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RESEARCH ARTICLE

MOLECULAR VARIATION AND GENETIC DIVERGENCE IN T. AMAN RICE GENOTYPES USING SSR MARKERS

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ABSTRACT

Transplanted (T.) aman rice (*Oryza sativa* L.) covers the largest cultivated area in Bangladesh while very little information on molecular level characterization of T. aman genotypes persists. The major aim of this study was to dissect molecular characterization and genetic diversity of 25 T. aman rice genotypes using twelve simple sequences repeats (SSRs) markers. The rice genotypes comprised local landraces that were collected from twelve districts of Bangladesh. A total of twelve SSR primer pairs covering all 12 chromosomes of rice were selected for the study. The molecular characterization, genetic diversity, population structure and principal component analysis (PCA) were estimated and the results revealed a total of fifty alleles across 12 loci ranged from 2 (RM411) to 7 (RM252) per locus. The maximum gene diversity exhibited in RM252 (0.7) while the minimum was in RM320 (0.15). The polymorphism information content (PIC) values ranged from 0.14 to 0.67 while RM252 and RM206 identified as the most suitable markers. Clustering based on unweight pair group method with arithmetic mean (UPGMA) analysis clustered 25 genotypes into six major clusters (I-VI) with similarity coefficient value of 0.34. Cluster V contained a maximum seven genotypes with 5 sub-groups. Again, the population structure displayed 3 populations namely population 1, 2 and 3. These populations were clustered into the 3 major groups in the Principle Component Analysis (PCA) 2D plot. Here, PC1 and PC2 contributed 32.7% variations. However, the overall hybridization suggested between the genotypes of the population 1 and 3, specifically between clusters I and cluster VI, and between the diverse parents such as, genotypes Bohi Trimota and Ranga, Bohi Trimota and Purple Rice-2, Bohi Trimota and Purple Rice-3. These diverse clusters and genotypes identified through SSR makers are lay foundation of molecular characterization of T. aman rice genotypes in Bangladesh and further marker-assisted studies will be suggested.

KEYWORDS

Cultivated, Genotypes, Markers, Polymorphism, Transplanted aman rice.

1. INTRODUCTION

Rice, *Oryza sativa* (2n=24) is the most important food crop of the developing world and the staple food of more than half of the world's population. The area of rice production in Bangladesh is approximately 11.53 million hectares (ha) producing 36.39 million (m) tons of rice annually (BBS, 2020). It is grown in three distinct overlapping seasons *viz.* Aus (April to August), Aman (August to December), and Boro (January to June), where the maximum 48.92% is covered by Aman rice (BBS, 2020).

In Bangladesh, the maximum varietal diversity exhibited in T. aman landraces of rice. This broader phenotypic and genotypic variability available among the landraces leads to a greater outlook of crop improvement (Chakravarthi and Naravaneni 2006). Due to high demand for food and extensive cultivation of modern high yielding varieties, rice diversity is in threatened condition (Ahmed et al., 2010). For improving heterosis over existing ones and developing a new crop cultivar, an ample amount of genetic diversity is imperative. The genetic diversity of a crop can be identified through phenotypic and molecular markers. The molecular markers have been proven to be a more efficient tool than the conventional phenotypic approaches (Thompson et al., 1998). The simple sequence repeats (SSR) or microsatellite markers are one of the powerful techniques used for DNA fingerprinting through Polymerase Chain Reaction (PCR). The PCR amplification of tandem repeat sequences that is greatly distributed in plant genomes known as SSR polymorphism which are highly polymorphic, reproducible, easy automate, co-dominant and locus specific markers in many plant species. (Cregan, 1992; Morgante and Olivieri, 1993).

