Bangladesh Rice J.27(2): 1-16, 2023, doi.org/10.3329/brj.v27i2.77686

Genetic Diversity Analysis in Boro Rice *(Oryza sativa* L.*)* Landraces of Bangladesh Using SSR Markers

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ABSTRACT

Genetic diversity and associations among 96 Boro rice landraces of Bangladesh were assessed using 12 SSR markers at the Genetic Resources and Seed Division of Bangladesh Rice Research Institute (BRRI). The number of alleles per locus ranged from 11 (RM163) to 41 (RM283), with a mean of 21 alleles. Polymorphism information content (PIC) ranged from 0.75 (RM163) to 0.95 (RM283) with an average of 0.891, showing a large variability among the tested landraces. PIC values showed that RM283 was considered as the best marker for identifying and evaluating the diversity of Boro rice landraces. The frequency of most common alleles at each locus ranged from 8% (RM283) to 34% (RM275, RM277). Nei's genetic distance based UPGMA dendrogram grouped the studied landraces into seven different clusters with a similarity coefficient of 0.11. Based on the dendrogram constructed using SSR markers, the accessions that are distant from each other in terms of genetic distance and diversity index (such as Pashusail and Tulsi Boro; Raja sail and Kali Boro; Bashful and Jamir; Begun bitchi and Boro deshi; Banjira and Bogra (Deshi); Jagli Boro and Lahi Boro; Bimion and Gorchi sail; Jhati sail and Khaia Boro; Tepi Boro and Jamir Boro) are strongly recommended for selection as parents in future breeding programmes aimed at developing high-yielding and stress-resilient rice variety in contribution to global food security. The results of this study could be useful for the identification of genetically diverse parents from the landraces for breeding programme aimed at varietal improvement.

Key words: Rice (*Oryza sativa* L.), boro, genetic distance, dendrogram, landrace, varietal improvement.

INTRODUCTION

Rice and food security are considered as the same thing in Bangladesh context (Brolley, 2015). Rice is an important staple food and accounts for about 78% of the country's total net cultivated area. The country achieves self-sufficiency to meet the rice demand of a population of 169.04 million with 11.55 million hectares of gross cultivated area (Kabir *et al.,* 2020; Nasim *et al.,* 2021). Bangladesh ranks fourth in the world in rice consumption, with an annual per capita availability of about 213.5 kg (FPMU Database, 2020). Current rice consumption is approximately 328.9 g per capita per day, providing approximately 60% of total calories and 50% of total protein for adults (HIES, 2022). About 48% of rural workers participate directly or indirectly in rice production for their livelihood. Rice is grown all year round in three seasons: Aus, Aman and Boro. Since independence, rice production has increased

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three-fold from approximately 11 MT in 1971–72 to about 36.6 MT in 2019–20 (BBS, 2020). The contribution of rice to the value of the crop sub-sector is about 70% (Mottaleb and Mishra, 2016).

Due to the great importance of this crop and its close connection to food security and local lifestyle and culture, Asian farmers have been selecting and cultivating a wide range of rice varieties for thousands of years. It is estimated by scientists that more than 1,40,000 rice varieties have been developed/selected/isolated in Asia. More than 1,32,000 germplasm are preserved in the world's largest rice genebank, the International Rice Research Institute (IRRI), located in the Philippines (http://irri.org/our-workresearch/genetic-diversity). A total of 9,006 varieties/landraces/cultivars/wild genotypes has been collected from indigenous and exotic sources and preserved in the BRRI Genebank. Genetic diversity is the primary component of any agricultural production system for selection, conservation, characterization, and appropriate use of germplasms (Emon and Ahammed, 2020). The lack of genetic diversity limits crop production and represents a serious problem in any breeding programme aimed at varietal development. Therefore, studying natural diversity or creating genetic diversity (if not exist) is a crucial step to increase yield. Rice breeders are using different types of germplasm like landraces, wild stock, commercially approved varieties etc. to explore the genetic diversity within the existing germplasm to increase yields. However, studies on the genetic diversity of different varieties and germplasm of Boro rice in Bangladesh are rare. Information about genetic diversity and the relationship among available germplasm is essential before taking any rational plant breeding programme. The genetic diversity of crop species can be determined using morphological, biochemical and molecular (DNA) markers (Rao, 2004). Several researchers have used morphological traits for diversity assessment and characterization of Bangladesh rice germplasm (Siddique *et al.,* 2011; Banik *et al.,* 2012; Khalequzzaman *et al*., 2012; Baktiar *et al.,* 2013; Siddique *et al.,* 2013; Islam *et al.,* 2014; Ahmed *et al.,* 2015a, 2015b; Kulsum *et al.,* 2015; Akter *et al.,* 2016; Biswash *et al.,* 2016; Siddique *et al.,* 2016a; Akter *et al.,* 2017; Islam *et al*., 2017; Akter *et al.,* 2018; Islam *et al.,* 2018a; Siddique *et al.,* 2018; Islam *et al.,* 2019; Muti *et al.*, 2020; Khalequzzaman *et al.,* 2022a; Khalequzzaman *et al.,* 2023). However, compared to biochemical markers, morphological markers are strongly affected by environmental factors and are therefore dependent on the environmental conditions during cultivation. The limitations associated with morphological and biochemical markers are overcome by molecular markers (Rao, 2004). Molecular characterization through the use of molecular markers is independent of environmental influences. This characterization can be performed with plant DNA from any growth stages (Tatikonda *et al.,* 2009). Among the various molecular markers, polymerase chain reaction (PCR)-based simple sequence-repeat (SSR) markers (Gianfranceschi *et al.,* 1998) have become popular in analyses of genetic diversity.

Molecular markers have been used successfully in registration activities such as cultivar and variety identification (Mailer *et al.,* 1994; Bligh *et al.,* 1999). An effective technique for DNA fingerprinting is the efficient PCR amplification of tandem repeat sequences that have long been known to be polymorphic and widespread in plant genomes, called simple sequence repeats (SSRs) or microsatellite polymorphisms (Cregan, 1992; Morgante and Olivieri, 1993). Motif mutations of and flanking

sequences as well as the distribution of microsatellites in a species' genome are used to reveal genetic variation and cultivar identity. In plants, SSRs have been shown to be highly informative and site-specific markers in many species (Akkaya *et al.,* 1992; Legarcrantz *et al.,* 1993; Wu and Tanksley, 1993, Rahman *et al.,* 2007). SSRs are becoming increasingly useful for integrating genetic, physical and sequence-based maps in rice, while providing breeders and geneticists with a powerful tool for linking phenotypic and genotypic variation.

