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Comparison among Clustering in Multivariate Analysis of Rice Using Morphological Traits, Physiological Traits and Simple Sequence Repeat Markers

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Abstract: A sample set of 21 rice varieties composed of 20 BRRI developed modern rice varieties and one local variety was selected to detect the genetic variation by using 13 morphological traits, 14 physiological traits and 34 SSR markers. All these three methods divide the varieties into five clusters and showed that there exist high genetic variations among the genotypes. Cluster analysis showed that there exists difference in grouping of varieties by morphological traits, physiological traits and SSR markers. Genotypes swapped among different clusters in different methods of clustering. In this study, SSR markers provides five clusters at 50% genetic similarity coefficient and it was found that grouping of genotypes corresponds well to their known pedigree data and aroma content. Physiological clustering was representation of photoperiod sensitivity, aroma producing ability and growth duration. It was also found that parent selections for breeding aspects using different methods differ from each other.

Key words: Genetic diversity · Multivariate analysis · SSR

INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop which supplies staple food for nearly 50% of the global population [1, 2]. It is the basis of food security and is intimately associated with traditional culture and customs of Bangladesh. Bangladesh is the fourth largest producer and consumer of rice in the world [1], with annual production of 33.317 million metric tons in an area of 11.359 million hectare of land [3]. In Bangladesh rice provides 75% of the calories and 55% of the protein in the average daily diet of the people [4]. Among Aus, Aman and Boro seasons, Aman occupied the highest area coverage (48.74 % of total rice cropped area) [3]. So, we have to give more attention for the improvement of T. Aman rice varieties to increase rice production in order to satisfy our population's need of food.

A clear understanding of genetic diversity is essential for the effective conservation and utilization of rice genetic resources [5]. Genetic diversity is a source of variation, the raw material for crop improvement work, essential to decrease crop vulnerability to abiotic and biotic stresses, ensure long-term selection gain in genetic improvement and promote rational use of genetic resources [6]. Diverse data sets including morphology [7], Physiology [8], isozymes [9], storage protein profiles [10] and DNA markers have been using to assess genetic diversity. Among these methods of diversity analysis, morphology, physiology and DNA markers [11] especially, simple sequence repeats (SSRs) have been widely using in different crop species, especially in rice. There are some reports to show differences and correlation among different methods of diversity study along with their clustering patterns

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[7, 12-16]. Our objective is to study the clustering patterns derived from mostly used three methods of diversity analysis of rice.

MATERIALS AND METHODS

The field experiment was conducted in the farm of Bangladesh Rice Research Institute (BRRI) regional station Sonagazi, Feni (under AEZ 18, Young Meghna Estuarine Floodplain) in T. Aman season, 2008 aiming to assess morphological and physiological diversity of T. Aman rice. A set of 21 genotypes were used in this study composed of 20 BRRI developed modern varieties and the most popular local variety called Rajasail (Table 1). The experiment was laid out in a RCBD with three replications. The dimension of an individual plot was 4.0 m × 5.0 m having plot to plot and block to block distance of 0.5 m and 1.0 m, respectively. Thirty days old seedlings were transplanted at the rate of two to three seedlings per hill with the spacing of 20 cm × 20 cm. All agronomic practices were done according to the recommendation of BRRI [17]. Morphological genetic variation was assessed on 13 traits; plant height (cm), panicle length (cm), maximum number of tillers/m^2, number of effective tillers/m^2, tiller mortality, number of spikelets/panicle, number of effective spikelets/panicle, number of ineffective spikelets/panicle, spikelet fertility, 1000-grain weight (g), phenotypic acceptability (PACP), straw yield (t/ha) and grain yield (t/ha). Cluster analysis was carried out by the software SPSS v17.0 (using Euclidean D^2 statistic).