The genetic variability, molecular characterization, identification of particular varieties and documentation have been carried out by SSRs in a number of crop species including rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), soybean (*Glycine max* L.), rapeseed (*Brassica napus* L.), potato (*Solanum tuberosum* L.) and other crops (Mau et al., 2017). Despite having in the major cereal crops, characterization, documentation, population structure and molecular diversity in T. aman rice genotypes grown in Bangladesh using SSR techniques are still poor. So, the research hypothesis focused on the characterization, population structure and diversity analysis of locally grown T. aman rice genotypes through PCR based SSR markers. Therefore, the present study was aimed; (i) to characterize the T. aman genotypes based on SSR markers, (ii) to assess the molecular genetic diversity among T. aman rice genotypes, (iii) to construct population structure and suggest suitable parents among the studied genotypes.

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2. Materials And Methods

2.1 Plant Materials

Twenty-five transplanted aman (T. aman) rice genotypes were collected from twelve districts namely Khulna, Barishal, Pirojpur, Madaripur, Kishorganj, Patuakali, Faridpur, Gazipur, Cumilla, Dhaka, Tangail, and Mymensingh in Bangladesh. The geographical location of each sampling area and the research station was demonstrated in Figure 1. The investigation was carried out at the Genetic Resource and Seed Division (GRS) of Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

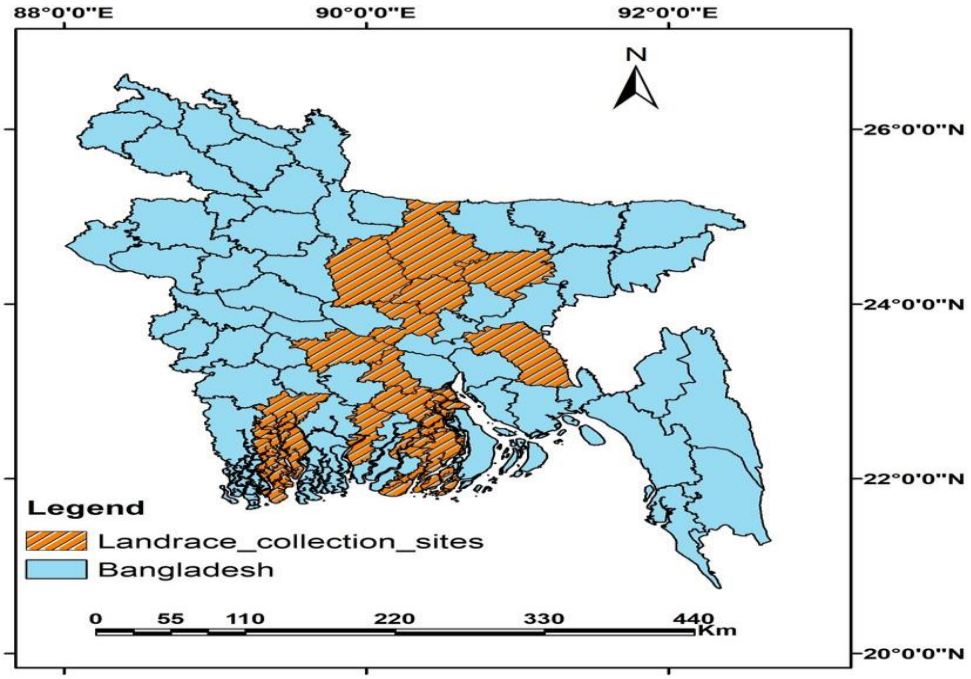

Figure 1: Map of Bangladesh showing genotype collection sites.

2.2 Genomic DNA Isolation and quantification

A healthy portion of fresh youngest leaves of three week's old T. aman rice genotypes was collected. DNA was extracted following the protocol described and stored at refrigerator (4°C) (Ferdous et al., 2012). In the following day, the DNA samples were subjected to RNase A treatment at the rate of 10 mg/mL and incubation at 37°C for 30 minutes. The quality of DNA was also checked by Thermo ScientificNanoDrop^TM1000 Spectrophotometer (Thermo Fisher Scientific, USA).

2.3 Simple sequences repeat (SSR) genotyping

A total of twelve simple SSR primer pairs (Sigma Aldrich, Germany) those covering all 12 chromosomes of rice were selected for the study presented in Table 1. The marker name, chromosomal positions, repeat motifs, primer sequences, expected length are stated in the Rice Genome database (http://www.gramene.org).

