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GENETIC DIVERSITY ANALYSIS OF RICE (*Oryza sativa* L.) LANDRACES THROUGH RAPD MARKERS

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Abstract

The molecular marker is a useful tool for assessing genetic variations and resolving cultivar identities. Information on genetic diversity and relationships among rice landraces from Bangladesh is currently very limited. Thirty-five rice genotypes including 33 landraces and 01 HYV of Bangladesh and 1 Indian landrace of particular interest to breeding programs were evaluated by means of random amplified polymorphic DNA (RAPD) technique. For molecular characterization, RAPD markers *viz.,* OPC 03, OPC 04 and OPA 01 gave reproducible and distinct polymorphic amplified products. A total of 20 RAPD bands were scored of which 15 polymorphic amplification products were obtained by using these arbitrary primers. The size of amplified fragments were ranged from 550 to 1775 bp. Based on analysis performed on a similarity matrix using UPGMA, 35 genotypes were grouped into 2 main clusters. Landrace Sylhet balam and Mota aman was totally different from other genotypes. The information will facilitate selection of genotypes to serve as parents for effective rice breeding programs in Bangladesh.

Keywords: Crop Diversity, Characterization, Genetic Distance, Genetic Identity, Polymorphic Loci

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Introduction

Rice (*Oryza sativa* L.) diversity consists of landraces, improved cultivars, hybrids and closely related wild relatives. Landraces are local crop varieties developed in primitive agricultural system, rather than being deliberately bred, selected by the farmers over many generations. Landraces of rice played a very important role in the local food security and sustainable development of agriculture, in addition to their significance as genetic resource for rice genetic improvement. To maintain crop diversity, collection, characterization and conservation of traditional landraces are vital. Bangladesh is a good source of landraces of rice. To formulate a sustainable breeding program precise knowledge about genetic divergence for yield components is a crucial one as varietal improvement depends mainly on the selection of parents with high genetic divergence in hybridization that is supposed to increase the chance of obtaining maximum heterosis and give broad spectrum of variability in segregating generations. PCR-based molecular marker technique RAPD analysis is a reliable tool for assessing genetic diversity. This technique use a single short oligonucleotide primer (9-10 bp) of arbitrary DNA sequence and polymerase chain reaction (PCR) mediated amplification of random fragment from genomic DNA. So, among the available DNA molecular techniques, RAPD has many advantages over others such as ease and rapidity of analysis, a relatively low cost, availability of large numbers of primers and the requirement of a very small amount of DNA for analysis (Weising *et al*., 1995). Advantages associated with RAPDs have made them a favorite marker technique in mapping, the determination of phylogenetic relationships, genetic diversity, and identification of cultivars and parents in a number of plant species. Since the RAPD technique involves enzymatic amplification of target DNA by PCR using arbitrary primers, it is also called Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) or DNA Amplification Fingerprinting (DAF). RAPD markers tend to estimate intra or inter genetic distances more distantly related individuals. In addition, no prior knowledge of sequence is required. Since primers can be chosen arbitrarily, any organism can be mapped with the same set of primers. These advantages make RAPD markers far easier to work. The objective of this present study was to evaluate genetic divergence of 35 rice genotypes with three RAPD markers.

Materials and Methods

The experiment was carried out at the Biotechnology Laboratory, Biotechnology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, during July 2012 to December 2012.

Plant materials

Thirty five rice genotypes including 33 landraces (i.e. Hati bajore, Malagoti, Kuchra, Enghi, Jamai naru, Hari, Dakh shail, Moina moti, Marish shail, Patnai, Bhute shallot, Kute patnai, Moghai balam, Sada gotal, Khak shail, Jota balam, Khainol, Hamai, Sylhet balam, Mota aman, Ghigoj, Piarjat, Lal biroi, Lalanamia, Golapi, Asam binni, Kakua binni, Ledra binni, Rotisail, Genggeng binni, Jolkumri, Mowbinni and Bogi) and 1 HYV of Bangladesh (i.e. Binadhan-8) and 1 Indian landrace (i.e. Nona bokhra) were used for genetic diversity analysis through RAPD markers.

DNA extraction

In order to carry out RAPD analysis, young, vigorously growing fresh leaf samples were collected from 25 days old seedling of each genotype and used as the source of genomic DNA. DNA was extracted from the leaves of each genotype using the Cetyl Trimethyl Ammonium Bromide (CTAB) mini-prep method. The simplified mini scale procedure for DNA isolation in PCR analysis developed at IRRI was followed. Confirmation of the DNA was done through electrophoresis on a 0.8% agarose gel and DNA quantification was through the spectrophotometer's (spectronic@ Genesisr™). Ten primers of random sequence were screened for amplification of the DNA sequences. Primers resulting in faint or irreproducible bands were excluded from subsequent analysis. A final subset of seven primers exhibiting good quality banding patterns and sufficient variability from where finally three primers were selected for further analysis.

PCR reaction and electrophoresis

PCR reactions were performed on each DNA sample in a 10µl reaction mix containing 1 µl Ampli *Taq* polymerase buffer (10X), 2.5 µl Primer (10 µM), 1 µl dNTPs (250 µM), 0.2 µl Ampli *Taq* DNA polymerase and 3.3 µl sterile deionized water. Two µl genomic DNA was added and finally, total volume was made 10 µl. DNA amplification was performed in an oil-free thermal cycler. The reaction mix was preheated at 94^{0}C for 3 minutes followed by 40 cycles of 1 min denaturation at 94^{0}C, 1 min annealing at 54^{0}C and elongation or extension at 72^{0}C for 2 minutes. After the last cycle, a final step for 7 minutes at 72^{0}C to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4^{0}C. Electrophoresis was carried out in 0.5 X TBE buffer on a 1.5 % agarose gel and amplified fragments were visualized by staining with ethidium bromide.

