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Genetic diversity assessment in Rice (*Oryza Sativa* l.) using agro-morphological traits

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Abstract
The study was conducted during 2024-25 using 48 rice genotypes at Agricultural Research Station, Radhanagari, Dist-Kolhapur, MS using agro-morphological traits under a randomized block design. Fourteen traits, including phenological, yield-contributing, grain quality and micronutrient traits, were evaluated. Substantial variability was observed for spikelets per panicle, number of panicles/m², 1000-grain weight, grain length, iron content and zinc content with high GCV and PCV values. High heritability coupled with high genetic advance as percent of mean was recorded for spikelets per panicle, 1000 grain weight, grain length, iron content, and zinc content, suggesting the effectiveness of direct selection. Broad-sense heritability was high for most traits, indicating predominance of additive gene action. Grain yield showed positive significant associations with plant height, panicle length, number of panicles/m², grain length, grain breadth, 1000-grain weight, iron content, zinc content and days to flowering. Path analysis revealed that grain breadth, days to flowering, length of panicle exerted strong direct effects on grain yield, while 1000-grain weight also contributed positively. Mahalanobis D² clustering grouped genotypes into three clusters: Cluster I (46 genotypes), Cluster II (RDN 9) and Cluster III (RDN 12). The highest inter-cluster distance was observed between Cluster I and Cluster III (47.87), indicating maximum divergence. Divergent genotypes such as RDN 9 and RDN 12 may serve as parents in future grain-yield and quality-oriented breeding programs.

Keywords: Genetic diversity, Rice, Grain yield, Micronutrient traits

Introduction
Rice (*Oryza sativa* L.) is the most important staple food crop for nearly half of the world's population and improving its yield and nutritional quality remains a major breeding objective. Genetic diversity is the foundation for crop improvement, influencing selection efficiency and heterosis exploitation. Agro-morphological traits continue to be widely used for diversity assessment due to their direct relevance to yield and grain quality, low cost, and environmental adaptability. Genetic variability parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, and genetic advance provide insight into the nature of gene action and the potential for improvement. Correlation and path coefficient analyses help identify traits with direct and indirect contributions to yield. Mahalanobis D² statistics remain a powerful multivariate approach to assess genetic divergence and identify divergent parents for hybridization. The present study was undertaken to evaluate genetic variability, trait associations, direct and indirect effects and genetic divergence among 48 rice genotypes based solely on agro morphological traits.

Materials and Methods
The experimental material used in this study comprised of forty-eight selected rice genotypes consisting of promising lines and improved varieties of rice laid in randomized block design (RBD) with two replications obtained from Agricultural Research Station, Radhanagari, Dist-Kolhapur during *Kharif* 2024-25. Five competitive plants from each genotype were randomly sampled in every replication to record observations on all yield and yield-attributing traits, except days to flowering. The traits evaluated included plant height (cm), panicle length (cm), spikelets per panicle, panicles per m², grain length (mm), grain breadth (mm), length-to-breadth ratio, 1000-grain weight (g), and grain yield per plot (kg). Days to 50% flowering were recorded on a plot basis. For Biochemical analysis paddy grains from each genotype were cleaned,

mortar and pestle. The powdered grain samples were used for biochemical estimations. Micronutrient concentrations, namely iron and zinc, were determined following the di-acid digestion method described by Zasoski and Burau (1977) [15]. Crude protein content was estimated using the Micro-Kjeldahl method. Amylose content was quantified according to the procedure outlined by (Williams *et al*.1958) [13]. These standardized protocols ensured accurate and reproducible measurement of key nutritional components in the rice genotypes. Mean values were subjected to analysis of variance to test the significance for each character as per methodology advocated by Panse and Sukhatme (1967) [10]. Phenotypic coefficient of variation (PCV) and Genotypic coefficient of variation (GCV) were calculated by the formula given by Burton (1952) [1], heritability in broad sense (h^2) by Burton and De Vane (1953) [2], and genetic advance i.e. the expected genetic gain were calculated by using the procedure proposed by Johnson *et al* (1955) [7]. The genetic divergence was estimated by Mahalanobis (1936) [9] D^2 statistics and the grouping of the genotypes into cluster were done by using Tocher's method (Rao 1952) [11].

