

Genetic Divergence in Rice Genotypes for Grain Micronutrients and Yield

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ABSTRACT

The current study aimed to assess the variability, heritability, and genetic diversity of grain characteristics, with a specific emphasis on grain zinc (Zn) and iron (Fe) content, across 31 different rice genotypes. The analysis of variance (ANOVA) revealed significant genotypic differences for all the traits studied. For each trait, the phenotypic coefficient of variation (PCV) consistently exceeded the genotypic coefficient of variation (GCV). Most traits demonstrated high heritability (> 60%), except for grain iron and zinc content. The number of grains per panicle trait exhibited the highest heritability at 93.20% and showed the greatest genetic advance as a percentage of the mean (52.57%), followed by test weight with a genetic advance of 33.516%. The 31 genotypes were categorized into six distinct clusters using the Euclidean method. The greatest inter-cluster distance was observed between cluster V and cluster III (366.88), indicating that genotypes from these clusters with significant differences in cluster means could be valuable for cross-breeding to produce superior recombinants. These genotypes hold potential for breeding programs aimed at improving Zn content alongside higher yield in rice.

Keywords: Biofortification, Genetic diversity, Heritability, Micronutrients, Rice, Variability

Highlights

- Rice serves as the main dietary staple for more than half of the global population.
- Malnutrition is a global challenge, impacting millions, especially in developing nations.
- Research focuses on identifying rice genotypes with significant genetic variability in grain micronutrient content (iron and zinc) with high yield potential.
- Boosting rice yield and nutritional quality strengthens global food security and helps fight malnutrition.

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INTRODUCTION

Rice (*Oryza sativa* L.) stands as the paramount staple crop globally, with Asia holding a preeminent position in production, encompassing 90% of the worldwide rice acreage (Schneider *et al.*, 2020). In the year 2019-20, India achieved a grain production of 112.43 Mt, yielding 2.7 t/ha. Despite India's notable rice production, its productivity lags behind that of major producers such as China (6.5 tons per hectare) and Indonesia (5.2 tons per hectare) (MoA and FW, GoI, 2019-20). Despite significant advancements in rice production, there remains an imperative to further enhance productivity to address the escalating demands of the expanding global population. Concurrently, there is a burgeoning apprehension about ensuring nutritional security, particularly pertaining to essential micronutrients, vitamins, and proteins. This concern arises due to the pervasive issue of micronutrient deficiency afflicting developing nations, exemplified by the situation in India (Bain *et al.*, 2013).

A diet deficient in essential minerals, particularly iron (Fe) and zinc (Zn), plays a significant role in contributing to hidden hunger or micronutrient malnutrition in developing countries (Welch and Graham, 2004). The World Health Organization (WHO) reported in 2022 that the prevalence of anemia among women aged 15 to 49 in 2019 remained similar to that of 2000. However, due to population growth, the total number of affected women increased from 492.9 million in 2000 to 570.8 million in 2019. Addressing this issue requires developing rice varieties that not only produce high yields but also contain adequate

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levels of essential micronutrients, thereby tackling both food security and nutritional challenges. In light of these hurdles and predicaments, the present study aimed to investigate variability, heritability, and genetic advances in yield-related traits, as well as grain Zn and Fe content. Additionally, the research sought to

identify genotypes with high grain yield and micronutrient (Zn & Fe) content among 31 rice genotypes, exploring their potential for use in crop improvement programs focused on enhancing both grain yield and micronutrient enrichment.

MATERIALS AND METHODS

Plant material and growth conditions

The current study was conducted at the Research Farm of RPCAU, located in Pusa, Bihar, during the *Kharif* season of 2020-2021. The experimental resources consisted of 31 rice genotypes (Table 1) obtained from the Harvest Plus program at ICRISAT, Hyderabad, which included the check variety, Rajendra Bhagwati. These genotypes were assessed using a Randomized Complete Block Design with two replications, adhering to standard cultural practices for puddled transplanted rice. Each entry was evaluated in experimental plots measuring 5 m².

