Exploring genetic variability and diversity in advanced breeding lines of rice for yield-attributing and grain quality traits

Abstract :

Genetic variability and diversity are crucial for enhancing yield and grain quality in rice (*Oryza sativa* L.). A field experiment was conducted during *Kharif*, 2023 at the Research cum Instructional Farm, Department of Genetics and Plant Breeding, College of Agriculture, IGKV, Raipur (C.G.). Thirty-three advanced breeding lines were evaluated for 30 yield-attributing and grain quality traits using a Randomized Block Design (RBD) with two replications. Analysis of variance revealed highly significant differences among the lines, indicating substantial genetic variability. Genetic diversity analysis through agglomerative hierarchical clustering based on Euclidean Distance grouped the lines into five distinct clusters. Cluster II was the largest (57.57%, n=19), followed by Cluster I (18.18%, n=6), while Clusters III, IV, and V contained 2, 5, and 1 line(s), respectively. Intra-cluster distances ranged from 0.00 (Cluster V) to 18.381 (Cluster IV), while the maximum inter-cluster distance was between Clusters I and V (76.364). Cluster III had the highest mean values for several traits, including grain yield per plant, making these lines promising candidates for hybrid breeding programs to improve yield and grain quality.

Key words: Rice, RBD, Advanced breeding lines, Variance, Genetic diversity, Agglomerative hierarchical clustering, Euclidean Distance, Intra-cluster, Inter-cluster.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops globally, serving as a staple food for over 3.5 billion people. It plays a crucial role in ensuring food security, particularly in Asia, which accounts for approximately 90% of the world's total rice production. More than 75% of the global rice supply is consumed in Asian countries, highlighting its critical importance to the region’s food security. Rice (*Oryza sativa* L.) is the primary staple food for one third of the world population and is often referred to as “Global Grain” (Ajmera *et al*., 2017). It thrives in hot and humid climates with high humidity, prolonged sunshine, and an adequate water supply. Nutritionally, rice is a significant source of carbohydrates, proteins, and essential micronutrients such as B vitamins, iron, and manganese.

In India, rice constitutes over 40% of the total food grain production and serves as a staple food for the majority of the population. The Chhattisgarh state, known as the "Rice Bowl of India," contributes approximately 5.26% of the country’s total rice production and houses a rich diversity of rice germplasm. By 2050, it is predicted that 160 million tons of rice needed to feed ever increasing Indian population (Muthu *et al*., 2020). Consequently, improving rice yield and quality remains a primary focus of national and international breeding programmes.

Grain yield improvement is the prime objective of plant breeders for several decades but demand for good quality of rice is also increased in current decade as living standard of people are being gradually improved (Rathi *et al*., 2010). Variability in yield and quality traits can arise due to differences in the genetic constitution of plant populations or environmental factors. Identifying and exploiting genetic variability is critical to selecting superior genotypes. The success of any breeding programme mainly depends on the quantum of genetic variability available for exploitation and the extent to which the desirable characters are heritable (Tiwari *et al*., 2011).

Genetic diversity is a fundamental prerequisite for crop improvement as it facilitates the production of superior recombinants by selecting parents with greater variability in key traits. Studies on genetic diversity help assess species diversity, genetic composition, and variability among genotypes, which are essential for identifying and conserving promising parental lines. Crosses between genetically diverse parents often result in higher heterosis, enhancing the potential for crop improvement. Thus, understanding genetic diversity and divergence is crucial for achieving systematic agricultural growth and enhancing the productivity and quality of rice. Therefore, this study aimed to evaluate 33 advanced breeding lines for yield and quality traits, providing insights into genetic variability, estimating key genetic parameters, and assessing genetic diversity to aid in the selection of superior genotypes for rice improvement.

Materials and Methods

The research experiment was conducted during the *kharif* season of 2023 at the Research cum Instructional Farm, Department of Genetics and Plant Breeding, College of Agriculture, IGKV, Raipur (C.G.). The experiment was laid out in Randomized Block Design using 33 genotypes or treatments in 2 replications for studying 11 yield-attributing traits and 19 grain quality traits. The statistical analysis was performed using Agri Analyze software for analysis of variance and XLSTAT software for genetic diversity analysis.