Results and Discussion

Results: The analysis of variance showed highly significant differences among the genotypes for all the traits studied, confirming the presence of substantial variability within the material. The genotypes also exhibited marked genetic divergence, indicating ample opportunity to identify and select superior and diverse genotypes. This diversity provides a strong foundation for breeding programmes aimed at improving the genetic yield potential of rice.

Genetic variability of rice

Substantial variability was observed among the genotypes for all traits, as indicated by the higher PCV values compared to GCV, though the differences were minimal, suggesting that most of the variation was genetic (Table 1). High GCV and PCV were recorded for spikelets per panicle, 1000-grain weight, number of panicles/m², and iron content, highlighting considerable scope for improvement through selection. Heritability estimates were high for the majority of traits, notably days to flowering, plant height, spikelets per panicle, grain length, grain breadth, 1000-grain weight, grain yield indicating strong genetic control. High heritability coupled with high genetic advance as percent of mean in spikelets per panicle, 1000-grain weight, iron content, and panicles/m² suggests that these traits are primarily governed by additive gene action and would respond effectively to direct selection. Among biochemical traits, amylose and zinc content showed moderate improvement potential, whereas protein content exhibited low genetic advance, indicating limited response to simple selection.

Correlation Analysis

A correlation analysis (Table 2) among yield and quality components revealed several significant associations important for rice improvement. Days to flowering showed a significant positive correlation with panicles/m² and zinc content, while exhibiting significant negative associations with grain size traits and protein content. Plant height and panicle length were significantly and positively correlated with grain yield, 1000-grain weight and grain dimensions, indicating their major contribution to productivity. The number of panicles/m² also showed a significant positive association with yield but had a significant negative correlation with spikelets per panicle, revealing a trade-off between these components. Grain length, grain breadth, and 1000-grain weight were mutually reinforcing and significantly associated with yield, highlighting grain size as a key determinant of productivity. Spikelets per panicle had significant negative correlations with grain size and panicle number. For micronutrients, zinc content showed a significant positive association with grain yield and iron content, whereas protein content exhibited significant negative correlations with zinc and yield. Overall, the study demonstrates that significant correlations involving plant height, panicle length, panicle number, grain size and zinc content can be effectively exploited in selection, while traits like protein content and spikelet number require balanced improvement to avoid yield penalties.

Path analysis

Path coefficient analysis (Table 3) revealed that several yield-contributing traits exerted significant direct and indirect effects on grain yield per plot. Length of panicle recorded the highest positive direct effect, followed by plant height, grain breadth, 1000-grain weight and zinc content, indicating their strong and favourable influence on yield and their importance as key selection criteria. Number of panicles/m² and grain length also contributed moderately through both direct and indirect pathways. In contrast, traits such as length-to-breadth ratio and protein content exhibited negative direct effects, suggesting their limited usefulness in direct yield improvement. Most traits, including days to flowering, spikelets per panicle, iron content and amylose content, influenced yield largely through indirect effects via major components such as panicle length, grain size and micronutrient traits. Overall, the path analysis highlights that improving panicle length, plant height, grain breadth, grain weight, and zinc content would be most effective for enhancing rice yield, while traits with negative or weak direct effects should be selected cautiously due to their indirect interactions.

