00	10.08	0.11	1.19	1.44
Genotype	107	22.68**	86.31**	5.03**	166.08**	54.64**
Error	107	0.30	7.32	0.18	0.86	0.46
CD (5 %)		1.09	5.36	0.84	1.84	1.35
CD (1 %)		1.44	7.10	1.12	2.43	1.78
CV		2.17	10.70	4.25	4.16	3.46

**: Significant at 1 % level

Distribution and Grouping of Genotypes

Evaluating the genetic diversity both between and within genotypes is essential for choosing the best parents to maximize heterosis and produce valuable recombinants. Using the Mahalanobis' D^2 statistic, cluster pattern revealed that 53 genotypes were present in cluster I, 29 genotypes were present in cluster II, 9 genotypes were present in cluster III, 10 genotypes were present in cluster IV, 5 genotypes were present in cluster V and 2 genotypes were present in cluster

VI indicating high degree of heterogeneity among the genotypes (**Table 2**). Similar results were obtained by Dey *et al*., (2020) where the 29 accessions are divided into six clusters.

Table 2: Clustering pattern of 108 red rice genotypes

Cluster	No of entries	Genotypes
I	53	BMR-10, BMR-11, BMR-12, Bolumallige, Halaga, Aishwarya, Mysore sanna, Nishmitha, BMR-1, Kandadivaalya, Athikaraya, BMR-4, BMR-15, BMR-16, BMR-17, BMR-18, BMR-19, BMR-20, BMR-21, BMR-22, BMR-23, BMR-24, BMR-25, BMR-26, BMR-27, BMR-28, BMR-29, BMR-30, BMR-31, BMR-32, BMR-33, BMR-34, BMR-35, BMR-5, BMR-3, KCP 1, MO-4, BMR-2, BMR-7, Lavanya, IR-64, Nagasampige, BMR-9, BMR-13, BMR-14, Kavalakannu, Kempusanna, Chakavoporiyat, Jyosthana, Mallige, Kuttimanja, Z.Gulvadisannakki, DRR Dhan 45.
II	29	Halagabatta, Karidadi, Kanjaddu, Doodaalur, Kartha, Pokkali, Bara ratnachoodi, Karikagga, Hallinga, Kempurajesh, Kalabatha, Karana, Kaavadari, Latha, KMT, MO-22, Karikantaka, Arundathi, Kabbaga, Nyaremind, Narunga, Akkalu, Neerambadi, Karimeesebhatta, Varadababanyasale, Kajejaya, Mundoni, Bilikannuhegga, Atire
III	9	Nereguli, Peetasale, Chittimuthyalu, Kalanamak, Balesuli, Navaraa, BPT-5204, Kasargodu, BMR-8
IV	10	BMR-6, Moradda, Maskath, Karavaikalanji, Misebhatta, Rakthashali, Dambarsali, Kiruvanna, Kempudadi, Karthika
V	5	Shahara, Karinesaru, Karikandaka, MO-23, Hallaga
VI	2	Kalavathi, Muttu

Intra and inter clusters distances

The intra and inter cluster D^2 values of yield, its related traits, grain zinc, iron and protein content studied are given in **Table 3**. The intra cluster distance ranged from 393.56 to 1843.40 when observed for diversity. Cluster VI was observed with maximum intra cluster distance *i.e*., 1843.40 followed by cluster V (1462.46), cluster IV (758.74), cluster III (631.24), cluster II (509.72) and cluster I (393.56) indicating that genotypes from these clusters were highly divergent meriting due consideration in selection of parents for hybridization. The greater the distance between two clusters, the wider would be the genetic diversity between genotypes of the clusters (cluster VI and cluster V), so the chances of developing good segregants by crossing the genotypes of these clusters are very high. The results were in alignment with the reports of (Vennela *et al*., 2017 and Chaudhary *et al*., 2023) in rice.

Table 3: Inter and intra cluster distances for yield, its attributing traits, grain zinc, iron and protein content in red rice genotypes

Clusters	I	II	III	IV	V	VI
I	**393.56**	1114.75	998.76	1849.04	1153.40	2401.83
II		**509.72**	872.56	895.50	2356.05	1462.29
III			**631.24**	1421.88	1977.36	1461.02
1V				**758.74**	2691.43	2167.76
V					**1462.46**	4178.22
V1						**1843.40**

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

Maximum inter cluster distance was found between cluster V and VI (4178.22) followed by cluster IV and V (2691.43), the 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 heterosis and crosses among these divergent genotypes are likely to produce desirable combinations for grain yield and grain zinc, iron and protein content. Whereas, the lowest inter cluster distance was found between cluster II and III (872.56) followed by cluster I and III (998.76) which indicates that the red rice genotypes falling into these clusters are genetically close. Similar results were reported by Gnaneswari *et al.,* (2023).

