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

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Abstract

The present investigation was conducted with forty rice genotype for consumer preferred eleven quality traits. The rice seeds were harvested from randomly selected plants which has grown in alpha lattice design with three replications during *Kharif* 2018. The 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. Using Mahalanobis D^2, all the 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 transgressivesegregants for desirable quality traits and also the importance of the genotypes present in these clusters for exploiting heterosis for the desirable traits of these clusters. The results of principal component analysis indicate that, almost 90% of variability present among the 40 rice genotypes was captured by the five principal component.

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

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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 important goal of rice breeder. The adaptability of any variety become crucial if it have significant rice quality traits. The consumer market has recently seen an increase in quality sensitivity. According to a survey of 11 main 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 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, kernel breath after cooking has a great importance in commercial rice production as it highly influences on the final output as well as the consumer demand which are directly contribute to the economic profitability of the rice cultivator.

Physicochemical properties of rice are determined based on amylose content, gel consistency and gelatinization temperature which is directly depend on cooking and eating qualities (Oko~~A. O.~~*et al*., 2012). In rice, eating and cooking qualities are mainly controlled by

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the physicochemical properties which greatly influence the consumer's affinity (Rohillaet al., 2000). Amylose content determine the texture of cooked rice. The term "resistant starch" refers to amylose, which is composed of linearly connected glucose molecules and is relatively difficult to digest. As a result, rice types with higher amylose starch content have a tendency to have lower glycemic indexes. It has been discovered that the amylose content of milled rice correlates favorably with cooked rice hardness values and unfavorably with stickiness values. The intermediate range of amylose content (21-25%) is desirable in Indian population concern. The eating and cooking quality of rice grains can therefore be considered as a significant critical quality component that must be prioritised in the country's future rice breeding programmes in order to meet market needs both locally and worldwide.

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

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In order to evaluate the variety of rice grain quality various genotypes on physical and physicochemical qualities, this study was carried out. 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 AgriculturalResearch 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 all the plant protection measures were followed. Observations regarding quality traits were recorded for seeds with a moisture content of 12-14% at the harvest maturity stage. The samples from each replication were taken into consideration.

Observation recorded:

The following physical and physiochemical observation were recorded:

1. **Grain length (GL):** 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).

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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 a period of 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 so that they do not 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 byJuliano, 1971. In a 100 ml volumetric flask, 100 mg of ground milled rice sample (sieved with a mesh size of 60) was placed. 1 ml of 95% ethanol and 9 ml of 1N sodium hydroxide were then added. After being heated in a boiling water bath to gelatinize the starch, the mixture was allowed to cool 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 up of distilled water. After thoroughly shaking the contents, let them stand for 20 minutes and then absorbance was taken at 620 nm using spectrometer.

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Statistical Analysis

Mahalanobis D^2 analysis was carried out for analysis of forty rice genotypes for all 11 characters, using the mean values of all recorded attributes from all genotypes in all replications. Tocher's optimization method, which Rao (1952) described, was used to group genotypes into different clusters. According to Singh and Choudhary, the

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D^{2}values for each combination were arranged in a table in ascending order (???? 1977). Each character was ranked according to how much they contributed to the divergence between two entries. For every combination of entries, each character was ranked from rank "1" (character with 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 summarise information about the relationships between various things.In present experiment forty genotypes were grouped into six clusters using Tocher's method (Singh and Choudhary, 1977) on the 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 whereas, VI had 4 genotypes.

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Range of average intra-cluster D^{2}values was 0.77 to 1.32 (Table2). 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 transgressivesegregants 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).

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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) andcluster III (1.27) respectively.Selection of parents from genetically homogeneous clusters should be avoided to maintain relatively broad genetic base. To get maximum amount of desire heterosis will be manifested in cross combinations involving the parents belonging to most divergent clusters for quality characters 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, therefore, can be used for making crosses. Considering the performance of cluster III, which 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 crossing programme.Simultaneous consideration of quality traits along with yield contributing traits for cluster analysis were done by Devi *et.al.* 2016,Asante*et al*., 2019 andKrishnaVeni *et al*., 2013.

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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)

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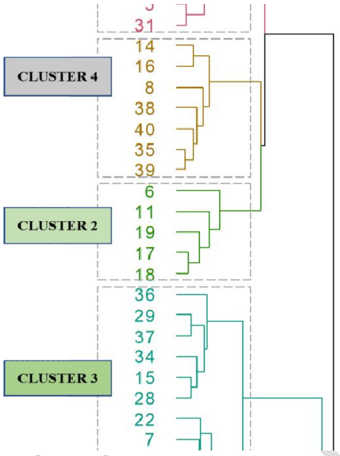

Fig.1 Dendrogram showing relationship among 40 rice (*Oryza sativa* L.) genotypes in six clusters based on Mahalanobis' D^2.

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 fivePCs (Table 4). PC1 contributed for 44.98% of the total variation with L/B (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 programme provides strong support for our findings.

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Table 4. Eigenvalues, per cent 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

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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
Amy.cnt	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 findingd were reported by Akinol TF *et al*., 2019, and Naik*et al*., 2021,

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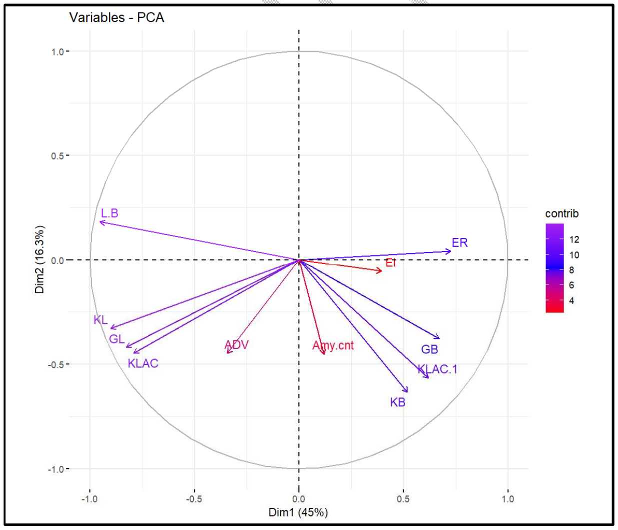

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Figure 2. Principle component analysis biplot traits

(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 has made ease to measure the magnitude of variability exist among the breeding materials. Choose of 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, therefore, can be used for making crosses as they have higher mean value for quality traits and higher intercluster 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 indicating they were negative correlated. Consideration of this estimates formulating the desired results for plant breeding will be benefit for further varietal improvement program.

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