Data analysis

The amplified bands were visually scored as present (1) and absent (0) separately for each individual primer. The scores of bands were pooled to create a single data matrix. This was used to estimate polymorphic loci, Nei's (1972) gene diversity, population differentiation (Gst), gene flow (N_m). Genetic distance (GD) and to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populations using a computer program, POPGENE (Version 1.31); (Yeh *et al*., 1997).

Results and Discussion

RAPD banding pattern

Ten primers were initially screened for their ability to produce polymorphic patterns and out of 10, three primers *viz.,* OPC 03, OPC 04 and OPA 01 gave reproducible and distinct polymorphic amplified products. DNA amplification from all the primers tested in this study was not consistently reproducible, is a very common feature of RAPD technique. A total of 20 RAPD bands were scored of which 15 (73.61%) polymorphic amplification products were obtained by using these arbitrary primers. This proportion of polymorphism was similar compared to previous RAPD analysis in rice genotypes by Skaria *et al.* (2011) who obtained 72.27% of polymorphic products. The size of the amplification products ranged from 550-1775 bp (Table 1). The selected 3 primers produced comparatively maximum number of high intensity band with minimal smearing, good technical resolution and sufficient variation among different cultivars. The dissimilar numbers of bands were generated by primer OPC 03, OPC 04 and OPA 01. Besides, the primer OPC-03 amplified maximum number of polymorphic bands (100%) while the primer OPA-01 generated the least (33.33%) polymorphic bands which were minimal in number. The banding patterns of 35 rice using primers OPC 03, OPC 04 and OPA 01 are shown in Figs. 1, 2 and 3, respectively. The DNA polymorphisms were detected according to the presence and absence of band. Absence of band may be caused by failure of primer to anneal a site in some individuals due to nucleotide, sequences difference or by insertions or deletions between primer sites (Clark and Lanigan, 1993). Frequencies of polymorphic loci (RAPD markers) in 35 rice genotypes were presented in Table 2.

Table 1. RAPD primers with corresponding bands score and their size range together with polymorphic bands observed in 35 rice genotypes

Primer code	Sequences (5´-3´)	Total number of bands scored	Size ranges (bp)	Number of polymorphic bands	Proportion of polymorphic loci (%)
OPC 03	GGGGGTCTTT	6	550-1650	6	100.00
OPC 04	CCGCATCTAC	8	600-1700	7	87.50
OPA 01	CAGGCCCTTC	6	600-1775	2	33.33
Total		20		15	220.83
Average		6.67		5.00	73.61

Genetic variation

The values of Nei's (1973) gene diversity and Shannon's information index for different accessions of rice across all loci are shown in Table 2. The estimate of Nei's (1973) genetic diversity for 35 genotypes of rice was 0.12 and Shannon's information index was 0.22. There was a high level of genetic variation among the studied genotypes of rice from the proportion of polymorphic loci point of view.

Table 2. Summary of Frequencies of polymorphic gene, genetic diversity statistics and Shanon information index for all loci in 35 rice genotypes

Loci	Gene frequency	Gene diversity (h)	Shanon information index (i)
OPC 03-1	0.8000	0.3200	0.5004
OPC 03-2	0.8857	0.2024	0.3554
OPC 03-3	0.9429	0.1078	0.2190
OPC 03-4	0.9429	0.1078	0.2190
OPC 03-5	0.9714	0.0555	0.1297
OPC 03-6	0.9143	0.1567	0.2925
OPC 04 -1	0.7714	0.3527	0.5375
OPC 04 -2	0.6000	0.4800	0.6730
OPC 04 -3	0.9714	0.0555	0.1297
OPC 04 -4	0.9714	0.0555	0.1297
OPC 04 -5	0.0000	0.0000	0.0000
OPC 04 -6	0.2286	0.3527	0.5375
OPC 04 -7	0.9714	0.0555	0.1297
OPC 04 -8	0.9714	0.0555	0.1297
OPA 01-1	0.0000	0.0000	0.0000
OPA 01-2	0.0857	0.1567	0.2925
OPA 01-3	0.0000	0.0000	0.0000
OPA 01-4	0.0000	0.0000	0.0000
OPA 01-5	0.0000	0.0000	0.0000
OPA 01-6	0.0286	0.0555	0.1297
Mean		0.1285	0.2203
St. Dev.		0.1420	0.2059

Genetic distance and genetic identity

Pair-wise comparisons of Nei's (1972) genetic distance (GD) between rice genotypes were computed from combined data for the three primers and the values ranged from 0.0000 to 0.9000 (Table 3). Comparatively higher genetic distance was observed between the genotypes Golapi vs. Bogi.