The polymerase chain reactions (PCR) were performed in a DNA 96-well thermal cycler. Before PCR, all the required reagents were counted, and a PCR cocktail was prepared. For each sample, a total volume of 10 µl reaction sample that contained 3µl DNA template, 4.0 µl Go Taq G2 Green Master Mix (Promega), 2µl Nuclease-Free Water, 10 µM concentrations of 0.5µl of each forward and reverse primers. In the thermal cycler, the PCR reactions were set as- initial denaturation for five minutes at 94°C followed by denaturation for 30 seconds at 95°C, annealing for 30 seconds at 55°C and extension for 25 seconds of 35 cycles where with a final extension for 5 minutes at 72°C. To observe the better resolutions of SSR marker bands, the PCR products were analyzed on 2% polyacrylamide gel electrophoresis (PAGE). In PAGE, 2.5 µl of amplified products of each sample separately were loaded and run the gel in 0.5X TBE (Tris base, boric acid and EDTA) buffer for 1.5-2.5 hours based on the allele size at 100 volts and 500 mA current. The gels were stained in 5 µl SYBR Safe DNA gel stain (10,000X concentration in DMSO, USA) with 200 ml 0.5X TBE buffer for 15 min and exposed to UV (ultra-violet) light through a gel documentation unit (XR System, Uvitec Cambridge, France) connected to a PC (window) for visualization under UV light.

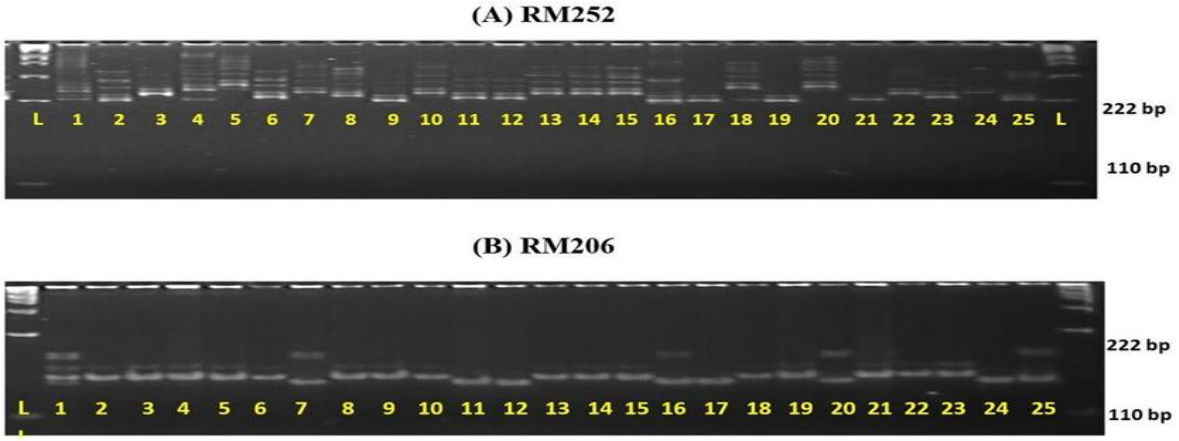

Figure 2: DNA fingerprinting of 25 T. aman rice germplasms using polyacrylamide gel electrophoresis.

Legends: 1=Uri Chadra, 2=Ranga Aman, 3=Betti Jona, 4=Bohi Trimota, 5=Shada Gabura, 6= Sotam, 7=Lal Binni, 8=Lal Mota, 9=Gabura, 10=Purple Rice-1, 11=Purple Rice-2, 12=Purple rice-3, 13=Aman Dhan-1, 14=Aman Dhan-2, 15=Aman Dhan-3, 16=Ijol Diga, 17=Bawoi Jhak, 18=Chini Sagar, 19=Bansha Pur, 20=Roshon Bok, 21=Pura Binni, 22=Telot, 23=Joli Amon, 24=Molla Diga, 25= Netpasha and L = 100 DNA ladder.