Microsatellites are abundant and well distributed throughout the rice genome (Akagi *et al.,* 1996; McCouch *et al.,* 1997; Wu and Tanksley, 1993). SSRs are codominant, recognize a high level of allelic diversity, and are efficiently characterized by PCR (McCouch *et al.,* 2002). Several researchers used molecular markers for diversity assessment and DNA fingerprinting of Bangladesh rice germplasm (Rahman *et al.,* 2006; Rahman *et al.,* 2007; Rahman *et al.,* 2008; Rahman *et al.,* 2010; Siddique *et al.,* 2014; Siddique *et al.,* 2016b, 2016c, 2016d; Khalequzzaman *et al.,* 2017; Siddique *et al.,* 2017; Islam *et al.,* 2018b; Islam *et al.,* 2021; Akter *et al.,* 2022; Khalequzzaman *et al.,* 2022b; Saha *et al.,* 2022; Khalequzzaman *et al.,* 2023). This study was undertaken with 96 Boro rice landraces and used molecular traits for characterization and diversity analysis. The objectives of this study were to evaluate the genetic diversity assessment of 96 Boro rice landraces and determine the genetic relationships exist among these landraces at molecular level.

MATERIALS AND METHODS

Plant materials

Ninety-six Boro rice landraces of Bangladesh were tested (Table 1). Five-gram seeds of each entry were first germinated and then sown into earthen pots for DNA extraction.

Table 1. Rice landraces used in this study with their provenance.

Landrace	BRRI accession no.	Place of collection	Variety	BRRI accession no.	Place of collection
Banajira	7	Barishal	Lafai	1969	Kishoreganj
Pashusail	54	Habiganj	Sail Boro	1970	Kishoreganj
Cunail	178	Tangail	Gochi	1971	Kishoreganj
Bhaturi	179	Tangail	Biron	1972	Kishoreganj
Dholi Boro	180	Tangail	Bogura	2251	Kishoreganj
Grugu Boro	182	Tangail	Lahaya	2252	Kishoreganj
Boro	253	Mymensingh	Chhola Boro	2258	Kishoreganj
Ausha Boro	254	Mymensingh	Kolisha Boro	2260	Kishoreganj
Jagli Boro	255	Mymensingh	Goa Bish	2261	Kishoreganj
Tepi Boro	258	Mymensingh	Madhab Sail	2264	Kishoreganj
Kali Boro	260	Mymensingh	Mogol Sail	2266	Kishoreganj
Kaiaka Boro	262	Mymensingh	Gola Tepi	2267	Kishoreganj
Poshu Sail	929	Sylhet	Lafa	2268	Kishoreganj
Gorchi Sail	932	Sylhet	Beun Bichi	3952	Netrakona

Landrace	BRRI accession no.	Place of collection	Variety	BRRI accession no.	Place of collection
Jhati Sail	933	Sylhet	Rata Boro	3959	Netrakona
Bimion	934	Sylhet	Kori Topa	3960	Kishoreganj
Soiler Peena	935	Sylhet	Lal Dengi	3962	Kishoreganj
Khaia Boro	936	Sylhet	Panpiag	3963	Kishoreganj
Boro Deshi	1405	Pabna	KN – 1B -361-1312-27- 1	3976	BRRI
Muktahar	1468	Dhaka	Choudhury Sail	3980	Sunamganj
Banajira	1470	Dhaka	Ashani	3981	Sunamganj
Bash ful	1471	Dhaka	Madanga	3982	Sunamganj
Am Boro	1472	Dhaka	Laitra Sail	3983	Sunamganj
Lahi Boro	1474	Dhaka	Fena Ful	3984	Sunamganj
Jamri Boro	1475	Dhaka	Kowla	3985	Sunamganj
Mukta har	1650	Dhaka	Madlai	3989	Habiganj
Bati Boro	1670	Dhaka	Lara	3991	Habiganj
Jamir	1706	Faridpur	Binni	3993	Habiganj
Kali Boro	1707	Faridpur	Lal Boro	3994	Habiganj
Bawoi	1708	Faridpur	Gachi Boro	3995	Habiganj
Khoea	1709	Faridpur	Bachi Boro	4000	Habiganj
Ulia	1711	Faridpur	Birain	4001	Habiganj
Solai	1713	Faridpur	Sona Rata	4002	Habiganj
Sada Boro (deshi)	1714	Faridpur	Naula topa	4003	Habiganj
Chaita Boro	1716	Khulna	Hunga Boro	4004	Habiganj
Isamoti	1790	Jashore	Bimon	4005	Habiganj
Jaista Boro	1792	Jashore	Gobi Sail	4006	Habiganj
Natel Boro	1793	Jashore	Nata Boro	4007	Habiganj
Guchi Boro	1796	Kishoreganj	Chaula Birain	4008	Habiganj
Lakhai	1800	Kishoreganj	Kala Birain	4009	Habiganj
Lakhai	1801	Kishoreganj	Badal Boro	4011	Sylhet
Bogra (Deshi)	1802	Kishoreganj	Jomir Sail	4012	Sylhet
Tupa	1811	Kishoreganj	Gopal Beri	4013	Habiganj
Begun Bitchi	1813	Kishoreganj	Muirol	4014	Habiganj
Pankaij	1817	Kishoreganj	Polash	4017	Habiganj
Bash Boro	1818	Kishoreganj	Gasbar	4202	Netrakona
Raja Sail	1819	Kishoreganj	Gumir Sail	4203	Netrakona
Tulsi Boro	1968	Kishoreganj	Jalda IRRI	4206	Brahmanbaria

SSR markers

Twelve SSR markers (Table 2) were used for the diversity analysis.

Table 2. List of the 12 simple sequence repeat (SSR) markers used in this study.

