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Principal Component and Cluster Analysis on Eating and Cooking Quality Parameters in Rice (*Oryza sativa* L.) Germplasm

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ABSTRACT: Eating and cooking quality traits in rice are complex traits such as grain size, shape, and texture of the cooked kernels parameters. These traits are influenced by various factors including genetic makeup, amylose content, gelatinization temperature, and processing techniques. Improving the grain quality parameters, especially the eating and cooking quality of rice has wide-ranging implications, including enhancing market competitiveness, increasing consumer satisfaction, and addressing nutritional needs. It also contributes to sustainable agriculture practices by optimizing resource utilization and reducing food waste. The present study was conducted to assess the divergence of fifty-five rice genotypes based on eight-grain quality traits. The results of principal component analysis (PCA) revealed that the four principal components (PC) accounted for approximately 82% of the total variability observed among the fifty-five rice genotypes. By employing Mahalanobis D^2 analysis, all fifty-five genotypes were categorized into nine clusters. The largest inter-cluster distance was observed between Cluster VII and Cluster VIII (36.67), followed by Cluster V and Cluster IX (34.75), and Cluster V and Cluster VI (32.78), indicating the presence of significant genetic diversity among the genotypes. The genotypes belonging to Cluster VII to Cluster VIII, Cluster V and Cluster IX, and Cluster V and Cluster VI can be considered for hybridization purposes, as they exhibit higher mean values for quality traits and greater inter-cluster distance, indicating increased diversity.

Keywords: Quality traits, Principal Component, Cluster and Genotypes.

INTRODUCTION

Rice serves as the primary staple food for a significant portion of the world's population, with more than half relying on it for sustenance. To meet the demands of an ever-growing population, crop geneticists and rice breeders are dedicated to the development of rice varieties that exhibit both high yields as well as superior grain quality parameters. Their efforts are directed towards creating rice cultivars that not only produce abundant harvests but also possess desirable traits and characteristics that contribute to enhanced nutritional value and overall eating experience. In the past few decades, advances in technology and the discovery and implementation of genes related to the "green revolution" and hybridization have led to significant improvements in rice yield (Pingali 2012; Chen *et al.*, 2019; Liu *et al.*, 2020; Wu *et al.*, 2020). Although rice yield research and breeding practices have made significant progress, there has been a considerable gap in the advancement of research and breeding practices related to rice quality. The increasing global demand for premium rice is raising concerns and creating a need to address the growing expectations of consumers and the expanding rice market. To meet this demand, there is a growing emphasis on breeding efforts to develop high-quality rice varieties. The quality of rice grains is closely associated with various characteristics such as seed size, composition, and overall grain quality. For example, the size of the hull surrounding the spikelet plays a crucial role in determining the size of the rice grain, which is an important agronomic trait. The size of rice grains not only influences the yield but also serves as a direct indicator of rice quality, which is determined by factors such as grain length, width, thickness, and L/B ratios. These characteristics significantly impact the appearance quality, cooking and eating quality of rice. In recent times, researchers have successfully cloned and studied several genes related to grain size (Li *et al.*, 2019). The endosperm of rice contains starch, which is the primary component consisting of two glucose

polymers known as amylose and amylopectin. The structure of amylose content (AC) in rice has a significant impact on its eating and cooking quality, as highlighted in the study conducted by Leng *et al*., (2014). Chemical and nutritional attributes including moisture, protein, fat, crude fibre, amylose content, and ash content exhibited significant differences among the rice varieties, indicating good grain quality for market value and adaptation of new varieties (Sulochana *et al.*, 2022). In commercial rice production, grain dimension quality traits play a vital role, including kernel length, kernel breadth, length-to-breadth ratio, elongation ratio, kernel length after cooking, and kernel breadth after cooking. These traits are crucial considerations in ensuring the desired quality of rice grains for the market. These traits significantly impact the final output and consumer demand, thereby affecting the economic profitability of rice cultivators. Principal component analysis (PCA) is a valuable multivariate analysis tool used to assess phenotypic diversity and identify genetically distinct clusters of genotypes. It is also effective in selecting important traits that contribute to the overall variation among genotypes. By employing PCA, genotypes can be naturally grouped, allowing for a precise examination of the differences between them. One of the key advantages of PCA is that it ensures each genotype is assigned to a single group, facilitating a clearer understanding of their characteristics (Khan *et al*., 2021). The 33 rice genotypes, including white and colored rice, using PCA to assess genetic divergence for quality traits. The analysis identified five principal components, explaining 78.26% of the total variability, with PC1 contributing the most (34.94%). In this study, a total of twenty-four genotypes were examined to assess thirteen grain quality traits. Principal Component Analysis (PCA) was employed to determine the relative contributions of these traits to the overall variability. Four components were identified with eigenvalues exceeding 1. These components, namely PC1, PC2, PC3, and PC4, accounted for 27.73%, 19.12%, 15.27%, and 10.12% of the variability, respectively (Sheela *et al.*, 2020).