Table 1: List of SSR Markers showing chromosome position, primer sequence and annealing temperature used in the study

Sl. No.	Primer Name	Chrom no.	Forward primer (5′–3′)	Reverse primer (5′–3′)	Annealing Temp. (°C)
1	RM212	1	CCACTTTCAGCTACTACCAG	CACCCATTTG	55
2	RM208	2	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	55
3	RM411	3	ACACCAACTCTTGCCTGCAT	TGAAGCAAAAACATGGCTAGG	55
4	RM252	4	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	55
5	RM26	5	GAGTCGACGAGCGGCAGA	CTGCGAGCGACGGTAACA	55
6	RM190	6	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG	55
7	RM320	7	CCAACACTGCCACTCTGTTC	GAGGATGGACACCTTGATCG	55
8	RM447	8	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC	55
9	RM205	9	CTGGTTCTGTATGGGAGCAG	CTGGCCCTTCACGTTTCAGTG	55
10	RM228	10	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC	55
11	RM206	11	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	55
12	RM1337	12	GTGCAATGCTGAGGAGTATC	CTGAGAATCTGGAGTGCTTG	55

2.4 Allele scoring and data analysis

In each of the SSR marker electrophoretic image, the size of the most intensive band (in nucleotide base pair) was determined with the help of 100 bp DNA ladder and Alpha Ease FC 5.0 software. The software package Power Marker 3.25 was used to estimate the allele number per locus, frequency of major allele, gene diversity and PIC values (Liu and Muse, 2005). The genetic distance between the rice genotypes was calculated with the help of "Nei distance 1983" (Page, 1996). The binary format of the alleles (allele presence of allele = "1" and absence of allele = "0") retrieved from the allele frequency using NTSYS-pc version 2.2 was used to establish a UPGMA dendrogram that revealed the distance-based relationship among the rice genotypes (Rohlf, 2002).

The population structure among the genotypes was computed using the software package version 2.3.3 where an admixture model-based clustering method was followed (Pritchard et al., 2000). Here, the clusters (k) were raged from 1 to 15 with 20 iterations at 100,000 burn-in period and 10,000 MCMC (Markov Chain Monte Carlo) replications. PCA was estimated, and the first two principal components were considered to construct 2D graph through Metabo Analyst software version 5.0 (McGill University, Montréal, QC, Canada).

3. Results

To characterize and observe the diversity pattern of studied rice genotypes, twelve microsatellite markers (SSRs) were used. All twelve SSRs revealed polymorphic bands after polymerase chain reaction and polyacrylamide gel electrophoresis analysis. The outcome of the research is presented under the following subheads.

3.1 Assessment of polymorphism from SSR Profiles

The twelve polymorphic SSR markers were analyzed across tested rice genotypes and summary statistics of the detected alleles are given in Table 2 and gel images of PCR amplified fragments using RM252 and RM206 markers are showed in Figure 2. 12 SSRs produced a total of 50 alleles across studied genotypes. The number of alleles per locus ranged from 2.0 to 7.0, with an average of 4.17. The marker RM252 produced the highest number of polymorphic alleles of seven (7) followed by RM26, RM205, RM228 and RM206 with five (5) each, respectively (Table 2). While RM411 produced least number of polymorphic alleles per locus of two (2). The most common allele frequency per locus ranged from 48% (RM252) to 92% (RM302). All tested genotypes shared a common major allele with an average, 69% at any given locus.

The gene diversity ranged from 0.15 to 0.70 with an average of 0.47. The maximum genetic diversity (0.70) was exhibited in locus RM252 and minimum (0.15) was recorded in locus RM302 (Table 2). The wide range of PIC among twelve SSRs exhibited that variation exists from 0.14 to 0.67 with an average of 0.43. The highest PIC value (0.67) was recorded for RM252, followed by RM206 (0.56), RM208 (0.49), RM228 (0.48), RM190 (0.46) and RM1337 (0.44).