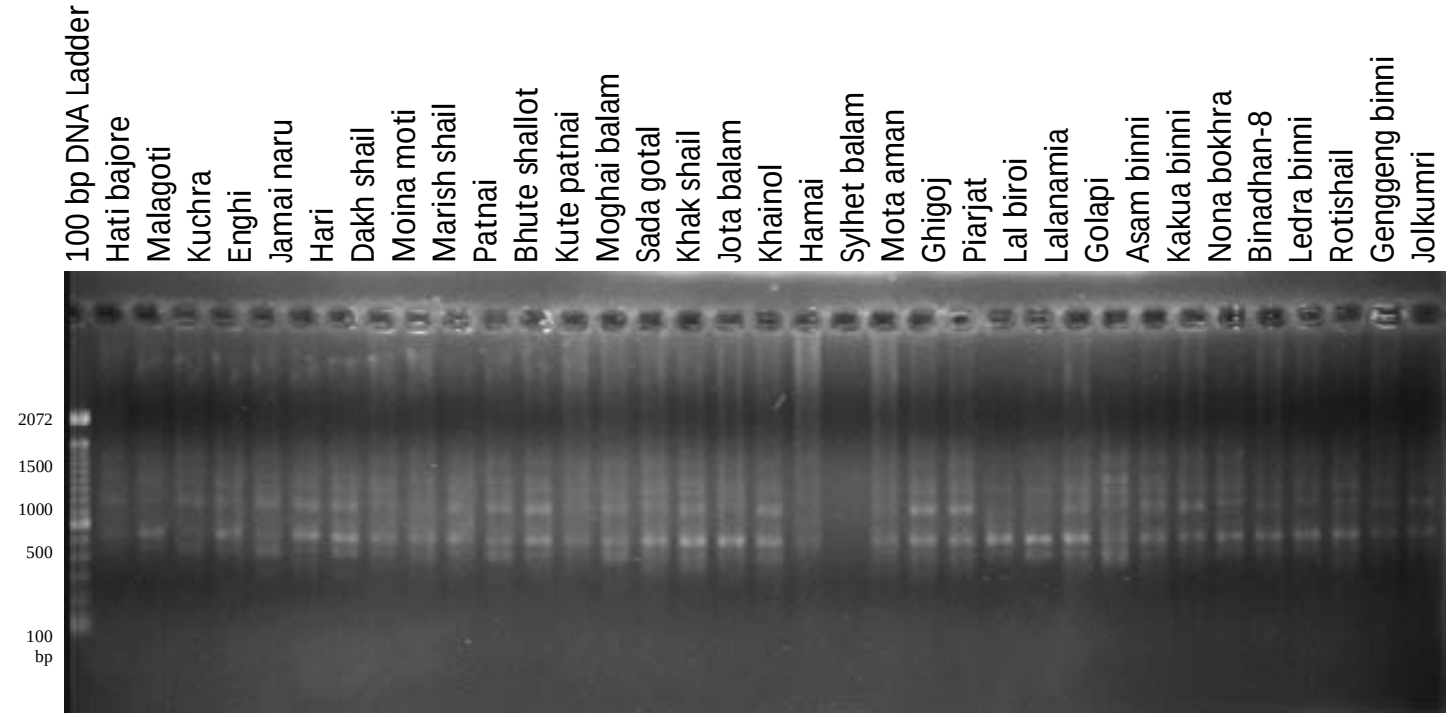

Fig. 1. RAPD profiles of different 35 rice genotypes using primer OPC-03

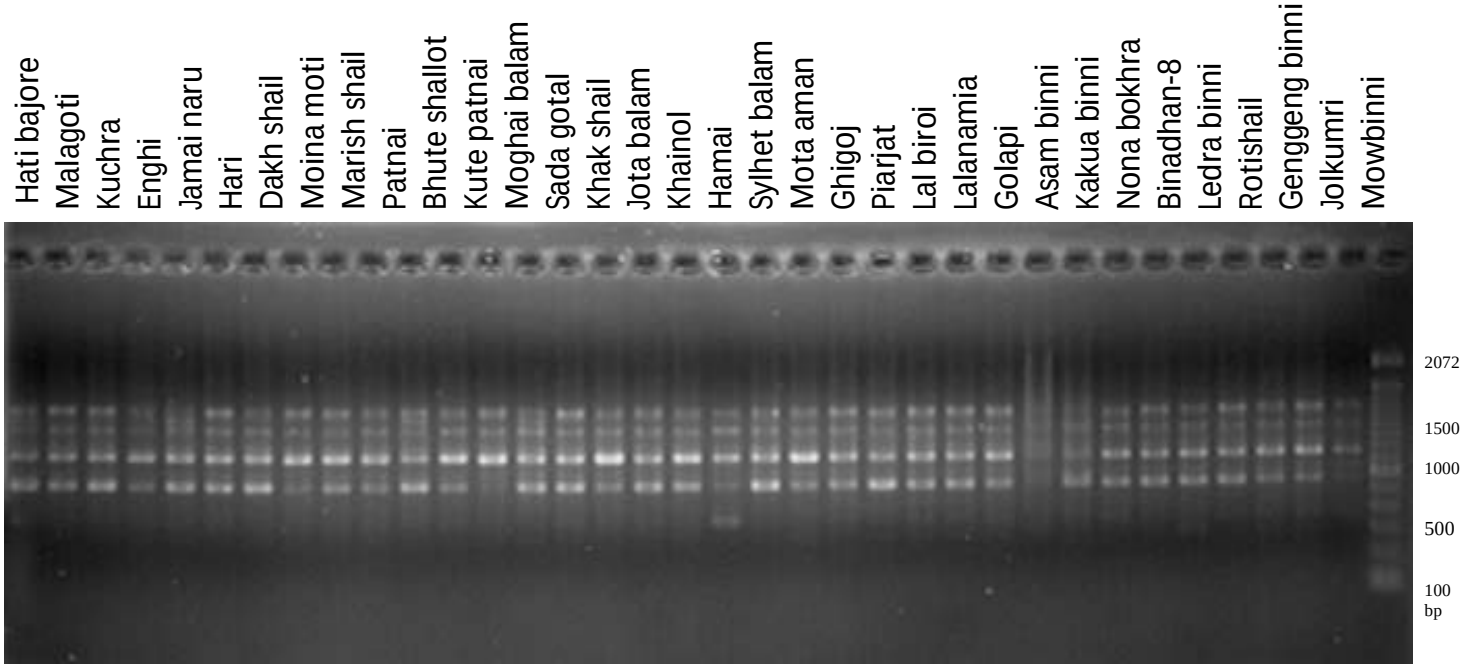

Fig. 2. RAPD profiles of different 35 rice genotypes using primer OPC-04

