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The Study of Genetic Divergence in Rice (Oryza Sativa L.) Genotypes with Grain Yield and Different Morpho-Physiological Traits using Mahalanobis' D^2 Analysis

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Abstract: The current study attempted to study genetic divergence in rice (Oryza sativa L.) genotypes with grain yield and different morpho-physiological traits among 18 rice varieties using Mahalanobis' D2 analysis. Keeping this in mind, the performance of the studied genotypes, as well as the association between various morpho-physiological and yield contributing traits among the genotypes, were evaluated in the field. The experiments were conducted from July to December 2018 at the Agronomy Field, Department of Agronomy and Agricultural Extension, Faculty of Agriculture, Rajshahi University. In terms of grain yield and other morpho-physiological traits, the genotypes differed significantly. The genotypes were classified into five clusters using Mahalonobis' D2 statistics and Tocher's method (Table 3). Cluster IV was the smallest of the clusters. Cluster l is the largest, with six genotypes. Clusters II and lV each contained three and two genotypes. The pattern of genotype distribution among various clusters reflected the significant genetic variability present in the genotypes, which may be due to adaptation of these genotypes to specific environmental conditions. Genotypes from different clusters are expected (inter-cluster). The intra (bold) and inter-cluster distances from D2 analysis (Table 4). Cluster I has the greatest intra cluster distance (0.956), while Cluster IV has the smallest (0.3583). The greatest inter-cluster distance is observed between cluster IV and (8.190), while the smallest inter-cluster distance is observed between cluster II and IV (2.924). In both vectors, the average inter-cluster distances were greater than the average intra-cluster distances, indicating the presence of greater genetic diversity among genotypes from different clusters than those from the same cluster. These findings indicated that these five traits contributed the most to the divergence among the eighteen rice genotypes. The results revealed that the clusters were remarkably distinct for the majority of the traits, indicating proper clustering. The most effective tiller, grain panicle, effective grain panicle, and days to maturity were found in Cluster V. The intra-cluster mean for grain yield was the lowest in this cluster. Cluster III had the lowest grain yield per acre. This was the cluster with the lowest initial grain yield. The intra-cluster mean for effective tiller and days to maturity was highest in this cluster. Cluster II had the smallest grain yield and the second smallest thousand grain weight. Cluster V had the shortest panicle length intra-cluster.

Keywords: Genetics divergence, clusters, Mahalonobis' D2 statistics, morpho-physiological traits, Tocher's method, grain panicle, genotypes.

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1. Introduction

One-third of the world's population relies on rice as a staple food. (RATNA RANI MAJUMDER, 2015). Rice (Oryza sativa L.) is the world's second most important cereal crop and a staple food for more than 60% of the global population, accounting for approximately 75% of their daily calorie and 55% of their protein intake. (M. Devi, 2019). One of the prerequisites for a successful breeding program in selecting genotypes with desirable characteristics is knowledge of the nature and magnitude of genetic variability. (Ayenew, 2020). Rice breeding and improved yield potential are viewed as important options for ensuring food security in Africa. (Sangaré, 2017).

Rice was introduced to Ethiopia in the 1970s, and the crop has been distributed quickly throughout the country. (Dejen Bekis, 2021). Rice is Africa's fastest growing food crop, and it is critical to food security and self-sufficiency in an increasing number of low-income food deficit countries. (Atsedemariyam Tewachew, 2019). The estimation of genetic diversity is an important factor in determining the source of genes for a specific trait among the available genotypes. The genetic diversity of the segregating population also aids in the selection of suitable types as parents and for commercial cultivation. (Kumar, 2017).

Salinity has a significant impact on rice production in southern Bangladesh. (Iffat Ara, 2019). Rice genetic resources are the primary source of rice breeding material, and they contribute significantly to global wealth creation and food security. (M.S. Ahmed, 2015). Drought is the most difficult abiotic stress on rice, affecting nearly a third of the total rice area in Asia and causing significant economic losses (Mounika Korada, 2021).

Crop variety diversity is critical for agricultural development in order to increase food production, alleviate poverty, and promote economic growth. (LAL, 2012). A clear understanding of genetic diversity and variety relationships is required for effective conservation and utilization of rice genetic resources (M. Mamunur Rahman, 2011).