In the present investigation, the aim was to study the nature and extent of genetic diversity among fifty-five rice genotypes for quality traits using PCA and cluster analysis. This analysis provides valuable insights into the patterns of variation and the relationships between different genotypes based on their quality traits. By applying PCA and cluster analysis, researchers can gain a comprehensive understanding of the genetic diversity present within the rice genotypes under investigation.

MATERIALS AND METHODS

The evaluation of all quality traits of the accessions was conducted as three replications at the laboratory of Karunya Institute of Technology and Sciences (Deemed University), Coimbatore in 2022-23. The experimental material consisted of fifty-five rice genotypes including Improved White Ponni, a popular high-yielding genotype known for its excellent cooking quality traits, which served as a reference or check variety. Table 1 provides detailed information about the genotypes examined in this investigation. The research focused on recording observations for five randomly selected quality characters, namely grain length, grain breadth, length-to-breadth ratio, kernel length, kernel breadth, kernel length after cooking, kernel breadth after cooking, linear elongation ratio, gelatinization temperature, and amylose content. To obtain the data for these quality traits, standard procedures were followed. Random grain samples were taken from each genotype of every replicate for accurate measurements. Manual measurements were performed on ten random samples of whole rice grains for length and breadth using a millimeter scale and graph.

Determination of Gelatinization Temperature (GT): GT was found using the alkali digestion test (Little *et al*., 1958). Six milled kernels that were whole and undamaged were selected and placed in a plastic bag. Ten milliliters of 1.7% KOH solution were added. The samples were arranged with enough space between the kernels to allow for spreading. The boxes were covered and incubated for 23 hours in a 30°C oven. The starchy endosperm was graded visually using a seven-point numerical spreading scale, which is a typical way of assessment for rice. According to the ASV score, rice grain GT can be separated into four groups: high (1-2), high-mid (3), intermediate (4-5), and low (6-7).

Determination of Amylose content (AC): The Cut Grain Dip (CGD) method was utilized to assess the gelatinization temperature of rice grains. This method involved horizontally cutting a single mature rice grain in half using a pair of scissors. The cut end of the grain was then dipped into different proportions of iodide (KI) and iodine (I) solutions. Specifically, the KI:I solutions were prepared by adding 6g of KI and maintaining an iodine content of 1g in 300 ml of water. In the experiment, five cut grains from each of the fifty-five genotypes were dipped into the solution. The duration of the entire process, from the start of dipping to the end, was measured using an electronic stopwatch (Agasimani *et al.*, 2013). The cut grain dip method (CGD) is a quick and accurate way to test for AC in rice grains. The currently accessible techniques call for expensive instruments like a spectrophotometer, auto-analyzer, and, more recently, a thermos cycler, which are frequently out of reach for developing nations due to the high costs of both their acquisition and maintenance. Since no expensive machinery or chemical reagents are needed, the technique is cost-effective. Among the various methods developed for detecting amylose content (AC) in rice, most of them were designed to be used with flour samples obtained from grains or tubers. However, the single kernel Cut Grain Dip (CGD) approach stands out as it eliminates the need for labor-intensive sample preparation. Moreover, this method is expected to provide more accurate results due to the reduced release of free fatty acids and phospholipids compared to techniques that utilize flour samples. Additionally, the CGD approach is time-efficient, as it can rapidly predict the AC of any germplasm or breeding lines within a few minutes (Agasimani *et al*., 2013).

