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Analysis of Genetic Diversity and Population Structure of Some Bangladeshi Rice Landraces and HYV

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Abstract

Information on the patterns of genetic variation and population structure is essential for rational use and efficient management of germplasms. It helps in monitoring germplasm and can also be used to predict potential genetic gains. Therefore, in the present study genetic diversity of 59 rice genotypes were assessed using 8 simple sequence repeat (SSR) primers. By the DNA profiling, a total of 114 alleles were detected. Allele number per/locus ranged from 9 to 27, with an average of 14.25. Average polymorphism information content (PIC) value was 0.857 with lowest 0.767 to highest 0.857. Mean gene diversity over all SSR loci was 0.870 with a range from 0.792 to 0.948. Fst values for each locus varied from 0.071 to 0.262. Genetic distance between the variety pair ranged from 0.33 to 1.0. The lowest genetic distance was found between Rajashili and Kumragori (2). Cluster and principal coordinate analysis (PCoA) analysis revealed similar pattern of variation. Marker RM11300 was found most polymorphic and robust among the accessions and can be widely used for rice germplasm characterization. The exclusive variability and unique feature of germplasm found in this study can be a gateway for both domestic and global rice improvement.

Keywords: Rice; Genetic diversity; SSR; DNA fingerprinting; Cluster analysis; AMOVA.

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1. Introduction

Rice landraces possess tremendous genetic variation. Evaluation of this terrific genetic diversity is very important for their rational use to diverse needs. It is not possible to meet

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up the striking demands of resource-restricted grower by the limited number of high-yielding and modern varieties [1]. Yields of improved rice varieties in favorable conditions have reached a plateau or even subsequently declined in many countries including Bangladesh. It is recommended that a large number of high performance varieties are required to accomplish specific socio-economic and agricultural needs. The analysis of population genetic parameters is thus thought to be useful in identifying the average genetic variation of a field/village/region. The differences in allele frequencies among different populations and the differences in the level of polymorphism among populations will serve greatly in this purpose [2]. Understanding the population genetic structure will also be helpful in monitoring diversity loss over time and space, and also for devising a rational conservation plan for management of farmer landraces on-farm. Many factors (pattern of evolution, breeding methods, ecological and geographical factors, past bottleneck, human etc.) affect the extent and distribution of genetic diversity in a crop species. Maximum amount of this variation of a species may be observed within individual populations, or divided into various populations. Therefore, balanced use of germplasm and its conservation and management requires proper understanding of genetic diversity and its distribution in various individuals and species. Recently it has been identified that still there is a remarkable amount of unexploited genetic diversity exist in the rice primary gene pool. This can be used for increasing variability in local rice and for adaptation to different agroecological settings [3-4]. Wild species of *Oryza* may also serve as a valuable source of potential allele for biotic and abiotic stress resistance [5-6]. A long history of traditional rice production across numerous environments in Bangladesh has led to a diverse array of local Bangladeshi rice varieties. Although a few varieties have been incorporated into modern breeding programs, the vast majority of traditional Bangladeshi germplasm remains uncharacterized and underutilized. Thus, in the present study 59 rice genotypes were evaluated with 8 simple sequence repeats marker for exploring the available variability. Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique genotypes that compliment existing cultivars.

2. Materials and Methods

The experiment was conducted in the plant biotechnology laboratory of plant breeding division of Bangladesh Institute of Nuclear Agriculture (BINA) from June 2010 to December 2011. Fifty nine rice genotypes comprising of land races, high yielding varieties and advanced breeding lines were selected for the experiment. The genotypes were collected from the Genetic Resources and Seed division of Bangladesh Rice Research Institute (BRRI) and from gene bank of Bangladesh Institute of Nuclear Agriculture (BINA). A list of the genotypes used in the study is given in Table 1.

Table 1. List of the genotypes used in the study.