Locus name	Chr.	Repeat motif	Forward primer	Reverse primer
RM 6	2	(AG)16	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC
RM 11	7	(GA)17	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG
RM 30	6	(AG)9A (GA)12	GGTTAGGCATCGTCACGG	TCACCTCACCACACGACACG
RM 44	8	(GA)16	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
RM 125	7	(GCT)8	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
RM 147	10	(TTCC)5 (GGT)5	TACGGCTTCGGCGGCTGATTCC	CCCCCGAATCCCATCGAAACCC
RM 163	5	(GGAGA)4 (GA)11C (GA)20	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT
RM 273	4	(GA)11	GAAGCCGTCGTGAAGTTACC	GTTTCCTACCTGATCGCGAC
RM 277	12	(GA)11	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG
RM 278	9	(GA)17	GTAGTGAGCCTAACAATAATC	TCAACTCAGCATCTCTGTCC
RM 283	1	(GA)18	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG
RM 287	11	(GA)21	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC

Genotyping protocol

Genomic DNA from each accession was extracted from fresh leaf tissues of 20-day-old plants according to a simple and modified protocol of Zheng *et al.,* 1995. The PCR was carried out in a reaction volume of 12.5 µl containing 5 to 25 ng of template DNA, 1.25 µl 10X PCR buffer without $MgCl_2$ (100 mM Tris-HCl pH 9.0 at 25°C, 500 mM KCl, 0.1% Triton® X-100 and H_2O), 1.5 µl of 25 mM $MgCl_2$, 0.25 µl of 10mM dNTP, 0.25 µl of 5 U/µl Taq polymerase enzyme, 0.625 µl each of 10 µM forward and reverse primers using a MJ Research single 96 well thermal cycler. To prevent evaporation, the mixture was overlaid with one drop of mineral oil. After an initial of 5 min denaturation at 94°C, each cycle consisted of 1 min denaturation at 94°C, 1 min of annealing at 55°C, and 2 min of extension at 72°C, with a final extension of 7 min at 72°C at the end of 35 cycles. The PCR products were then mixed with bromophenol blue gel loading dye and were analyzed by electrophoresis on 8% polyacrylamide gel using mini vertical polyacrylamide gels for high-throughput manual genotyping (CBS Scientific Co. Inc., CA, USA). 2.5 µl of amplification products were resolved by running gel in 1xTBE

buffer for 2-2.5 hrs depending upon the allele size at a voltage of approximately 75 volts and 180 mA current. The gels were run with 0.5 mg/ml ethidium bromide stained and documented with UVPRO (Uvipro Platinum, EU) gel documentation device. Microsatellite markers or simple sequence repeat (SSRs) were used for DNA analysis (Temnykh *et al.,* 2001; McCouch *et al.,* 2002).

Data analysis

The size (in nucleotide base pairs) of the most amplified band for each microsatellite marker was determined by its migration relative to the molecular weight marker (50-bp DNA ladder) using Alpha Ease FC 5.0 software. SSR marker alleles were analyzed using Power Marker software. Summary statistics, including number of alleles per locus, major allele frequency, genetic diversity, polymorphism information content (PIC) values etc., were obtained using Power Marker software version 3.25 (Liu and Muse, 2005). Allele frequency data from the Power Marker software were used to export the data into a binary format, with presence scored as 1 and absence or missing observation scored as 0 of unique and shared bands for each marker allele-genotype combination for further analysis with NTSYS-pc version 2.2 (Rohlf, 2002). The similarity matrix was calculated with the Simqual subprogram using the Dice coefficient, then the cluster analysis was carried out with the SHAN subprogram using the UPGMA clustering method (UPGMA algorithm computed following 'Hierarchical cluster analysis') as implemented in NTSYS-pc version 2.2. For an unrooted phylogenetic tree, genetic distance was calculated using the 'Nei distance' (Rohlf, 2002), followed by phylogeny reconstruction based on the UPGMA method using neighbor-joining in Power Marker with tree viewed using TREEVIEW Win32 version 1.66 (Page, 1996). Finally, NTSYS-pc was used to construct a UPGMA (unweighted pair group method with arithmetic averages) dendrogram based on the Nei's (Nei and Takezaki, 1983) distance-based showing the genetic interrelationship among the genotypes. The genetic distance was calculated using the 'Nei genetic distance' (Nei, 1972; Rohlf, 2002).

RESULTS

Overall SSR diversity

Ninety-six rice landraces were successfully amplified with 12 SSR markers, where primer pairs called loci and DNA bands as alleles. A total of 252 alleles were detected in 12 SSR markers from 96 rice landraces. The size of the amplicons ranged from 42 to 165bp. The number of alleles per locus ranged from nine (RM163) to 41 (RM283) with a mean of 21. The largest range of band sizes was found for RM6 (102-165) followed by RM283 (80-160), RM273 (46-159), RM278 (87-158) and RM11 (57-137). Among the 30 SSR markers, the highest number of alleles (41) were found for RM283 followed by RM11 (25); RM6 and RM278 (24); RM44 and RM273 (21); RM125 and RM 277(18); RM30 and RM287 (17); RM147 (15); RM163 (11) (Table 3). The PIC values ranged from 0.759 (RM163) to 0.959 (RM283) with a mean of 0.759, indicating that the SSR primers used in this study were efficient and polymorphic. The PIC values for other markers were 0.93 (RM6, RM11), 0.92 (RM278), 0.91 (RM44), 0.90 (RM273), 0.89 (RM125, RM287), 0.87 (RM147, RM277), and 0.83 (RM30) respectively (Table 3). The PIC value revealed that RM283 was the best marker for 96 rice landraces. Figure 1 shows the DNA profiles of 96 Boro landraces with the SSR marker RM283.

Table 3. Allele number, allele size, frequency, genetic diversity and PIC for 12 SSR markers in 96 Boro rice landraces.

Marker	Chr. no	Position (cM)	Allele no.	Allele size (bp)	Allele frequency (%)	Genetic diversity	PIC value
RM 6	2	29.57	24	151.60	11.46	0.9397	0.9364
RM 11	7	47	25	120.88	10.42	0.9397	0.9364
RM 30	6	125.4	17	73	21.88	0.8520	0.8354
RM 44	8	2.88	21	97.09	13.54	0.9193	0.9137
RM 125	7	24.8	18	104	18.75	0.8932	0.8841
RM 147	10	20.68	15	87.53	22.92	0.8880	0.8788
RM 163	5	91.4	11	51.09	34.38	0.7867	0.7594
RM 273	4	94.4	21	119	14.58	0.9143	0.9080
RM 277	12	57.2	18	119.5	23.96	0.8850	0.8757
RM 278	9	77.5	24	140.79	13.54	0.9325	0.9286
RM 283	1	31.4	41	119.95	8.33	0.9609	0.9595
RM 287	11	68.6	17	106.47	17.71	0.8924	0.8830
Mean			21		17.62	0.9003	0.8916