Analysis of Variance (ANOVA)

The analysis of variance (ANOVA) was done on the basis of model described by Cochran and Cox (1950) for randomized complete block design.

$$Y_{ij} = \mu + g_i + r_j + e_{ij}$$

Where, Y_{ij} = phenotypic observation of $i\ th$ genotype and $j\ th$ replication

μ = general mean

g_i = effect of i^{th} genotype

r_j = effect of j^{th} replication

e_{ij} = random error associated with i^{th} genotype and j^{th} replication.

The level of significance was tested at 5% and 1% levels of probability against error degree of freedom using F table values given by Fisher and Yates (1963).

Genetic Diversity

Genetic diversity analysis was done by agglomerative hierarchical clustering, based on the similarity or dissimilarity between objects, often calculated from quantitative traits using a Euclidean distance matrix, which was calculated by the formula,

$$ED_{jk} = \sqrt{\sum_{i=1}^{n} \left(X_{ij} - X_{ik}\right)^2}$$

where, ED_{jk} = distance between genotypes j and k,

X_{ij} and X_{ik} = phenotype traits values of the ith character for genotypes j and k, respectively,

n = number of phenotype traits used to calculate the distance.

The distance matrix from phenotype traits was used to construct Dendrogram based on the Unweighted Pair-group Method with Arithmetic Means (UPGMA). The results of cluster analysis were presented in the form of Dendrogram.

Intra-cluster distance was measured by taking the average distance between all the points in the cluster. Inter-cluster distances were measured by considering the minimum, maximum, or average distance between all points in two different clusters. In addition, mean ED was calculated for each genotype by averaging of a particular genotype to the other genotypes. The per cent contribution of characters towards genetic divergence was calculated according to Singh and Choudhary.

Results and Discussions

For all the yield and quality traits studied, the analysis of variance revealed highly significant differences among all the 33 advanced breeding lines considered under the investigation, indicating substantial genetic variation within the material. The results of analysis of variance for yield and quality traits are provided in table 1. The F-test for all yield and quality traits showed that the mean sum of squares was highly significant at the 1% level of significance. Significant genetic variability for various yield and quality related traits in rice was also documented by Dhanwani *et al.* (2013), Ketan and Sarkar (2014), Abebe *et al.* (2017), Gupta *et al.* (2021) and Parihar *et al* (2023).

Table 1: Analysis of variance for yield attributing and grain quality traits in advanced breeding lines of rice

S. No	Sources of variance	Mean sum of squares		
		Replication (df=1)	**Genotypes (df=32)**	**Error (df=32)**
1.	Days to 50% flowering	0.5455	25.0568**	2.4830
2.	Plant height (cm)	68.0558	132.3867**	17.7355
3.	Number of effective tillers per plant	0.0183	3.6670**	0.5561
4.	Panicle length (cm)	2.0599	3.7437**	0.5456
5.	Length of flag leaf (cm)	12.4976	12.8907**	3.8351
6.	Breadth of flag leaf (cm)	0.0070	0.0506**	0.0018
7.	Spikelet fertility percent	9.5076	132.0401**	9.0414
8.	100 seed weight (g)	0.0932	0.4858**	0.0350
9.	Biological yield per plant (g)	16.0728	88.9665**	10.8658
10.	Harvest Index (%)	2.1456	26.5379**	8.8395
11.	Grain yield per plant (g)	5.2137	24.0553**	2.3682
12.	Hulling percentage	0.2293	3.6906**	0.6402
13.	Milling percentage	9.8909	6.7981**	2.4311
14.	Paddy length (mm)	0.0024	1.1671**	0.0112
15.	Paddy breadth (mm)	0.0014	0.0904**	0.0057
16.	Paddy L/B ratio	0.0024	0.2091**	0.0093
17.	Brown rice length (mm)	0.0006	0.6878**	0.0119
18.	Brown rice breadth (mm)	0.0074	0.0351**	0.0049
19.	Brown rice L/B ratio	0.0070	0.1386**	0.0105
20.	Kernel length (mm)	0.0606	0.5419**	0.0162

21.	Kernel breadth(mm)	0.0055	0.0408**	0.0017
22.	Kernel L/B ratio	0.00002	0.1246**	0.0037
23.	Kernel length after cooking (mm)	0.0218	1.8609**	0.0071
24.	Kernel breadth after cooking (mm)	0.0014	0.0720**	0.0045
25.	Cooked rice L/B ratio	0.00001	0.1809**	0.0056
26.	Elongation ratio	0.0014	0.0533**	0.0012
27.	Gel consistency	0.9697	1307.599**	0.2666
28.	Alkali spreading value	0.0152	1.4669**	0.1089
29.	Amylose percentage	0.3068	165.9233**	0.0743
30.	Head rice recovery (%)	19.0517	119.1791**	9.8395