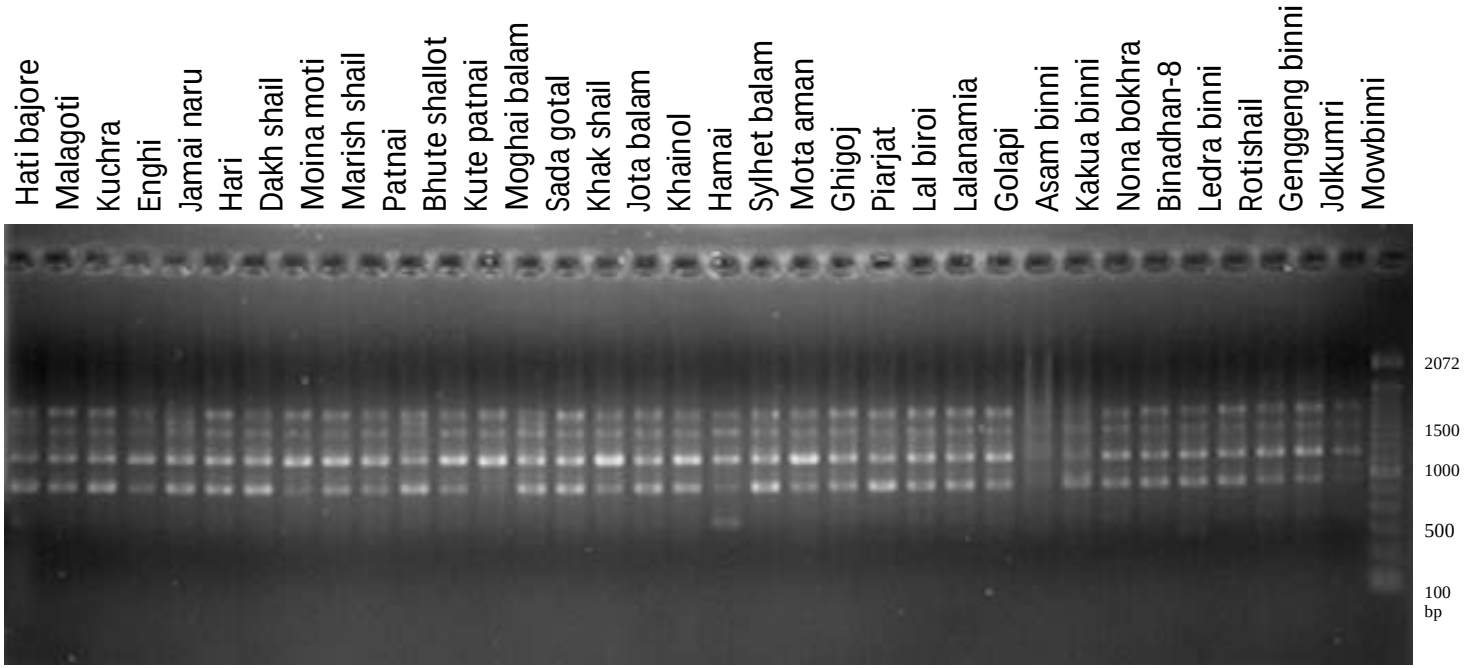

Fig. 3. RAPD profiles of different 35 rice genotypes using primer OPA-01

Table 3. Summary of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between 35 rice genotypes