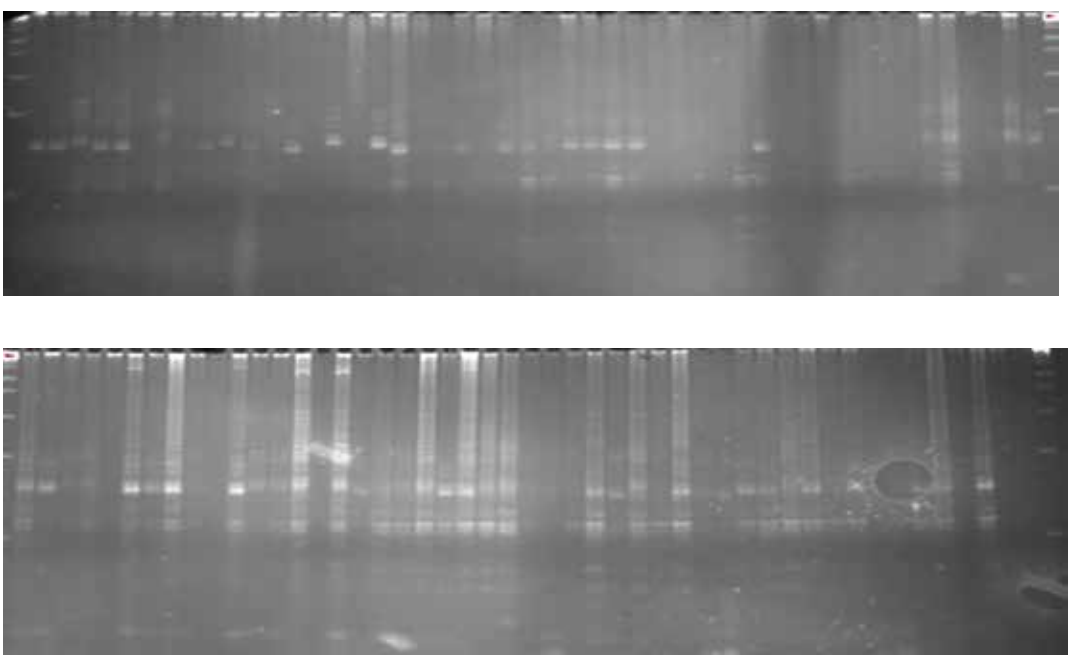

Fig. 1. DNA profile of 96 Boro landraces with RM283 (Number 1-96 represents rice landraces presented in Table 1).

Genetic distance analysis

Results based on genetic distance observed in the unrooted neighbour-joining tree produced seven clusters in 96 landraces (Fig. 2). The largest numbers of landraces (25) were found in cluster I, then in clusters VII (19), V (17), II and III (10), IV (9) and the least number in cluster VI (6).

UPGMA clustering was performed to group the landraces into a dendrogram. From this dendrogram, 96 rice landraces were classified into seven major clusters at a coefficient of 0.11, and the value of similarity coefficient ranged from 0.06 to 0.58. Cluster IV had 34 landraces and is the largest group of seven clusters, followed by cluster VI with 19 landraces; cluster II included 18 landraces; cluster I included 12 landraces; cluster III and VII included five landraces; cluster V included three landraces. The genetic differences between the 96 landraces were greatest for most genotypes (Fig. 3).

Genetic similarity analysis using the UPGMA cluster model was different for the landraces tested compared to that of unrooted neighbour-joining tree.

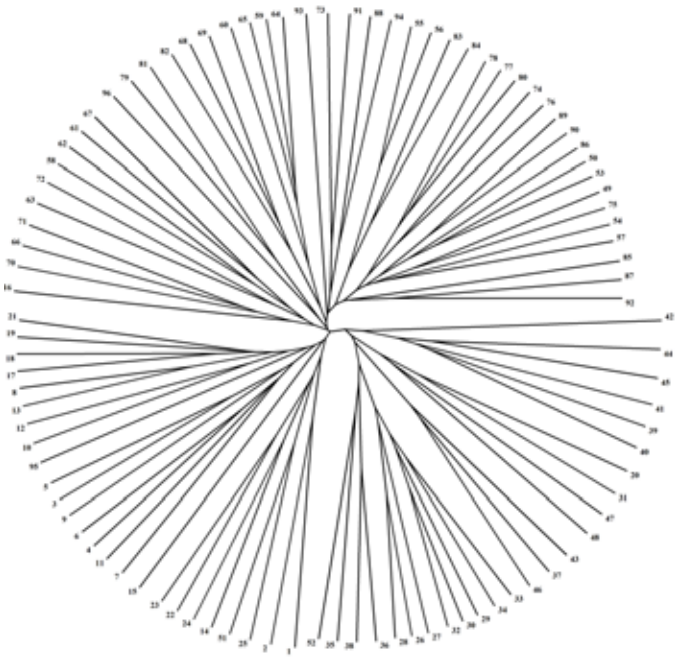

Fig. 2. An unrooted neighbour-joining tree showing the genetic relationships among 96 Boro rice landraces in Bangladesh (Number 1-96 represents rice landraces presented in Table 1).

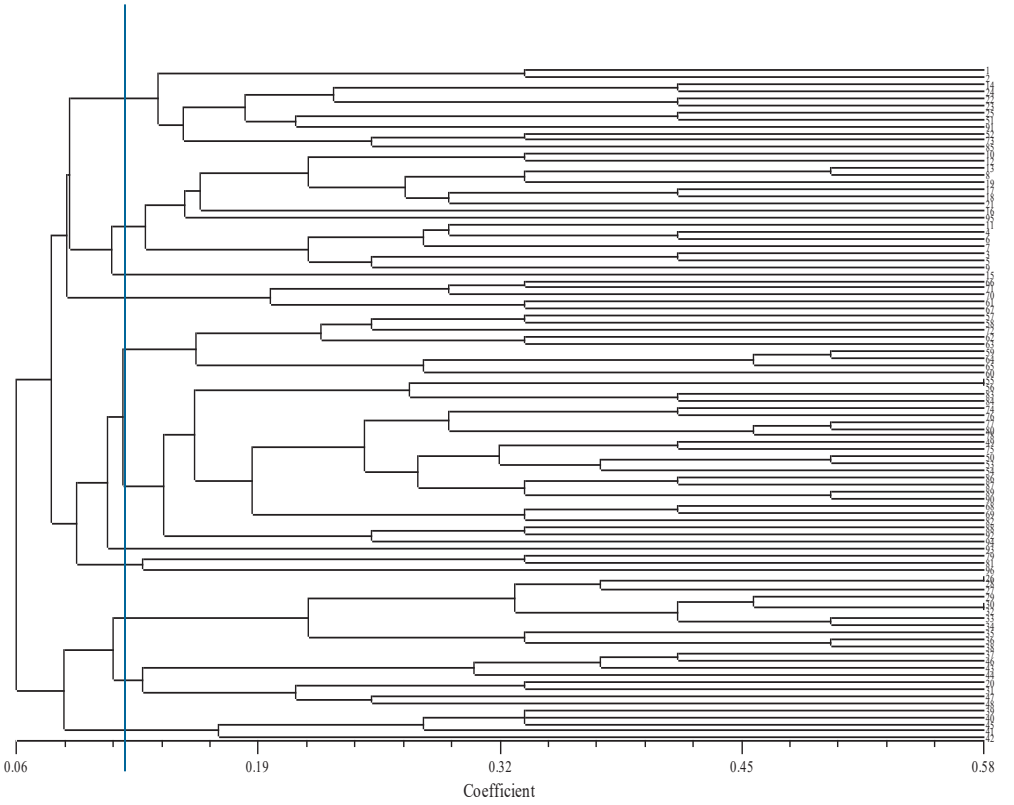

Fig. 3. Dendrogram of UPGMA cluster showing the genetic relationships between 96 Boro rice landraces of Bangladesh based on the alleles detected by 12 SSR markers (Number 1-96 represents rice landraces presented in Table 1).