Table 2: Numbers of alleles, allele size range, frequency, gene diversity, PIC for 12 microsatellite markers (SSRs) in 25 T. aman rice genotypes

S/No	Marker	Chro. No.	Position (cM)	Motif*	Allele No.	Unique Allele	Size range (bp)	Size (bp)	Freq (%)	Gene diversity	PIC
1	RM212	1	148.7	(CT)24	4	-	117-144	144	72.00	0.45	0.42
2	RM208	2	186.4	(CT)17	3	-	163-179	179	60.00	0.56	0.49
3	RM411	3	127.9	(GTT)7	2	-	110-116	110	72.00	0.40	0.32
4	RM252	4	99	(CT)19	7	3	202-240	202	48.00	0.70	0.67
5	RM26	5	118.8	(GA)15	5	-	97-122	122	72.00	0.46	0.44
6	RM190	6	7.4	(CT)11	4	-	110-123	123	68.00	0.50	0.46
7	RM302	7	62.4	(AT)11GTAT(GT)13	3	2	220-243	220	92.00	0.15	0.14
8	RM447	8	124.6	(CTT)8	3	-	108-122	108	76.00	0.39	0.35
9	RM205	9	114.7	(CT)25	5	2	116-161	116	76.00	0.41	0.39
10	RM228	10	130.3	(CA)6(GA)36	5	1	113-156	156	68.00	0.51	0.48
11	RM206	11	102.9	(CT)21	5	2	129-169	169	56.00	0.61	0.56
12	RM1337	12	0	(AG)21	4	1	185-222	222	68.00	0.49	0.44
	Max.				7	3			92.00	0.70	0.67
	Min.				2	1			48.00	0.15	0.14
	Total				50	11			828.00	5.63	5.97
	Mean				4.17	1.0			69.00	0.47	0.43

Note: Chro. No. = Chromosome number, Freq. =Frequency, PIC=Polymorphic Information Content

3.2 Pairwise genetic distance

A dissimilarity matrix based on the "Nei genetic distance dissimilarity 1983" was estimated and are shown in Table 3. According to this, the pairwise dissimilarity ranged from 0.00 to 0.92 (0% to 92%) with an average of 0.45 (45%). Bohi × Ranga, Bohi × Purple Rice-2, Bohi × Purple Rice-3, exhibited the highest genetic dissimilarity of (92%). The minimum genetic dissimilarity was observed between Aman Dhan-2 × Aman Dhan-1, Bansha Pur × Aman Dhan-3 of 0% (i.e., 100% similarity). Here, Aman Dhan-1, -2 and -3 were collected from Gazipur and Bansha Pur was collected from its adjacent district Tangail.

Table 3: Nei's Genetic Dissimilarity Distance Between the pair of T. aman rice Genotypes obtained from 12 Microsatellite used in 25 T. aman rice genotypes

