Divergence study of rice (*Oryza sativa* L) genotypes for consumer-preferred quality traits.

ABSTRACT

Aim: To study the divergence of forty rice genotypes for consumer-preferred eleven quality traits.

Design, Place and Duration of the study: The rice seeds were harvested from randomly selected plants grown in alpha lattice design with three replications during *Kharif* season (start in June and end in October) 2018 at Agricultural research farm, Banaras Hindu University, Varanasi UP, India.

Methodology: The data were analysed using biometrical tools - Mahalanobis D^2 and Principal Component Analysis (PCA). The studied traits include, - grain length, grain breadth, kernel length, length to breadth ratio, kernel breadth, kernel length after cooking, kernel breadth after cooking, elongation ratio, elongation index, amylose content and alkali digestion value.

Results: Using Mahalanobis D^2, all forty genotypes were distributed into six clusters. The maximum inter-cluster distance was recorded between Cluster II to cluster V (5.76), followed by cluster V and cluster III (5.71), and cluster II and cluster VI (5.57) which indicated the existence of high genetic diversity among genotypes in these clusters and, therefore, crosses between the genotypes of these clusters could yield desirable transgressive isolates for desirable quality traits and the importance of the genotypes present in these clusters for exploiting heterosis for the desirable traits of these clusters. The PCA indicates that the five principal components (PC) captured almost 90% of variability present among the 40 rice genotypes.

Conclusion: The genotypes belonging to Cluster II to cluster V, followed by cluster V and cluster III, and cluster II and cluster VI, can be used for making crosses as they have higher mean values for quality traits and higher inter-cluster distance for greater diversity.

Keywords: Genetic divergence, rice, quality traits, principal component, Mahalanobis D^2

Introduction:

"Rice (*Oryza sativa* L.) is the most important crop in the world as it is present in the daily dishes of two-thirds of the world's population" (Sala *et al*., 2013). Developing the variety with better yield is one of the foremost vital goals of rice breeders. The adaptability of any variety becomes crucial if it has significant rice quality traits. The consumer market has recently seen an increase in quality sensitivity. According to a survey of 11 major rice-growing nations, grain quality is the second-most important breeding goal behind production (Juliano and Duff, 1991). Various rice products are always in demand by both, poor to rich people, and the requirement of quality accords with country, continent, state, and even person to person.

Grain dimension quality traits i.e. kernel length, kernel breath, length to breadth ratio, elongation ratio, kernel length after cooking, and kernel breadth after cooking have great importance in commercial rice production as it highly influences one final output as well as the consumer demand which directly contributes the economic profitability of the rice cultivator.

The Physicochemical properties of rice are determined based on amylose content, gel consistency and gelatinisation which depends on cooking and eating qualities and greatly influences the consumer's affinity (Oko A. O. *et al*., 2012, Rohilla *et al*., 2000). Amylose content determines the texture of cooked rice. The term "resistant starch" refers to amylose, composed of linearly connected glucose molecules and relatively difficult to digest. As a result, rice types with higher amylose starch content tend to have lower glycemic indexes. It has been discovered that there is positive correlation between the amylose content of milled rice c and hardness values of cooked rice, and negative correlation between the amylose conetn and stickiness values. The intermediate range of amylose content (21-25%) is desirable in the Indian population. The eating and cooking quality of rice grains can therefore be considered a significant critical quality component that must be prioritized in the country's future rice breeding programs to meet market needs worldwide.

"To achieve this objective, the breeder must identify diverse parents with high genetic variability for combining desirable characters. Therefore, the knowledge of genetic diversity is essential for undertaking any recombination breeding program. Multivariate statistical techniques that assess several variables on each individual under investigation are frequently used to genetic diversity. Principal component analysis (PCA) and cluster analysis have been demonstrated to be particularly helpful in selecting genotypes for breeding programs that satisfy a plant breeder's objectives" (Mohammadi and Prasanna, 2003)

This study was carried out to evaluate the variety of rice grain quality, various genotypes on physical and physicochemical qualities. The results of this study will be hugely beneficial for both, customers and future rice breeding operations.