People prefer certain types of rice for a variety of reasons. These characteristics included grain shape and appearance, aroma, texture, and the absence of chalk (Lalita Kumari, 2022). Genetic diversity is a powerful tool for determining genotype clustering patterns, which can then be used to select appropriate parental genotypes for hybridization in order to develop high yielding potential varieties (M.Z. Islam, 2019).

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2. MATERIALS AND METHODS

Under the following headings, a brief description of the experimental site and duration, experimental treatment, materials, sampling procedure and technologies used for production, data collection, recording, and statistical analysis are explained.

2.1. Description of the experimental site

2.1.1. Location and duration of the experiment

The experimental field was located on the western side of the University of Rajshahi's Department of Agronomy and Agricultural Extension. The experimental field was situated at 24°22362 N latitude and 88°38272 E longitudes, at an elevation of 20m above sea level to High Ganges River Flood Plain AEZ-11.

2.1.2. Soil and Climate

The land was medium in elevation, with silty loam soil. The soil's pH level was 6.75.

During the kharif season, the climate was characterized by relatively high temperatures and rainfall, while the Rabi season was characterized by low temperatures and little rain fall.

2.2. Experimental material

In the current study, eighteen rice varieties were used. The varieties are listed in the table below 1.

TABLE 1: the list of the genotypes used in the experiment with their sources

SL. NO	VARIETIES	SOURCES
1.	BR11	BRR1
2.	DRR46	BRR1
3.	BRR1 dhan79	BRR1
4.	CR DHAN 405	BRR1
5.	BRR1 dhan 51	BRR1
6.	BRR1 dhan78	BRR1

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7.	CSR 43	BRR1
8.	BRR1 dhan66	BRR1
9.	DRR 42	BRR1
10.	BRR1 dhan52	BRR1
11.	DRR 39	BRR1
12.	BRR1 dhan56	BRR1
13.	CR dhan801	BRR1
14.	BINA 11	BRR1
15.	BINA 7	BRR1
16.	BRR1 dhan57	BRR1
17.	DRR 44	BRR1
18.	BRR1 dhan71	BRR1

BRR1= Bangladesh Rice Research Institute

2.3. Experimental design

The experiment was run with three replications using a Randomized Complete Block Design (RCBD). Each plot was 2.5 m by 2 m in size (5 m2.). There were a total of 18 x 3 = 54 plots.

2.4. Recording of data

Data on each individual plant was collected using ten randomly selected plants from each of the TEN rows of each plot in each block. Days to 50% flowering and days to physiological maturity were two of the field-observed studied characters. The remaining characters were recorded after harvesting.

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The data were recorded on the following traits:

a) Days to 50% flowering: Days from sowing to flowering were recorded when 50% of the plants in each plot flowered.

b) Days to maturity: Days on plot were recorded from sowing to the time when approximately 90% of the plant was ready for harvesting.

c) Plant height (cm): The length of five main culms of five randomly selected plants was measured from the ground to the tip of its panicle, and an average was taken.

d) Number of total tillers m^{-2}: The number of tillers (effective or ineffective) on each hill was counted, and the average of the five hills was calculated.

e) Effective tiller number m^{-2}: The average number of panicle bearing tillers was counted from each of the five randomly selected plants in the sample.

f) Number of non-effective tillers m^{-2}: The panicles, which had no grain, were regarded as non-effective tillers.

g) Panicle length (cm): The distance (cm) from the last node of the rachis to the tip of the main panicle was randomly selected from each plant and averaged.

h) Grain panicle $^{-1}$: the number of grains per panicle

i) Number of filled grain panicle^{-1}: The spikelets with kernels were considered to be filled grain and were counted from one randomly selected panicle from each plant, with the average taken.

j) Number of unfilled grain panicle^{-1}: The spikelet without the kernel was considered unfilled grain, and it was counted from one randomly selected panicle from each plant, with the average taken.

k) Thousand grain weight (g): Thousand grain weight of each plot was taken proper cleaning and sun drying.

l) Grain Yield per m^{-2} (kg): Total gain weight of each plot was taken after proper cleaning and drying for at least two days.

m) Straw weight. Straw weight of each plot was taken.

