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Parent Selection for Transplanted Aman Rice Breeding by Morphological, Physiological and Molecular Diversity Analysis

[1,2]M. Mamunur Rahman, [3]M.G. Rasul, [4]M.K. Bashar, [5]M.A. Syed and [6]M.R. Islam

[1]Graduate School of Natural Science and Technology,
Kanazawa University, Kakuma, Kanazawa, Japan.
[2]Bangladesh Rice Research Institute, Regional Station, Sonagazi, Feni, Bangladesh.
[3]Department of Genetics and Plant Breeding, BSMRAU, Gazipur-1706, Bangladesh.
[4]Bangladesh Rice Research Institute (BRRI), Gazipur-1701, Bangladesh.
[5]Bangladesh Rice Research Institute, Regional Station, Comilla, Bangladesh.
[6]Department of Genetics and Plant Breeding, SAU, Dhaka-1207, Bangladesh.

Abstract: The research work was conducted to assess the morphological, physiological and molecular divergence among 21 T. Aman rice cultivars at Bangladesh Rice Research Institute (BRRI), aiming parent selection for breeding program. Data were collected on 13 morphological and 14 physiological traits and were analyzed by GENSTAT v 5.5 software. Molecular diversity was determined using 34 microsatellite or simple sequence repeat (SSR) markers. All of the above three methods grouped the genotypes into five clusters. Cluster analysis showed that there exist difference in genetic distances of varieties determined by morphological and physiological traits and SSR markers. Morphological and physiological diversity analyses suggest four common genotypes as parent for future breeding program (BRRI dhan33, BRRI dhan38, BRRI dhan44, BRRI dhan46). Three genotypes (BRRI dhan33, BRRI dhan38 and BRRI dhan46) have been suggested as parent commonly by these three methods. These differences in suggesting parents by different methods are due to the swopping tendency of varieties to different clusters in different methods. Parental genotypes could be selected from BR11, BR23, BR25, BRRI dhan33, BRRI dhan38, BRRI dhan44, BRRI dhan46 and Rajasail according to breeding objective.

Key words: Parent selection % Rice breeding % SSR marker % Crop improvement

INTRODUCTION

Asian cultivated rice (*Oryza sativa* L.) is an important cereal crop consumed exclusively by humans which was domesticated from its wild ancestor about 11,500 years ago; it supplies staple food for nearly 50% of the global population [1]. In many developing countries, rice is the basis of food security and is intimately associated with traditional culture and customs in local regions [2]. Rice of subcontinent area especially Bangladeshi rice is of three types depending on their growing season e.g. Aus, Aman and Boro [3]. Among Aus, Aman and Boro seasons, the prospects of T. Aman rice is to be given more emphasis over the others due to some of its suitability in Bangladesh perspective. Such as, T. Aman occupied the highest area coverage (48.74 % of total rice cropped area) [4], it is cultivated in rainy season based on rain water, no need of irrigation (only supplementary irrigation may be needed in some cases), soil salinity of coastal area become very low in this season due to heavy rainfall, land should not to be undertaken for other crops during this season which may provide more land area for growing of rice. So, we have to give more attention for the improvement of T. Aman rice varieties to increase rice production in order to satisfy our increasing population's need of food.

Corresponding Author: M. Mamunur Rahman, Bangladesh Rice Research Institute, Regional Station, Sonagazi, Feni, Bangladesh.

Genetic diversity in the available gene pool is the foundation or the raw material of all plant improvement programs. The availability of transgressive segregants in any breeding program also depends upon effectively inclusion of parents. Selection of parents based on genetic divergence has been successfully utilized in different crop species [5-7]. So, precise information on the nature and degree of genetic divergence of the parents is the prerequisite of an effective breeding program. Among different methods of diversity analysis, morphological [8], physiological [9] and molecular [10] diversity analysis is mostly used. Molecular marker technology provides a powerful tool for determining genetic variation in rice varieties [11, 12]. Therefore, SSR markers have been extensively used for studying rice germplasm for diversity analysis, germplasm conservation or utilization [1, 13, 14]. Now-a-day, DNA markers are the most promising technique used for diversity estimation in rice. The aim of the present study is to suggest parents for future breeding program by diversity analysis using morphological and physiological traits and SSR markers.