1. Hurkun	11. Malbhog	21. Kumragori (2)	31. Katari Bhog	41. Mal Bhog	51. Binashail
2. Beti Balam	12.Jabra	22. Saita	32. Amon Chala	42. Banshi Raj (2)	52. Iratom 24
3. Balam	13. Tilokkajol	23. Shil Komol	33. Raktachamhara	43. Depo (2)	53. Bina Dhan-7
4. Lalgati (2)	14. Birpala	24. Rajasaili	34. Path Jak	44. Pathor Chapa	54. PBSAL-655
5. Horkuch (2)	15. Khirkun	25. Sal-Kele (2)	35. Jhur Dhan	45. Kalojira	55. PBRC-STL-15
6. Dudsail	16. Lalmuta	26. Kalokuli	36. Mohishkani	46. Modhu Sail	56. PBRC-30
7. Patnai (3)	17. Sadamuta	27. Bachoi Kolom	37. Lechya	47. Hida (2)	57. PBRC-37
8. Brtichikon	18. Chengai Dhan	28. Kochoi	38. Sita Bhog	48. Nagra	58. PBRC-STL-20
9. Kamranga	19. Bota	29. Mowa	39. Gosta	49. Binadhan-4	59. PBSAL-656
10. Khi-Pala	20. Hurkun	30. Kumragoir	40. Bet	50. Binadhan-6	

Table 2. Summary of microsateelite markers used in the study.

Primer name	Repeat Motif*		Primer sequence
RM10720	$(TA)_{34}$	For.	GCAAACGTCTACGTGAGAAACAAGC
		Rev.	GCATGTGGTGCCTTAACATTTGG
RM10772	$(TATC)_{10}$	For.	GCACACCATGCAAATCAATGC
		Rev.	CAGAAACCTCATCTCCACCTTCC
RM11300	$(GA)_{26}$	For.	GGTGAGGGAGGTACCGAACTAGG
		Rev.	AACTAGGGCGCTGGGAGAGG
RM11757	$(TTG)_{48}$	For.	GCTTGTTGCCTGTGAACAGTAGC
		Rev.	TGTCAGCATGCAACATCAATCC
RM443	$(GT)_{10}$	For.	GATGGTTTTCATCGGCTACG
		Rev.	AGTCCCAGAATGTCGTTTCG
OSR14	$(GA)_n$	For.	AAATCCACGCACACTTTGCG
		Rev.	AGGTAAACGAGCTTGAGGTG
RM28102	$(TA)_{48}$	For.	CACTAATTCTTCGGCTCCACTTTAGG
		Rev.	GTGGAAGCTCCGAGAAAGTGC
RM28502	$(CTT)_{16}$	For.	CGAGCAGATCTGATGTCGTCTTCC
		Rev.	CTTTGCTTTGCATGCCTCACG

SSR analysis

Fresh leaves from 20-days old seedlings were used for DNA extraction followed by CTAB mini-prep method [7]. Eight selected SSR primers from rice chromosomes 1, 2 and

12 were used for the survey. The total PCR reaction volume was 15 µl, composed of 2.0 µl genomic DNA, 1.5 µl 10X PCR buffer (Tris with 15 mM MgCl2, Conc. 10X), 0.75 µl dNTPs, 1.0 µl forward primer, 1.0 µl reverse primer, 0.5 µl Taq DNA polymerase (conc. 5 U/µl) and 8.25 µl sterile deionized water. Samples were subjected to the following thermal profile for amplification in a thermocycler: after the initial 7 min at 95°C, SSR marker amplification comprised 10–15 touchdown cycles of 94°C for 30 s, annealing for 30s, decreasing the temperature by 0.5°C per cycle until the specified annealing temperature was reached (55^{o}C for all the markers), and 72°C for 30s. This was then followed by 25–35 cycles of amplification with the specified annealing temperature, and a final extension at 72°C for 10min. After amplification, the PCR tube was stored at 4°C until electrophoresis. Visualization of amplification products were accomplished on a 3% agarose gel in 0.5 X TBE buffer. The agarose gels were stained with ethidium bromide solution for 20-25 min. The stained agarose gel was illuminated by UV-trans-illuminator and photographed for assessing the DNA profiles.

Analysis of SSR data

The allele size at each microsatellite locus was scored in base pairs by using Alpha EaseFC 4 software. The summary statistics including the number of alleles per locus, gene diversity and polymorphism information content (PIC) values were determined using the software POWER MARKER version 3.25 [8]. Allele molecular weight data were also used to determine the genetic distance for phylogeny reconstruction based on the UPGMA method as implemented in POWER MARKER with the tree viewed using TREEVIEW [9]. Principal Coordinate (PCoA) and AMOVA analysis was performed using the program GENALEX 6.4 [10].

Table 3. Data on number of alleles, number of rare alleles, allele size range, gene diversity, Fst and Polymorphism Information Content (PIC) found among 59 rice genotypes for 8 microsatellites (SSR).