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2.5. Multivariate analysis (D^2 statistics)

GENSTAT 5.13 and Microsoft Excel 2010 software were used for multivariate analysis, which included four techniques: principal component analysis, principal coordinate analysis, cluster analysis, and canonical vector analysis.

2.5.1. Principal component analysis (PCA)

The PCA technique was used to investigate the relationships between nine quantitative traits. The correlation matrix (obtained from the sum of squares and products matrix of the traits) and genotype scores were used to compute the principal component (obtained from the first component and the succeeding component with latent roots greater than unity).

The latent roots are referred to as 'Eigen values. The PCA technique was used to investigate the relationships between nine quantitative traits. The correlation matrix (obtained from the sum of squares and products matrix of the traits) and genotype scores were used to compute the principal component (obtained from the first component and the succeeding component with latent roots greater than unity).

The latent roots are referred to as 'Eigen values. ‘Principal coordinate analysis (PCO).PCO was used to calculate the inter genotype distance and provide the shortest distance between each pair of N points using a similarity matrix with all P dimensions (Rahman, Genetic diversity of muskmelon using multivariate technique., 2016)

2.5.2. Cluster analysis (CA)

D2 statistics (developed by Mahalanobis in 1928 and 1936 and expanded by Rao in 1952) were used to perform cluster analysis, which divides genotypes into more or less homogeneous groups based on the data set. D2 is the sum of the squares of the differences in any two populations for each uncorrelated variable (obtained by transforming correlated variables through Pivotal condensation method). Non-hierarchical and hierarchical classifications were used for clustering. D2 statistics are defined as –

$D^2_x = \sum_{k=1}^{P} (\lambda^{ij}) d_i d_j$

Where, X = Number of metric in point

P = Number of populations or genotypes

λ^{ij} = The matrix reciprocal to the common dispersion matrix

$d_i d_j$ = The differences between the mean values of the two genotypes for the i^{th} and j^{th} traits respectively

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In simpler form, D^2 statistic is defined by the following formula-

$D^2 = \sum_{i=1\ to\ x}^{p} d_i^2 = \sum_{i=1\ to\ x}^{p} (y_i^j - y_j^k) \qquad (j \neq k)$

Where, y = Uncorrelated variable which varies from i=1 to X

X = Number of traits.

Superscripts j and k to y = a pair of any two genotypes

Cluster analysis was carried out using the computer software GENSTAT 5.13, which searched for optimal values of the chosen criterion. The algorithm classified the genotypes into the required number of groups and then transferred genotypes from one group to another as long as this transfer improved the value of the criterion.

When no more transfers could be found to improve the criterion, the algorithm moved on to a second stage, which looked at the effect of swooping two genotypes from different groups, and so on.

2.5.3. Canonical vector analysis (CVA)

CVA is a type of multivariate analysis in which canonical vectors and roots represent different axes of differentiation and the amount of variation accounted for by each of these axes are derived. Canonical vector analysis identifies linear combinations of original variability that maximize the ratio of between-group to within-group variation, resulting in functions of the original variables that can be used to differentiate between groups. In this analysis, a series of orthogonal transformations is used to maximize the ratio of among group variation to within group variation.

2.5.4. Computation of average intra-cluster distance

The average intra-cluster distance for each cluster was calculated by averaging all possible D2 values within a cluster's members as determined by PCO. The formula used to calculate the average intra-cluster distance was as follows: Intra-cluster distance = $\frac{\sum D^2}{n}$

Where,

$\sum D^2$ = The sum of distances between all possible combinations (n) of the genotypes included in a cluster.

n = Number of all possible combination

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3. Results and Discussion

The experimental data were collected and analyzed to Study of genetic divergence in rice (Oryza sativa L.) genotypes with grain yield and different morpho-physiological traits using Mahalanobis' D2 analysis

3.1. Multivariate analysis (D^2 statistics)

3.1.1. Principle component analysis

The eigen values of the principal component analysis are frequently used to determine how many factors to keep. The first six axes accounted for 99.09% of total variation among the nine traits describing 18 rice genotypes, while the first two accounted for 82.87% (Table 2), implying that these components were highly responsible for genetic divergence in the current materials. The importance of the largest contributor to total variation is reflected in principal component analysis (G. Rakesh, 2015).

