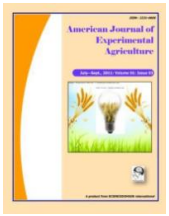

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Partitioning of Rice (*Oryza sativa* L.) Genotypes Based on Morphometric Diversity

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Authors' contributions

This work was carried out in collaboration between all authors. Author AH wrote the protocol, designed the study, executed the field experiment, performed the statistical analyses, and wrote the first draft of the manuscript. Authors SNB and AHKR revised the protocol, supervised the experiment, and helped to manage the analyses of the study. Author LH managed the literature searches and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The Objectives of this study were to partition the rice genotypes into different clusters, identification of heterotic groups, and most important traits contributing to divergence to utilize them for specific objective-oriented breeding programs in future.
Study Design: The experiment was set out in randomized complete block design with three replications.
Place and Duration of Study: The experiment was carried out at experimental farm of Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Bangladesh during *Aus* (Kharif) season of 2012.
Methodology: The diversity among sixty rice genotypes and contribution of thirteen traits towards diversity were analyzed using Mahalanobis's D^2 statistics and principal component analysis.
Results: Analysis of variance showed highly significant variation among the genotypes for all the traits. Cluster analysis based on D^2 values exhibited seven distinct clusters. The highest intra-

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cluster distance (21.95) was observed in cluster II whereas that was lowest (7.62) for cluster VI. Maximum inter-cluster distance was observed between cluster III & VII (46.75) followed by cluster II & VII (42.91), cluster V & VII (38.48), and cluster III & VI (30.87). In all cases inter-cluster distance was higher than the intra-cluster distance suggesting wider diversity among the genotypes. All the short duration genotypes with high yield, high tiller number per hill and more filled grain per panicle were grouped in cluster VII whereas tall, long duration genotypes with low yield, wider flag leaf area, long panicle and more unfilled grain per panicle were grouped in cluster II. Cluster III composed of long duration & moderate yielded genotypes, but cluster V composed of genotypes with long duration and high yield. First three principal components explained about 81% of the total variation. Results of PCA suggested that traits such as number of filled grains per panicle, number of unfilled grains per panicle, flag leaf area, plant height and days to maturity were the principal discriminatory characteristics.

Conclusion: The studied rice genotypes showed considerable divergence for most of the traits. These results can now be used by the breeders to develop rice varieties having desirable characteristics and new breeding strategies for rice improvement.

Keywords: Rice; D^2 statistics; cluster analysis; principal component analysis.

1. INTRODUCTION

Rice (*Oryza sativa* L.), belonging to the family gramineae, is the main source of nutrition for 50% of world population [1] and supplies staple food for one third of the global population [2]. Among the most cultivated cereals in the world, it ranks second next to wheat [3]. Bangladesh being an agro-based and over populated country where peoples mainly depend on rice for their livelihood. The demand for rice is increasing day by day as nearly three million people being added each year to the total population [4]. Therefore, to meet the food demand of the growing population and to achieve food security, the present production level need to be increased, which is possible through heterosis breeding and other innovative breeding approaches.

Magnitude of heterosis depends on the choice of appropriate parental lines [5]. Chances of attaining higher heterosis and wide range of variability in segregating generations can be increased by the inclusion of more diverse parents in hybridization program [6,7]. However, in some cases crossing between moderately diverse parents also have displayed maximum heterosis [8,9]. Precise assessment of the levels and patterns of genetic diversity facilitates the exploration of genetic variability in germplasm [10], identification of diverse parental combinations to create segregating progenies with maximum genetic variability for further selection [11] and introgression of desirable genes from wild to adapted high yielding germplasm [12]. Thus information about genetic diversity simplifies the selection of parental genotypes from random populations before attempting crosses and hence saving time and resources [13] and also helps to make decision on management procedures of the germplasm.

With the advancement of multivariate statistics, the assessment of degree of divergence among biological populations and relative contribution of different traits to total divergence have now become possible. In the bid to generate genotypes having desirable attributes, the breeder would like to choose genetically distant parents for hybridization. The process of classification of germplasm using Mahalanobis's D^2 statistics can help to practical reach [14]. In this regard, different methods were used for classification [15], but a procedure combining principal component and D^2 analysis was found expedient [16]. From this point of view, the present investigation was done using sixty rice genotypes to assess the genetic diversity for determining the heterotic groups and the relative contribution of different traits towards the total divergence for further management and utilization of those lines in hybridization program using Mahalanobis's D^2 statistics and Principal Component Analysis (PCA).