Locus	No. of alleles	*Rare alleles	Allele Size ranges(bp)	Difference (bp)	F_{st} value	Gene Diversity	PIC Value	No. of private allele
RM11300	27	19	113-488	375	0.083	0.9486	0.946	20
RM10772	13	5	269-387	118	0.174	0.8641	0.851	11
RM10720	13	7	103-194	91	0.071	0.8296	0.811	8
RM28102	9	5	87-139	52	0.262	0.7921	0.767	6
RM28502	10	2	105-146	41	0.100	0.8470	0.831	4
RM11757	18	8	320-522	202	0.205	0.9279	0.923	13
RM443	11	3	104-160	56	0.094	0.8587	0.843	9
OSR14	13	4	125-280	155	0.157	0.8963	0.887	8
Mean	14.25	7	---	---	0.143	0.870	0.857	9.875

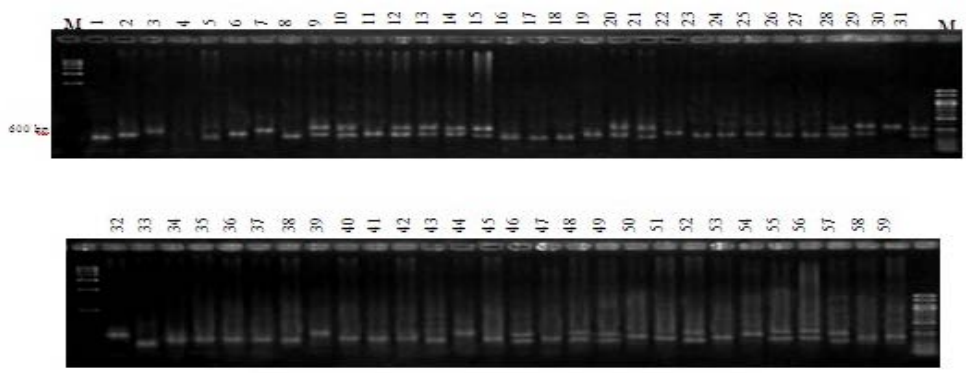

Fig. 1. Microsatellite profile of 59 rice genotype with RM11300.

Legend

Lane 10: Khi-pala	Lane 11 : Malbhog	Lane 21: Saita	Lane 31: Amon Chala	Lane 41: Banshi Raj_2	Lane 51: PBRC-30
Lane 9: Kamranga	Lane 12 : Jabra	Lane 22: Shil Komol	Lane 32: Raktachamhara	Lane 42: Depo_2	Lane 52: Bina Dhan-7
Lane 8: Britichikon	Lane 13 : Tilok kajol	Lane 23: Rajasail	Lane 33: Path Jak	Lane 43: Pathor Chapa	Lane 53: PBRC-37
Lane 7: Patnai_3	Lane 14 : Birpala	Lane 24: Sal-Kele_2	Lane 34: Jhur Dhan	Lane 45: Modhu Sail	Lane 54: PBRC-STL-20
Lane 6: Dudsail	Lane 15 : Khirkun	Lane 25: Kalokul	Lane 35: Mohishkani	Lane 44: Kalojira	Lane 55: Bina Dhan-4
Lane 5: Horkuch_2	Lane 16 : Lalmuta	Lane 26: Bachoi Kolom	Lane 36: Lechya	Lane 46: Hida	Lane 56: Binashil
Lane 4: Lalgati_2	Lane 17 : Sadamuta	Lane 27: Kochoi	Lane 37: Sita Bhog	Lane 47: Nagra	Lane 57: PBSAL-656
Lane 3: Balam	Lane 18 :Chengai Dhan	Lane 28: Mowa	Lane 38: Gosta	Lane 48: Khoya Motor	Lane 58: Iratom-24
Lane 2: Beti Balam	Lane 19 : Bota	Lane 29: Kumragoir	Lane 39: Bet	Lane 49: PBSAL-655	Lane 59: Bina dhan-6
Lane 1: Hurkun	Lane 20 : Kumragori_2	Lane 30: Katari Bhog	Lane 40: Mal Bhog	Lane 50: PBRC-STL-15	

3. Results

Genetic diversity

Eight SSR primer pairs were used for the genetic diversity analysis of 59 rice accessions. Detailed information of these primers is shown in Table 2. A total of 114 alleles were detected among the 59 rice germplasms. The average number of allele per single marker was 14.25, with a range of 9 (RM28102) to as many as 27 (RM11300) (Table 3). The representative amplification profiles (RM11300) are shown in Fig. 1. Rare alleles were observed at all of the SSR loci in one or more of the 59 accessions with an average of seven rare alleles per locus and a total of 53 (46%) across all the loci (Table 3). An allele observed in less than 5% of the 59 accessions was considered to be rare [11]. Highest level of gene diversity (0.9486) was observed in loci RM11300 and the lowest one (0.7921) in loci RM28102 with a mean diversity of 0.8706 (Table 3).