Table2: Eigen values (latent roots) and percent contribution of traits towards divergence in 18 rice genotypes

Principal component axes	Eigen values	Contribution (%)	Cumulative contribution (%)
ETM^2	2.1866	65.20	65.20
PL	0.5926	17.67	82.87
GP^{-1}	0.2761	8.23	91.10
EGP^{-1}	0.1192	3.55	94.65
TGW	0.0955	2.85	97.5
DF	0.0533	1.59	99.09
DM	0.0180	0.54	99.63
PH	0.0108	0.32	99.95
GY	0.0018	0.05	100

ETM= Effective tiller M^2
PL= Panicle Length
GP = Grain $Panicle^{-1}$
EGP = Effective Grain $Panicle^{-1}$
TGW = Thousand Grain Weight
DF = Days to Flowering
DM = Days to Maturity
PH = Plant Height
GY = Grain Yield

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3.1.2. Non-hierarchical clustering

In terms of grain yield and other morpho-physiological traits, the genotypes differed significantly. The genotypes were classified into five clusters using Mahalonobis' D2 statistics and Tocher's method (Table 3). Cluster IV was the smallest of the clusters. The genotypes differed significantly in terms of grain yield and other morpho-physiological traits. Using Mahalonobis' D2 statistics and Tocher's method, the genotypes were classified into five clusters (Table 3). Cluster IV was the tiniest of the bunch.

Table 3: Distribution of 18 rice genotypes among the five clusters based on mahalonobis' D^2 value

Cluster	Number of genotypes	% of total entries	Genotypes
I.	6	33.33	DR 46, BRRI dhan 79, BRRI dhan 66, BRRI dhan 52, CR dhan 801, BRRI dhan 57
II.	3	16.66	BRRI dhan 78,BRRI dhan 56, BRRI dhan 71
III.	3	16.66	CR dhan 405,CSR 43, DRR 42
IV.	2	11.11	BR 11, DRR 39
V.	4	22.22	BRRI dhan 51, BINA dhan 11, BINA dhan 7, DRR 44

3.1.3. Canonical vector analysis

Genotypes from different clusters are expected (inter-cluster). The intra (bold) and inter-cluster distances from D2analysis (Table 4). Cluster I has the greatest intra cluster distance (0.956), while Cluster IV has the smallest (0.3583). The greatest inter-cluster distance is observed between cluster IV and (8.190), while the smallest inter-cluster distance is observed between cluster II and IV (2.924). The average inter-cluster distances were greater than the average intra-cluster distances, indicating that there was more genetic diversity among genotypes from different clusters than among genotypes from the same cluster. Similar findings were made by (Ramesh chandra, 2007)in rice.

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Table 4: Average intra (bold) and inter cluster distances (D^2) for 18 rice genotypes

	I.	II.	III.	IV.	V.
I.	**0.956**				
II.	6.157	**0.638**			
III.	6.504	4.772	**0.664**		
IV.	5.690	2.924	7.233	**0.3583**	
V.	3.504	7.394	5.137	8.190	**0.831**

3.1.4. Contribution of traits towards divergence

The trait that contributes the most to the divergence is emphasized when deciding on the cluster for further selection and when selecting parents for hybridization (Rahman, GENETIC DIVERSITY IN SPRING WHEAT GENOTYPES UNDER DROUGHT STRESS IN BANGLADESH., 2013). Table 5 shows the contributions of traits to divergence obtained from Canonical Variate Analysis (CVA). Negative values for traits in any of the vectors indicated that the trait contributed less to divergence, whereas positive values indicated a greater contribution.

Panicle length, days to flowering, and grain yield all had negative values in both vectors, indicating that these three traits contributed the least to total divergence. The important traits responsible for genetic divergence in vector I (the major axis of differentiation) were 1000-grains weight. Days to maturity, thousand grain weight, effective grain panicle-1, grain panicle, and effective tiller, on the other hand, had positive values in vector II (the second axis of differentiation). These findings indicated that these five traits contributed the most to the divergence among the eighteen rice genotypes. The result suggests that the divergence in current materials due to these five characteristics provides a good opportunity for improvement (Rahman, GENETIC DIVERSITY IN SPRING WHEAT GENOTYPES UNDER DROUGHT STRESS IN BANGLADESH., 2013)investigated genetic diversity and discovered that days to maturity and 1000 grain weight contributed to total divergence.