2. MATERIALS AND METHODS

A field experiment was conducted to study the extent of genetic diversity in rice for growth parameters and yield contributing traits. Sixty genotypes of rice were evaluated under field condition during the period from March to December, 2012, at the experimental farm of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh

in Randomized Complete Block Design (RCBD) with three replications. Twenty six days old seedlings were transplanted in 2 m^2 plot with inter-row and intra-row spacing of 20 × 15 cm. All recommended practices were followed to maintain uniformity in plant population and to ensure good crop growth. Five representative plants for each treatment in each replication were randomly selected to record days to panicle initiation, plant height (cm), flag leaf area (cm^2), number of effective tillers per hill, panicle length (cm), number of filled grains per panicle and number of unfilled grains per panicle. Traits related to growth duration such as days to 50% flowering, days to flowering completion, days to milking stage, days to dough stage, days to maturity and yield per plot were computed on plot basis. For calculation of days to panicle initiation five main (primary) tillers from five randomly selected hills of each plot for each replication were collected at maximum tillering stage. The collected tillers were then exposed and the growing zone of first node was examined carefully under stereomicroscope and the length of the newly developed panicle was measured using a millimeter graph paper. Then the stage of panicle development was determined by the guidelines described in Manual for Hybrid Rice Seed Production [17]. The days to panicle initiation was recorded as days from sowing to first stage of panicle development using the following formula:

$$DPI = T_n - T_1$$

Here,

DPI = Days to panicle initiation
T_n = Days from sowing to observed stage of panicle development
T_1 = Time gap between 1st and observed stage of panicle development according to manual

For calculating flag leaf area, twenty flag leaves were clipped from randomly selected plants from different plots and their areas were measured by leaf area meter. The length and breadth of those flag leaves were measured by using meter scale. Then K-value was measured by using the following formula:

K-value = Leaf area measured by leaf area meter / (leaf length x leaf breadth)

Finally the flag leaf area of five tillers from five randomly selected hills of each plot was measured by using the following formula and average was taken

Flag leaf area = Length× Breadth× K-value

To test the significance of variation among the genotypes for each trait, the analysis of variance was performed using the plant breeding statistical program PLABSTAT, version 2N [18]. The Mahalanobis's generalized distance (D^2) and principal component analysis were done using statistical software R, version 3.1.1 [19]. The clustering of the lines was done following the Tocher's method [20].

3. RESULTS AND DISCUSSION

The analysis of variance (Table 1) revealed that the genotypes differed significantly (*P*=.01) for all the traits studied, indicating the presence of remarkable genetic variability among them. This implied that it would be sensible to classify the lines on the basis of degree of divergence considering all the thirteen studied traits.

Depending upon the range of diversity, 60 genotypes were grouped into seven clusters (Table 2). The distribution pattern revealed maximum number of genotypes (16 genotypes) in cluster I whilst cluster V included minimum number of genotypes (3 genotypes). Cluster II, III, IV, VI and VII comprised of 4, 10, 9, 11 and 7 genotypes, respectively. The less number of genotypes in the clusters V and II was probably due to small number of traits as well as duplication effect of the traits included in the study. In this connection, in case of hierarchical and dynamic clustering, the frequency of genotypes in a given cluster is increased by increasing the number of traits under study [21]. In all the cases the inter-cluster distances were greater than the intra-cluster distances (Table 3) suggesting wider diversity among the genotypes of the distant groups. The similar results regarding inter and intra-cluster distances have been described in different crops by various researchers [22-26].

The intra-cluster divergence was maximum in cluster II (21.95) and minimum in cluster VI (7.62), indicating that the genotypes in cluster II were more heterogeneous than those in cluster VI. The intra-cluster distances were comparatively high for all the seven clusters with the range of 7.62 to 21.95, indicating heterogeneous nature of the genotypes within each cluster. In previous experiment, homogeneous nature of the genotypes within the

clusters for irrigated traditional and modern rice germplasm was reported [27], which is contrarily to the present findings. The possible reason could be variation in number of genotypes and traits studied in present experiment with that of the previous one.