Recording of observation

Data for thirteen quantitative traits were recorded. Data on traits viz., PH- Plant height, DFF- Days to 50% flowering, NPP- Number of panicles per plant, PL- Panicle length, FLL- Flag leaf length, NGP- Number of grains per panicle, 1000GW- Test weight, KL- Kernel length, KB- Kernel breadth, L/B- Kernel length to breadth ratio and YPP- Yield per plant were recorded following Standard Evaluation System, IRRI, while data on FeC- Grain iron content, ZnC- Grain zinc content was recorded through Energy Dispersive X-Ray Fluorescence (ED-XRF).

Statistical Analysis

The mean data for each trait were subjected to analysis of variance using the method described by Panse and Sukhatme

Table 2: Analysis of variance (ANOVA) for 13 quantitative traits in rice

Traits	Mean squares		
	Replication (df = 1)	Treatment (df = 30)	Error (df = 30)
Plant height (cm)	63.347	337.410**	34.368
Days to 50% flowering	8.532	236.180**	21.866
Number of panicles/ plants	1.966	6.917**	6.917
Panicle length (cm)	0.147	13.858**	1.906
Flag leaf length (cm)	2.326	42.841**	4.171
Number of grains/panicles	82.825	7290.506**	255.884
Test weight (g.)	2.643	22.590**	1.410
Kernel length (mm)	0.032	0.741**	0.167
Kernel breadth (mm)	0.013	0.070**	0.012
Kernel length to breadth ratio	0.015	0.294**	0.059
Grain iron content (ppm)	0.488	3.696*	1.862
Grain zinc content (ppm)	43.111	29.049*	14.527
Grain yield per plant (g.)	3.504	19.198**	1.814

** - Significant at $\alpha=0.01$, * Significant at $\alpha=0.05$

(1978). The Genotypic (GCV) and Phenotypic (PCV) coefficients of variation were calculated following the approach of Burton (1952). Broad-sense heritability was estimated based on the method of Lush (1940), and the Genetic Advance as a percentage of the mean was determined according to Johnson *et al.*, (1955). Genetic divergence among the 31 genotypes was analyzed using D² analysis as proposed by Mahalanobis (1936). Genotype clustering was carried out using the Euclidean method with Windostat version 9.2 software from Indostat services.

RESULTS AND DISCUSSION

The statistical technique of Analysis of Variance (ANOVA) was applied to evaluate the treatment means across all traits. A perusal of Table 2 reveals that the mean squares of treatment exhibited statistical significance at $\alpha = 0.01$ for all traits, except for Grain iron content and Grain zinc content, where significance was observed at $\alpha = 0.05$ (treatment & error df = 30) and the mean squares of replication were found to lack significance (replication df = 1, error df = 30). It suggests that the genotypes differ significantly for all the traits and the experimental material is suited for further genetic study. The result from ANOVA is pertinent as the experimental material consists of diverse rice genotypes collected from different rice breeding centers of the country, each having a differed lineage and adapted to different agroecologies. Similar findings have been reported by various workers working in rice with different sets of genotypes (Das and Borthakur, 1974; Dhanwani *et al.*, 2013; Nachimuthu *et al.*, 2014; Abebe *et al.*, 2017 and Parihar *et al.*, 2017).

The examination of variation coefficients shown in Fig. 1 indicates that the calculated values for the Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) demonstrate minimal differences across all traits studied,

Table 1: List of 31 rice genotypes used for research

S. No.	Genotype	S. No.	Genotype
1.	R-RGY-IS-110	17.	R-RH2-M1-93
2.	R-RGY-MH-113	18.	CR Dhan 311
3.	R-RHZ-MB-119	19.	CR 2818-1-11-1-B-1-1-2-B-1
4.	R-56 (Protagene)	20.	CR 2819-1-5-3-2B-12-1
5.	R-RHZ-IB-80	21.	R-RGY-RH-16
6.	CGZR-1	22.	MTU 1356
7.	CR Dhan 310	23.	MTU 1357
8.	R-RHZ-SM-14	24.	CSR HZR 17-41
9.	MI 156	25.	JDP 2520-2-4-1
10.	Samba Mahsuri	26.	NVSR 522
11.	DRR Dhan 45	27.	CSR HZR 17-8
12.	MI 127	28.	CSR HZR 17-42
13.	IR 64	29.	BPT 3137
14.	CGZR-2	30.	BPT 3144
15.	R-RHZ-IH-82	31.	Rajendra Bhagwati
16.	DRR Dhan 49		