Statistical analysis. The software used for conducting principal component analysis was STAR (Statistical Tool for Agricultural Research) version 2.0.1, which was developed by the International Rice Research Institute (IRRI). The recorded data were then subjected to D^2 statistics analysis, which was devised by Mahalanobis (1936). The analysis of D^2 was done with R-Package) (https://cran.r-project.org)

RESULTS AND DISCUSSION

Principal component analysis. The variability for all the major contributors was computed by using Principal Component Analysis (PCA). The Scree plot, as illustrated in Fig. 1, provided an understanding of the percentage of variation explained by the Eigen Values and Principal Components. It was evident from the graph that the highest variation was observed in PC1, PC2, PC3, and PC4, as reported by Gour *et al.* (2017) ; Sheela *et al.* (2020). This indicates that these principal components captured a significant amount of variability in the dataset. The Principal Component Analysis revealed a broad distribution of scores for the eight quality traits, indicating a wide range of diversity. By representing the variance structure through a few linear combinations of the variables, the analysis allowed for the assessment of the proportion of variability present in the dataset. In the current study, the analysis revealed that four principal components, which had eigenvalues greater than one, were considered reliable. These four components collectively accounted for 82 percent of the total variability observed in the dataset, as shown in Table 2. With an Eigenvalue of 2.91, PC 1 displayed 36% variability, and the graph for the following PCs rapidly declined. The first principal component, referred to as the "GT and length factors," showed a strong positive association with GT (0.26) and negative associations with GL (−0.50), KL (−0.47), and KLC (-0.45). The second principal component, known as the "breadth and AC factors," exhibited positive loadings for KB (0.57) and GB (0.55) and negative loadings for AC (−0.39), KLC (-0.26), and KBC (-0.20). The third principal component, referred to as the "KB and AC factor," had positive loadings for KB (0.51) and AC (0.53) and a negative loading for GT (−0.53). The fourth principal component, known as the "AC factor," displayed positive loadings for AC (0.50), GT (0.37), KL (0.33), and GL (0.19), and a negative loading for KBC (−0.66) (Table 3). The biplot diagram (Fig. 2), which represents the distribution and nature of diversity, illustrates the relationship between PC1 and PC2. The loading plot indicated that both variables and genotypes exhibited a high degree of variance (Ravi *et al*., 2018).

In this investigation, three components had Eigenvalues that were higher than 1.0. Eigenvalues for PCA 1, 2, and 3 were 2.91, 1.58, and 1.12, respectively. These three components had variances of 36, 20, and 14%, respectively. In combination, they accounted for 70% of the genotype variability included in the diversity analysis. A study assessed the variation among 40 rice genotypes using PCA analysis and showed that five principal components accounted for approximately 90% of the total variability. The traits included grain size, cooking characteristics, amylose content, and gelatinization temperature (Singla *et al.*, 2022). Sudeepthi *et al*. (2020); Salem *et al*. (2021) reported findings were comparable in nature. The biplot diagram (Fig. 2) and the comparison between genotypes and parameters revealed significant levels of variation. This study will be valuable in identifying the variables that contribute to the variability and selecting suitable genotypes for breeding and crop improvement in terms of grain quality attributes.

Cluster Analysis. In this study, the D2 analysis revealed the grouping of the fifty-five genotypes into nine clusters, as depicted in Fig. 3 and 4. Cluster I consisted of twenty genotypes, while Cluster II comprised thirteen genotypes. Additionally, Cluster III included three genotypes, while Cluster V, Cluster VII, and Cluster IX each contained two genotypes. Further, seven genotypes were grouped under Cluster IV, five under Cluster VI, and one under Cluster VIII respectively (Table 4). Based on the analysis, the genotypes were classified into clusters according to the percentage contribution of the traits and it was observed that the GT (38.84%) followed by AC (32.76%) were the major contributors to divergence in this population (Table 5). Thus, we could observe the presence of variability for GT and AC in the collected genotypes. Hence, these traits could be improved by selection in future breeding programs for cooking quality estimations.

The distance between clusters and within clusters are measured to assess the level of dissimilarity among genotypes. Among the clusters, a high inter-cluster distance was observed between Cluster VII and Cluster VIII (36.67), followed by Cluster V and Cluster IX (34.75), and Cluster V and Cluster VI (32.78). This indicates a higher degree of genetic diversity among Cluster VII and VIII genotypes. However, the clusters with the lowest inter-cluster distance were Cluster III and Cluster IV, indicating a higher similarity between the genotypes within those clusters (6.03). This exhibits genetic similarity and closeness prevailing among these clusters. From the diverse Clusters VII and VIII, we could utilize the genotypes for future hybridization programs to develop genotypes with high cooking quality traits (Table 6). Although the genotypes were distributed among different clusters, there were few variations within the clusters for the traits other than the major contributing traits. The observed variability in all recorded traits among the genotypes indicates the presence of diversity. Cluster II exhibited the highest intra-cluster distance (5.68), followed by Cluster I (4.96) and Cluster VI (4.22). This suggests a greater genetic diversity among the genotypes within Cluster II, Cluster I, and Cluster VI for all the traits. However, the intra-cluster distance was low and it comprised the genotype KRG49 in Cluster VIII. Thus, this genotype is observed to share genetic similarity for the cooking quality characterization. Gayin *et al*. (2015); Devi *et al.* (2016); Singla *et al*. (2022); Shi *et al*. (2022) also reported comparable results regarding the observed variation among different rice genotypes for cooking

quality traits. These studies support the findings of the present investigation, highlighting the presence of significant genetic diversity within rice genotypes in relation to cooking quality attributes.