Regarding the inter-cluster distance, cluster III showed maximum distance from cluster VII (46.75) followed by the distance between cluster II and VII (42.91), cluster V and VII (38.48) and cluster III and VI (30.87) which reflected wider diversity among these clusters. Minimum distance was found between the genotypes of the cluster I and VI (12.40) followed by the genetic distance between clusters IV and VI (13.56) (Table 3).

Table 1. Analysis of variance (ANOVA) for 13 traits of 60 rice genotypes

Traits	Sources of variation (Mean of square)		
	Replication (d.f.= 2)	Genotype (d.f.=59)	Error (d.f.=118)
DPI	38.046	249.725**	2.411
DF	1.250	288.547**	0.900
DFC	1.572	292.202**	0.730
DMI	2.067	288.766**	1.010
DD	11.872	290.943**	1.629
FLA	371.772	355.217**	24.094
DM	32.706	332.058**	3.299
PH	156.606	408.012**	14.069
ETN	0.617	5.702**	2.289
PL	3.517	12.649**	1.810
FG	385.089	1355.493**	49.761
UFG	192.772	356.943**	17.558
YPP	0.012	0.202**	0.003

DPI: days to panicle initiation, DF: days to 50% flowering, DFC: days to flowering completion, DMI: days to milking stage, DD: days to dough stage, FLA: flag leaf area (cm^2), DM: days to maturity, PH: plant height (cm), ETN: number of effective tillers per hill, PL: panicle length (cm), FG: number of filled grains per panicle, UFG: number of unfilled grains per panicle, YPP: yield per plot (kg), d.f.: degrees of freedom

*** Significant at 1% level*

Table 2. Clustering of 60 rice genotypes based on D^2 statistics

Cluster no.	No. of genotypes	Name of genotypes
I	16	G1, G9, G10,G15, G20, G21, G22, G35, G36,G39,G41, G45, G46, G52, G53, G59
II	4	G2, G3, G5, G19
III	10	G4,G7,G8, G11,G12,G14,G43,G44,G58, G60
IV	9	G6, G17, G26, G30, G31, G40, G47, G51, G57
V	3	G13, G16, G32
VI	11	G18, G23, G24, G25, G27, G29, G33, G37, G38, G42,G48
VII	7	G28, G34, G49, G50, G54, G55, G56

Table 3. Intra and inter cluster average distances among 60 rice genotypes

Cluster	I	II	III	IV	V	VI	VII
I	**11.81**	27.01	25.92	13.81	20.21	12.40	24.77
II		**21.95**	18.81	19.77	20.37	27.50	42.91
III			**11.08**	22.92	16.86	30.87	46.75
IV				**9.62**	19.95	13.56	29.81
V					**13.83**	25.01	38.48
VI						**7.62**	20.14
VII							**11.30**

Values in bold illustrate the intra cluster distance and others show inter cluster distance

Mean performance of different clusters for studied traits (Table 4) reflected that all the short duration genotypes were grouped into cluster VII whereas cluster III included long duration genotypes indicating maximum contribution of growth duration towards the divergence between cluster III and VII. Again all the high yielding genotypes with high number of filled grains per panicle were grouped into cluster VII whereas cluster II included low yielding genotypes with less number of filled grains per panicle indicating maximum contribution of these traits towards the divergence between cluster II and VII. The cluster V was divergent from cluster VII mainly due to days to panicle initiation, plant height, flag leaf area and number of effective tillers per hill indicating maximum contribution of these traits towards the divergence. The cluster III was divergent from cluster VI mainly due to days to 50% flowering, days to maturity and flag leaf area. In this context, traits contributing maximum towards the divergence should be given greater emphasis for deciding the type of cluster for the purpose of further selection and the choice of parents for hybridization [28].