**Significance at 1% level of probability

Based on genetic dissimilarities the genotypes were grouped in to five distinct clusters according to agglomerative hierarchical analysis. The Euclidean distance matrix of 528 pair of genotypes estimated from different yield attributing and quality traits were used to construct dendrogram based on the Unweighted Pair-group Methods with Arithmetic Means (UPGMA). The advanced breeding lines were tabulated beside their respective clusters and presented in Table 2. This indicates the presence of significant genetic diversity among the advanced breeding lines studied. The genotypes grouped under same cluster had similarity for many traits but dissimilarity to other genotypes in other clusters with one or more traits. Similarly, Garg *et al*. (2011) grouped 48 diverse rice genotypes into 5 clusters, Majumder *et al*. (2015) grouped 30 rice genotypes into 5 clusters and Sree *et al*. (2023) grouped 40 diverse rice genotypes into 5 distinct clusters. The dendrogram representing thirty three advanced breeding lines distributed into five major clusters and distinguished by different colours was shown in Figure 1.

Table 2: Clustering pattern among 33 advance breeding lines of rice for both yield and quality traits

Cluster Number	No. of genotypes	Percent of genotypes	Advanced breeding lines
I	6	18.18%	R 2370-96-2-9-1 **[1]**, R 2496-483-1-582-1 **[15]**, R2757-4-1 **[24]**, R2737-54-1 **[29]**, R2757-55-1 **[30]**, Karma Mahsuri (C) **[32]**
II	19	57.57%	R 2546-C5-3-472-1 **[2]**, R 2406-184-2-112-1 **[4]**, R 2404-164-2-87-1 **[5]**, R 2698-150-1 **[6]**, R 2542-C1-8-442-1 **[7]**, R 2296-31-1 **[8]**, R 2550-C9-3-500-1 **[11]**, R 2699-19-1 **[12]**, R 2701-45-1 **[13]**, R 2697-169-1 **[16]**, R 2406-182-1-106-1 **[17]**, R 2296-30-1 **[18]**, R 2697-140-1 **[19]**, R 2406-182-2-107-1 **[20]**, R2737-98-1 **[22]**, R2737-3-1 **[26]**, R2737-25-1 **[27]**, R2737-26-1 **[28]**, IGKV R 1244 (C) **[33]**
III	2	6.06%	R 2506-485-1-584-1 **[3]**, IR17A2192 **[14]**
IV	5	15.15%	R 2405-171-91-1 **[9]**, R 2326-108-1-61-1 **[21]**, R2764-25-1 **[23]**, R2764-35-1 **[25]**, Narendra 359 (C) **[31]**
V	1	3.03%	R 2545-C4-6-468-1 **[10]**

The average intra and inter-cluster distances among five clusters were tabulated and presented in Table 3. Highest inter-cluster distance was observed between Clusters I and V (76.364) followed by Clusters I and II (63.178), III and V (56.890) and Clusters IV and V (55.025) suggesting wide diversity between these clusters. The least inter-cluster distance was observed between Clusters III and IV (25.812) followed by Clusters I and III (31.773), I and IV (33.236) and Clusters II and V (34.866) indicating close relationship between the genotypes of these clusters. The genotypes within each cluster were closer to each other than the genotypes in different clusters. The intra-cluster distances were lesser than the inter-cluster distances, which was implying that the genotypes within a cluster tend to diverse less from each other. The intra-cluster distance was ranged from 9.739 in Cluster III to 18.381 in Cluster IV.