The level of polymorphism among the 59 genotypes was evaluated by calculating PIC values for each of the 8 SSR loci. The PIC values varied widely among loci and ranged from a low of 0.767 (RM28102) to a high of 0.946 (RM11300) per marker and averaged of 0.857 (Table 3). Genetic differentiation (Fst) values were found in the ranges of 0.071 to 0.262 for each marker with an average of 0.143 (Table 3).

Table 4. Data on number of different allele, effective allele, shannon's information index, private allele, Polymorphism information content and Gene diversity Of different groups of population.

Population	No. different allele (Na)	No. effective allele	Shannon's information index	Average no. private allele	PIC	Gene diversity
Local cultivars	13.000	8.237	2.214	8.875	0.8367	0.8520
HYV	3.375	3.032	1.049	0.375	0.5271	0.5738
Breeding line	4.000	3.512	1.255	0.625	0.6196	0.6728

*HYV= High yielding variety

In order to compare the genetic diversity, we divided all the germplasm accessions into three groups' viz., Landrace cultivars (LC), high yielding varieties (HYV) and Crossing line (CL). The effective number of allele per group ranged from 8.23 (LC) to 3.51 (CL). The genetic diversity in terms of Shannon's information index was highest (2.214) in traditional varieties and lowest (1.225) in breeding lines. 13 different alleles were found in the LC group and the value for HYV and CL group was 3.375 and 4.0 respectively. Average number of private allele (allele found in a single population) [12] among the groups ranged from 8.875 to 0.375 (Table 4). The highest number of private allele (7) were observed in the accessions Beti Balam, Horkhuch (2), Britichikon, Tilok Kajol, Chengai Dhan, Sal Kele (2), Bet, Modhusail and lowest (2) were captured in the variety Nagra. Most of the private alleles were found in the landrace population. HYV and Breeding lines exhibited only one private allele throughout the eight loci.

Genetic distance based analysis

A dendrogram was constructed based on the Nei's [13] genetic distance using UPGMA algorithm. The UPGMA cluster analysis led to the grouping of the 59 varieties in three major clusters (Fig. 2). Each cluster distinguishes the genotypes clearly from the other. Group I is comprised of high yielding varieties and breeding lines. Some traditional local cultivars were also included in this group. These are Khoya Motor, Hida 2, Lalgati 2, Birpala, Sadamuta. Group I is divided into two subgroups. Group II was diverse and divided into several subgroups. All other accessions were clustered in Group III. The grouping and sub-groping of the accessions within the different cluster was basically in accordance with their genealogies. Genetic distance of the studied germplasm was ranged from 0.33 to 1.00 (data not shown) which indicates magnitude of diversity among the studied germplasms. The lowest genetic distance was between the variety pair Rajashili and kumragori (2) and those between Nagra and Pathor Chapa.

Principle coordinate analysis

In the principal coordinate analysis (PCoA), the first three co-ordinates explained 58.76% of the total variation, with 23.90 % explained by the first co-ordinate and 18.06 % by the second co-ordinate (Fig. 3). The first PCoA separates cluster II from the rest with an exception of landrace Raktachamhara (cluster I) and the second one separates cluster I from cluster III. In cluster III, the second PCoA separates Bina dhan 7, Hida (2) and Khoya Motor from the rest (Fig. 2). In the cluster II the first PCoA separates Banshi Raj (2) from the rest. The grouping obtained through PCoA confirmed that obtained by UPGMA cluster analysis.