MATERIALS AND METHODS

The field research was conducted using 21 T. Aman rice cultivars (Table 1) composed of 20 BRRI developed high yielding varieties and one most popular local variety (Rajasail) at BRRI regional station, Sonagazi, Feni. The experiment was laid out in RCBD with three replications. The individual plot size was 3.0 m. × 5.0 m having plot to plot and block to block distance of 0.5 m and 1.0 m, respectively. A fertilizer rate of 60-25-30 kg ha^{-1} of N-P-K in the form of urea, TSP and MP, respectively was applied. 30 days old seedlings were transplanted with the spacing of 20 cm × 20 cm. Data were recorded on13 morphological traits consisting of 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). Fourteen physiological traits were also recorded consisting of seedling vigor (mg/cm), days to flowering (50 %), panicle exsertion rate (%), flag leaf area (cm^2), days to maturity, LAI at panicle initiation and at flowering using length-width method [15]. CGR at panicle initiation and at flowering were measured following Radford [15].

Relative growth rates (RGR) at panicle initiation and at flowering were measured as growth rate per unit plant biomass following Tanaka *et al.* [16]. Net assimilation rates (NAR) at panicle initiation and at flowering were calculated using the formula of Kubota *et al.* [17].

Genetic diversity in respect of morphological and physiological traits was analyzed using GENSTAT v 5.5 software program where clustering was done using non-hierarchical classification using covariance matrix. The genetic divergence between two genotypes was calculated using following formula proposed by Mahalanobis [18]:

$$pD^2 = W^{ij}(xG^1_i - x^{-2}_i)(xG^1_j - x^{-2}_j)$$

Where:

pD^2 = Genetic divergence between two genotypes.

W^{ij} = The inverse of estimated variance and co-variance matrix.

x_i and x_j = The multiple measurements available on each individual.

Cluster analysis was also carried out using thirty four microsatellite or simple sequence repeat (SSR) markers distributed in 12 chromosomes (Table 2).

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

Sl. No.	Variety code	Name of the variety	Year of release	Sl. No.	Variety code	Name of the variety	Year of release
01.	V1	BR3	1973	12.	V12	BRRI dhan33	1997
02.	V2	BR4	1975	13.	V13	BRRI dhan34	1997
03.	V3	BR5	1976	14.	V14	BRRI dhan37	1998
04.	V4	BR10	1980	15.	V15	BRRI dhan38	1998
05.	V5	BR11	1980	16.	V16	BRRI dhan39	1999
06.	V6	BR22	1988	17.	V17	BRRI dhan40	2003
07.	V7	BR23	1988	18.	V18	BRRI dhan41	2003
08.	V8	BR25	1992	19.	V19	BRRI dhan44	2005
09.	V9	BRRI dhan30	1994	20.	V20	BRRI dhan46	2007
10.	V10	BRRI dhan31	1994	21.	V21	Rajasail	Local
11.	V11	BRRI dhan32	1994				variety