Material and Methods:

The material of the present experiment consists of forty rice genotypes sown at Agricultural Research Farm, Banaras Hindu University, Varanasi, Uttar Pradesh, India during *Kharif* - 2018. The 25 days old seedlings were transplanted in Alpha Lattice design by adopting the spacing of 25 x 15 cm and recommended package of practices, and following all plant protection measures. At harvest maturity stage, with the moisture content of 12-14%, the observed seed quality trates were recorde. The samples from each replication were taken into consideration.

Observation recorded:

The following physical and physiochemical observations were recorded:

1. **Grain length (GL):** The length of 10 grains from its base to tip was recorded (mm).
2. **Grain breadth (GB):** Grain breadth was measured at the widest point of the 10 grains (mm)
3. **Kernel length (KL):** Length of 10 dehulled (after milling) grains from its base to tip was recorded (mm).

4. **Kernel breadth (KB):** Kernel breadth was measured at the widest point of the 10 kernels (mm).
5. **L/B ratio (LBBC):** Value was obtained by dividing kernel length by kernel breadth before cooking.
6. **Kernel length after cooking (KLAC):** Kernel length after cooking for 10 minutes in a water bath was recorded (mm).
7. **Kernel breadth after cooking (KBAC):** Kernel breadth after cooking for 10 minutes in a water bath was recorded (mm).
8. **Elongation ratio (ER):** Kernel length (mm) of 10 grains after cooking was divided by kernel length (mm) before cooking.
9. **Elongation Index (EI):** It was obtained by dividing by L/B ratio of the cooked kernel by L/B ratio of the raw kernel.
10. **Alkali digestion value / Gelatinization Temperature (ADV/GT)):** The spread of the 6 milled rice kernels in 1.7 percent KOH solution for 23 hours was rated as per the Standard Evaluation System for Rice (IRRI, 1996). Six milled kernels were placed in 10 ml of 1.7 percent KOH solution in a Petri dish and arranged in a manner not to touch each other and were allowed to stand for 23 hours at 30 ^{0}C to score spreading on a 1-7 scale.
11. **Amylose content (Amylcnt):** Amylose content (%) was calculated using the simplified procedure described by Juliano, 1971. In a 100 ml volumetric flask, 100 mg of ground milled rice sample (sieved with a mesh size of 60) was placed. One ml of 95% ethanol and 9 ml of 1N sodium hydroxide were added. After being heated in a boiling water bath to gelatinize the starch, the mixture was cooled for an hour before being thoroughly mixed with distilled water up to the volume. Starch solution (5 ml), 1 ml of 1N acetic acid, and 2 ml of iodine solution were added to a 100 ml volumetric flask and the volume was made with distilled water. After thoroughly shaking the contents, let them stand for 20 minutes and then absorbance was taken at 620 nm using a spectrometer.

Statistical Analysis

Mahalanobis D^2 values using Tocher's method was used to carry out analysis of forty rice genotypes for all 11 characters, using the mean values of all recorded attributes from all genotypes in all replications Rao (1952). According to Singh and Choudhary (1977), the D^2 values for each combination were arranged in a table in ascending order. Each character was ranked according to how much they contributed to the divergence

between the two entries. For every combination of entries, each character was ranked from rank "1" (character with a highest mean difference) to rank "p." (character with the lowest mean difference).

Result and Discussion:

Cluster Analysis:

The study of genetic diversity and the creation of core subsets for classifying accessions with comparable characteristics into a single homogenous category are two common uses for cluster analysis. By combining related units, clustering can also be utilized to communicate and summarize information about the relationships between various things. In the present experiment, “forty genotypes were grouped into six clusters using Tocher’s method” (Singh and Choudhary, 1977) based on Mahalanobis D^2 values. Clusters with their genotypes are presented in table 1. Cluster I had nine genotypes, Cluster II had five genotypes, Cluster III had thirteen, cluster IV had seven genotypes, cluster V had two genotypes, VI had 4 genotypes.