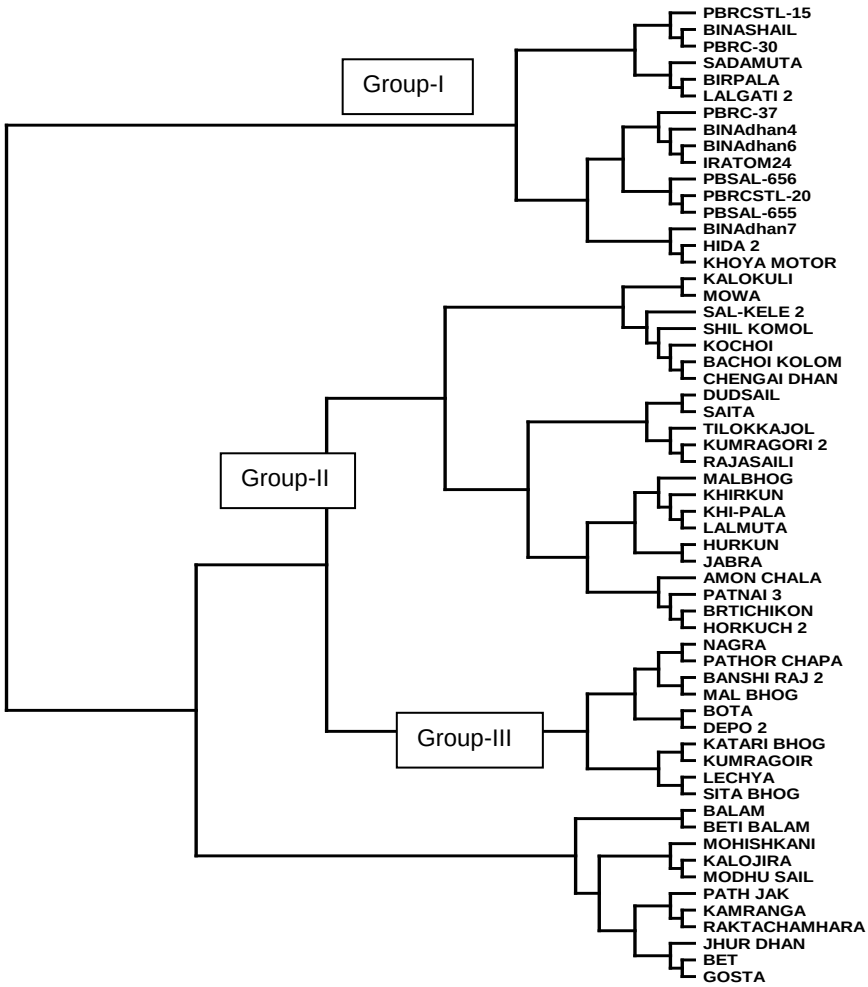

Fig. 2. Dendogram of 59 rice germplasm derived from UPGMA cluster analysis based on Nei [1983] genetic disease.

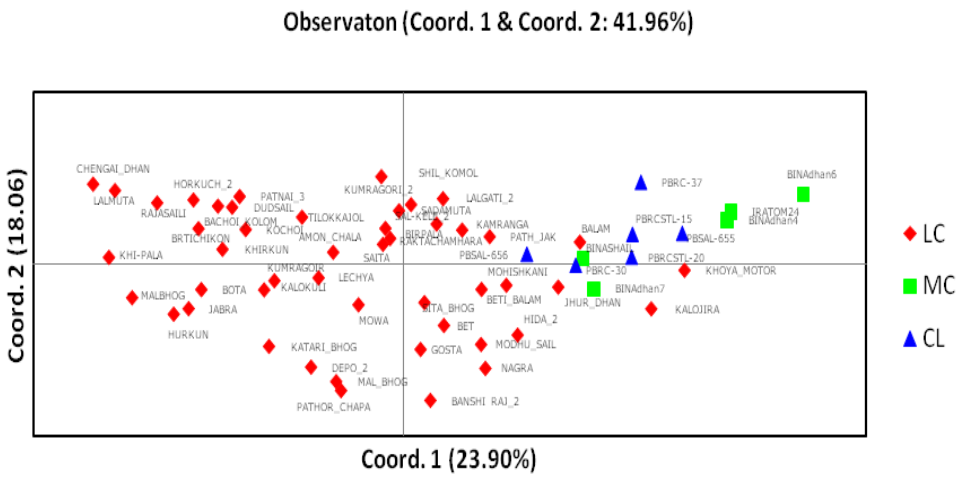

Fig. 3. Scatter plot of the first and second principal co-ordinates of 59 rice genotypes as found in principal coordinate analysis.