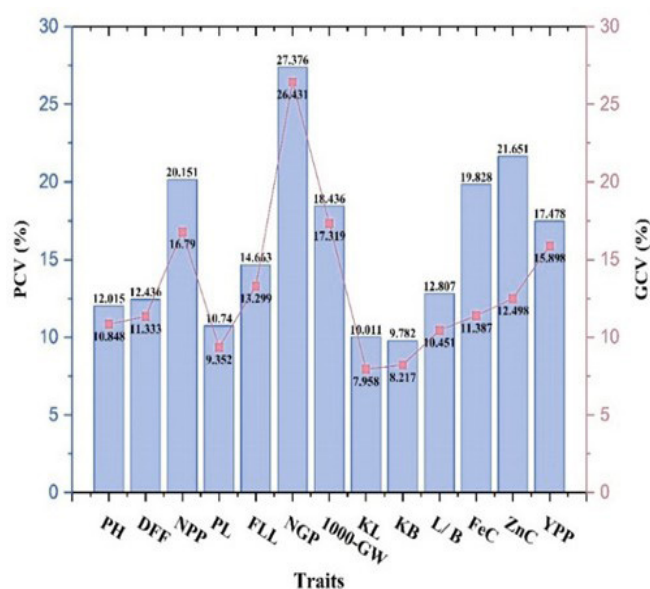


Fig. 1: Graphical representation of coefficient of variation (PCV & GCV) for 13 quantitative traits under study

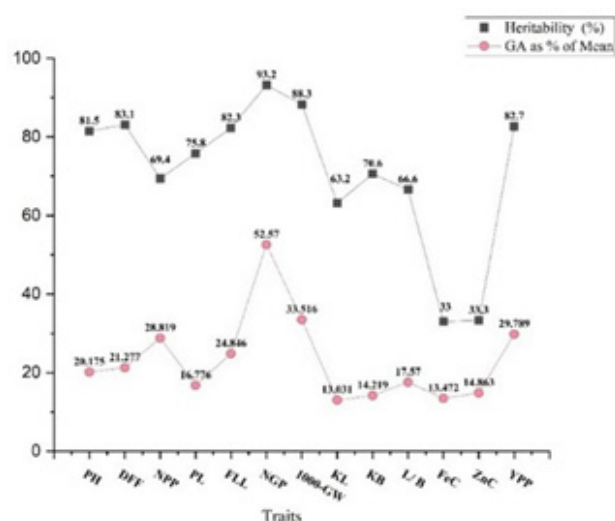


Fig. 2: Graphical representation of heritability and genetic advance as of percentage mean for 13 quantitative traits under study.

