Genetic diversity in some aromatic and nonaromatic maintainer lines based on principal component analyses for hybrid rice (*Oryza Sativa* L.) breeding

Abstract:

Genetic diversity of 21 maintainer lines of rice was studied under rainfed condition through Mahalanobis D^2 statistic for grain yield and yield contributing characters. The lines were grouped into five clusters. The inter-cluster distances were higher than intra-cluster distances indicating wider genetic diversity among the lines of different clusters. The intra-cluster distances were lower in all the cases reflecting homogeneity of the lines within the clusters. The principal component analysis showed that the first four components with vector values > 1 contributed 88.86 % of the total variations. The highest number (08) of lines was constellated in cluster V and the lowest (1) in cluster II. The intra- and inter-cluster distances were the maximum in cluster V (0.76) and between clusters II and V (18.10), respectively and the minimum in cluster IV (0.51) and between clusters I and III (4.12), respectively. The maximum value of inter cluster distance indicated that the lines belonging to cluster V were far diverged from those of cluster II. So, it is expected in our results that parent's selection for hybridization from the clusters V and II may give the desirable heterosis for heterotic rice hybrids. Besides, the cluster mean revealed that the crosses between the lines of cluster I with clusters II and V would exhibit high heterosis for maximum good characters. Hence, only effective tiller number had maximum contribution towards genetic divergence. Besides, evaluation of yield contributing characters demonstrated that the maintainer lines under the present study possessed a considerable genetic diversity. Therefore, maintainer lines need to be conserved in gene bank for future hybrid rice breeding programs.

*Key words***:** Genetic diversity, maintainer lines, morphological characters

Introduction:

Bangladesh agriculture involves food production for 163.65 million people from merely 8.75 million hectares of agricultural land. More food will be required in future because of increasing population. It is the staple food for the people of Bangladesh, constituting over 91% of the food grain production, and providing 62% of the caloric and 46% of the protein intake in the average daily diet [1]. Rice production area in Bangladesh about 11.4 million hectares (ha) of land in which 51.64 million tons of rice is produced [2]. Alarmingly, decreasing resources (e.g. land, labour, soil health and water), and increasing climate vulnerability (e.g., drought, salinity, flood, heat and cold) appeared as the great challenges to keep the pace of food production in the background of increasing population. Sufficient rice production is the key to ensure food security in Bangladesh.

Hybrids may offer to break through the yield ceiling of semi-dwarf rice began in 1964. The discovery of CMS in rice [3] suggested that breeding could develop a commercially viable F_1 hybrid. Currently, hybrid rice technology is considered as a viable option to increase rice yield globally [4]. China is the first country to exploit heterosis commercially in rice. During late 90's, about 55% of the rice area in China was under hybrid rice, producing 66% of the total rice production [5]. Presently, hybrid rice covers 70% of the total rice area which is about 20 million hectares. The most promising hybrids yielded 20-30% [6] and 15-20% [7] higher than the best conventional and modern rice varieties, respectively.

Genetic diversity is a powerful tool to determine the genetic discrimination among the genotypes which can be used to select appropriate parental genotypes for hybridization to develop high yielding potential variety [8]. The necessity of principal component analysis (PCA), principal coordinate analysis (PCO), non-hierarchical clustering and canonical vector analysis (CVA) for measuring the degree of divergence has been established by several investigators in rice and other crops [9-11]. In three lines system (A/B/R) of heterosis breeding in rice, genetic diversity of the lines is very important. If we develop or improve parental lines (A/B/R) of hybrid rice based on diversity we can find most diverse parents that will help developing heterotic hybrid combinations. About one hundred sixty (160) hybrid rice varieties have been released in Bangladesh from National Seed Board (NSB) under various private companies and public organizations. Hence, information on variability in respect of yield and it contributing traits in required to be assessed for its improvement. The present study was, therefore, undertaken to assess the genetic diversity of 21 aromatic and non aromatic maintainer lines of rice for developing new hybrid rice varieties in local conditions.

Materials and Methods

Experimental Site
The experiment was conducted at the research farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during July to December (T. Aman season) 2013. Geographically, the place is located at 24°09' N latitude and 90°26' E longitude with an elevation of 8.4 meters from the sea level and is characterized by subtropical climate. The soil of the experimental site was shallow Red Brown Terrace type.