Genotype	G1	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G2	G20	G21	G22	G23	G24	G25	G3	G4	G5	G6	G7	G8	G9
G1	0.00																								
G10	0.17	0.00																							
G11	0.50	0.58	0.00																						
G12	0.58	0.67	0.08	0.00																					
G13	0.33	0.33	0.58	0.50	0.00																				
G14	0.33	0.33	0.58	0.50	**0.00**	0.00																			
G15	0.17	0.17	0.42	0.50	0.17	0.17	0.00																		
G16	0.42	0.42	0.42	0.50	0.33	0.33	0.25	0.00																	
G17	0.50	0.42	0.67	0.75	0.67	0.67	0.50	0.58	0.00																
G18	0.50	0.50	0.58	0.58	0.42	0.42	0.33	0.42	0.58	0.00															
G19	0.17	0.17	0.42	0.50	0.17	0.17	**0.00**	0.25	0.50	0.33	0.00														
G2	0.33	0.42	0.67	0.75	0.58	0.58	0.42	0.67	0.58	0.67	0.42	0.00													
G20	0.50	0.50	0.33	0.33	0.42	0.42	0.33	0.25	0.67	0.33	0.33	0.75	0.00												
G21	0.33	0.25	0.58	0.50	0.17	0.17	0.17	0.42	0.58	0.42	0.17	0.58	0.42	0.00											
G22	0.25	0.33	0.42	0.50	0.33	0.33	0.17	0.42	0.58	0.50	0.17	0.33	0.50	0.33	0.00										
G23	0.25	0.25	0.50	0.50	0.17	0.17	0.08	0.33	0.58	0.33	0.08	0.50	0.33	0.17	0.25	0.00									
G24	0.50	0.58	0.33	0.42	0.58	0.58	0.42	0.33	0.67	0.58	0.42	0.67	0.25	0.58	0.42	0.50	0.00								
G25	0.33	0.42	0.58	0.67	0.50	0.50	0.33	0.58	0.33	0.42	0.33	0.58	0.67	0.42	0.42	0.42	0.67	0.00							
G3	0.33	0.42	0.75	0.67	0.42	0.42	0.42	0.67	0.67	0.58	0.42	0.67	0.67	0.25	0.50	0.42	0.75	0.42	0.00						
G4	0.75	0.67	**0.92**	**0.92**	0.67	0.67	0.67	0.67	0.83	0.75	0.67	**0.92**	0.75	0.67	0.75	0.67	0.83	0.83	0.58	0.00					
G5	0.50	0.58	0.67	0.67	0.58	0.58	0.58	0.75	0.67	0.67	0.58	0.50	0.58	0.67	0.42	0.58	0.83	0.67	0.58	0.75	0.00				
G6	0.33	0.42	0.58	0.58	0.42	0.42	0.33	0.58	0.58	0.50	0.33	0.58	0.58	0.42	0.33	0.25	0.67	0.42	0.33	0.67	0.50	0.00			
G7	0.50	0.50	0.50	0.50.	0.42	0.42	0.33	0.25	0.58	0.42	0.33	0.67	0.25	0.42	0.42	0.33	0.33	0.67	0.67	0.67	0.75	0.58	0.00		
G8	0.58	0.50	0.83	0.83	0.58	0.58	0.50	0.75	0.58	0.67	0.50	0.75	0.67	0.50	0.58	0.42	0.75	0.67	0.42	0.33	0.67	0.42	0.67	0.00	
G9	0.25	0.33	0.58	0.67	0.42	0.42	0.33	0.50	0.67	0.67	0.33	0.42	0.67	0.50	0.33	0.42	0.67	0.58	0.58	0.83	0.50	0.42	0.67	0.75	0.00

Note: Where 'G' represent the Genotypes as**:** 1=Uri Chadra, 2=Ranga Aman, 3=Betti Jona, 4=Bohi Trimota, 5=Shada Gabura, 6= Sotam, 7=Lal Binni, 8=Lal Mota, 9=Gabura, 10=Purple Rice-1, 11=Purple Rice-2, 12=Purple rice-3, 13=Aman Dhan-1, 14=Aman Dhan-2, 15=Aman Dhan-3, 16=Ijol Diga, 17=Bawoi Jhak, 18=Chini Sagar, 19=Bansha Pur, 20=Roshon Bok, 21=Pura Binni, 22=Telot, 23=Joli Amon, 24=Molla Diga, 25= Netpasha

3.3 Clustering pattern and genetic distance of T. aman rice genotypes

The genetic relationship using UPGMA dendrogram are shown in Figure 3. Here, the UPGMA dendrogram was generated by using similarity coefficient values of 25 T. aman rice genotypes. According to the Figure 3, six different clusters were revealed with the similarity coefficient 0.00 to 0.30. The cluster V comprised of maximum seven genotypes namely; Chini Sagar, Betti Jona, Sotam, Gabura, Gabura, Uri Chadra and Purple Rice-1. These genotypes were originated from seven different districts viz., Tangail, Barisal, Madaripur, Faridpur.