Range of average intra-cluster D^2 values was 0.77 to 1.32 (Table 2). Maximum intra cluster distance was shown by cluster V (1.32). While, cluster IV had minimum intra cluster value (0.77). Intra cluster values of 1.26, 1.07, 1.06, 0.92 were possessed by cluster I, cluster II, cluster III, cluster V and cluster VI respectively. The cluster V had high intra-cluster distance indicate wide diversity among genotypes present in cluster. The maximum inter-cluster distance was recorded between Cluster II to cluster V (5.76), followed by cluster V and cluster III (5.71), and cluster II and cluster VI (5.57) which indicated the existence of high genetic diversity among genotypes in these clusters, and therefore crosses between the genotypes of these clusters could yield desirable transgressive segregants for desirable quality traits. The minimum inter-cluster was recorded by cluster IV and cluster III (2.96), followed by cluster IV and cluster II (3.03), cluster II and cluster I (3.36), and cluster VI and cluster VII (3.39).

Cluster means of all the quality traits are presented in Table 3. The cluster mean showed different values for all the eleven traits studied. Cluster III had shown the highest mean value for GL (10.05), KL (7.41), LBBC (3.58), KLAC (9.52). The desirable intermediate range of Amycnt (21-25%) was reported by cluster I (23.13), cluster III (23.44) and cluster IV (22.71). The lower value is desirable for grain breadth, kernel breadth, kernel breadth after cooking and elongation ratio and has been found by cluster III (2.32), cluster III (2.09),

cluster IV (2.33) and cluster III (1.27) respectively. “Selection of parents from genetically homogeneous clusters should be avoided to maintain relatively broad genetic base. The maximum amount of desired heterosis will be manifested in cross combinations involving the parents belonging to most divergent clusters for quality characters and is important objective for a plant breeder” (Naik *et al*., 2021). By considering this, genotypes belonging to Cluster II to cluster V, followed by cluster V and cluster III, and cluster II and cluster VI, can be used for making crosses. Cluster III has shown desirable estimates for different quality traits. Naik *et al*., 2021 studied the experiment with combination of physical (KL, KB, KLAC, KBAC, EI, ER) and physiochemical quality traits (ADV and amylcnt) and suggested more divergent cluster for breeding program.. Simultaneous consideration of quality traits along with yield contributing traits for cluster analysis were done by Devi *et.al.* 2016, Asante *et al*., 2019 and Krishna Veni *et al*., 2013.

Table 1. Cluster composition of forty rice genotypes.

Cluster	**Number of Genotypes**	**Name of Genotypes**
1	9	BD105, Swarna, Karhani, URG-22, Sambhamahsuri, HUR3022, HUR105, URG-30, DRR Dhan 48
2	5	Dudhkander, URG-19, IR 85850-AC 157-1, IR 91143-AC 293-1, URG-24
3	13	IR 95133:1-B-16-14-GBS-P1-2-2, IR 95133:1-B-16-14-10-GBS-P5-2-3, IR 95133:1-B-16-14-GBS-P1-2-3, IR15M1633, IR 82475-110-2-2-1-2, IR15M1689, IR64, Sathi, IR 95133:1-16-14-10-GBS-P6-1-5, IR 91143-AC 290-1, IR 91143-AC 239-1, IR15M1546, IR 99642-57-1-1-1-B
4	7	M-48, M-399, URG-1, BRRIdhan 64 , HURZ-3, IR15M1537, BRRIdhan 72
5	2	Nagina-22, BG-102
6	4	IR 95133:1-B-16-14-10-GBS-P5-1-3, HURZ-1, DRR Dhan 45, MTU1010

Table 2. Inter-cluster and Intra-cluster (diagonal) D^2 of forty rice genotypes

	C1	**C2**	**C3**	**C4**	**C5**	**C6**
C1	1.26	3.36	4.11	2.95	4.22	4.40

C2		1.07	3.78	3.03	**5.76**	5.57
C3			1.06	2.96	5.71	3.59
C4				0.77	3.83	3.39
C5					1.32	3.84
C6						0.92

Table 3. Average performance of clusters for ten quality traits in forty rice genotypes.