Cluster mean analysis

Indirect selection for crop nutrition and yield improvement relies on selecting nutrition and yield related traits that have a direct positive impact on nutrition and yield. This selection should focus on clusters exhibiting the highest mean values for these traits. The cluster mean values for all the studied traits varied across the different clusters (**Table 4**). Cluster I exhibited the highest mean value for the trait 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 zinc content. Cluster VI recorded the highest mean values for plant height, test weight and iron content. Hence, high grain yield related traits and high grain zinc, iron and protein content genotypes in cluster III, IV, V and VI can be utilized in hybridization for developing high grain zinc, iron and protein content rice varieties. Red rice genotypes can be selected from these clusters based on their favourable mean values for desired traits, and can used in hybridization programs aimed at producing segregants with higher yield and nutritional quality. The results are in accordance with the reports of Nirosha *et al*., (2016).

Table 4: Cluster means for yield, its attributing traits, grain zinc, iron and protein content in red rice genotypes

Clusters	Days to maturity	Plant height (cm)	Panicle length (cm)	Productive tillers per plant	Number of grains per panicle	Test weight (g)	Protein content (g/100g)	Zinc content (ppm)	Iron content (ppm)	Grain yield per plant (g)
I	133.96	82.56	21.30	10.22	105.68	25.69	8.02	18.18	19.44	26.03
II	133.10	115.68	24.72	9.44	107.19	26.10	8.03	24.18	16.67	24.98
III	138.16	103.27	23.13	12.72	115.38	21.37	8.12	20.16	24.27	24.05
IV	130.40	122.78	25.30	9.60	101.80	25.99	9.32	39.01	19.45	25.01
V	132.40	69.10	20.76	12.40	92.20	23.21	8.18	29.83	23.09	22.52
VI	136.75	129.50	23.77	7.50	111.50	27.27	6.76	12.22	28.44	21.03

Contribution of yield, its attributing traits, grain zinc, iron and protein content towards total divergence

The per cent contribution of each trait towards total divergence was assessed and presented in **Table 5**. The maximum contribution towards divergence was made by plant height (49.5 %) followed by zinc content (19.0 %), iron content (11.5 %), test weight (7.4 %), days to maturity (3.3 %), protein content (3.2 %), number of grains per panicle (3.0 %) and number of productive tillers per plant (2.6 %). Results suggest that these traits can be considered while selecting parents for hybridization programme. The least contribution to divergence was made by panicle length (0.6 %) and grain yield per plant (0.4 %). Similar findings were observed by Manohara and Singh (2013) for plant height, Purusothoaman and Geetha (2014) for zinc content.

Table 5: Contribution of yield, its attributing traits, grain zinc, iron and protein content towards total divergence

Character	Per cent contribution
Plant height (cm)	49.5
Zinc content (ppm)	19.0
Iron content (ppm)	11.5
Test weight (g)	7.4
Days to maturity	3.3
Protein content (g/100g)	3.2
Number of grains per panicle	3.0
Number of productive tillers per plant	2.6
Panicle length (cm)	0.6
Grain yield per plant (g)	0.4

Conclusion

The results indicated that the genotypes in cluster VI (1843.40) and V (1462.46) had the largest intra-cluster distance, indicating significant genetic variation among the red rice genotypes within these clusters and therefore future breeding programs can focus on hybridizing the genotypes from diverse clusters as parents for developing desirable segregants for grain yield and high grain zinc, iron and protein content. Maximum inter cluster distance was found between cluster V and VI (4178.22) followed by cluster IV and V (2691.43). Genotypes like BMR-6, Moradda, Maskath, Karavaikalanji, Misebhatta, Rakthashali, Dambarsali, Kiruvanna, Kempudadi, Karthika are in cluster IV, Shahara, Karinesaru, Karikandaka, Hallaga in cluster V and Kalavathi, Muttu in cluster VI have a broad genetic diversity. Among all the genotypes BMR-2 (45.4 g/plant) and BMR-13 (30.28 g/plant) were found to have highest yield potential. Some of the genotypes were identified as high yielders with good protein, zinc and iron content respectively, such as Karimeesebhatta (21.15 g/plant, 11.11 %, 32.35 ppm and 13.75 ppm), Doodaalur (40.6 g/plant, 5.44 %, 34.70 ppm and 13.85 ppm) and Mundoni (39.7 g/plant, 9.88 %, 32.75 ppm and 13.95 ppm).

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