Divergence

Mahalanobis D^2 analysis grouped the 48 rice genotypes into three genetically distinct clusters (Table 4), indicating substantial divergence within the population. Cluster I was the largest, containing 46 genotypes, while Cluster II and Cluster III each consisted of a single, highly distinct genotype. The intra-cluster distance was highest in Cluster I ($D^2 = 23.10$), suggesting considerable variability within this group, whereas Clusters II and III showed zero intra-cluster distance due to single-genotype representation. The inter-cluster distances were highest between Clusters I and II ($D^2 = 47.84$) and Clusters I and III ($D^2 = 47.87$), followed by Clusters II and III ($D^2 = 38.34$), indicating wide genetic divergence and opportunities for hybridization between these groups (Table 5). Cluster mean performance (Table 6) revealed notable differences among clusters. Cluster I showed moderate performance for most yield traits, including high spikelets per panicle (169.11), panicles/m² (249.28), and grain yield (2.36 kg/plot). Cluster II, represented by a single genotype, exhibited superior grain length (6.8 mm), grain breadth (2.8 mm), length-breadth ratio (2.44) and the highest 1000-grain weight (33.25 g), along with higher amylose content (23.89%). Cluster III

displayed early flowering, shorter grains (4.35 mm) and low amylose content (16.40%), but showed relatively higher zinc content (21.6 mg/100 g). Overall, the presence of high inter-cluster divergence and distinct cluster means suggests that crossing genotypes from Clusters I and II or Clusters I and III may produce heterotic combinations and broaden the genetic base for yield and nutritional traits in rice.

Discussion

The presence of high phenotypic and genotypic variability for key traits such as spikelets per panicle, 1000-grain weight, grain size traits and micronutrient contents indicates a rich genetic base among the evaluated genotypes. Similar results were reported by recent studies emphasizing substantial morphological diversity in rice by Kumar *et al.* (2018) [8]. High heritability and high genetic advance for grain size traits and micronutrient contents suggest the predominance of additive gene action, aligning with findings by Kumar *et al.* (2018) [8]. Positive significant correlations of grain yield with panicle traits, grain size and micronutrient content align with earlier reports Thorat *et al.* (2019) and Williams *et al.* (2021) [12, 14]. Path analysis highlighted grain length as the most important direct contributor, consistent with findings by Dhurai *et al.* (2016) [6]. The D^2 analysis revealed strong genetic divergence between RDN 9, RDN 12 and the main cluster. Therefore, crosses involving RDN 9 × elite Cluster I and RDN 12 × Cluster I genotypes may produce transgressive segregants. Similar clustering patterns with isolated single-genotype clusters were reported by Devi *et al.* (2016) and Devi *et al.* (2019) [4, 5], confirming the suitability of divergent parents for hybridization. Overall, the study identified key traits and divergent genotypes that can serve as valuable breeding resources for improving rice yield and quality.

Table 1: Estimates of genetic variability parameters for 14 different characters

Sr. No.	Character	Mean	Range	GCV (%)	PCV (%)	h^2 % (B.S)	Genetic advance	GA as percent mean (%)
1	Days to Flowering (No.)	101.54	89-118	5.64	5.77	95.7	11.55	11.37
2	Plant Height (cm)	94.75	69-133	11.41	11.76	94.2	21.63	22.82
3	Length of Panicle (cm)	21.85	16.9-25.8	7.07	8.117	76.0	2.77	12.70
4	Spikelets per Panicle (No.)	169.00	92-274	26.27	26.44	98.8	90.91	53.79
5	Number of Panicle/m^2 (No.)	248.50	101-353	20.04	22.00	83.0	93.46	37.61
6	Grain Length(mm)	6.03	4.30-7.98	13.59	13.65	99.0	1.68	27.86
7	Grain Breadth (mm)	2.11	1.72-2.80	10.61	10.67	98.8	0.45	21.72
8	Length : Breadth Ratio	2.88	1.62-3.74	13.93	14.05	98.2	0.82	28.44
9	1000 Grain Weight(gm)	19.56	11.4-33.8	26.83	26.99	98.8	10.75	54.95
10	Grain yield/Plot(kg)	2.36	1.20-3.50	17.17	20.96	67.1	0.68	28.97
11	Amylose content (%)	21.85	19.0-24.7	6.05	6.34	91.2	2.60	11.91
12	Protein content (%)	7.58	7.08-8.26	3.09	3.97	60.7	0.37	4.971
13	Iron content (mg/100 g)	2.18	0.61-4.37	38.66	41.65	86.2	1.61	73.94
14	Zinc content (mg/100 g)	2.82	1.73-3.55	12.36	13.68	81.7	0.65	23.03