Hierarchical cluster analysis (HCA) was done to show the average linkage between the genotypes through a dendrogram (Fig. 1). Fourteen physiological data were recorded from the same plots to assess physiological genetic diversity among these varieties. The traits consists of seedling vigor (mg/cm), days to flowering (50%), panicle exertion rate (%), flag leaf area (cm^2), days to maturity, LAI at panicle initiation and at flowering using length-width method [18]. CGR at panicle initiation and at flowering were measured following Radford [19]. Relative growth rates (RGR) at panicle initiation and at flowering were measured as growth rate per unit plant biomass following Tanaka *et al.*[20]. Net assimilation rates (NAR) at panicle initiation and at flowering were calculated using the formula of Kubota *et al.* [21]. Clustering and HCA was carried out as described in morphological section above. Molecular diversity was assessed using 34 Simple Sequence Repeat (SSR) markers (Table 2) in 2009, in the laboratory of plant breeding division of BRRI, Gazipur. Seeds were germinated in germination chamber and after 3 days, germinated seedlings were sown in pots. Then the pots were kept in the net house. DNA was collected from the leaf of 28 days old seedlings following modified miniscale protocol. Molecular weight for each amplified allele was measured in base pair using Alpha-Ease FC 5.0 software. Genetic distance was calculated using the "Nei distance" [22]. The allele frequency data from PowerMarker version 3.25 [23] was used to export the data in binary format (allele presence="1" and allele absence = "0") for

Table 1: List of rice (*Oryza sativa* L.) varieties used in this study

SL. No.	Name of variety	Year of release	Parents/crosses
01	BR3	1973	IR506-1-133 × Latisail
02	BR4	1975	IR20 × IR5-114-3-1
03	BR5	1976	Progeny selection from Badshahvog
04	BR10	1980	IR20 × IR5-114-3-1
05	BR11	1980	IR20 × IR5-47-2
06	BR22	1988	BR51-46-5 × Nizersail
07	BR23	1988	DA29 × BR4
08	BR25	1992	IR26 × PAJAM
09	BRRI dhan30	1994	IR2058-78-1-3-2-3 × BR4
10	BRRI dhan31	1994	BR11 × ARC10550
11	BRRI dhan32	1994	BR4 × BR2662
12	BRRI dhan33	1997	BG388 × BG367-4
13	BRRI dhan34	1997	Selection (Acc. No 4341)
14	BRRI dhan37	1998	Bashmati (D) × BR5
15	BRRI dhan38	1998	Bashmati (D) × BR5
16	BRRI dhan39	1999	BR1185-2B-56-2-1-1×BR1674-28-3-1-1
17	BRRI dhan40	2003	BR10 × IR4595-4-1-15
18	BRRI dhan41	2003	BR23 × BR1185-2B-16-1
19	BRRI dhan44	2005	BR10 × BRRI dhan31
20	BRRI dhan46	2007	BR11 × ARC14766 × Swarnolota
21	Rajasail	Local variety	Selection

Table 2: SSR markers used the determination of molecular diversity of 21 rice varieties