Plant Materials
 A total of twenty one (21) maintainer lines of rice (Table 1) were collected from Department of Plant breeding and Genetics, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU). Pre-germinated seeds were sown in the seed bed.

Experimental Design and Setting the Experiment
The experiment was conducted following a randomized complete block design with three replicates for each treatment. Thirty days old seedlings of each maintainer lines of rice were transplanted on the 15 August, 2013 using single seedling per hill in 2.4m^2 plot with 25 cm and 20 cm space between rows and plants, respectively.

Intercultural Operations
Fertilizers were applied @ 80:60:40: 12 kg N, P, K and S per hectare. All the fertilizers except N were applied at final land preparation. Nitrogen was applied in three equal splits, at 15 days after transplanting (DAT), at 35 DAT and just before flowering. Intercultural operations and pest control measures were done as and when necessary.

Data Collection
Data were collected on flag leaf area (cm^2), days to flowering, days to maturity, panicle length (cm), effective tiller number, plant height (cm), filled grains per panicle, grain length(mm), grain breadth (mm), 1000- grain weight (g), yield per hill (g).

Aroma Test
Aroma was detected by sniffing and was scored as nonscented, lightly scented and scented following 1.7% KOH based method [12].

THE
EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

Data Analysis
Mean data for each character was subjected to multivariate analysis techniques *viz*. Principal component analysis (PCA), Principal coordinate analysis (PCO), Canonical vector analysis (CVA) and Cluster analysis (CLSA) using GENSTAT 5.5 software.

Principal component analysis (PCA)
Principal component analysis is one of the multivariate techniques to know the interrelationships among several characters and can be done from the sum of squares and product matrix for the characters. Principal components were computed from the correlation matrix and genotypic scores obtained for the first component and succeeding components with latent roots greater than unity [13]. The latent roots are called "Eigen values". The first component has the property of accounting for maximum variance. The PCA displays most of the original variability in a smaller number of dimensions, since it finds linear combinations of a set of variate that maximize the variation contained within them. Contributions of the different characters towards divergence are discussed from the latent vectors of the two principal components.

Principal coordinate analysis (PCO)
Principal coordinate analysis is equivalent to PCA but it is used to calculate inter unit distances. Through the use of all dimensions of p it gives the minimum distances between each pair of the N points using similarity matrix [14]. Inter-distances between genotypes were studied by PCO.

Canonical variate analysis (CVA)
CVA complementary to D^2-statistic is a sort of multivariate analysis where canonical vectors and roots representing different axes of differentiation and the amount of variation accounted for by each of such axes, respectively are derived. Canonical variate analysis finds linear combination of original variability that maximizes the ratio of between groups to within groups variation, thereby giving functions of the original variables that can be used to discriminate between the groups. Thus in this analysis, a series of orthogonal transformations sequentially maximize the ratio of among groups to within group variations.

Cluster analysis (CLSA)
Genotypes were divided into groups on the basis of a data set into some number of mutually exclusive groups. The clustering was done using non-hierarchical classification. In Genstat, the algorithm is used to search for optimal values of the chosen criterion. The optimal values of the criteria followed by some initial classification of the genotypes into required number of groups, the algorithm repeatedly transfers genotypes from one group to another so long as such transfer improved the value of the criterion. No further transfer can be found to improve the criterion. The algorithm switches to second stages that examine the effect swapping two genotypes of different classes, and so on.

Computation of average Intra-cluster distance
Computation of Average Intra-Cluster distance for each cluster was calculated by taking possible D^2 values within the members of a cluster obtained from the PCO after the clusters are formed. The formula utilized was $\Sigma D^2/n$, where ΣD^2 is the sum of distances between all possible combinations (n) of the genotypes included in a cluster. The square root of the average D^2 values represents the distance (D) within cluster.

Results and Discussion
Eigen values (latent roots) and percentage of total variation accounted for them obtained from principle component analysis are offered in Table 2. The result exposed that the first four components in the PCA with eigen values >1, contributed 88.86

% of the total variations among the rice genotypes for 12 morphological characters. Islam et al. [9] observed that the first nine axes accounted about 90% of the total variations by PCA in 113 aromatic and fine grain rice landraces. On the other hand, Akter et al. [15] and Chakravorty et al. [16] observed the contribution of 83.46 and 75.9% of the first six and four components, respectively to the total variation in rice.