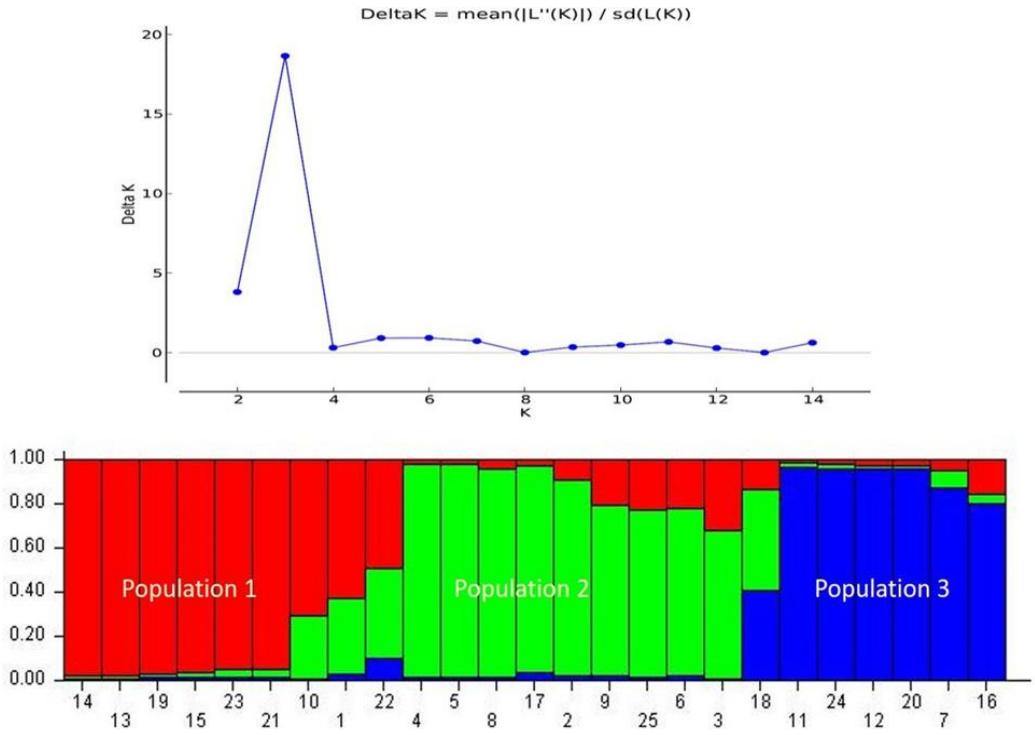

Figure 3: An UPGMA cluster dendrogram showing the genetic relationships of 25 T. Aman rice germplasms

Mymensingh, Khulna, and Gazipur respectively. Here, Betti Jona and Sotam showed 13.01% dissimilarity and Uri Chadra and Purple Rice-1 showed 7.03% dissimilarity. Cluster IV consisted of genotypes Purple Rice-2, Purple rice-3, Molla Diga, Lal Binni, Ijol Diga and Roshon Bok which showed maximum 20% dissimilarity between two major sub-clusters. Here, Molla Diga and Ijol Diga originated from Dhaka and rest genotypes under this cluster are derived from Gazipur, Cumilla, Kishorgonj and Tangail. Joli Amon, Aman Dhan-3, Bansha Pur, Pura Binni, Aman Dhan-1 and Aman Dhan-2 formed another single cluster VI which had the additional sub-cluster within it. Here, three genotypes Aman Dhan-1, Aman Dhan -2 and Aman Dhan -3 originated from same district Gazipur. Again, Pura Binni and Joli Aman genotypes extract from same district and revealed minimum distance.

Here, cluster I consisted of Bohi Trimota and Lal Mota which showed 10.01% dissimilarity between them and originated from Pirojpur and Patuakali. Cluster II consisted of Ranga Aman and Shada Gabura which showed 20% dissimilarity between them. Ranga Aman and Shada Gabura originated from Khulna and Faridpur, respectively. Cluster III consisted of Bawoi Jhak and Netpasha which showed 10.01% dissimilarity between them and originated from Dhaka.