Sl. No.	Primer	Sequence of the primer		Chromosome Number	Position (MB)
		Forward	Reverse		
01.	RM05	CACACTCCCATGCTAACAACTGG	CATCAAGAAGAGCAGTCCTGTGC	01	24.30
02.	RM490	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG	01	-
03.	RM1287	CCATTTGCAGTATGAACCATGC	ATCATGCAATAGCCGGTAGAGG	01	10.80
04.	RM3412	TGATGGATCTCTGAGGTGTAAAGAGC	TGCACTAATCTTTCTGCCACAGC	01	11.60
05.	RM8094	AAGTTTGTACACATCGTATACA	CGCGACCAGTACTACTACTA	01	11.20
06.	RM7075	GCGTTGCAGCGGAATTTGTAGG	CCCTGCTTCTCTCGTGCAGTCG	01	15.10
07.	RM10696B	TCCAGATCAACCAGCACATC	CCTGAAGGG.AGGGAGTATTTG	01	-
08.	RM10696	CCTTCGACTCCATGAAACAAACG	TCTCTTTGCCCTAACCCTATGTCC	01	11.00
09.	RM10713	ATGAACCCGGCGAACTGAAAGG	CTGGCTCCCTCAAGGTGATTGC	01	11.20
10.	RM10720	GCAAACGTCTACGTGAGAAACAAGC	GCATGTGGTGCCTTAACATTTGG	01	11.40
11.	RM10927	TGGATCCCACTAATCCAAATGC	GAAAGACTCCTTCCAATGTTAGGC	01	15.70
12.	RM279	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	02	2.90
13.	RM424	GATTCCACGTCAGGATCTTCTGG	GCTCACCAGTTGAGATTGAAAGG	02	11.38
14.	RM489	GAACAGGGACACAATGATGAGG	GACGATCGGACACCTAATTACAGC	03	4.30
15.	RM6266	CACCTTCTTGAGAAGCTCCTTCG	GACATCGAGAGCGAGGACAGC	03	23.60
16.	RM401	GCATGAGCTGCTCTCATTATTGTCC	GAAACGAACCAAACGTTCATCG	04	13.20
17.	RM1155	GACAGGGAGTGTGGCAACTATGC	GATCACAGACAATCATGGGTTGG	04	20.50
18.	RM1024	AACTGCCATCTCTGAAACTCTGC	CATCTCACTTCAGAAGGATCATAGCC	05	1.20
19.	RM289	TTCCATGGCACACAAGCC	CTGTGCACGAACTTCCAAAG	05	7.78
20.	RM469	TTACGTGATCACACAGGCTCTCC	AAGCTGAACAAGCCCTGAAAGG	06	0.60
21.	RM20224	AGTATGAAAGTCGGTGACGATGG	GAGATGTCACGTCTTCACTTAGGG	06	20.60
22.	RM5371	GCAGAGGATGCCCACTTAATTCC	GGGCTAGCTTTAGCTGCGTTGC	06	25.40
23.	RM436	ATTCCTGCAGTAAAGCACGG	CTTCGTGTACCTCCCCAAAC	07	22.09
24.	RM455	CCACAAATTAATCCGGATCACACC	AGCATTGTGCAATCACGAGAAGG	07	22.30
25.	RM38	ACGAGCTCTCGATCAGCCTAGC	CACTCCATGGAAGAGGCAAGC	08	2.10
26.	RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC	08	24.14
27.	RM566	AATATGGTGGCGCGTACATCC	TGATCGAGCCAACAACAACTGG	09	14.70
28.	RM242	AAACACATGCTGCTGACACTTGC	TTACTAGATTTACCACGGCCAACG	09	18.60
29.	RM258	CTCCCTGGCCTTTAAAGCTGTCG	GACGAACAGCAGCAGAAGAGAAGC	10	17.60
30.	RM590	GAGATCGAGGAGGAGGTGAGG	AGTACTGCCGATCATATGGAAGC	10	22.60
31.	RM3428	GCCATTGACACCAAATGATCACC	GGCATATAAGGTCCATGGTGAATTGG	11	13.40
32.	RM286	CTGGCCTCTAGCTACAACCTTGC	AAACTCTCGCTGGATTCGATAGG	11	0.38
33.	RM17	GGAGAAAGAGAGGTGATCCTTTCC	CATGTCTTGGTGAGTGATGTTGC	12	26.95
34.	RM463	GAGGATTAATTAGCGTGTGACC	GTCGTGACATCTACTCAAATGG	12	22.09

(Source: http://www.gramene.org)

analysis with NTSYS-pc version 2.2 [24]. A similarity matrix was calculated with the Simqual subprogram using the Dice coefficient, followed by cluster analysis with the SAHN subprogram using the UPGMA clustering method as implemented in NTSYS-pc was used to construct a dendogram showing relationship among the genotypes.