Table 2: SSR markers used for the determination of molecular variation

Sl. No.	Primer	Sequence of the primer		Motif	Chromosome location	Position (MB)
		Forward	Reverse			
01.	RM05	CACACTCCCATGCTAACAACTGG	CATCAAGAAGAGCAGTCCTGTGC	(AG)15	01	24.3
02.	RM490	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG	(CT)13	01	-
03.	RM1287	CCATTTGCAGTATGAACCATGC	ATCATGCAATAGCCGGTAGAGG	(AG)17	01	10.8
04.	RM3412	TGATGGATCTCTGAGGTGTAAAGAGC	TGCACTAATCTTTCTGCCACAGC	(CT)17	01	11.6
05.	RM8094	AAGTTTGTACACATCGTATACA	CGCGACCAGTACTACTACTA	(AT)31	01	11.2
06.	RM7075	GCGTTGCAGCGGAATTTGTAGG	CCCTGCTTCTCTCGTGCAGTCG	(ACAT)13	01	15.1
07.	RM10696B	TCCAGATCAACCAGCACATC	CCTGAAGGG.AGGGAGTATTTG	-	01	-
08.	RM10696	CCTTCGACTCCATGAAACAAACG	TCTCTTTGCCCTAACCCTATGTCC	(CT)11	01	11.0
09.	RM10713	ATGAACCCGGCGAACTGAAAGG	CTGGCTCCCTCAAGGTGATTGC	(AGA)12	01	11.2
10.	RM10720	GCAAACGTCTACGTGAGAAACAAGC	GCATGTGGTGCCTTAACATTTGG	(TA)34	01	11.4
11.	RM10927	TGGATCCCACTAATCCAAATGC	GAAAGACTCCTTCCAATGTTAGGC	(CT)10	01	15.7
12.	RM279	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	(GA)16	02	2.9
13.	RM424	GATTCCACGTCAGGATCTTCTGG	GCTCACCAGTTGAGATTGAAAGG	(CAT)9	02	11.38
14.	RM489	GAACAGGGACACAATGATGAGG	GACGATCGGACACCTAATTACAGC	(ATA)8	03	4.3
15.	RM6266	CACCTTCTTGAGAAGCTCCTTCG	GACATCGAGAGCGAGGACAGC	(CTC)9	03	23.6
16.	RM401	GCATGAGCTGCTCTCATTATTGTCC	GAAACGAACCAAACGTTCATCG	(CT)15	04	13.2
17.	RM1155	GACAGGGAGTGTGGCAACTATGC	GATCACAGACAATCATGGGTTGG	(AG)13	04	20.5
18.	RM1024	AACTGCCATCTCTGAAACTCTGC	CATCTCACTTCAGAAGGATCATAGCC	(AC)13	05	1.2
19.	RM289	TTCCATGGCACACAAGCC	CTGTGCACGAACTTCCAAAG	G11(GA)16	05	7.78
20.	RM469	TTACGTGATCACACAGGCTCTCC	AAGCTGAACAAGCCCTGAAAGG	(AG)15	06	0.6
21.	RM20224	AGTATGAAAGTCGGTGACGATGG	GAGATGTCACGTCTTCACTTAGGG	(CT)25	06	20.6
22.	RM5371	GCAGAGGATGCCCACTTAATTCC	GGGCTAGCTTTAGCTGCGTTGC	(TC)13	06	25.4
23.	RM436	ATTCCTGCAGTAAAGCACGG	CTTCGTGTACCTCCCCAAAC	(TAA)5	07	25.8
24.	RM455	CCACAAATTAATCCGGATCACACC	AGCATTGTGCAATCACGAGAAGG	(TTCT)5	07	22.3
25.	RM38	ACGAGCTCTCGATCAGCCTAGC	CACTCCATGGAAGAGGCAAGC	(GA)16	08	2.1
26.	RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC	(CT)21	08	24.14
27.	RM566	AATATGGTGGCGCGTACATCC	TGATCGAGCCAACAACAACTGG	(AG)15	09	14.7
28.	RM242	AAACACATGCTGCTGACACTTGC	TTACTAGATTTACCACGGCCAACG	(CT)26	09	18.6
29.	RM258	CTCCCTGGCCTTTAAAGCTGTCG	GACGAACAGCAGCAGAAGAGAAGC	(GA)21 (GGA)3	10	17.6
30.	RM590	GAGATCGAGGAGGAGGTGAGG	AGTACTGCCGATCATATGGAAGC	(TCT)10	10	22.6
31.	RM3428	GCCATTGACACCAAATGATCACC	GGCATATAAGGTCCATGGTGAATTGG	(CT)18	11	13.4
32.	RM286	CTGGCCTCTAGCTACAACCTTGC	AAACTCTCGCTGGATTCGATAGG	(GA)21	11	0.38
33.	RM17	GGAGAAAGAGAGGTGATCCTTTCC	CATGTCTTGGTGAGTGATGTTGC	(GA)21	12	26.95
34.	RM463	GAGGATTAATTAGCGTGTGACC	GTCGTGACATCTACTCAAATGG	(TTAT)5	12	22.09