Acce.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	****	0.9500	1.0000	0.9500	0.9000	0.9500	0.9000	0.9000	0.9000	0.9500	0.8500	0.9000	0.9500	0.8500	0.9000	0.9000	0.9000	0.9500
2	0.0513	****	0.9500	0.9000	0.8500	1.0000	0.8500	0.8500	0.9500	0.9000	0.8000	0.8500	0.9000	0.8000	0.8500	0.8500	0.9500	0.9000
3	0.0000	0.0513	****	0.9500	0.9000	0.9500	0.9000	0.9000	0.9000	0.9500	0.8500	0.9000	0.9500	0.8500	0.9000	0.9000	0.9000	0.9500
4	0.0513	0.1054	0.0513	****	0.9500	0.9000	0.9500	0.9500	0.8500	0.9000	0.9000	0.9500	0.9000	0.9000	0.9500	0.9500	0.8500	0.9000
5	0.1054	0.1625	0.1054	0.0513	****	0.8500	0.9000	0.9000	0.8000	0.8500	0.9500	0.9000	0.8500	0.9500	0.9000	0.9000	0.8000	0.8500
6	0.0513	0.0000	0.0513	0.1054	0.1625	****	0.8500	0.8500	0.9500	0.9000	0.8000	0.8500	0.9000	0.8000	0.8500	0.8500	0.9500	0.9000
7	0.1054	0.1625	0.1054	0.0513	0.1054	0.1625	****	1.0000	0.9000	0.9500	0.9500	1.0000	0.9500	0.9500	1.0000	1.0000	0.9000	0.9500
8	0.1054	0.1625	0.1054	0.0513	0.1054	0.1625	0.0000	****	0.9000	0.9500	0.9500	1.0000	0.9500	0.9500	1.0000	1.0000	0.9000	0.9500
9	0.1054	0.0513	0.1054	0.1625	0.2231	0.0513	0.1054	0.1054	****	0.9500	0.8500	0.9000	0.9500	0.8500	0.9000	0.9000	1.0000	0.9500
10	0.0513	0.1054	0.0513	0.1054	0.1625	0.1054	0.0513	0.0513	0.0513	****	0.9000	0.9500	1.0000	0.9000	0.9500	0.9500	0.9500	1.0000
11	0.1625	0.2231	0.1625	0.1054	0.0513	0.2231	0.0513	0.0513	0.1625	0.1054	****	0.9500	0.9000	1.0000	0.9500	0.9500	0.8500	0.9000
12	0.1054	0.1625	0.1054	0.0513	0.1054	0.1625	0.0000	0.0000	0.1054	0.0513	0.0513	****	0.9500	0.9500	1.0000	1.0000	0.9000	0.9500
13	0.0513	0.1054	0.0513	0.1054	0.1625	0.1054	0.0513	0.0513	0.0513	0.0000	0.1054	0.0513	****	0.9000	0.9500	0.9500	0.9500	1.0000
14	0.1625	0.2231	0.1625	0.1054	0.0513	0.2231	0.0513	0.0513	0.1625	0.1054	0.0000	0.0513	0.1054	****	0.9500	0.9500	0.8500	0.9000
15	0.1054	0.1625	0.1054	0.0513	0.1054	0.1625	0.0000	0.0000	0.1054	0.0513	0.0513	0.0000	0.0513	0.0513	****	1.0000	0.9000	0.9500
16	0.1054	0.1625	0.1054	0.0513	0.1054	0.1625	0.0000	0.0000	0.1054	0.0513	0.0513	0.0000	0.0513	0.0513	0.0000	****	0.9000	0.9500
17	0.1054	0.0513	0.1054	0.1625	0.2231	0.0513	0.1054	0.1054	0.0000	0.0513	0.1625	0.1054	0.0513	0.1625	0.1054	0.1054	****	0.9500
18	0.0513	0.1054	0.0513	0.1054	0.1625	0.1054	0.0513	0.0513	0.0513	0.0000	0.1054	0.0513	0.0000	0.1054	0.0513	0.0513	0.0513	****
19	0.4308	0.5108	0.4308	0.3567	0.4308	0.5108	0.4308	0.4308	0.5978	0.5108	0.5108	0.4308	0.5108	0.5108	0.4308	0.4308	0.5978	0.5108
20	0.4308	0.5108	0.4308	0.5108	0.5978	0.5108	0.4308	0.4308	0.4308	0.3567	0.5108	0.4308	0.3567	0.5108	0.4308	0.4308	0.4308	0.3567
21	0.0513	0.1054	0.0513	0.1054	0.1625	0.1054	0.0513	0.0513	0.0513	0.0000	0.1054	0.0513	0.0000	0.1054	0.0513	0.0513	0.0513	0.0000
22	0.0513	0.1054	0.0513	0.1054	0.1625	0.1054	0.0513	0.0513	0.0513	0.0000	0.1054	0.0513	0.0000	0.1054	0.0513	0.0513	0.0513	0.0000
23	0.1625	0.1054	0.1625	0.2231	0.2877	0.1054	0.1625	0.1625	0.0513	0.1054	0.2231	0.1625	0.1054	0.2231	0.1625	0.1625	0.0513	0.1054
24	0.1625	0.2231	0.1625	0.2231	0.2877	0.2231	0.1625	0.1625	0.1625	0.1054	0.2231	0.1625	0.1054	0.2231	0.1625	0.1625	0.1625	0.1054
25	0.1054	0.1625	0.1054	0.1625	0.2231	0.1625	0.1054	0.1054	0.1054	0.0513	0.1625	0.1054	0.0513	0.1625	0.1054	0.1054	0.1054	0.0513
26	0.1054	0.0513	0.1054	0.1625	0.2231	0.0513	0.1054	0.1054	0.0000	0.0513	0.1625	0.1054	0.0513	0.1625	0.1054	0.1054	0.0000	0.0513
27	0.1054	0.1625	0.1054	0.0513	0.1054	0.1625	0.0000	0.0000	0.1054	0.0513	0.0513	0.0000	0.0513	0.0513	0.0000	0.0000	0.1054	0.0513
28	0.1054	0.1625	0.1054	0.0513	0.1054	0.1625	0.0000	0.0000	0.1054	0.0513	0.0513	0.0000	0.0513	0.0513	0.0000	0.0000	0.1054	0.0513
29	0.0513	0.1054	0.0513	0.1054	0.1625	0.1054	0.0513	0.0513	0.0513	0.0000	0.1054	0.0513	0.0000	0.1054	0.0513	0.0513	0.0513	0.0000
30	0.1054	0.1625	0.1054	0.0513	0.1054	0.1625	0.0000	0.0000	0.1054	0.0513	0.0513	0.0000	0.0513	0.0513	0.0000	0.0000	0.1054	0.0513
31	0.2877	0.3567	0.2877	0.2231	0.2877	0.3567	0.2877	0.2877	0.4308	0.3567	0.3567	0.2877	0.3567	0.3567	0.2877	0.2877	0.4308	0.3567
32	0.0513	0.1054	0.0513	0.1054	0.1625	0.1054	0.0513	0.0513	0.0513	0.0000	0.1054	0.0513	0.0000	0.1054	0.0513	0.0513	0.0513	0.0000
33	0.0513	0.1054	0.0513	0.1054	0.1625	0.1054	0.0513	0.0513	0.0513	0.0000	0.1054	0.0513	0.0000	0.1054	0.0513	0.0513	0.0513	0.0000
34	0.2231	0.1625	0.2231	0.2877	0.3567	0.1625	0.2231	0.2231	0.1054	0.1625	0.2877	0.2231	0.1625	0.2877	0.2231	0.2231	0.1054	0.1625
35	0.2231	0.1625	0.2231	0.2877	0.3567	0.1625	0.2231	0.2231	0.1054	0.1625	0.2877	0.2231	0.1625	0.2877	0.2231	0.2231	0.1054	0.1625

Table 3. Contd.