RESULTS AND DISCUSSION

Clustering of Genotypes Found in Morphological Diversity Analysis: Cluster analysis based on morphological traits provides five clusters (Figure 1) at a level of 12.8. Clustering pattern showed that cluster I is composed of the highest number of genotypes (13) followed by cluster II consisting of 3 genotypes, cluster III and cluster IV consisting of 2 genotypes and cluster V consisting of one genotype. Same clustering pattern was previously reported in several reports [13, 16, 25-27]. Cluster I includes all genotypes whose ancestral record are directly or indirectly related to IR 20 (Table 1) except BR10. Three other genotypes named BR22, BR25 and BRRI dhan34 were also in this cluster. BR10 undergoes to cluster II instead of cluster I. Cluster II includes two more varieties (BRRI dhan33 and BRRI dhan39) developed from 2 distinct crossing (Table 1). Two aromatic rice genotypes (BR5 and BRRI dhan37) formed cluster IV. Cluster V is composed of three genotypes of apparently diverged genetic background, one is aromatic (BRRI dhan38), one is local moderately saline tolerant (Rajasail) and the rest one is BR3, a variety which can be grown exceptionally all over three rice seasons in Bangladesh with high yield potentiality. According to [28], selection of one parent

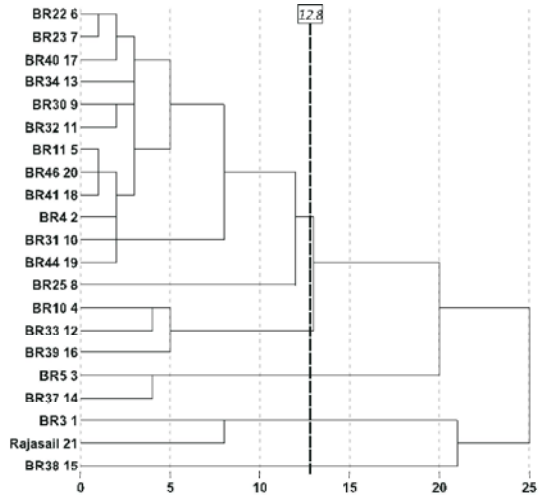

Fig. 1: Dendrogram of 21 rice cultivars based on 13 morphological characters

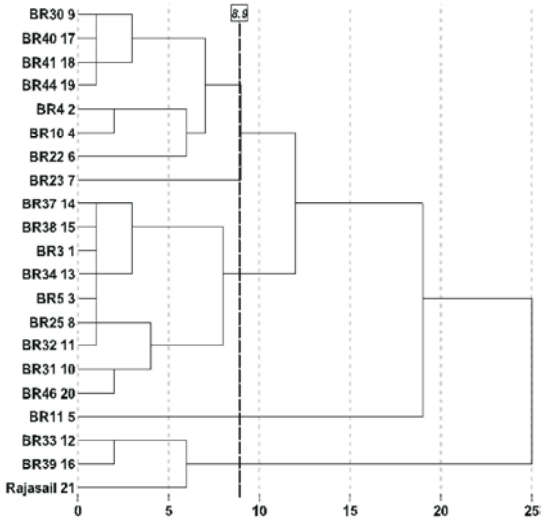

Fig. 2: Dendrogram of 21 rice cultivars based on 14 physiological characters

from each cluster and crossing them by a series of diallel cross were proved to be highly fruitful. So if we want to select one genotype from each cluster then BR11, BRRI dhan33, BR5, BR3 and BRRI dhan38 could be selected from cluster I, cluster II, cluster III, cluster IV and cluster V, respectively.