Table 2: Genotypic correlation coefficients of 13 characters of 48 genotypes of rice on grain yield

	Days to flowering (No.)	Plant height (cm)	Length of panicle (cm)	Spikelets per panicle (No.)	Number of panicle/m^2 (No.)	Grain length (mm)	Grain breadth (mm)	Length: breadth ratio	1000 grain weight (gm)	Amylose content (%)	Protein content (%)	Iron content (mg/ 100 g)	Zinc content (mg/ 100 g)	Grain yield/ plot (kg)
Days to flowering (No.)	**1**	-0.0108	-0.0857	-0.1300	0.3934**	-0.1495	-0.0661	-0.0816	-0.1922	0.1017	-0.2043*	0.212*	0.3622**	0.206*
Plant height (cm)		**1**	0.5329**	0.227*	0.0999	0.0872	0.3822**	-0.1596	0.2563*	-0.0266	0.1067	0.1702	0.1548	0.3772**
Length of panicle (cm)			**1**	0.0558	0.2599*	0.2898**	0.2951**	0.0606	0.2986**	0.1253	-0.2214*	0.199	0.3202**	0.4334**
Spikelets per panicle (No.)				**1**	-0.4083**	-0.1846	-0.1812	-0.0253	-0.2323*	0.0266	0.2063*	-0.213*	-0.2758**	0.1209
Number of panicle/m^2 (No.)					**1**	0.2206*	0.2272*	0.0348	0.246*	-0.1322	-0.2348*	0.3733**	0.3832**	0.2293*
Grain length(mm)						**1**	0.207*	0.745**	0.8486**	-0.0361	-0.1309	-0.0341	0.1685	0.1949
Grain breadth (mm)							**1**	-0.4914**	0.6101**	-0.1254	-0.2358*	0.0731	0.3355**	0.3809**
Length: Breadth ratio								**1**	0.3367**	0.0822	0.0653	-0.0753	-0.0777	-0.0926
1000 grain weight(gm)									**1**	-0.0395	-0.2331	-0.0319	0.28	0.2979**
Amylose content (%)										**1**	-0.1284	0.0554	0.0392	0.0475
Protein content (%)											**1**	0.2363*	-0.5319	-0.0846
Iron content (mg/100 g)												**1**	0.1859	0.0492
Zinc content (mg/100 g)													**1**	0.3099**

*Significant at 5% level; ** Significant at 1% level

Table 3: Direct (diagonal) and indirect (above and below diagonal) path effects of different characters towards grain yield at genotypic level in rice