Acce.	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	0.6500	0.6500	0.9500	0.9500	0.8500	0.8500	0.9000	0.9000	0.9000	0.9000	0.9500	0.9000	0.7500	0.9500	0.9500	0.8000	0.8000
2	0.6000	0.6000	0.9000	0.9000	0.9000	0.8000	0.8500	0.9500	0.8500	0.8500	0.9000	0.8500	0.7000	0.9000	0.9000	0.8500	0.8500
3	0.6500	0.6500	0.9500	0.9500	0.8500	0.8500	0.9000	0.9000	0.9000	0.9000	0.9500	0.9000	0.7500	0.9500	0.9500	0.8000	0.8000
4	0.7000	0.6000	0.9000	0.9000	0.8000	0.8000	0.8500	0.8500	0.9500	0.9500	0.9000	0.9500	0.8000	0.9000	0.9000	0.7500	0.7500
5	0.6500	0.5500	0.8500	0.8500	0.7500	0.7500	0.8000	0.8000	0.9000	0.9000	0.8500	0.9000	0.7500	0.8500	0.8500	0.7000	0.7000
6	0.6000	0.6000	0.9000	0.9000	0.9000	0.8000	0.8500	0.9500	0.8500	0.8500	0.9000	0.8500	0.7000	0.9000	0.9000	0.8500	0.8500
7	0.6500	0.6500	0.9500	0.9500	0.8500	0.8500	0.9000	0.9000	1.0000	1.0000	0.9500	1.0000	0.7500	0.9500	0.9500	0.8000	0.8000
8	0.6500	0.6500	0.9500	0.9500	0.8500	0.8500	0.9000	0.9000	1.0000	1.0000	0.9500	1.0000	0.7500	0.9500	0.9500	0.8000	0.8000
9	0.5500	0.6500	0.9500	0.9500	0.9500	0.8500	0.9000	1.0000	0.9000	0.9000	0.9500	0.9000	0.6500	0.9500	0.9500	0.9000	0.9000
10	0.6000	0.7000	1.0000	1.0000	0.9000	0.9000	0.9500	0.9500	0.9500	0.9500	1.0000	0.9500	0.7000	1.0000	1.0000	0.8500	0.8500
11	0.6000	0.6000	0.9000	0.9000	0.8000	0.8000	0.8500	0.8500	0.9500	0.9500	0.9000	0.9500	0.7000	0.9000	0.9000	0.7500	0.7500
12	0.6500	0.6500	0.9500	0.9500	0.8500	0.8500	0.9000	0.9000	1.0000	1.0000	0.9500	1.0000	0.7500	0.9500	0.9500	0.8000	0.8000
13	0.6000	0.7000	1.0000	1.0000	0.9000	0.9000	0.9500	0.9500	0.9500	0.9500	1.0000	0.9500	0.7000	1.0000	1.0000	0.8500	0.8500
14	0.6000	0.6000	0.9000	0.9000	0.8000	0.8000	0.8500	0.8500	0.9500	0.9500	0.9000	0.9500	0.7000	0.9000	0.9000	0.7500	0.7500
15	0.6500	0.6500	0.9500	0.9500	0.8500	0.8500	0.9000	0.9000	1.0000	1.0000	0.9500	1.0000	0.7500	0.9500	0.9500	0.8000	0.8000
16	0.6500	0.6500	0.9500	0.9500	0.8500	0.8500	0.9000	0.9000	1.0000	1.0000	0.9500	1.0000	0.7500	0.9500	0.9500	0.8000	0.8000
17	0.5500	0.6500	0.9500	0.9500	0.9500	0.8500	0.9000	1.0000	0.9000	0.9000	0.9500	0.9000	0.6500	0.9500	0.9500	0.9000	0.9000
18	0.6000	0.7000	1.0000	1.0000	0.9000	0.9000	0.9500	0.9500	0.9500	0.9500	1.0000	0.9500	0.7000	1.0000	1.0000	0.8500	0.8500
19	****	0.7000	0.6000	0.6000	0.6000	0.7000	0.6500	0.5500	0.6500	0.6500	0.6000	0.6500	0.6000	0.6000	0.6000	0.5500	0.5500
20	0.3567	****	0.7000	0.7000	0.7000	0.8000	0.7500	0.6500	0.6500	0.6500	0.7000	0.6500	0.5000	0.7000	0.7000	0.7500	0.7500
21	0.5108	0.3567	****	1.0000	0.9000	0.9000	0.9500	0.9500	0.9500	0.9500	1.0000	0.9500	0.7000	1.0000	1.0000	0.8500	0.8500
22	0.5108	0.3567	0.0000	****	0.9000	0.9000	0.9500	0.9500	0.9500	0.9500	1.0000	0.9500	0.7000	1.0000	1.0000	0.8500	0.8500
23	0.5108	0.3567	0.1054	0.1054	****	0.9000	0.8500	0.9500	0.8500	0.8500	0.9000	0.8500	0.6000	0.9000	0.9000	0.8500	0.8500
24	0.3567	0.2231	0.1054	0.1054	0.1054	****	0.9500	0.8500	0.8500	0.8500	0.9000	0.8500	0.7000	0.9000	0.9000	0.8500	0.8500
25	0.4308	0.2877	0.0513	0.0513	0.1625	0.0513	****	0.9000	0.9000	0.9000	0.9500	0.9000	0.7500	0.9500	0.9500	0.9000	0.9000
26	0.5978	0.4308	0.0513	0.0513	0.0513	0.1625	0.1054	****	0.9000	0.9000	0.9500	0.9000	0.6500	0.9500	0.9500	0.9000	0.9000
27	0.4308	0.4308	0.0513	0.0513	0.1625	0.1625	0.1054	0.1054	****	1.0000	0.9500	1.0000	0.7500	0.9500	0.9500	0.8000	0.8000
28	0.4308	0.4308	0.0513	0.0513	0.1625	0.1625	0.1054	0.1054	0.0000	****	0.9500	1.0000	0.7500	0.9500	0.9500	0.8000	0.8000
29	0.5108	0.3567	0.0000	0.0000	0.1054	0.1054	0.0513	0.0513	0.0513	0.0513	****	0.9500	0.7000	1.0000	1.0000	0.8500	0.8500
30	0.4308	0.4308	0.0513	0.0513	0.1625	0.1625	0.1054	0.1054	0.0000	0.0000	0.0513	****	0.7500	0.9500	0.9500	0.8000	0.8000
31	0.5108	0.6931	0.3567	0.3567	0.5108	0.3567	0.2877	0.4308	0.2877	0.2877	0.3567	0.2877	****	0.7000	0.7000	0.6500	0.6500
32	0.5108	0.3567	0.0000	0.0000	0.1054	0.1054	0.0513	0.0513	0.0513	0.0513	0.0000	0.0513	0.3567	****	1.0000	0.8500	0.8500
33	0.5108	0.3567	0.0000	0.0000	0.1054	0.1054	0.0513	0.0513	0.0513	0.0513	0.0000	0.0513	0.3567	0.0000	****	0.8500	0.8500
34	0.5978	0.2877	0.1625	0.1625	0.1625	0.1625	0.1054	0.1054	0.2231	0.2231	0.1625	0.2231	0.4308	0.1625	0.1625	****	1.0000
35	0.5978	0.2877	0.1625	0.1625	0.1625	0.1625	0.9000	0.1054	0.2231	0.2231	0.1625	0.2231	0.4308	0.1625	0.1625	0.0000	****