Principal coordinate analysis

Two-dimensional and three-dimensional graphical views of principal coordinate analysis (PCoA) showed the spatial distribution of landraces along two and three principal axes. The landraces Bhaturi, Dholi boro, Boro, Ausha boro, Mukta har, Bati boro, Jamir, Kali boro, Khoea, Ulia, Solai, Isamoti, Lakhai, Panpiaj, KN-1B-361-1312-27-1, Ashani, Sona rata, Nata boro and Muirol were found far from the center of the cluster, while the remaining landraces were found approximately around the centroid. (Fig. 4 and 5).

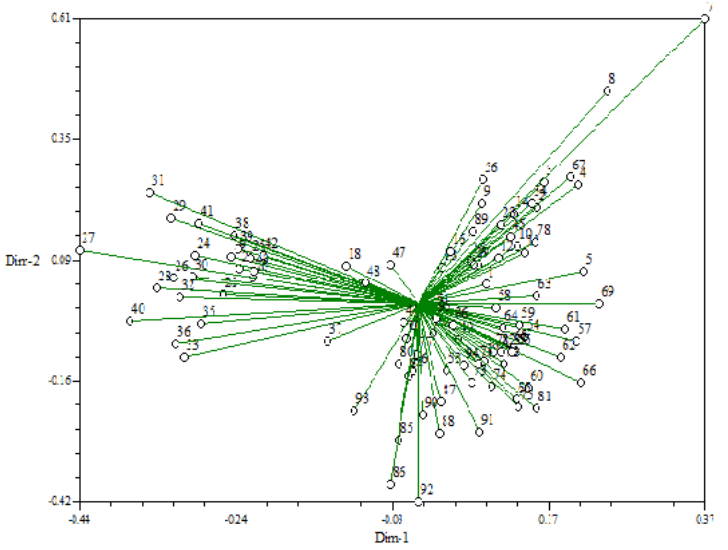

Fig. 4. Two-dimensional principal coordinate analysis (PCoA) view of 12 SSR markers in 96 Boro rice landraces (Number 1-96 represents rice landraces presented in Table 1).

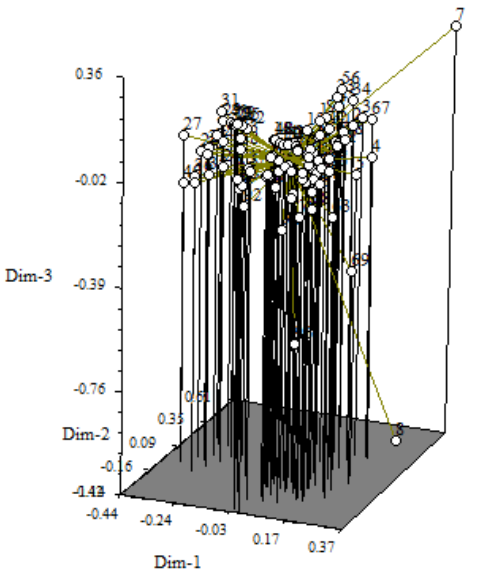

Fig. 5. Three-dimensional principal coordinate analysis (PCoA) view of 12 SSR markers in 96 Boro rice landraces (Number 1-96 represents rice landraces presented in Table 1).

DISCUSSION

The study of genetic diversity is of utmost importance in applied plant breeding to select parental genotypes in a plant breeding programme. Effective germplasm evaluation provides a scientific basis for parents/donor selection for varietal improvement, and selection of genotypes for specific agro-ecological conditions (Kumar *et al.,* 2012). Analysis of diversity at the molecular level using PCR-based markers is considered as an efficient and rapid method for identifying the associations and/or differences between genotypes (Schulman, 2007). Microsatellites are becoming increasingly popular and are suitable for large-scale analyses of genetic diversity and breeding studies (Brown and Kresovich, 1996; Joshi *et al.,* 2000). Competent and consistent use of molecular markers such as SSRs to study the genetic diversity of any food crop requires the selection and use of primers that provide clear, distinct, reliable and sufficient information required to study divergence within a crop (Arolu *et al.,* 2012). In our studies, the number of polymorphic loci detected by the primer combination varied depending on the primer. SSR markers amplified distinct band patterns in 96 Boro rice landraces, and each marker showed polymorphism.

The observed PIC values ranged from 0.759 (RM163) to 0.959 (RM283) and the mean was 0.759, which is consistent with the findings of Siddique *et al.,* (2014), who estimated genetic diversity in T. Aman rice germplasm collections using SSR markers, where PIC ranged from 0.65 to 0.91. The allele frequency ranged from 8.33% (RM283) to 34.38% (RM163) with an average of 17.62, which is consistent with the results of Siddique *et al.,* (2016d), who estimated the genetic diversity of the T. Aman rice germplasm collections using SSR markers, ranging from 8.33% to 22.92% with an average of 15.89. Gene diversity varied from 0.78 to 0.96 and its average value was 0.90, which also indicated the presence of adequate genetic diversity (Table 3). This average value was higher than that of Zai-quan *et al.,* (2012) and Siddique *et al.,* (2016b). Improved determination of relationships between 96 accessions was enabled by UPGMA cluster analysis using SSR markers. Seven major groups were found with a similarity coefficient of 0.11 in which rice accessions were mostly clustered together (Fig. 3), and the similarity coefficient ranged from 0.06 to 0.58. Siddique *et al.,* (2016d) also observed seven groups of T. Aman rice, whose similarity coefficient ranged from 0.06 to 0.75. It should be noted that SSRs formed seven groups, but in the subgroup of IV, the number of accessions was the highest (34) and the reason can be explained by the fact that SSR markers can target a larger number of repetitive sequences, in particular in the centromeric region that can significantly influence the classification pattern (Parsons *et al.,* 1997). These clustering patterns confirm the reliability of SSR markers for the genetic diversity analysis among Boro rice landraces. The principal coordinate analysis (PCoA) showed that landraces that were far from the centroid were more genetically diverse, while landraces that were close to the centroid had similar genetic backgrounds. Hence, the cluster analysis was largely supported by PCoA.