The contribution of different traits towards the divergence was measured through principal component analysis (Table 5). 'Proper values' measure the importance and contribution of each component to total variance, whereas each coefficient of proper vectors indicates the degree of contribution of every original variable with which each principal component is associated. The higher the coefficients, regardless of the direction (positive or negative), the more effective they will be in discriminating between genotypes. For determining the number of factors to extract, scree test was done. According to scree test (Fig. 1), first three components were found most effective which accounted for more than 80% of total variation (Table 5), giving a clear idea of the structure underlying the variables analyzed. However, to determine the cutoff limit for the co-efficients of the proper vectors, the criteria that treated co-efficients greater than 0.3 as having a large effect, while traits having a coefficient less than 0.3 were considered not to have important effects on the overall variation observed in the present study.

The first principal component accounted for more than 56% of total variance, whereby days to panicle initiation, days to 50% flowering, days to flowering completion, days to milking stage, days to dough stage and days to maturity were the variables that contributed most negatively (Table 5). As a result, the first component differentiated those genotypes that flower and mature earlier in the season. The first component identified mainly phenological variables presenting negative contributions. Similar results for days to 50% flowering and days to maturity in rice were also observed by the previous researchers [29,30]. The second principal component accounted for more than 16% of total variance. Variables highly and negatively correlated were number of filled grain per panicle and yield per plot. In contrast, number of unfilled grains per panicle contributed most positively. Thus, the second principal component differentiated the high yielding genotypes. The third principal component accounted for 8% of total variance and was associated with flag leaf area, plant height, number of effective tillers per hill and panicle length, thus differentiating those genotypes with good architecture. These findings agree with Caldo et al. [31], who reported that maturity, heading, plant height, culm length, leaf length, and tillering ability were the major factors contributing to the variation of parental lines of modern Philippine rice cultivars.

It is expected that maximum amount of heterosis will be manifested in cross combinations involving the parents belonging to the most divergent clusters. There are also reports that selection of parents for hybridization should be from two clusters having wider inter-cluster distance to get maximum variability in the segregating generations and subsequent selection of ideotypes [32,33]. In the present study, relatively high genetic distance existed between cluster III and VII, II and VII followed by V and VII, III and VI. Keeping in view the above observations, crossing between the genotypes from cluster III and VII will give heterosis for growth duration and breeder can choose desirable one (short, intermediate, long) from the segregating generations as these two clusters are diversified from each other mostly for growth duration contributing traits. Again to get heterosis in hybrid and variations in segregating generations for yield per plot & filled grain per panicle; days to panicle initiation, plant height & number of effective tillers per hill; 50% flowering, days to maturity & flag leaf area crossings between the genotypes from cluster II & VII; V & VII; III & VI, respectively are more sensible.

Table 4. Cluster mean for different traits among 60 rice genotypes

Cluster no.	DPI	DF	DFC	DTM	DTD	FLA	DM	PH	TN	PL	FG	UFG	YPP
I	65.88	95.13	99.73	105.06	112.13	45.40	126.15	101.29	8.75	26.08	106.71	23.40	0.70
II	76.25	106.17	109.92	114.84	122.42	70.33	137.42	120.00	6.75	28.17	59.67	61.00	0.21
III	80.63	111.03	115.33	120.83	128.43	59.73	142.83	108.33	8.40	27.33	102.84	23.63	0.60
IV	67.59	97.67	102.11	107.74	115.15	49.93	128.92	103.07	8.44	26.18	70.00	35.93	0.35
V	72.33	105.56	110.00	114.00	121.66	67.56	137.00	130.00	5.89	25.33	116.78	24.78	0.82
VI	60.67	91.00	95.79	101.33	109.79	39.91	122.94	97.79	8.51	24.70	85.67	26.33	0.50
VII	53.62	80.76	84.29	89.71	96.67	38.67	109.67	99.57	9.52	23.19	115.33	23.90	0.87

DPI: days to panicle initiation, DF: days to 50% flowering, DFC: days to flowering completion, DMI: days to milking stage, DD: days to dough stage, FLA: flag leaf area (cm2), DM: days to maturity, PH: plant height (cm), ETN: number of effective tillers per hill, PL: panicle length (cm), FG: number of filled grains per panicle, UFG: number of unfilled grains per panicle, YPP: yield per plot (kg)