Genetic differentiation and rate of migration among subdivided population

Nei's analysis of gene diversity in subdivided populations presented the gene flow (Nm+) value of 0.000 and the proportion of total genetic diversity (Gst) was 1.0000. Hardy-Weinberg expectation of average heterozygosity in sub- population (Ht) was 0.1285, whereas the heterozygosity (Hs) was 0.0000 (Table 4) (McDermott and McDonald, 1993).

Table 4. Summary of genetic variation statistics across all loci

Loci	Sample Size	Ht	Hs	Gst	Nm+
OPC 03-1	35	0.3200	0.0000	1.0000	0.0000
OPC 03-2	35	0.2024	0.0000	1.0000	0.0000
OPC 03-3	35	0.1078	0.0000	1.0000	0.0000
OPC 03-4	35	0.1078	0.0000	1.0000	0.0000
OPC 03-5	35	0.0555	0.0000	1.0000	0.0000
OPC 03-6	35	0.1567	0.0000	1.0000	0.0000
OPC 04 -1	35	0.3527	0.0000	1.0000	0.0000
OPC 04 -2	35	0.4800	0.0000	1.0000	0.0000
OPC 04 -3	35	0.0555	0.0000	1.0000	0.0000
OPC 04 -4	35	0.0555	0.0000	1.0000	0.0000
OPC 04 -5	35	0.0000	0.0000	****	****
OPC 04 -6	35	0.3527	0.0000	1.0000	0.0000
OPC 04 -7	35	0.0555	0.0000	1.0000	0.0000
OPC 04 -8	35	0.0555	0.0000	1.0000	0.0000
OPA 01-1	35	0.0000	0.0000	****	****
OPA 01-2	35	0.1567	0.0000	1.0000	0.0000
OPA 01-3	35	0.0000	0.0000	****	****
OPA 01-4	35	0.0000	0.0000	****	****
OPA 01-5	35	0.0000	0.0000	****	****
OPA 01-6	35	0.0555	0.0000	1.0000	0.0000
Mean	35	0.1285	0.0000	1.0000	0.0000
St. Dev.		0.0202	0.0000		

H_t = Hardy-Weinberg average heterozygosity expected in sub-population
H_s = Hardy-Weinberg average heterozygosity obtained in sub-population
G_{st} = Co-efficient of gene differentiation
Nm = Estimate of gene flow from Gst or Gcs. e.g., $N_m = 0.5(1-G_{st})/G_{st}$
****= Infinity
The number of polymorphic loci is : 15
The percentage of polymorphic loci is : 75.00

UPGMA Dendrogram

A dendrogram was constructed based on Nei's (1972) genetic distance following the Unweighted Pair Group Method of Arithmetic Means (UPGMA). The 35 genotypes of rice were grouped into 2 main clusters namely cluster 1 and cluster 2 (Fig. 4).

Genotypes Sylhet balam and Mota aman was included in cluster 2. Cluster 2 i.e. Sylhet balam and Mota aman was totally different from other genotypes. So, genetic relationship was not present between cluster 1 genotypes with cluster 2.

Genotypes belong in cluster 1 were Hati bajore, Moghai balam, Golapi, Malagoti, Sada gotal, Asam binni, Kuchra, Khak shail, Kakua binni, Enghi, Jota balam, Nona bokhra, Jamai naru, Khainol, Binadhan-8, Hari, Hamai, Ledra binni, Dakh shail, Rotisail, Moina moti, Genggeng binni, Marish shail, Ghigoj, Jolkumri, Patnai, Piarjat, Mowbinni, Bhute shalot, Lal biroi, Bogi, Kute patnai and Lalanamia.