Analysis of molecular variance

Analysis of molecular variance showed that the maximum percentage of variation present was among individuals within groups (91%) followed by 9% within the groups (Fig. 4). No variation was observed within individuals. The variation present among the landraces was greater than the variation present within the landraces.

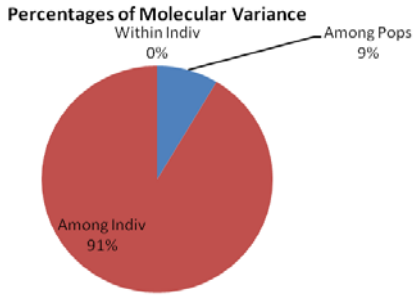

Fig. 4. Pie chart of analysis of molecular variance (AMOVA).

5. Discussions

One main cause of eradication of plant genetic resources has been the adaptation of narrowly based advanced varieties for intensive cultivation practices. Variation in landraces is helpful for broadening the crop gene pool [14]. Diversity exploration among plant landraces made necessary by the failure of the green revolution to be sustained.

Little was known about the relationship between Bangladeshi agronomic crop cultivars in general and rice landraces in particular on the basis of SSR analysis. Here, eight microsatellite markers were used to assess the genetic diversity of 59 genotypes of rice.

The numbers of alleles detected per microsatellite markers were high (ranged from 9 to 27 with an average of 14.5) and the results were nearly similar to other studies [15-16]. But, the allele numbers are, on a per locus basis, much larger than the previous reports [1, 17]. This may be for inclusion of higher number of landrace accessions. Still, the high level of SSR polymorphism that has been detected here could be attributed primarily to the diverse origins of the accessions studied.

In the present study higher PIC values (varied from 0.76 to 0.94) were observed than the earlier works [17-18] but are quite comparable to other studies [19-21]. High PIC values can be due to the fact that rice accessions used in this study were collected from diverse regions of Bangladesh so that more variability might exist in such population. Higher average allele number and greater PIC values compared to other studies suggest that the germplasms posses unique diversity. This huge diversity could be readily used in broadening the genetic basis of existing cultivars. Almost all the markers in the present were highly polymorphic but RM11300 was robust in capturing the diversity. It revealed the highest PIC value, number of allele and number of private allele (Table 3). This means that this marker is well distributed throughout the rice genome and could routinely be used for molecular characterization of rice germplasms.

The number of private alleles detected in this study was high (Table 3) and similar to the previous reports [22] but less than other findings [15]. Variation in number of private alleles found in this study may be due to the variable periods of genetic isolation during their evolutionary history of the studied accessions. The presence of alleles unique to a specific landrace population indicates an inimitable genetic variability at certain loci. This information is valuable to categorize accessions with exclusive genetic variability, whose selection can increase the allele richness of gene banks. Genotypes with private allele can be utilized in breeding programs as donor parent to develop more adapted variety. They can also be used to intensify the genetic basis of existing cultivars of rice.

Analysis of molecular variance also showed that maximum variability (Fig. 4) present among the individuals within a group rather than among the group. As the variation present among the landraces is greater than the variation present within the landraces, it is important to conserve and utilize as many landrace populations as possible. The result is consistent with the previous report by [6] where they obtain 91.2% variation among the individuals within the group but quite contradictory with the other results [1, 23]

Here the lowest genetic diversity was observed in the HYV group and highest in landrace group. This is because; decline in diversity is considerably greater for genes involved in domestication than neutral and random markers [24].The result is quite consistent with result of [25] where they found that Sorghum landraces conserve the 80% of the diversity observed in the wild. In this study, highest genetic distances were seen between the traditional cultivars and HYVs. This can be explained by the fact that in one side the land races and on the other side the HYVs have been involved. The distance has been generated during the process of the development of HYVs. The generated distance

can further be used for inclusion of gene source from these traditional varieties to more HYVs [17].

6. Conclusion

Bangladeshi rice cultivars used in the present study have not been examined previously in terms of genetic diversity using molecular markers. The SSR analysis of rice landrace and HYV in this study indicated sufficient polymorphism to fully differentiate the inter- and intra-population diversity. The study also confirmed the value of microsatellite loci for genetic diversity studies of rice landraces found in earlier studies. The diversity and the unique features of the Bangladeshi rice-landrace collections examined in this study could be quite relevant to both domestic and global rice development.

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