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

Seeds were germinated in germination chamber and after 3 days, germinated seedlings were sown in pots. Then the pots were kept under the net house. DNA was collected from the leaf of 28 days old seedlings following modified miniscale protocol to isolate total genomic DNA. 1.5 µl 10x PCR buffer, 1.0 µl dNTPs, 0.5 µl P_F (Primer forward), 0.5 µl P_R (Primer reverse), 0.5 µl Taq polymerase enzyme and 10.0 µl double distilled water (a total of 12.5 µl volume) PCR components were used per reaction for the optimized protocol for microsatellite analysis. 0.5 % polyacrylamide gel was used for gel electrophoresis for 45 minutes to 75 minutes depending upon the allele size at around 100 mA current. The gels were stained for 30-35 minutes in dark using Syber safe staining solution and were documented using UVPRO Alpha Innotech gel documentation unit. Molecular weight for each amplified allele was measured in base pair using Alpha-Ease FC 5.0 software. The allele frequency data from Power Marker version 3.25 [19] was used to export the data in binary format (allele presence="I" and allele absence = "0") for analysis with NTSYS-pc version 2.2 [20]. Genetic distance was calculated using the "Nei distance" [21]. 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.

RESULTS AND DISCUSSION

Parent Selection Considering Morphological Diversity: Morphological diversity analysis produced five clusters of 21 rice varieties considering thirteen morphological traits (Figure 1). The distribution pattern of genotypes to different clusters indicated that the maximum number of genotypes (7) were included in cluster V followed by cluster II (5), cluster I (4), cluster III (3) and cluster IV (2).

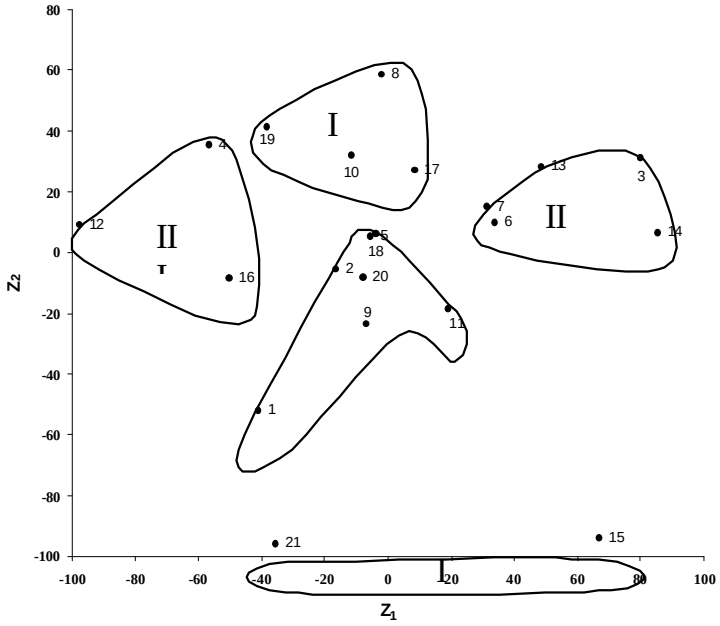

Fig. 1: Scattered diagram of T. Aman rice cultivars based on morphological diversity