Clustering of Genotypes Found in Physiological Diversity Analysis: Fourteen physiological characters based clustering provides five clusters (Figure 2) among 21 rice genotypes. Cluster III is composed of the highest genotypes (9) followed by cluster I (7 genotypes), cluster V (3 genotypes) and cluster II and cluster IV is composed of one genotypes each (Figure 2). The genotypes comprising cluster I is characterized by photoperiod sensitivity (BR4, BR10, BR22, BRRI dhan30, BRRI dhan40, BRRI dhan41 and BRRI dhan44). Cluster II is composed of only one genotype (BR23) which is a strong photo period sensitive with slender grain type rice. All aromatic rice varieties are under the cluster III (BR5, BR25, BRRI dhan34, BRRI dhan37 and BRRI dhan38). They are also characterized by high photosensitivity [29]. Another highly photosensitive variety BRRI dhan46 also undergoes to this cluster. Three photoperiod insensitive variety BR3, BRRI dhan30 and BRRI dhan31 have also placed in this cluster. Cluster IV consists of only one genotype, the mega variety of T. Aman rice, BR11. Lacal rice genotype, Rajasail clustered with BRRI dhan33 and BRRI dhan39. These three varieties are very short durated variety and remarkably earlier then the other varieties and have bold type grain. If we select one genotype from each cluster then BRRI dhan44, BR23, BRRI dhan46, BR11 and BRRI dhan33 could be selected from cluster I, cluster II, cluster III, cluster IV and cluster V, respectively for breeding aspects.

Clustering of Genotypes Found in Molecular Diversity Analysis: Molecular clustering provides five clusters at 50 % dissimilarity level (Figure 3). In this study, the grouping of cultivars based on SSR polymorphisms corresponds well to their known pedigree data. Thirteen rice varieties formed cluster I, where BR4, BR10, BR11, BR22, BR23 and BRRI dhan30 formed one sub-cluster where all these varieties, except BR22, were related by their ancestry directly with IR20 or indirectly through BR4. These varieties are also moderately photosensitive except BRRI dhan30. BRRI dhan31, BRRI dhan32, BRRI dhan39, BRRI dhan40, BRRI dhan41 and BRRI dhan44 form second sub-cluster and they are indirectly related to the ancestral genotype of IR20 through BR10, BR11 or BR4 (exception BRRI dhan39). These genotypes are photoperiod insensitive except BRRI dhan40 and BRRI dhan41. A strongly photoperiod sensitive genotype, BRRI dhan46 formed the third sub cluster which is also indirectly related with IR20. After all, excluding BRRI dhan33 and BRRI dhan39, all genotypes under this cluster are related with IR20 in their ancestral record.

BR3, plant type and yield similar with BRRI dhan33, but developed from distinct crosses were placed under the cluster II. BR25, which has a long plant type and developed from a cross between two completely divergent parents (IR26 × PAJAM) that have no relation to the other varieties from breeding records, formed a distinct cluster. Rajasail, a moderately saline tolerant local variety grown