Based on the degree of divergence, 21 lines were grouped into five clusters on the basis of cluster analysis (Table 3). Maximum 8 lines were grouped into the cluster V followed by 6 in cluster IV, 4 in cluster III and 2 in cluster I. The cluster II contained the lowest (1) number of lines.

Intra and inter-cluster distances are presented in Table 4. There were marked variations in intra-cluster distances which ranged from 0.51 to 0.76.The highest intra-cluster distance was observed in cluster V (0.76) where the lowest intra-cluster distance was observed in cluster IV (0.51). Such results indicated that the lines in the cluster V was more diverged than cluster IV.

The highest inter-cluster distance was recorded between cluster II and V (18.10) followed by clusters I and II (17.26). The lowest inter-cluster distance was observed between cluster I and III (4.12) followed by cluster III and IV (5.52). The maximum value of inter cluster distance indicated that the lines belonging to cluster V was far diverged from those of cluster II. The minimum inter cluster divergence was observed between cluster I and III (4.12) indicating that the lines of these clusters were genetically closed. So, it is expected in our results that parent's selection for hybridization from the clusters V and II may give the desirable heterosis for heterotic rice hybrids. On the other hand, the inter cluster distances in all the clusters were higher than the intra cluster distances suggesting wider genetic diversity among the genotyps of different groups. The results were in agreement with Islam [17], Siddique [18] and Ahmed [19].

The mean performance of 12 characters in five clusters was presented in Table 5. The data revealed that different clusters exhibited the highest and lowest mean values of individual characters and none of the single cluster showed the highest or lowest mean values of all the characters. The highest cluster means for effective tiller number, grain length, length breadth ratio and yield obtained from cluster I. The lowest cluster means for days to flowering, days to maturity, panicle length and yield were found in cluster V. The highest means for filled grains per panicle and panicle length was found in cluster II and III, respectively. Thus the results suggest that the crosses between the lines belonging to the cluster I with clusters II and V might be selected as parents for future breeding program depending upon the objectives. Ahmed [19] and Islam [20] earlier reported similar trend of conclusions on rice germplasm using D^2 statistics.

Contributions of the characters towards divergence are presented in Table 6. The canonical vector analysis revealed that the vectors (vector I and II) were positive only for effective tiller number indicating maximum contribution of the character was to the total divergence. Similar findings were obtained by Akter et al. [21]. The other characters namely flag leaf area and grain length had the positive value for vector I, similarly, filled grains per panicle was found positive for vector II which revealed that those characters was contributed less toward the total genetic divergence.

Conclusions:
Genetic diversity is fundamentally important for developing heterotic rice hybrid. Results of this study indicated that maintainer lines are diversed and grouped into five clusters. Considering the magnitude of genetic distance through the multivariate analysis, IR58025B, IR62829B, IR6888B, Elai B, Straw TAPL-500B, DubsailB, DakshahiB were selected due to their greater divergence that would offer a good scope to develop elite CMS line(s) by improvement of maintainer for the

exploitation of better heterosis in hybrid rice. Besides, characterizations of the identified maintainer lines need to be done using SSR markers.

Table 1. List of 21 maintainer lines of rice with their source and origin

Sl. No.	Maintainer Lines	Season	Source	Origin	1.7% KOH (aroma)
1	TilkapurB	T.Aman	BSMRAU	Bangladesh	LS
2	GopalbhogB	T.Aman	BSMRAU	Bangladesh	S
3	Kamini soruB	T.Aman	BSMRAU	Bangladesh	S
4	ElaiB	T.Aman	BSMRAU	Bangladesh	NS
5	Lal SoruB	T.Aman	BSMRAU	Bangladesh	S
6	BaoibhogB	T.Aman	BSMRAU	Bangladesh	S
7	DesikatariB	T.Aman	BSMRAU	Bangladesh	S
8	Raduni pagalB	T.Aman	BSMRAU	Bangladesh	LS
9	Sugandhi dhanB	T.Aman	BSMRAU	Bangladesh	NS
10	Straw TAPL-500B	T.Aman	BSMRAU	Bangladesh	S
11	KhazarB	T.Aman	BSMRAU	Iran	NS
12	NoyanmoniB	T.Aman	BSMRAU	Bangladesh	S
13	DubsailB	T.Aman	BSMRAU	Bangladesh	LS
14	DakshahiB	T.Aman	BSMRAU	Bangladesh	NS
15	Kalijira12B	T.Aman	BSMRAU	Bangladesh	S
16	Kalijira2B	T.Aman	BSMRAU	Bangladesh	S
17	IR58025B	T.Aman	BSMRAU	Philippines	NS
18	IR62829B	T.Aman	BSMRAU	Philippines	NS
19	BRRI1B	T.Aman	BSMRAU	Bangladesh	NS
20	Gan46B	T.Aman	BSMRAU	China	NS
21	IR6888B	T.Aman	BSMRAU	Philippines	NS