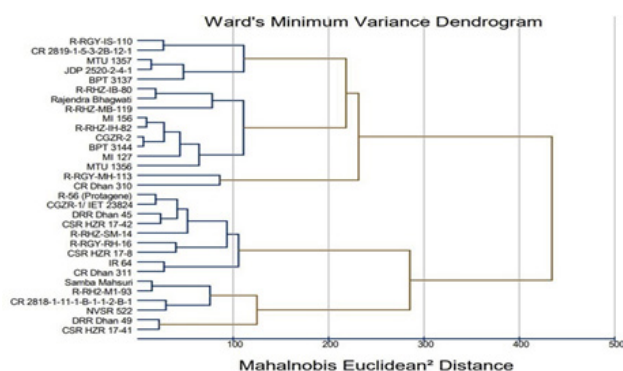


Fig. 3: Dendrogram showing the clustering pattern of 31 genotypes

suggesting a limited impact of environmental factors on phenotype. Specifically, GCV and PCV values for Kernel breadth (8.217 and 9.782), Kernel length (7.958 and 10.011), and Panicle length (9.352 and 10.74) were less than 10%, consistent with the discoveries of Singh *et al.* (2011). Traits such as Plant height (10.848 and 12.015), days to 50% flowering (11.333 and 12.436), number of panicles per plant (16.79 and 20.151), Flag leaf length (13.299 and 14.663), test weight (17.319 and 18.436), Kernel length to breadth ratio (10.451 and 12.807), Grain iron content (11.387 and 19.828), Grain zinc content (12.498 and 21.651) and Grain yield per plant (15.898 and 17.478) exhibited moderate GCV and PCV values ranging from 10% to 20%. Notably, similar observations regarding, number of panicles per plant, test weight, and days to 50% flowering have been documented by Khan *et al.* (2009) and Raza *et al.* (2019). Conversely, the number of grains per panicle (26.431 and 27.376) displayed high GCV and PCV values (>20%), consistent with the findings of Bekele *et al.*, (2013). The minimal disparity between PCV and GCV across all traits in our investigation suggests that the phenotypic variation observed is predominantly due to genetic factors rather than environmental influences.

The results obtained from the evaluations of heritability and genetic advance as a percentage of the mean (GAM) illustrated in Fig. 2 indicate that characteristics such as number of grains per panicle (93.2 and 52.57), Grain yield per plant (82.7 and 29.789), Test weight (88.3 and 33.516), number of panicles per plant (69.4 and 28.819), Flag leaf length (82.3 and 24.846), Plant height (81.5 and 20.175), and Days to 50% flowering (83.1 and 21.277) demonstrate significant heritability (>60%) and substantial GAM (>20%). These findings suggest that these traits hold the potential for direct selection to influence their modification in the desired direction, ultimately leading to an enhancement in grain yield (Agrawal, 2003; Girma *et al.*, 2018). Conversely, other traits, including Panicle length (75.8 and 16.776), Kernel length to breadth ratio (66.6 and 17.57), Kernel

Table 3: Clustering pattern of 31 genotypes based on Euclidean distance of 13 quantitative traits in rice evaluated during Kharif- 2021

Cluster No.	No. of genotypes within the cluster	Genotypes in cluster
I	5	R-RGY-IS-110, CR 2819-1-5-3-2B-12-1, MTU 1357, JDP 2520-2-4-1, BPT 3137
II	9	R-RHZ-IB-80, Rajendra Bhagwati, R-RHZ-MB-119, MI 156, R-RHZ-IH-82, CGZR-2, BPT 3144, MI 127, MTU 1356
III	2	R-RGY-MH-113, CR Dhan 310
IV	9	R-56 (Protogene), CGZR-1/ IET 23824, DRR Dhan 45, CSR HZR 17-42, R-RHZ-SM-14, R-RGY-RH-16, CSR HZR 17-8, IR 64, CR Dhan 311
V	4	Samba Mahsuri, R-RH2-M1-93, CR 2818-1-11-1-B-1-1-2-B-1, NVSR 522
VI	2	DRR Dhan 49, CSR HZR 17-41