Figure 1: The dendrogram based on UPGMA clustering method showing genetic relationships among 33 rice genotypes for all traits

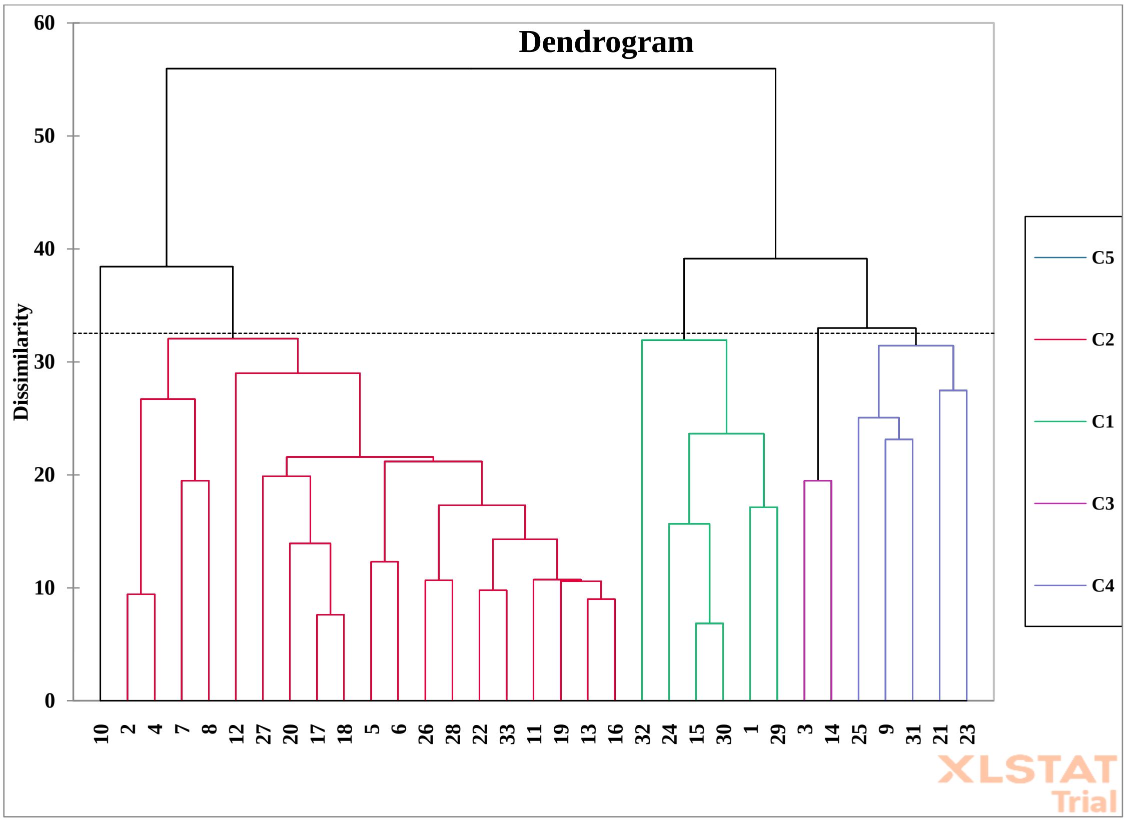

Table 3: Average intra-cluster (diagonal) and inter-cluster distance values

Cluster Number	I	II	III	IV	V
I	**15.649**	63.178	31.773	33.236	76.364
II		**16.480**	43.779	36.519	34.866
III			**9.739**	25.812	56.890
IV				**18.381**	55.025
V					**0**

The cluster mean values of all the traits assessed were shown in Table 4 along with percent contribution towards genetic diversity. Cluster I did not show highest mean for any trait, whereas, Cluster II showed highest means for traits paddy length (9.445mm), brown rice length (7.176mm), brown rice L/B ratio (3.061) and kernel length (6.974mm). Cluster III manifested maximum number of highest cluster means for various characters including grain yield per plant (22.820g) such as number of effective tillers per plant (9.100), panicle length (26.265cm), breadth of flag leaf (1.550cm), spikelet fertility percent (87.065%), 100 seed weight (3.070g), biological yield (4.930g), kernel length after cooking (11.475mm), cooked rice L/B ratio (3.475) and elongation ratio (1.725). Cluster IV and V exhibited same number of highest cluster mean for various characters**.** Traits with highest cluster mean under Cluster IV were days to 50% flowering (100.20), length of flag leaf (29.578cm), harvest index (48.198%), hulling percentage (80.430%), milling percentage (70.186%), paddy L/B ratio (3.642), kernel L/B ratio (3.284) and head rice recovery (52.900%). Traits with highest cluster mean under Cluster V were plant height (129.670cm), paddy breadth (2.900mm), kernel breadth (2.400mm), brown rice breadth (2.350mm), kernel breadth after cooking (3.400mm), gel consistency (97.250), alkali spreading value (4.500) and amylose percentage (51.340). Cluster III had maximum number of highest desirable cluster means including grain yield and hence, could be directly selected and utilized in breeding programmes. Similar findings were observed by Devi (2016), Solanki *et al*. (2019) and Bekis *et al*. (2021) under cluster mean analysis.