The genotypes of cluster 1 again divided into two sub-cluster 1 and sub-cluster 2. Sub-cluster 1 consisted of genotypes Hati bajore, Moghai balam, Golapi, Malagoti, Sada Gotal, Asam binni, Kuchra, Khak shail, Kakua binni, Enghi, Jota balam, Nona bokhra, Jamai Naru, Khainol, Binadhan-8, Hari, Hamai, Ledra binni, Dakh shail, Moina moti, Genggeng binni, Marish shail, Ghigoj, Jolkumri, Patnai, Piarjat, Mowbinni, Bhute shalot, Lal biroi, Bogi, Kute patnai and Lalanamia. Sub-cluster 2 formed by only one genotype Rotisail, genetic relationship was present between sub-clusters.

The genotypes of sub-cluster 1 again divided into two sub-sub-cluster 1 and sub-sub-cluster 2. Sub-sub-cluster 1 consisted of genotypes Hati bajore, Moghai balam, Golapi, Malagoti, Sada gotal, Asam binni, Kuchra, Khak shail, Kakua binni, Enghi, Jota balam, Nona bokhra, Jamai naru, Khainol, Binadhan-8, Hari, Hamai, Ledra binni, Dakh shail, Moina Moti, Genggeng binni, Marish shail, Ghigoj, Jolkumri, Patnai, Piarjat, Bhute shalot, Lal biroi, Kute patnai and Lalanamia. Sub-sub-cluster 2 formed by two genotypes Mowbinni and Bogi.

Genotypic variations based on molecular characterization indicated that genotypes belonging to different clusters depend on their genetic components itself, but not at geographical origin at all. Therefore, it could be concluded that for further research program, especially for hybridization, genotype could be selected from different clusters will be provided maximum heterosis regarding yield.

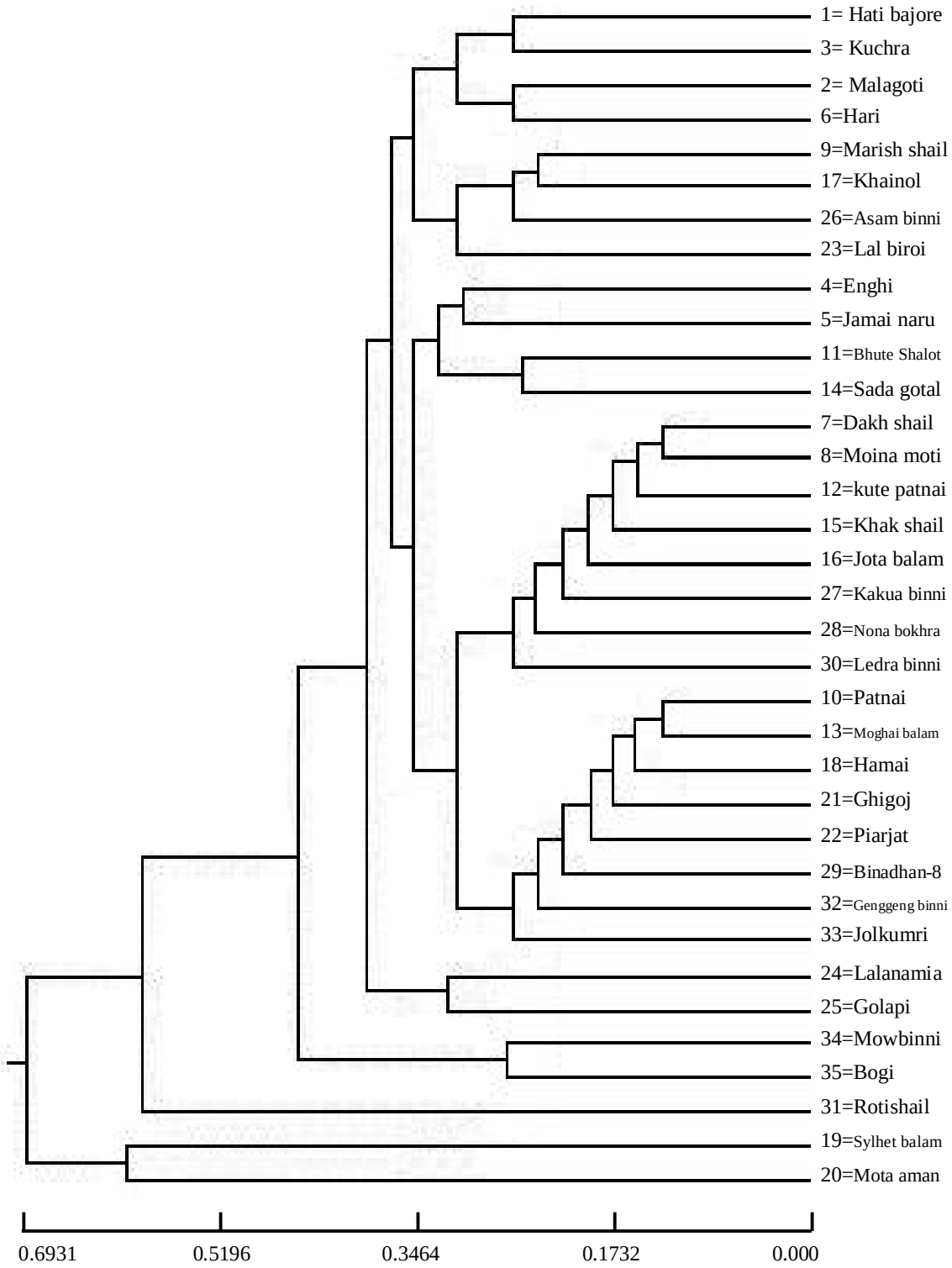

Fig. 4. Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Neis's (1972) genetic distance, summarizing data on differentiation in 35 rice genotypes according to RAPD analysis.

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