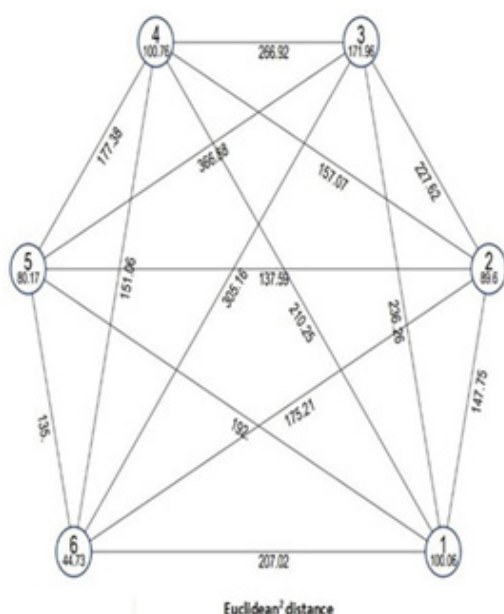


Fig. 4: Cluster diagram showing intra and inter-cluster distance for 06 cluster

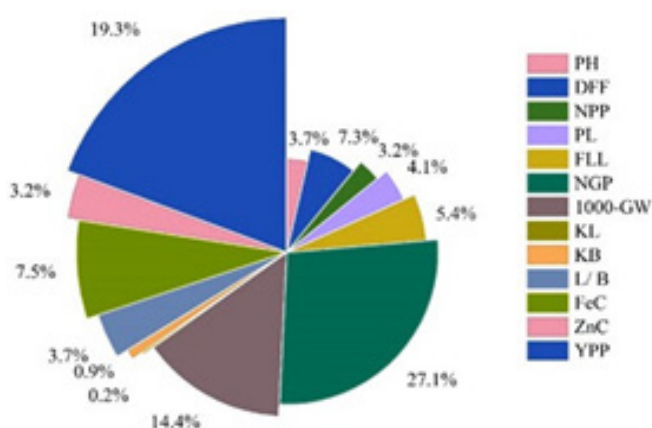


Fig. 5: Contribution of 13 quantitative traits towards genetic divergence

Table 4: Inter- and intra-cluster distance (D2) of clusters formed by 31 genotypes evaluated during Kharif- 2021

Clusters	I	II	III	IV	V	VI
I	100.06	147.75	236.26	210.25	192.00	207.02
II		89.60	227.62	157.07	137.59	175.21
III			171.96	266.92	366.88	305.16
IV				100.76	177.38	151.06
V					80.17	135.00
VI						44.73

breadth (70.6 and 14.219), and Kernel length (63.2 and 13.031), demonstrate high heritability but low GAM. This observation may be attributed to the influence of environmental factors on trait expression. Consequently, simple selection strategies may

not yield significant improvements in these traits, as evidenced by prior research (Mall *et al.*, 2005; Khan *et al.*, 2009; Brar *et al.*, 2011; Kumar *et al.*, 2020; Vanlalrinnngama *et al.*, 2023). According to the outcomes, an inference can be made from the present research, the selection of traits for those with the greatest values of GCV, PCV, heritability, and Genetic Advance, such as number of grains per panicle, would be most helpful to achieve higher yield.

When the replicated data on each trait for all the genotypes were subjected to D² analysis, it became apparent that the experimental materials exhibited significant diversity. Utilizing Euclidean methods, thirty-one genotypes were organized into six clusters, as detailed in Table 3 and dendrogram showing the clustering pattern in Fig. 3. The distribution of genotypes varied across clusters, with the highest number of genotypes (9 genotypes) found in Cluster II and IV, followed by 5 genotypes in cluster I and 4 genotypes in cluster V, while cluster III and VI had the least number of genotypes (2 genotypes). Genotypes within the same cluster showed less divergence compared to those in separate clusters. Table 4 outlines the cluster means for all the studied traits. Notably, Cluster V displayed superior mean performance for traits such as number of panicles per plant and particularly for grain yield per plant. On the other hand, Cluster IV showed optimal mean performance for traits like grain Fe content and grain Zn content, indicating the potential suitability of genotypes from this cluster as parent candidates for improving grain yield, grain Fe, and Zn content. Insights into the intra-cluster and inter-cluster distances among all six clusters derived from this investigation are provided in Table 5 and Fig. 4. The maximum intra-cluster distance was found within cluster III (171.96) comprising 2 genotypes, followed by cluster IV (100.76) with 9 genotypes, then cluster I (100.06) with 5 genotypes, and cluster II (89.60) with 9 genotypes having the minimum intra-cluster distance. Inter-cluster distances are further outlined in descending order, highlighting the maximum Euclidean² distance between cluster III and V (366.88), followed by between cluster III and VI (305.16), between cluster III and IV (266.92), between cluster I and III (236.26), between cluster II and III (227.62), between cluster I and IV (210.25), between cluster I and VI (207.02), between cluster I and V (192.00), between cluster IV and V (177.th), between cluster II and VI (175.21), between cluster II and IV (157.07), between cluster IV and VI (151.06), between cluster I and II (147.75), between cluster II and V (137.59) and the minimum between cluster V and VI (135.00). Crosses among individuals selected from these cluster pairs are expected to yield diverse segregants, enabling effective selection, as the clustering pattern reflects genotype diversity. Utilizing parents from diverse clusters facilitates the accumulation of favourable alleles within a single genetic background. Noteworthy reports from rice researchers, including Rayala *et al.*, (2016), Ashok *et al.*, (2017), Behera *et al.*, (2018), Tripathy (2020), and Singh *et al.*, (2020), support this approach. The distribution of traits' contributions to the overall divergence is depicted in Figure 5. Notably, the trait Number of grains per panicle made the largest contribution (27.10%) to genetic divergence, followed by Grain yield per plant" (19.35%), Test weight (14.41%), and the least contribution was attributed to Kernel length (0.22%). When selecting parents for specific traits in breeding programs, it is advisable to choose from diverse clusters characterized by