Regarding the cluster means for all traits, cluster VIII performed superior with the highest mean for GL (9.56), GB (3.11), KL (7.00), KLC (10.67), GT (7.00), and AC (28.00). Additionally, it was observed that Cluster V displayed the highest average value for KB (3.10), while Cluster III exhibited the highest mean value for KBC (3.64) (Table 7). In comparison with the genotypes, the varieties KRG49, KRG55, and KRG56 recorded higher means for four major AC attributing traits with higher AC. Among all the genotypes, KRG49, KRG55, and KRG56 could be identified as promising genotypes for major AC-attributing traits. Therefore, they could be utilized in rice quality improvement programs for the development of genotypes with higher AC.

Table 1: List of rice genotypes used for evaluation.

Sr. No.	Accession Name	Sr. No.	Accession Name	Sr. No.	Accession Name
1.	KRG 1	19.	KRG 36	37.	IR 64 *sub1*
2.	KRG 2	20.	KRG 39	38.	FL 474
3.	KRG 3	21.	KRG 43	39.	CHOKKIRIYA 5
4.	KRG 4	22.	KRG 49	40.	KAVUNGIN POOTHALA
5.	KRG 5	23.	KRG 51	41.	KALYANIKUTTY
6.	KRG 6	24.	KRG 52	42.	MATTACHEMBAVE
7.	KRG 7	25.	KRG 53	43.	HARYANA BASMATHI
8.	KRG 8	26.	KRG 54	44.	VELLARIKAYAMA
9.	KRG 9	27.	KRG 55	45.	SWARNA *sub 1*
10.	KRG 10	28.	KRG 56	46.	FL 478
11.	KRG 23	29.	KRG 58	47.	SWARNA MALLI
12.	KRG 25	30.	KRG 59	48.	VELLATHOWAN
13.	KRG 28	31.	JADATHI	49.	MYSOORE MATTA
14.	KRG 31	32.	AMBALAVAYAL	50.	BHADRA
15.	KRG 32	33.	CHENTHONDI	51.	GOPIKA
16.	KRG 33	34.	GOURI	52.	ADUKKAN
17.	KRG 34	35.	SREYAS	53.	OTTANOORI
18.	KRG 35	36.	PONKARUVA	54.	NMS 2
				55.	WHITE PONNI (IMP)

Table 2: Eigenvalues, Percentage of variation, and Cumulative percentage for principal components.

Principal Components	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Standard deviation	1.71	1.26	1.06	0.95	0.75	0.65	0.55	0.43
Proportion of Variance	0.36	0.20	0.14	0.11	0.07	0.05	0.04	0.02
Cumulative Proportion	0.36	0.56	0.70	0.82	0.89	0.94	0.98	1.00
Eigen Values	2.91	1.58	1.12	0.91	0.56	0.43	0.30	0.19

Table 3: Eigenvectors of first four principal components to variation in rice varieties

Traits	PC1	PC2	PC3	PC4
GL	-0.50	0.03	-0.31	0.19
GB	-0.32	0.55	0.02	0.02
KL	-0.47	0.10	0.04	0.33
KB	-0.15	0.57	0.51	-0.17
KLC	-0.45	-0.26	-0.25	0.05
KBC	-0.34	-0.20	-0.11	-0.66
GT	0.26	0.33	-0.53	0.37
AC	-0.13	-0.39	0.53	0.50

Table 4: Distribution of fifty-five genotypes among different clusters based on D^2 analysis with the cooking quality traits.