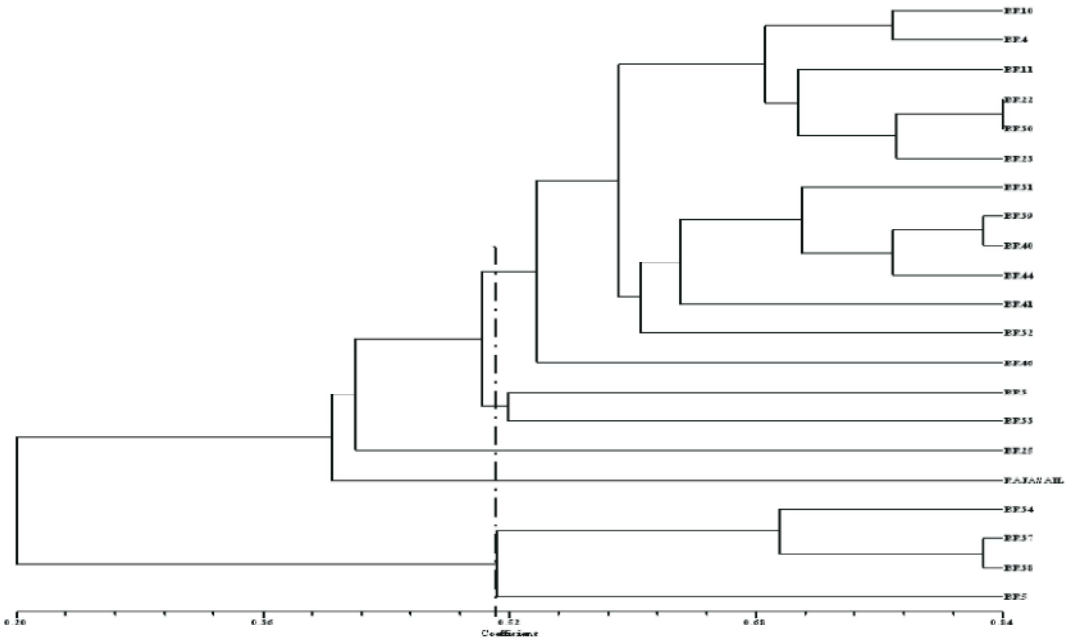

Fig. 3: A UPGMA cluster dendrogram showing the genetic relationships among 21 rice cultivars based on the alleles detected by 34 SSR markers

mainly in salinated coastal ecosystems [30], which has no relation to the other varieties in the pedigree record, formed fourth cluster. Interestingly, all the aromatic rice varieties clustered in the same group. These aromatic rice varieties were strongly photoperiod sensitive and were developed by crossing with Basmati (a local aromatic rice of India) or by improving the local aromatic germplasm (Table 1). Among these aromatic rice varieties, BRRI dhan37 and BRRI dhan38 were developed from the same cross (Bashmati (D) × BR5) and showed maximum similarity (83 % similarity). Several study with DNA markers also found similar result for aromatic rice [31-34]. If we select one variety from each cluster for breeding aspect, then we may select BR11, BRRI dhan33, BR25, Rajasail and BRRI dhan38 from cluster I, cluster II, cluster III, cluster IV and cluster V, respectively.

Comparison of Clustering Pattern: All the three methods assessed high level of genetic variations and divide the genotypes into five clusters. The clustering pattern is different among t hese methods. Among these three methods of diversity analysis, molecular diversity provides the maximum genetic differences among the test genotypes followed by morphological diversity analysis and physiological diversity was the least method to describe diversity among the genotypes. Several reports suggested that molecular diversity provide remarkably higher estimates of genetic diversity than morphological or physiological methods [6, 13, 35, 36].

Genotypes also swapped from one cluster to another cluster among different methods and this pattern is somewhat irregular. Differences in clustering pattern and swapping of genotypes among different clusters in different methods of diversity analysis have reported by a number of studies [16, 27, 37-39]. These differences is not an indicator of the failure or limitation or weakness of the methods [15]. These results may be due to the diversity at the molecular level, which may not reflect in the diversity at the morphological or physiological level, as described by Karhu *et al*. [40]. If the number of markers might be used as several thousand and the morphological or physiological traits would contain all possible parameters, then the provided diversity might be very near. Another possible reason for this variation in clustering might be the environmental influence and genotype environment interaction. Compared to morphological and physiological characteristics, the DNA genome provides direct comparison of genetic diversity at the DNA level, are phenotypically neutral and are not modified by environment and management practices [6]. Morphological and physiological characters are the ultimate expression of molecular constitution of a variety

where a number of biochemical processes is involved. So different types of clustering in different methods is not unusual [27].

For future breeding program, parents are selected from diverged clusters with better agronomic performance or from each cluster one parent is selected and then the selected genotypes are subjected to crossing [28]. Since selections of parents are based on the clustering pattern and genotypes swapped from one method to another method, so selection of parents will differ from one method to another method. Some parents may be identified by all these methods (BR11 and BRRI dhan33), but most of them differ from one method to another method. The objective of the study will define which method of genetic variation is to be used. For example, if the objective is germplasm conservation, then molecular diversity is preferable. It mainly depends on the insight capacity of breeder or the experimenter and efficiency is involved with precise estimation of the variables and efficiency of variable selection.

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