Table 5- Cluster Mean of 13 quantitative traits in rice evaluated during Kharif- 2021

W Cluster	PH (cm)	DFF	NPP	PL (cm)	FLL (cm)	NGP	1000-GW (g.)	KL (mm)	KB (mm)	L/B	FeC (ppm)	ZnC (ppm)	YPP (g.)
Cluster I	122.87	100.90	10.73	28.10	33.50	261.20	15.26	6.05	2.06	2.96	7.41	18.69	18.11
Cluster II	110.39	88.39	9.43	25.26	34.89	254.22	20.29	6.72	2.04	3.31	8.57	23.24	18.08
Cluster III	132.00	81.25	7.92	23.42	38.83	153.08	15.93	6.52	1.95	3.46	8.60	17.10	14.70
Cluster IV	113.13	83.00	9.98	26.30	27.83	188.11	18.65	6.82	2.09	3.29	9.31	23.79	17.58
Cluster V	99.04	98.13	11.46	26.67	36.92	258.92	20.29	7.16	2.13	3.39	7.00	18.24	24.23
Cluster VI	115.75	114.75	10.42	26.17	33.83	163.50	21.38	7.40	2.15	3.48	8.78	22.20	18.57

significant inter-cluster distances and noticeable variations in cluster mean values for the designated traits. Moreover, while selecting parents for trait improvement, the importance of yield should not be underestimate

CONCLUSION

Significant variation is present among the genotypes for every trait, according to the analysis of variance (ANOVA). Traits such as plant height, number of panicles per inflorescence, number of grains per panicle, days to 50% flowering, length of flag leaf, zinc content in grain, yield of grain per plant, and test weight displayed high levels of genetic advance as a percentage of mean, high heritability, and high GCV and PCV values, suggesting that these traits could be effectively selected for modification in the desired direction of selection.

The clustering of genotypes resulted in the formation of 6 clusters, encompassing all 31 genotypes. The distances between the clusters were found to be higher in comparison to the distances within the clusters, indicating significant variability among the genotypes. Cluster III and V exhibited the greatest inter-cluster distances, while clusters V and VI displayed the lowest inter-cluster distances. The two clusters consisting of multiple genotypes also showed sufficient intra-cluster distance. This genotype can be used for breeding in the improvement of Zn with higher yield, suggesting its potential to be utilized in biofortification programmes.

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AUTHOR CONTRIBUTION

Mr. Mithilesh Kumar Singh designed the experiment and Ms. Namata and Mr. Bishawajit Kumar performed the experiment, organized the data, and prepared the manuscript. Ms. Shalu Kumari, Mr. Banshidhar and Mr. Digvijay Singh have reviewed the data along with the whole manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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