Clusters	Number of genotypes	Name of genotypes
I	20	KRG 1, KRG 7, KRG 9, KRG 34, KRG 36, KRG 39, KRG 43, KRG 51, KRG 53, KRG 54, KRG 59, JADATHI, GOURI, KAVUNGINPOOTHALA, HARYANA BASMATHI, SWARNAMALLI, BHADRA, ADUKKAN, NMS 2 and WHITE PONNI (IMP)
II	13	KRG 2, KRG 8, KRG 10, KRG 35, KRG 58, SREYAS, IR 64 *sub1*, FL 474, VELLARIKAYAMA, FL 478, VELLATHOWAN, GOPIKA and OTTANOORI
III	3	KRG 3, KRG 33 and AMBALAVAYAL
IV	7	KRG 4, KRG 5, KRG 25, KRG 28, PONKARUVA, KALYANIKUTTY and MATTACHEMBAVE
V	2	KRG 6 and CHOKKIRIYA 5
VI		KRG 23, KRG 52, CHENTHONDI, SWARNA *sub1* and MYSOORE MATTA
VII	2	KRG 31 and KRG 32
VIII	1	KRG 49
IX	2	KRG 55 and KRG 56

Table 5: Contribution of characters toward genetic divergence.

Trait	% contribution
GL	5.46
GB	3.80
KL	3.73
KB	4.15
KLC	8.43
KBC	2.83
GT	38.84
AC	32.76

Table 6: Average inter and intra-cluster D^2 values among nine clusters.

	C1	C2	C3	C4	C5	C6	C7	C8	C9
C1	**4.96**	16.73	8.25	8.73	21.30	12.29	23.45	13.54	9.72
C2		**5.68**	14.71	9.82	27.74	13.27	25.99	8.73	21.02
C3			**0.89**	6.03	8.66	17.30	25.42	9.90	14.61
C4				**2.07**	12.03	12.83	16.63	12.94	16.59
C5					**2.41**	32.78	24.35	27.50	34.75
C6						**4.22**	19.41	15.11	12.12
C7							**1.48**	36.67	31.95
C8								**0.00**	7.90
C9									**1.82**

Table 7: Cluster mean values for the cooking quality traits.

Traits	I	II	III	IV	V	VI	VII	VIII	IX
GL	7.90	8.18	8.21	7.57	7.59	7.38	5.45	9.56	9.17
GB	2.39	2.66	2.99	2.51	2.68	2.07	1.84	3.11	2.17
KL	5.75	5.72	6.33	5.66	5.72	4.92	3.67	7.00	6.34
KB	1.99	2.07	2.46	2.12	3.10	1.72	1.62	1.67	1.50
KLC	9.26	8.96	9.33	8.69	8.40	8.80	7.07	10.67	10.13
KBC	3.56	3.47	3.64	2.93	3.20	3.31	2.54	3.20	3.74
GT	21.55	27.08	21.33	26.00	23.00	27.20	28.00	28.00	22.50
AC	6.35	2.69	5.33	4.57	5.50	4.20	5.00	7.00	7.00

GL- Grain length; GB- Grain breadth; KL- Kernel length; KB- Kernel breadth; KLC- Kernel length after cooking; KBC- Kernel breadth after cooking; AC- Amylose content; GT-Gelatinization temperature.

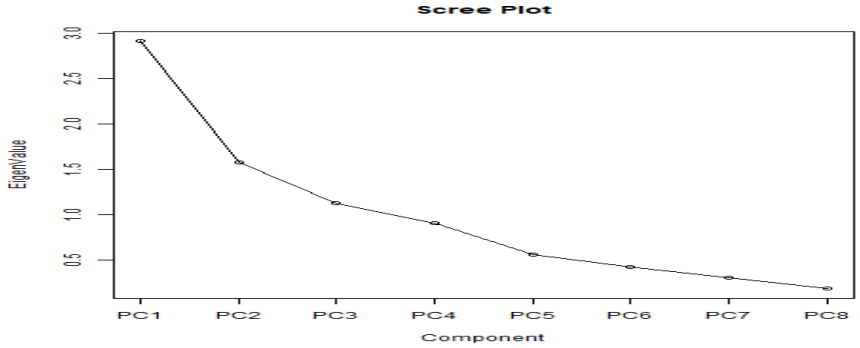

Fig. 1. Scree plot diagram constructed using eight principal components.