Cluster	I	II	III	IV	V	VI
GL	8.16	8.54	10.05	8.67	7.63	9.75
GB	2.44	3.09	2.32	2.74	2.84	2.44
KL	5.8	5.81	7.41	6.3	5.59	7.17
KB	2.13	2.28	2.09	2.37	2.43	2.09
LBBC	2.87	2.57	3.58	2.68	2.32	3.45
KLAC	7.87	8.39	9.52	8.41	7.70	9.37
KBAC	2.37	2.75	2.33	2.57	2.85	2.28
EI	1.23	1.20	1.16	1.24	1.18	1.21
ER	1.37	1.43	1.27	1.34	1.38	1.31
ADV	2.23	3.53	4.06	4.18	3.17	3.56
Amycnt	23.13	25.11	23.44	22.71	19.77	20.33

(GL: grain length, GB: grain breadth, KL: Kernel length, KB: Kernel breadth, KLAC: Kernel length after cooking, KBAC: Kernel breadth after cooking. EI: Elongation Index, ER: Elongation ratio, ADV: Alkali digestion value, Amylcnt: Amylose content)

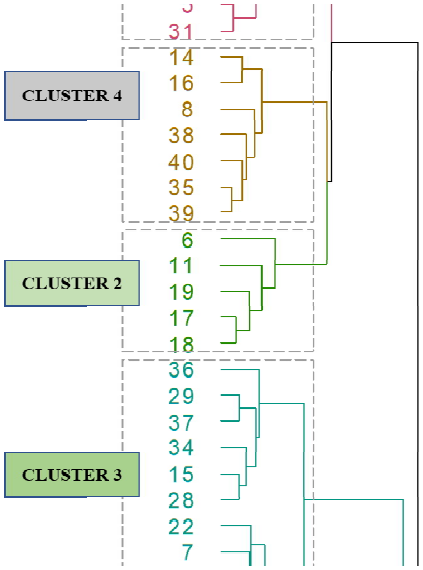

Fig.1 Dendrogram showing relationship among 40 rice (*Oryza sativa* L.) genotypes in six clusters based on Mahalanobis' D^2.

Principle component analysis

PCA was used to access the variation and relationships among the eleven studied characters. Almost 90% of variability present among the 40 rice genotypes was captured by the five Principal Components (PC) (Table 4). PC1 contributed for 44.98% of the total variation with LBBC (18.34) having the highest and Amylcnt (0.28) with the lowest loading. PC2 accounted 16.28% of the total variation with KB (22.73) having the highest and ER (0.10) with the lowest loading.

Furthermore,11.10% variation was explained by PC3 with EI (46.87) and KL (0.02), having highest and lowest loading, respectively. The remaining two PC, *i.e.,* PC4 and PC5 were captured 10.31% and 7.12% variability. The PC4 has highest loadings for amylcnt (25.89) while, the lowest loadings for KBAC (0.29). Similarly, in the case of PC5, amylcnt (44.61) had the highest loadings and LBBC has zero percent loading. The variables included in the first PC which explained 44.98% of total variance thus showed high importance for primary selection in under-studied rice breeding lines. Ashok *et al*., 2017, Naik *et al*., 2021 demonstration of the use of factor analysis for efficient selection criteria in rice breeding mprogram provides strong support for our findings.

Table 4. Eigenvalues, percent variance, cumulative variance percent and estimated compound matrix in principle components

Source	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	4.95	1.79	1.22	1.13	0.78	0.57
Variance percent	44.98	16.28	11.10	10.31	7.12	5.20
Cumulative variance percent	44.98	61.26	72.36	82.67	89.79	94.98
GL	13.82	9.79	0.87	2.08	2.49	4.77
GB	9.12	8.00	1.72	4.16	0.19	12.68
KL	16.43	6.07	0.02	4.02	0.05	0.01

KB	5.44	22.38	2.52	22.38	0.23	6.46
LBBC	18.34	1.87	1.08	1.87	0.00	1.38
KLAC	12.67	11.14	3.01	0.58	1.16	16.15
KBAC	7.75	17.85	18.00	0.29	0.68	5.37
ER	10.63	0.10	17.04	1.96	5.49	19.52
EI	3.14	0.16	46.87	20.98	0.09	4.60
Amycnt	0.28	11.39	7.37	25.89	44.61	6.32
ADV	2.38	11.25	1.50	15.78	45.01	22.73

(GL: grain length, GB: grain breadth, KL: Kernel length, KB: Kernel breadth, KLAC:Kernel length after cooking, KBAC: Kernel breadth after cooking, EI: Elongation Index, ER: Elongation ratio, ADV: Alkali digestion value, amylcnt: Amylose content)