Parent selection can be done in a number of fashions depending upon the objective of the breeding program. Inclusion of more diverse parents in hybridization is supposed to increase the chance of obtaining maximum heterosis and gives broad spectrum of variability in segregating generations [22-24]. It suggests distant parents for inclusion in breeding program. According to Falconer [25], parent is to be selected on the basis of specific objectives considering positive common criterion as additional benefit. Jagadev *et al*. [26] opined that breeding program perform better if parents are selected considering magnitude of genetic distance, contribution of different characters towards the total divergence and magnitude of cluster means for different characters are kept in consideration. Moreover, selection of one parent from each cluster and crossing them by a series of diallel cross were proved to be highly fruitful [27]. Among the proposed selection procedures, selection of one genotype from each group/cluster bearing better traits and considering positive common criterion as additional benefit may be done which is now being widely used in plant breeding. Considering this view, from morphological diversity analysis we may select BRRI dhan44 from cluster I, BR23 from cluster II, BRRI dhan33 from cluster III, BRRI dhan38 from cluster IV and BRRI dhan46 from cluster V. All the proposed varieties are high yielder. Among them BRRI dhan38 is aromatic fine rice variety which is much distantly positioned in the clustered diagram (Figure 1).

Parent Selection Considering Physiological Diversity: Analyzing 14 physiological traits by Genstat v 5.5 program, 21 T. Aman rice varieties were clustered into five groups (Figure 2). This clustering pattern was different from morphological clustering. So suggested parents by this analysis is a little different than that of morphological analysis.

Here, the maximum number of genotypes (6) were included in cluster I followed by cluster IV (5), cluster II (4), cluster III (3) and cluster V (3). If we consider one genotype from each cluster then we may select BRRI dhan44 from cluster I, BRRI dhan46 from cluster II, BRRI dhan33 from cluster III, BRRI dhan38 from cluster IV and BR11 from cluster V. This method has suggested 4 parents similar with morphological analysis but differed by suggesting BR11 instead of BR23. In scattered diagram, BR11 placed in distant position compared to other varieties (Figure 2).

Parent Selection from Molecular Diversity: Compared to morphological and physiological diversity analysis, molecular diversity analysis provides different clustering pattern. Here all the fine and aromatic rice varieties were grouped in a same cluster. DNA genome provides more powerful source of genetic polymorphism [28] and allows direct comparison of genetic diversity at DNA level, are phenotypically neutral, allow scoring of plants at any developmental stage and are not modified by environment and management practices [29].

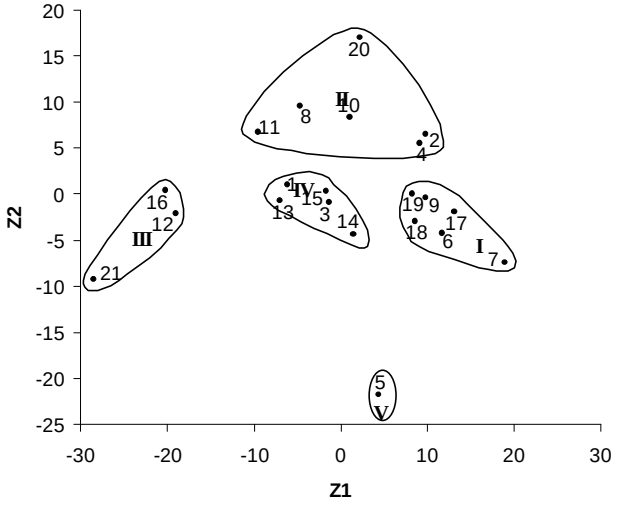

Fig. 2: Scattered diagram of T. Aman rice cultivars based on physiological diversity