NS: Nonscented, **LS:** Light scented and **S:** Scented, **TAPL:** Transplant Aman Pure Line, **BSMRAU:** Bangabandhu Sheikh Mujibur Rahman Agricultural University

Table 2. Eigen values and percent of variation for 12 principal component axes in 21 maintainer lines of rice

Traits	Principal component axes	Latent roots	Variation (%)	Cumulative % of variation
Flag leaf area(cm^2)	PC 1	4.89	40.77	40.77
Days to flowering	PC 2	2.75	22.95	63.72
Days to maturity	PC 3	1.73	14.43	78.15
Panicle length (cm)	PC 4	1.26	10.51	88.86
Effective tiller number	PC 5	0.55	4.57	93.23
Plant height (cm)	PC 6	0.35	2.90	96.13
Filled grains per panicle	PC 7	0.23	1.88	97.03
Grain length (mm)	PC 8	0.11	0.90	97.93
Grain breadth (mm)	PC 9	0.07	0.59	98.52
Length breadth ratio	PC 10	0.05	0.42	98.94
1000 grain weight (g)	PC 11	0.01	0.05	99.99
Yield per hill (g)	PC 12	0.004	0.01	100

Table 3. Distribution of 21 maintainer lines of rice into five clusters

Cluster	No. of Lines	% total	Name of maintainer lines
I	2	9.52	GopalbhogB, KaminisoruB
II	1	4.76	BaoibhogB
III	4	19.05	TilkapurB,DesikatariB,Raduni pagalB,NoyanmoniB
IV	6	28.57	LalSoruB,StrawTAPL500B,DubsailB, DakshahiB,Kalijira12B, Kalijira2B
V	8	38.10	ElaiB,SugandhidhanB,KhazarB,IR58025B,IR62829B,BRRI1B ,Gan46B,IR6888B

Table 4. Intra (bold) and inter-cluster distances (D^2) for 21 maintainer lines of rice

Clusters	I	II	III	IV	V
I	**0.61**	17.26	4.12	8.73	13.75
II		**0.69**	15.48	11.26	18.10
III			**0.62**	5.52	14.35
IV				**0.51**	15.62

V				0.76	

Table 5. Cluster means for 12 quantitative characters in 21 maintainer lines of rice

Characters	I	II	III	IV	V
Flag leaf area(cm^2)	32.61	36.34	33.46	39.71	40.55
Days to flowering	98.00	102.00	98.00	106.00	86.00
Days to maturity	127.00	130.00	128.00	135.00	114.00
Panicle length (cm)	27.71	28.30	29.08	28.40	23.69
Effective tiller number	11.00	10.00	9.00	8.00	9.00
Plant height (cm)	139.17	141.07	155.50	159.02	104.36
Filled grains per panicle	106.00	204.00	133.00	156.00	108.00
Grain length (mm)	10.22	6.58	7.74	7.27	8.94
Grain breadth (mm)	2.16	2.28	2.20	2.56	2.32
Length breadth ratio	4.67	2.88	3.53	2.87	3.94
1000 grain weight (g)	16.24	10.22	12.13	14.93	18.63
Yield per hill (g)	13.09	10.78	10.97	12.18	9.93

Table 6. Relative contributions of the 12 characters of 21 maintainer lines to the total divergence

Characters	Vector 1	Vector 2
Flag leaf area(cm^2)	0.041	-0.520
Days to flowering	-0.412	-0.087
Days to maturity	-0.382	-0.071
Panicle length (cm)	-0.392	-0.065
Effective tiller number	0.113	0.143
Plant height (cm)	-0.319	-0.142
Filled grains per panicle	-0.386	0.038
Grain length (mm)	0.292	-0.321
Grain breadth (mm)	-0.119	-0.008
Length breadth ratio	0.313	-0.461
1000 grain weight (g)	0.239	-0.461
Yield per hill (g)	-0.101	-0.381

Authors' contributions:
All authors are contributed equally in this research work.

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THE

EXPERIMENT

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