The LBBC, GL, KL, KB, GB, KLAC, KBAC and ER had higher vector length indicating the presence of large variability, while remaining traits namely, ADV, Amyl.cnt and EI had smaller vector lengths indicating low variability (Figure 2). Vector LBBC and ER has formed an angle with any other trait. Traits, EI, GB, KBAC, KB and amylcnt these traits positively correlated with each other. While, vector of LBBC and ER, LBBC and EI, KL and ER were diverged and form a large angle (close to 180°), which indicating they were negative correlated. The vector of ER meets with EI, GB, KBAC, KB and amylcnt, also, LBBC meets vectors of KL, GL and KLAC almost at 90° indicating non-significant or low negative association with these traits. Similar experimental findings were reported by Akinol TF *et al*., 2019, and Naik *et al*., 2021,

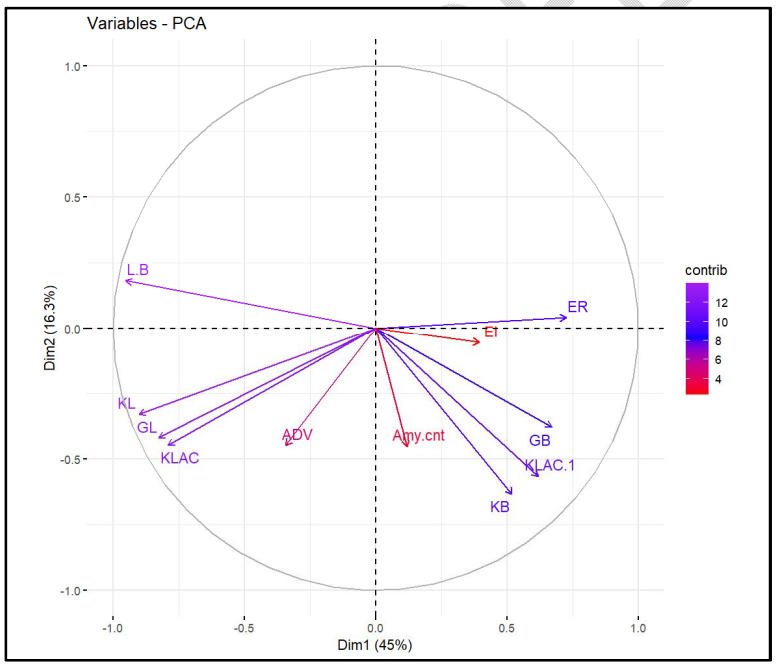

Figure 2. Principle component analysis biplot traits

(GL: grain length, GB: grain breadth, KL: Kernel length, KB: Kernel breadth, KLAC: Kernel length after cooking, KLAC-1: Kernel breadth after cooking. EI: Elongation Index, ER: Elongation ratio, ADV: Alkali digestion value, Amylcnt: Amylose content)

Conclusion:

Biometrical technique made it easy to measure the magnitude of variability among the breeding materials.

Selecting contrasting parent lines for hybridization as reflected from D^2 statistics and PCA , would ensure greater chances of obtaining high heterotic hybrids and broad spectrum of variability in segregating progenies. Therefore, the genotypes belonging to Cluster II to cluster V, followed by cluster V and cluster III, and cluster II and cluster VI, can be used for making crosses as they have higher mean value for quality traits and higher inter cluster distance for greater diversity. Hence, by considering the criteria of yield performance, these genotypes can be further utilized in breeding programs for consumer preferred traits. The result of PCA indicate that 90% of diversity is apprehended by first five PCs. Also, EI, GB, KBAC, KB and amylcnt these traits positively correlated with each other. While, the traits, LBBC and ER, LBBC and EI, KL and ER were diverged and form a large angle (close to 180°), which is indicating they were negatively correlated. Consideration of this estimates for formulating the desired results in plant breeding will be benefitial for further varietal improvement program.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all the authors. Authors ARK, SKS and JM designed the study and wrote the protocol. Authors, ARK, AS, SS and KM performed the statistical analysis and wrote the first draft of manuscript. Authors DKS, SVH, APB and PKM managed the further analyses and improvement in the manuscript and also, managed the literature searches. All authors read and approved the final manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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