CONCLUSION

The results obtained in this study provided useful implications for establishing the sovereignty of the gene pool of Boro rice landraces in Bangladesh. This study showed a high level of genetic diversity in Boro rice, suggesting that SSR markers were very effective in detecting polymorphism in this ecosystem. To expand the genetic base and

improve Boro rice, landrace with the lowest genetic similarity can be selected as parents. Hybridization must, therefore, be carried out between the genotypes of two distant populations. Taking into account all these criteria and the results of diversity analysis based on markers that are distant from each other due to their genetic relatedness (like Pashusail and Tulsi boro; Raja sail and Kali boro; Bashful and Jamir; Begun bitchi and Boro deshi; Banjira and Bogra (Deshi); Jagli boro and Lahi boro; Bimion and Gorchi sail; Jhati sail and Khaia boro; Tepi boro and Jamir boro) can be selected as parents for a further breeding programmes. This will lead to generate greater diversity and prebreeding lines will be developed with greater variabilities. Hopefully, this endeavour will increase genetic diversity in non-elite lines which will ultimately be helpful for achieving accelerated rate of genetic gain for different traits in breeding programmes.

AUTHORS' CONTRIBUTION

MAS and MK generated the idea; MAS and MZI developed methodology; MFRP, MAS, MHKB and AB gathered data; ESMHR and MSA supervised the study; MAS carried out analysis and wrote the manuscript; and PSB reviewed the manuscript.

ACKNOWLEDGEMENT

The authors are grateful to coordinated sub-project on "Characterization of important plant genetic resources" supported by the World Bank through NATP-SPGR, Bangladesh Agricultural Research Council, for financial support of this research.

DECLARATION OF INTERESTS

The authors wish to confirm that there are no known conflicts of interest concerning this publication.

REFERENCES

Ahmed, M S, S Parveen, M H K Baktiar and M A Siddique. 2015a. Study of qualitative agro-morphological characters of Dhaliboro rice germplasm. *Eco-friendly Agril. J.* 8 (8): 91-96.

Ahmed, M S, S Parveen, M Z Islam and M A Siddique. 2015b. Characterization of Dhaliboro rice (*Oryza sativa* L.) germplasm of Bangladesh for morpho-physicochemical traits. *Intl. J. BioRes.* 19 (1): 1-11.

Akagi, H, Y Yokozeki, A Inagaki and T Fujimura. 1997. Highly polymorphic microsatellites of rice consist of AT repeats, and a classification of closely related cultivars with these microsatellite loci. *Theor. Appl. Genet.* 94: 61-67.

Akkaya, M S, A A Bhagwat and P B Cregan. 1992. Length polymorphism of simple sequence repeat DNA in soybean, Genetics 132: 1131-1139.

Akter, N, M Z Islam, M A Siddique, T Chakrabarty, M Khalequzzaman and M A Z Chowdhury. 2016. Genetic diversity of Boro rice (*Oryza sativa* L.) landraces of Bangladesh. Bangladesh *J. Pl. Breed. Genet.,* 29 (2): 33-40.

Akter, N, M Z Islam, A Bhuiyan, M A Siddique and M Khalequzzaman. 2017. Distinctness of 45 irrigated rice (*Oryza sativa* L.) landraces of Bangladesh through agro-morphological traits. *Eco-friendly Agril. J.* 10 (7): 100-107.

Akter, N, H Begum, M Z Islam, M A Siddique and M Khalequzzaman. 2018. Genetic diversity in Aus rice (*Oryza sativa* L.) genotypes of Bangladesh. *Bangladesh J. Agril Res.* 43 (2): 253-266.

Akter, M B, A Mosab-Bin, M Kamruzzaman, R Reflinur, N Nahar, M S Rana, M I Hoque and M S Islam. 2022. Morpho-molecular diversity study of rice cultivars in Bangladesh. *Czech Journal of Genetics and Plant Breeding,* 58 (2): 64–72. https://doi.org/10. 17221/69/2021-CJGPB.

Arolu, I W, M Y Rafii, M M Hanafi, T M M Mahmud and M A Latif. 2012. Molecular characterizations of *Jatropha curcas* germplasm using inter simple sequence repeat (ISSR) markers in Peninsular Malaysia. *Aust. J. Crop Sci.* 6 (12): 1666–1673.

Banik, S, M Z Islam, M A Haque, M A Siddique, A Mallik and M A Siddiquee. 2012. Physico-chemical properties based genetic diversity of T. Aman rice genotypes. *Eco-friendly Agril. J.* 5 (7): 69-72.

Baktiar, M H K, M A Samad, M Khalequzzaman, N Sharma and M A Siddique. 2013. Morphological characterization of local Aus rice germplasm (*Oryza sativa* L.). *Eco-friendly Agril. J.* 6 (10): 238-242.

BBS (Bangladesh Bureau of Statistics). 2020. Yearbook of agricultural statistics of Bangladesh. Government of Bangladesh, Dhaka.

Biswash, M R, M Sharmin, N M F Rahman, T Farhat and M A Siddique. 2016. Genetic diversity in modern T. Aman rice varieties of Bangladesh (*Oryza sativa* L.*). Sains Malaysiana* 45 (5): 709–716.

Bligh, H F J, N W Blackhall, K J Edwards and A M McClung. 1999. Using amplified fragment length polymorphisms and simple sequence length polymorphisms to identify cultivars of brown and white milled rice. *Crop Sci.* 39: 1715-1721.

Brolley, M. 2015. Rice security is food security for much of the world. Rice Today. International Rice Research Institute (IRRI), DAPO Box 7777, Metro Manila, Philippines. pp. 30–32.

Brown, S M and S Kresovich. 1996. in: A.H. Paterson (Ed.), Genome mapping in plants, Clandes, New York, 1996, pp. 85–93.

Cregan, P B. 1992. Simple sequence repeats DNA length polymorphisms. Probe 2: 18-22.

Emon, R M and G J Ahammed. 2020. Germplasm and genetic diversity studies in rice for stress response and quality traits. In: Rice research for quality improvement: genomics and genetic engineering, 47–60. Singapore: Springer. https://doi.org/10.1007/ 978-981-15-4120-9_3. ISBN: 978-981-15-4120-9.