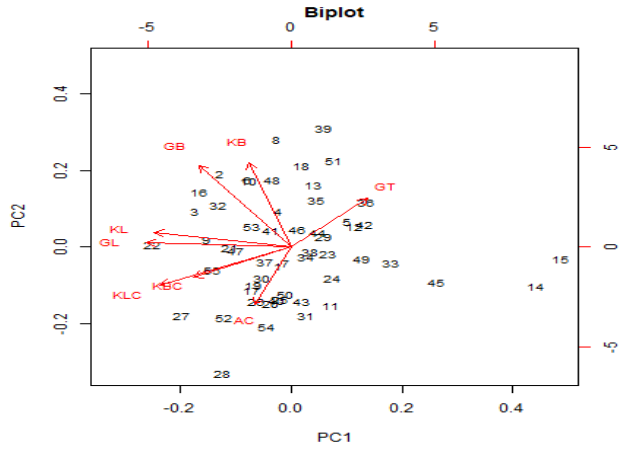

Fig. 2. Biplot diagram of PC1 and PC2.

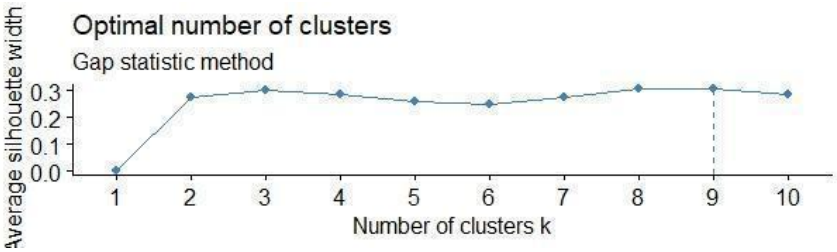

Fig. 3. Optimal number of cluster.

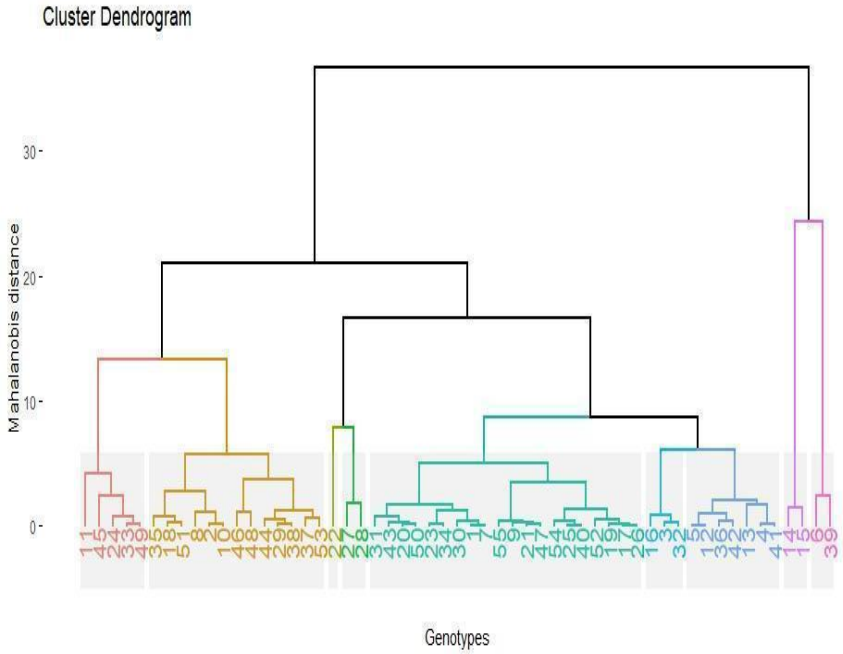

Fig. 4. Dendrogram showing the relationship among fifty-five rice (*Oryza sativa* L.) genotypes in nine clusters based on Mahalanobis' D^2.

CONCLUSIONS

In this study, fifty-five rice genotypes were analysed for their cooking quality traits and were grouped into nine clusters based on their percent contribution to the traits. GT and AC were found to be the major contributors to genetic diversity. High inter-cluster distances between some clusters indicated higher genetic diversity and potential for future breeding programs. Cluster VIII was found to be the best-performing cluster, and three genotypes (KRG49, KRG55, and KRG56) were identified as promising candidates for improving amylose content. Varieties with intermediate amylose contents are typically favored in Indian circumstances because they appear dry and fluffy and maintain their soft feel even after cooling. In conclusion, these findings have practical implications for rice breeding programs aimed at developing high-quality rice varieties.

FUTURE SCOPE

In the future, there could be a continued focus on developing rice varieties with intermediate amylose contents that meet the preferences of Indian consumers. This could involve further research on the genetic basis of amylose content and its correlation with other quality traits, as well as breeding programs to develop new varieties with improved cooking and eating qualities. Furthermore, it is important to emphasize the cultivation and utilization of high-quality rice varieties, both at local and global levels, to address the increasing need and promote sustainable agricultural approaches.

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Conflict of Interest. None.

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