Observations	Days to flowering	Plant height (cm)	Length of panicle	Spikelets per panicle	Number of panicle/m^2	Grain length	Grain breadth	Length: Breadth ratio	1000 grain weight (gm)	Amylose content	Protein content	Iron content	Zinc content	Grain yield/ plot
Days to flowering	**0.304**	-0.004	-0.0265	-0.0309	0.0425	-0.3886	0.1038	0.2306	-0.0021	0.0169	-0.0732	0.0458	0.0757	0.2060
Plant height (cm)	-0.004	**0.0412**	0.1647	0.0539	0.0108	0.2266	-0.6	0.4511	0.0027	-0.0044	0.0382	-0.0367	0.3230	0.3772
Length of panicle	-0.0261	0.0219	**0.3091**	0.0133	0.0281	0.7532	-0.4633	-0.1713	0.0032	0.0209	-0.0794	-0.0432	0.0669	0.4334
Spikelets per panicle	-0.0395	0.0093	0.0173	**0.2375**	-0.0441	-0.4799	0.2845	0.0715	-0.0025	0.0044	0.0739	0.0460	-0.0576	0.1209
Number of panicle/ m^2	0.1196	0.0041	0.0803	-0.0097	**0.108**	0.5733	-0.3567	-0.0982	0.0026	-0.0220	-0.0841	-0.0806	0.0801	0.2293
Grain length	-0.0455	0.0036	0.0896	-0.0438	0.0238	**2.5991**	-0.3249	-2.1058	0.0091	-0.006	-0.0469	0.0074	0.0352	0.1949
Grain breadth	-0.0201	0.0157	0.0912	-0.043	0.0245	0.5379	**-1.5698**	1.3890	0.0065	-0.0209	-0.0845	-0.0158	0.0701	0.3809
Length: Breadth ratio	-0.0248	-0.0066	0.0187	-0.006	0.0038	1.9365	0.7714	**-2.8263**	0.0036	0.0137	0.0234	0.0163	-0.0162	-0.0926
1000 grain weight	-0.0584	0.0106	0.0923	-0.0552	0.0266	2.2055	-0.9577	-0.9516	**0.0107**	-0.0066	-0.0835	0.0069	0.0585	0.2979
Amylose content	0.0309	-0.0011	0.0387	0.0063	-0.0143	-0.0939	0.1969	-0.2322	-0.0004	**0.1664**	-0.0460	-0.0120	0.0082	0.0475
Protein content	-0.0621	0.0044	-0.0684	0.0490	-0.0253	-0.3401	0.3701	-0.1844	-0.0025	-0.0214	**0.3584**	-0.0510	-0.1111	-0.0846
Iron content	0.0644	0.007	0.0618	-0.0506	0.0403	-0.0885	-0.1147	0.2129	-0.0003	0.0092	0.0847	**-0.2158**	0.0388	0.0492
Zinc content	0.1101	0.0064	0.099	-0.0655	0.0414	0.4379	-0.5266	0.2197	0.0030	0.0065	-0.1906	-0.0401	**0.2089**	0.3099

Table 4: Distribution of 48 rice genotypes into different clusters

Cluster No	Number of genotypes included	Genotypes
I	46	RDN 1, RDN 2, RDN 3, RDN 4, RDN 5, RDN 6, RDN 7, RDN 8, RDN 10, RDN 11, RDN 13, RDN 14, RDN 15, RDN 16, RDN 17, RDN 18, RDN 19, RDN 20, RDN 21, RDN 22, RDN 23, RDN 24, RDN 25, RDN 26, RDN 27, RDN 28, RDN 29, RDN 30, RDN 31, RDN 32, RDN 33, RDN 34, RDN 35, RDN 36, RDN 37, RDN 38, RDN 39, RDN 40, RDN 41, Phule Radha, Phule Kolam, Indrayani, Bhogavati, Phule Samruddhi, DRR 48, DRR 63
II	1	RDN 9
III	1	RDN 12

Table 5: Average intra and inter cluster D^2 of 3 clusters formed from 48 genotypes

Clusters	I	II	III
I	23.1	47.84	47.87
II		0	38.34
III			0

Table 6: Mean performance of different clusters in rice

Cluster	Days to flowering	Plant height (cm)	Length of panicle	Spikelets per panicle	Number of panicle/m^2	Grain length (mm)	Grain breadth (mm)	Length: breadth ratio	1000 grain weight (g)	Grain yield/plot (kg)	Amylose (%)	Protein (%)	Iron content (mg/100 g)	Zinc content (mg/100 g)
I	101.5	93.7	21.8	169.1	249.2	6.0	2.0	2.9	19.3	2.36	21.8	7.59	2.17	2.82
II	99	129	23.8	125	246	6.8	2.8	2.4	33.2	2.38	23.8	7.57	2.26	3.00
III	102	105.5	21.0	208	215	4.3	2.6	1.6	16.4	2.55	21.6	7.69	2.82	3.00

Conclusion

Significant genetic variability exists among the 48 rice genotypes for yield and quality traits. High heritability coupled with high genetic advance for spikelets per panicle, 1000-grain weight, grain length, iron and zinc contents indicates the effectiveness of selection. Grain yield was mainly influenced by grain size, spikelets per panicle, panicles/m² and 1000-grain weight. Mahalanobis D² analysis identified RDN 9 and RDN 12 as the most divergent genotypes. These can be effectively used as parents in breeding programs aiming for improved grain yield, quality and micronutrient content.

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