FPMU. 2020. Bangladesh Food Situation Report, April-June 2020. Food planning and monitoring unit, Ministry of food, Government People's Republic of Bangladesh, Dhaka, Bangladesh. Vol. 121.

Gianfranceschi, L, N Seglias, R Tarchini, M Komjanc and C Gessler. 1998. Simple sequence repeat for the genetic analysis of apple. *Theor. Appl. Genet.* 96 (8): 1069–1076.

HIES (Household Income and Expenditure Survey). 2022. Bangladesh Bureau of Statistics. Government of Bangladesh, Dhaka.

Islam, M Z, M A Siddique, E S M H Rashid, M S Ahmed and M Khalequzzaman. 2014. Genetic diversity in Sadajira rice (*Oryza sativa* L.) germplasm. *The Agriculturists J.* 12 (1): 26-32.

Islam, M Z, M Khalequzzaman, M A Siddique, N Akter, M S Ahmed and M A Z Chowdhury. 2017. Phenotypic characterization of jhum rice (*Oryza sativa* L.) germplasm collected from Rangamati district in Bangladesh. *Bangladesh Rice Journal* 21 (1): 47-57.

Islam, M Z, N Akter, T Chakrabarty, A Bhuiya, M A Siddique and M Khalequzzaman. 2018a. Agro-morphological characterization and genetic diversity of similar named Aromatic rice (*Oryza sativa* L.) landraces of Bangladesh. *Bangladesh Rice Journal* 22 (1): 45-56.

Islam, M Z, M Khalequzzaman, M F R K Prince, M A Siddique, E S M H Rashid, M S Ahmed, B R Pittendrigh, M P Ali, *et al.,* 2018b. Diversity and population

structure of red rice germplasm in Bangladesh. PLoS ONE 5: e0196096.

Islam, M Z, M A Siddique, N Akter, M F R K Prince, M R Islam, M Anisuzzaman and M A K Mian. 2019. Morpho-molecular divergence of restorer lines for hybrid rice (*Oryza sativa* L.) development. Cereal Research Communications 47 (3): 531–540.

Islam, M Z, M Khalequzzaman, T Chakrabarty, N Akter, M F R K Prince, A Bhuiya and M A Siddique. 2021. Genetic diversity and population structure of similar named aromatic rice (*Oryza sativa* L.) landraces of Bangladesh. *SAARC J. Agric.,* 19 (2): 57-71. DoI: https://doi.org/10.3329/sja.v19i2.57826.

Joshi, S P, V S Gupta, R K Aggarwal, P K Ranjekar and D S Brar. 2000. Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus Oryza. *Theor Appl. Genet.* 100: 1311–1320.

Kabir, M S, M Salam, A Islam, M A R Sarkar, M Mamun, M Rahman, *et al.,* 2020. Doubling rice productivity in Bangladesh: A way to achieving SDG 2 and moving forward. *Bangladesh Rice Journal.* 24: 1–47. https://doi.org/10.3329/BRJ.V24I2.53447.

Khalequzzaman, M, M A Siddique and M K Bashar. 2012. Rice genetic resources conservation and utilization in Bangladesh. Genetic Resources and Seed Division, Gazipur, Bangladesh. pp. 50-60.

Khalequzzaman, M, M Z Islam, M A Siddique, M F R K Prince, E S M H Rashid and M S Ahmed. 2017. Genetic diversity in Aus rice landraces of Bangladesh using SSR markers. *Bangladesh J. Pl. Breed. Genet.,* 30 (1): 11-20.

Khalequzzaman, M, T Chakrabarty, M Z Islam, E S M H Rashid, M F R K Prince and M A Siddique. 2022b. Assessment of mean performance, variability, traits association and path coefficient in transplanted Aman rice (*Oryza sativa* L.) landraces. *Asian Journal of Biology,* 16 (4): 27-39.

Khalequzzaman, M, M Z Islam, M F R K Prince, E S M H Rashid and M A Siddique. 2022a. Genetic diversity and population structure of Boro rice landraces of Bangladesh. Rice Research: Open Access, 10 (6): 1-9.

Khalequzzaman, M, T Chakrabarty, M Z Islam, E S M H Rashid, M F R K Prince and M A Siddique. 2023. Deciphering genetic variability, traits association, correlation and path coefficient in selected Boro rice (*Oryza sativa* L.) landraces. *Asian Journal of Biology,* 19 (2): 33-45.

Kulsum, M U, M J Hasan, M A Siddique, F Begum and Z Akond. 2015. Genetic diversity in selected rice genotypes (*Oryza sativa* L.). *Bangladesh J. Agri.* 38-40: 63-72.

Kumar, H, G Kaur and S Banga. 2012. Molecular characterization and assessment of genetic diversity in sesame (*Sesamum indicum* L.) germplasm collection using ISSR markers. *J. Crop Improve.* 26: 540–557.

Lagercrantz, U, H Ellegren and L Andersson. 1993. The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res. 21: 1111-1115.

Liu, K and S V Muse. 2005. PowerMarker: Integrated analysis environment for genetic marker data. Bioinformatics 21: 2128–2129.

Mailer, R J, R Scarth and B Fristensky. 1994. Discrimination among cultivars of rapeseed (Brassica napus L.) using DNA polymorphism amplified from arbitrary primers. *Theor. Appl. Genet.* 87: 697-704.

McCouch, S R, L Teytelman, Y Xu, K B Lobos, K Clare, M Walton, B Fu, R Maghirang, Z Li, Y Xing, *et al.,* 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9: 199-207.

Morgante, M and A M Olivieri. 1993. PCR-amplified microsatellites as markers in plant genetics. *Plant J.* 3: 175-182.

Mottaleb, K A and A K Mishra. 2016. Rice consumption and grain-type preference by household: a Bangladesh case. *J Agric Appl Econ* 48: 298–319.

Muti, S A A, M I Hoque, M M Islam, M A Siddique and M S Islam. 2020. Morpho-molecular characterization and screening of rice (*Oryza sativa* L.) genotypes for salinity tolerance at seedling stage. SAARC *Journal of Agriculture* 18(2): 1-15.

Nasim, M, A Khatun, M Kabir, A Mostafizur, M Mamun, M Sarkar, *et al.,* 2021. Intensification of cropping through utilization of fallow period and unutilized land resources in Bangladesh. *Bangladesh Rice Journal*. 25: 89–100. https://doi.org/10.3329/BRJ.V25I1.55181.