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Table 5: Latent vectors for nine traits genotypes.

Traits	Vector I	Vector II
ETM	0.1357	0.0310
PL	-0.7677	-0.1795
GP	0.1086	0.0797
EGP	0.0202	0.0558
TGW	1.4603	0.2674
DF	-0.4016	-0.2622
DM	0.4743	0.3094
PH	0.0269	-0.0280
GY	-1.6096	-0.0120

ETM= Effective tiller M^2
PL= Panicle Length
GP = Grain Panicle
EGP = Effective Grain Panicle
TGW = Thousand Grain Weight
DF = Days to Flowering
DM = Days to Maturity
PH = Plant Height
GY = Grain Yield

3.1.5. Intra-cluster mean

The results revealed that the clusters were remarkably distinct for the majority of the traits, indicating proper clustering. The most effective tiller, grain panicle, effective grain panicle, and days to maturity were found in Cluster V. The intra-cluster mean for grain yield was the lowest in this cluster. Cluster III had the lowest grain yield per acre. This was the cluster with the lowest initial grain yield. The intra-cluster mean for effective tiller and days to maturity was highest in this cluster. Cluster II had the smallest grain yield and the second smallest thousand grain weight. Cluster V had the shortest panicle length intra-cluster.

Table 6: Cluster means for the 9 traits of 18 rice genotypes

Traits	Cluster mean				
	I.	II.	III.	IV.	V.
ETM	226.67	187.89	180.55	192.17	236.59
PL	24.98	25.07	24.56	28.12	24.12
GP^{-1}	145.74	149.12	94.92	178.94	116.08
EGP^{-1}	129.38	113.25	84.22	149.11	100.39
TGW	26.13	24.40	29.40	26.10	27.90
DF	67.17	63.78	61.33	76.00	67.00
DM	99.83	96.67	97.11	103.00	98.75

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PH	100.33	98.55	87.24	106.64	91.73
GY	6.36	4.92	3.68	6.77	5.38

ETM= Effective tiller M^2
PL= Panicle Length
GP = Grain Panicle
EGP = Effective Grain Panicle
TGW = Thousand Grain Weight
DF = Days to Flowering
DM = Days to Maturity
PH = Plant Height
GY = Grain Yield

4. Conclusion

In terms of grain yield and other morpho-physiological traits, the genotypes differed significantly. The genotypes were classified into five clusters using Mahalonobis' D2 statistics and Tocher's method (Table 3).

Cluster IV was the smallest of the clusters. Cluster l is the largest, with six genotypes. Clusters II and lV each contained three and two genotypes. The pattern of genotype distribution among various clusters reflected the significant genetic variability present in the genotypes, which may be due to adaptation of these genotypes to specific environmental conditions.

Genotypes from different clusters are expected (inter-cluster). The intra (bold) and inter-cluster distances from D2analysis (Table 4). Cluster I has the greatest intra cluster distance (0.956), while Cluster IV has the smallest (0.3583).

The greatest inter-cluster distance is observed between cluster IV and (8.190), while the smallest inter-cluster distance is observed between cluster II and IV (2.924). The average inter-cluster distances were greater than the average intra-cluster distances, indicating that there was more genetic diversity among genotypes from different clusters than among genotypes from the same cluster.

Within both vectors these findings indicated that these five traits contributed the most to the divergence among the eighteen rice genotypes. The results revealed that the clusters were remarkably distinct for the majority of the traits, indicating proper clustering. The most effective tiller, grain panicle, effective grain panicle, and days to maturity were found in Cluster V. The intra-cluster mean for grain yield was the lowest in this cluster.

Cluster III had the lowest grain yield per acre. This was the cluster with the lowest initial grain yield. The intra-cluster mean for effective tiller and days to maturity was highest in this cluster. Cluster II had the smallest grain yield and the second smallest thousand grain weight. Cluster V had the shortest panicle length intra-cluster.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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