Among all the traits under study, gel consistency (53.08%) had maximum contribution towards diversity followed by amylose percentage (25.08%), brown rice length (3.87%), kernel breadth after cooking (2.58%), brown rice breadth (2.31%), brown rice L/B ratio (1.79%), kernel breadth (1.22%), grain yield per plant (1.22%), harvest index (1.06%) and the least contribution was observed by spikelet fertility percent (0.01%) followed by number of effective tillers per plant (0.03%), length of flag leaf (0.03%) and milling percentage (0.04%).

Table 4: Average performance of advanced breeding lines for each cluster for different characters along with percent contribution of traits

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Percentage contribution
Days to 50% flowering	100.17	98.42	98.00	100.20	98.00	0.1%
Plant height (cm)	106.757	116.029	127.770	112.616	**129.670**	0.16%
Number of effective tillers per plant	8.333	7.955	**9.100**	7.070	7.100	0.03%
Panicle length (cm)	24.153	25.076	**26.265**	24.118	23.110	0.09%
Length of flag leaf (cm)	28.788	29.456	27.695	**29.578**	27.860	0.03%
Breadth of flag leaf (cm)	1.400	1.506	**1.550**	1.320	1.250	1.01%
Spikelet fertility percent	84.003	85.465	**87.065**	84.900	79.120	0.01%
100 seed weight (g)	2.848	2.851	**3.070**	2.328	2.060	0.15%
Biological yield per plant (g)	40.963	45.306	**49.930**	35.470	35.910	0.57%
Harvest Index (%)	48.152	45.181	45.510	**48.198**	43.870	1.06%
Grain yield per plant (g)	19.632	20.502	**22.820**	17.034	15.750	1.22%
Hulling percentage	78.728	79.223	79.730	**80.430**	80.350	0.1%
Milling percentage	68.680	68.503	68.955	**70.186**	66.990	0.04%
Paddy length (mm)	8.467	**9.445**	9.075	9.410	9.250	0.37%
Paddy breadth (mm)	2.750	2.853	2.650	2.610	2.900	0.71%
Paddy L/B ratio	3.087	3.323	3.420	**3.642**	3.190	0.33%
Brown rice length (mm)	6.442	**7.176**	6.825	7.130	7.100	3.87%
Brown rice breadth (mm)	2.283	2.350	2.250	2.170	2.350	2.31%

Brown rice L/B ratio	2.827	**3.061**	3.040	3.298	3.020	1.79%
Kernel length (mm)	6.325	**6.974**	6.725	6.920	6.900	1.03%
Kernel breadth(mm)	2.217	2.287	2.300	2.110	2.400	1.22%
Kernel L/B ratio	2.857	3.058	2.925	**3.284**	2.880	0.61%
Kernel length after cooking (mm)	10.800	11.189	**11.475**	11.040	10.650	0.04%
Kernel breadth after cooking (mm)	3.250	3.332	3.300	3.210	3.400	2.58%
Cooked rice L/B ratio	3.323	3.374	**3.475**	3.430	3.140	0.68%
Elongation ratio	1.710	1.611	**1.725**	1.596	1.540	0.71%
Gel consistency	33.542	95.342	53.750	62.500	97.250	53.08%
Alkali spreading value	4.250	3.237	3.750	3.500	4.500	0.75%
Amylose percentage	17.987	22.777	21.020	15.818	51.340	25.08%
Head rice recovery (%)	39.692	44.531	45.505	**52.900**	39.560	0.28%

Note: Bold digits represent highest mean values for that particular traits and mean values which are not favourable are not bold.

Conclusion

Highly significant genetic variation was observed among genotypes for all traits studied, indicating a strong potential for selecting superior genotypes for yield and quality improvement. Cluster II with maximum genotypes and intra-cluster distance, suitable for within-cluster hybridization, while inter-cluster crosses between Cluster I and V, Cluster I and II were likely to produce broader genetic recombination because of their maximum genetic divergence. Cluster III exhibited maximum number of highest cluster mean values including grain yield per plant (g). Therefore, the genotypes R 2506-485-1-584-1 and IR17A2192 under Cluster III could be directly utilised in hybrid breeding programmes for obtaining improved grain yield and quality.

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