Table 5. Principal components (PCs) for thirteen traits in 60 rice genotypes

	PC1	PC2	PC3
Eigen value	7.85	2.27	1.21
% Variance	56.10	16.20	8.60
Cumulative (%) total variance	56.10	72.30	80.90
Co-efficient vector			
Days to panicle initiation	-0.337	-0.126	-0.116
Days to 50% flowering	-0.349	-0.110	-0.082
Days to flowering completion	-0.349	-0.104	-0.087
Days to milking stage	-0.348	-0.099	-0.108
Days to dough stage	-0.346	-0.093	-0.118
Flag leaf area (cm^2)	-0.221	0.126	0.557
Days to maturity	-0.343	-0.096	-0.120
Plant height (cm)	-0.197	0.079	0.456
Number of Effective tillers per hill	0.098	-0.123	-0.305
Panicle length (cm)	-0.181	0.170	0.339
Number of filled grains per panicle	0.087	-0.570	0.305
Number of unfilled grains per panicle	-0.107	0.515	0.104
Yield per plot (kg)	0.125	-0.514	0.305
% Variation explained	56.10	16.20	8.60

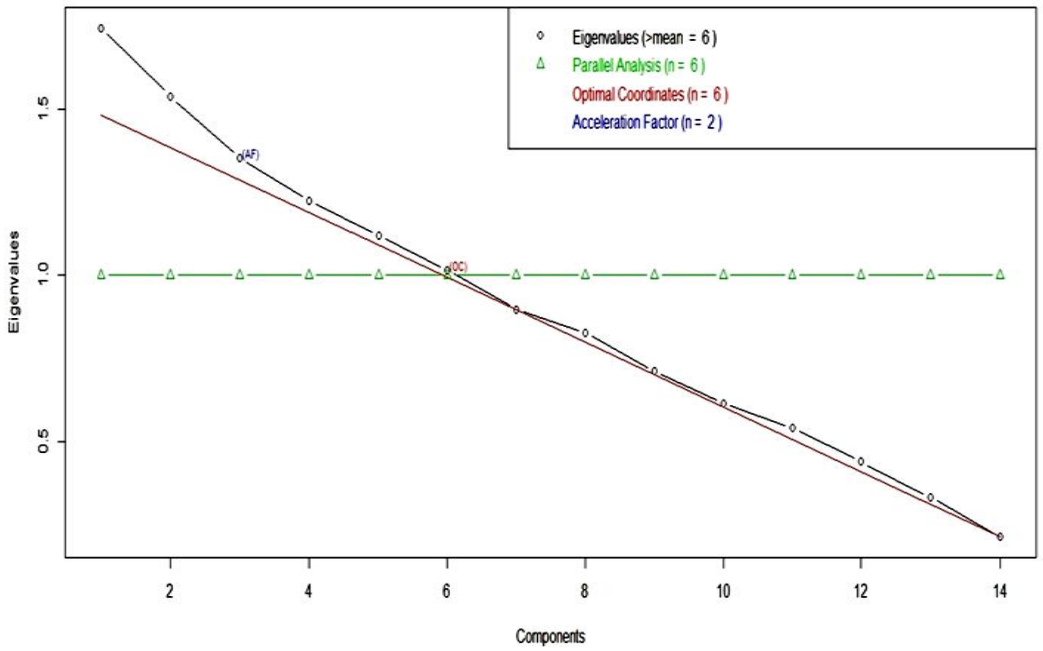

Fig. 1. Scree plot of principal component analysis of 60 genotypes of rice

Several reports showed that the parents separated by the medium magnitude of divergence also show high heterosis [34]. So genotypes from clusters differentiated from each other with medium magnitude of divergence are also important. Breeder can also perform hybridization among the genotypes of the same cluster having high intra-cluster distance to get heterosis and variation. Again for improving several desirable traits, combination breeding could be done among the genotypes from different clusters.

4. CONCLUSION

The multivariate analysis using Mahalanobis's D^2 statistics in association with principal component analysis clearly displayed the existence of wide diversity among the studied rice genotypes. Cluster analysis based on morphological traits suggested that the genotypes could be grouped, such assemblages are valuable for breeders to identify suitable parents for further breeding program to improve the specific traits. As morphological variations are influenced by environmental factors also, molecular markers can be used for further confirmation of such groupings.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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