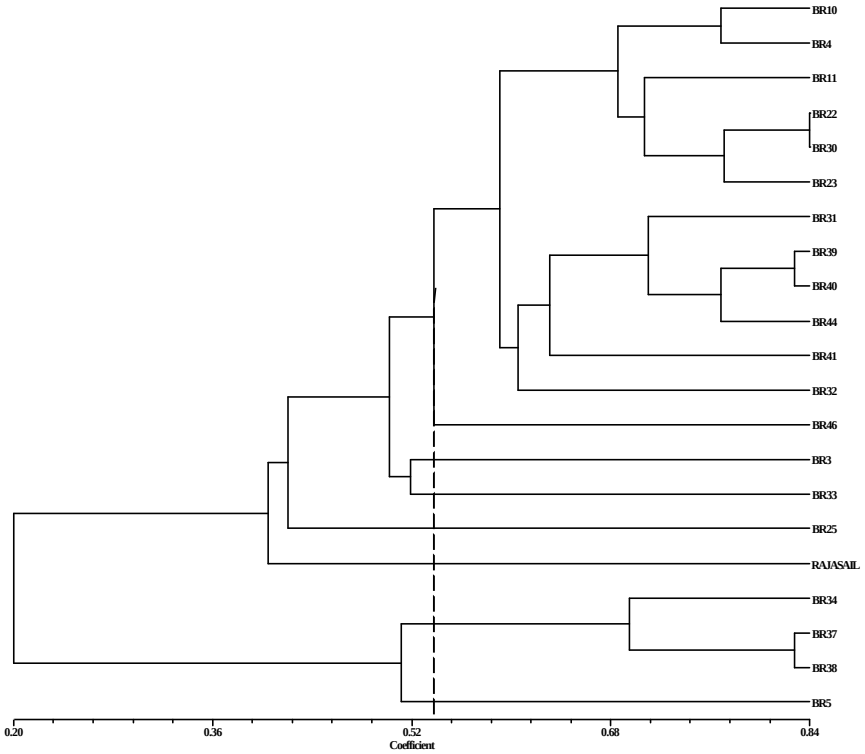

Fig. 3: A UPGMA cluster dendrogram showing five clusters among T. Aman rice cultivars based on the alleles detected by 34 SSR markers.

A number of studies reported that DNA markers are the most promising technique used in diversity analysis and breeding programs [30-32]. In this study, the grouping of cultivars based on SSR polymorphisms corresponds well to their known pedigree data also. However, five groups were obtained by molecular diversity analysis of the test varieties at a similarity coefficient of 0. 50. We may select BRRI dhan46 from cluster I, BRRI dhan33 from cluster II, BR25 from cluster III, Rajasail from cluster IV and BRRI dhan38 from cluster V. Here performances of varieties were considered from the morphological and physiological characters for selecting the best genotype from several genotypes of a cluster.

Comparison of Parent Selection: Among morphological, physiological and molecular diversity analysis, morphological and physiological diversity provide very close selection of parents. Both of these methods suggested four genotypes for selection as parental genotype (BRRI dhan33, BRRI dhan38, BRRI dhan44 and BRRI dhan46). Morphological diversity proposed BR23 where as physiological diversity proposed BR11as another parent.

These varieties were the most diverged genotypes in their respective analysis. On the other hand, molecular diversity suggested BRRI dhan33, BRRI dhan38 and BRRI dhan46 as parent similar with morphological and physiological diversity, but suggested BR25 and Rajasail instead of considering BR11 or BR23 or BRRI dhan44.

This trend of difference in parent selection is due to the differences in clustering pattern by these three methods. Dias *et al* [33] also found 5 clustering both in morphological and molecular (using SSR marker) diversity analysis with swapping genotypes suggesting different parents for breeding program in different analysis. Han-yong *et al.* [34] reported five clusters in each case using morphological traits, allozymes and SSR markers with different varietal distribution which is similar to this study. Several reports suggested swapping of varieties in clustering by different methods, based on which parent selection will also be different [35-37].

BRRI dhan33, BRRI dhan38 and BRRI dhan46 have been suggested as parent in all three methods of diversity analysis. The question is about BR11, BR23, BR25, BRRI dhan 44 and Rajasail. Among these varieties which variety/varieties is to be included for breeding program? It mainly depends on the purpose and thinking of the breeder. If breeder wants to get early maturing, bold type grain and medium saline tolerant genotype then Rajasail might be a better choice. If slender rice along with relatively tall plant stature and high yield is desired, then BR23 and BR25 could be selected. If higher grain yield with bold grain type and short plant stature is the purpose, then BR11 and BRRI dhan44 would be prioritized. However, Selection of parents from the above genotypes followed by cross in a diallele fashion may provide higher yielding segregants.

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