Nei, M. 1972. Genetic distance between populations. Am. Natur. 106: 283-292.

Nei, M and N Takezaki. 1983. Proc. World Congr. *Genet. Appl. Livest. Prod*. 21: 405p.

Page, R D. 1996. TreeView: an application to display phylogenetic trees on personal computers. Comput. Mol. Biol. 12: 357–358.

Parsons, J B, H T Newbury, M T Jackson and B V Ford-Lloyd. 1997. Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. Mol. Breeding 3: 115–125.

Rahman, L, M R Molla, S Sultana, M N Islam, N U Ahmed, M S Rahman, M Nazim-ud-Dowla, M Shah-E-Alam and M S Alam. 2006. Plant Varieties of Bangladesh-Morphological and Molecular characterization for plant variety protection. *Bangladesh J. Agril*. Sci. 33(2): 215-225.

Rahman, L, M R Molla, S Sultana, M N Islam, N U Ahmed, M S Rahman and M Nazim-ud-Dowla. 2007. PLANT VARIETIES OF BANGLADESH: Morphological and Molecular Characterization, Seed Wing, Ministry of Agriculture, Government of the Peoples' Republic of Bangladesh, Vol. 1, 2007, 486 p.

Rahman, L, M N Islam, M S Rahman, M S Islam, M Shah-e-Alam and M K Bashar. 2008. Characterization of 94 rice (*Oryza sativa* L.) varieties of Bangladesh based on microsatellite loci. *Bangladesh J. Agril. Sci.* 35 (1): 97-112.

Rahman, L, M K Bashar, M A K Mian, M A Siddique, E S M H Rashid, N Haque and M S Islam. 2010. Plant Varieties of Bangladesh: Morphological and Molecular Characterization, Seed Wing, Ministry of Agriculture, Government of the People's Republic of Bangladesh, Volume-4, Part-Rice, 2010, pp. 1-235.

Rao, N K. 2004. Plant genetic resources: advancing conservation and use through biotechnology. *Afr. J. Biotechnol.* 3: 136–145.

Rohlf, F. 2002. NTSYS-pc: Numerical taxonomy and multivariate analysis system, 2.2 edn., Department of Ecology and Evolution, State University of NY, Stony Brook.

Saha, S R, A Ferdausi, L Hassan, M A Haque, S N Begum, F Yasmin and W Akram. 2022. Rice landraces from haor areas of Bangladesh possess greater genetic diversity as revealed by morpho-molecular approaches along with grain quality traits. Cogent Food & Agriculture 8:1,

DOI: 10.1080/23311932.2022.2075130.

Schulman, A H. 2007. Molecular markers to assess genetic diversity. *Euphytica* 158: 313–321.

Siddique, M A, E S M H Rashid, M Khalequzzaman, M Z Islam, M S Ahmed and M H K Baktiar. 2010. Genetic diversity of local rainfed rice (*Oryza sativa* L.). *Bangladesh J. Pl. Breed. Genet.* 23 (2): 41-46.

Siddique, M A, M Z Islam, M Khalequzzaman and M S Ahmed. 2011. Genetic diversity in rice (*Oryza sativa* L.) landraces of hilly areas in Bangladesh. *Bangladesh J. Pl. Breed. Genet.* 24 (2): 25-30.

Siddique, M A, M Khalequzzaman, M Z Islam, M S Ahmed and E S M H Rashid. 2013. Genetic diversity in local Boro rice (*Oryza sativa* L.) genotypes of Bangladesh. *Bangladesh J. Pl. Breed. Genet.* 26 (1): 19-24.

Siddique, M A, E S M H Rashid, M Khalequzzaman, M K Bashar and L R Khan. 2014. Molecular Characterization and Genetic Diversity in T. Aman Landraces of Rice (*Oryza sativa* L.) Using Microsatellite Markers. *Thai J. of Ag. Science.* 47 (4): 211-220.

Siddique, M A, M Khalequzzaman, M Z Islam, M H K Baktiar and M S Ahmed. 2016a. Genetic diversity assessment of quantitative traits in landraces of Aus rice. *Bangladesh J. Pl. Breed. Genet.,* 29 (2): 17-24.

Siddique, M A, M Khalequzzaman, M M Islam, K Fatema and M A Latif. 2016b. Molecular characterization and genetic diversity in geographical indication (GI) rice (*Oryza sativa* L.) cultivars of Bangladesh. *Brazilian J. Bot.* 39 (2):631–640 (DOI 10.1007/s40415-016-0271- 1)

Siddique, M A, M Khalequzzaman, M Z Islam, E S M H Rashid, M H K Baktiar and M A Z Chowdhury. 2016c. Genetic diversity in Aus rice genotypes using ILP markers. *Bangladesh Rice J.* 20 (2):13-19.

Siddique, M A, M Khalequzzaman, K Fatema, M Z Islam, M M Islam and M A Z Chowdhury. 2016d. Molecular characterization and genetic diversity of Aman rice (*Oryza sativa* L.) landraces in Bangladesh. *Bangladesh Rice J.* 20 (2):1-11.

Siddique, M A, M Khalequzzaman, K Fatema, M Z Islam, M H K Baktiar and M M Islam. 2017. DNA Fingerprinting and Genetic Diversity in Aus Rice (*Oryza sativa* L.) Landraces of Bangladesh. *Bangladesh Rice J.* 21 (1):59-65.

Siddique, M A, M Z Islam, M Khalequzzaman and A Bhuiya. 2018. Agro-Morphological characterization of geographical indication rice cultivars grown in Bangladesh. The Experiment 45 (4): 2598-2610.

Tatikonda, L, S P Wani, S Kannan, N Beerelli, T K Sreedevi, D A Hoisington, *et al.,* 2009. AFLP-based molecular characterization of an elite germplasm collection of *Jatropha curcas* L., a biofuel plant. Plant Sci. 176: 05–513.

Temnykh, S, G DeClerck, A Lukashova, L Lipovich, S Cartinhour and S R McCouch. 2001. Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.), frequency, length variation, transposon associations and genetic marker potential, *Genome Res.* 11: 1441–1452.

Wu, K S and S D Tanksley. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol. Gen. *Genet*. 241: 225–235.

Zai-quan, C, Y Fu-you, L Ding-qing, Y Teng-qiong, F Jian, Y Hui-jun, *et al.,* 2012. Genetic diversity of wild rice species in Yunnan province of China. *Rice Sci*. 19 (1): 21–28.

Zheng, K, N Huang, J Bennet and G S Khus. 1995. PCR-based marker assisted selection in rice breeding, IRRI, Manila, Philippines, pp. 16-18.