3.4. Population structure

The population structure of 25 rice genotypes was assessed using Bayesian based approach. The estimated membership fractions of the 25 genotypes for different k values, ranged from 2 to 15 (Figure 4). The best Evanno's ΔK was 3, which indicated that the entire population evaluated could be clustered into three groups 1 (36%), 2 (40%), and 3 (24%). Here, the membership fractions were used to classify the populations either pure or an admixture type. A Significant proportion of pure population were revealed by population 1 (66.67%), population 2 (50%) and population 3 (66.67%). The remaining portions in each sub-population were admixture.

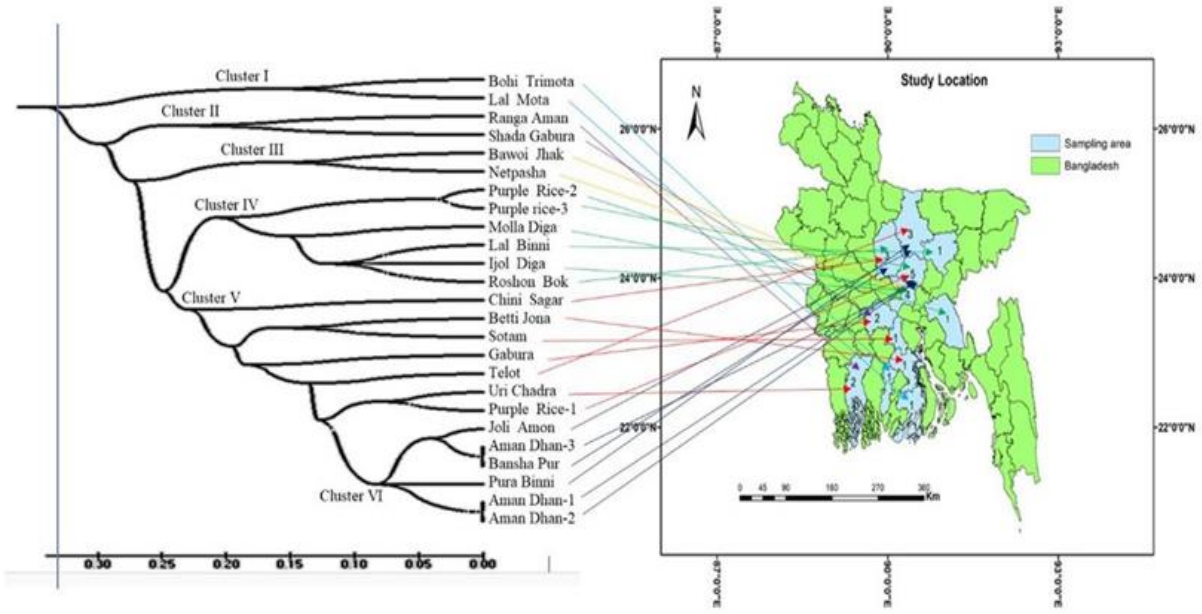

Figure 4: The structure analysis showed highest Link values and relationships of 25 T. aman rice germplasms

Genetic variation in three populations evolved from 25 rice genotypes was tested using fixation index (FST) statistics. Here, the FST population values were 0.5789 for population 1, 0.0216 for population 2, and 0.4349 for population 3, with an average of alpha 0.0912 representing the significant differences among the population structure. We calculated average distances (expected heterozygosity) between individuals in same populations. The average intra-population distances in the same cluster were 0.1100 for population 1, 0.3201 for population 2 and 0.2027 for population 3. The maximum genetic distance (0.0792) was exhibited between population 1 and 3, and the minimum genetic distance was revealed between population 1 and 2 (0.0398).

3.5 Principal component analysis (PCA)

Principal Component Analysis associations among 25 genotypes were computed using PCA method. Here, first principal component (PC1), and the second principal component (PC2) explained 19.0% and 13.7 % of total variation, respectively and localization of genotypes in a 2D PCA plot indicates the genetic distances among tested genotypes (Figure 5). Genotype Chini Sagar, Ijol Diga, Lal Binni, Purple Rice-2, Purple rice-3, Molla Diga and Roshon Bok, included in an unique group those originated from population 3. Population 1 and population 2 were mixed with genotypes Uri Chadra, Purple Rice-1, Netpasha and Bawoi Jhak.

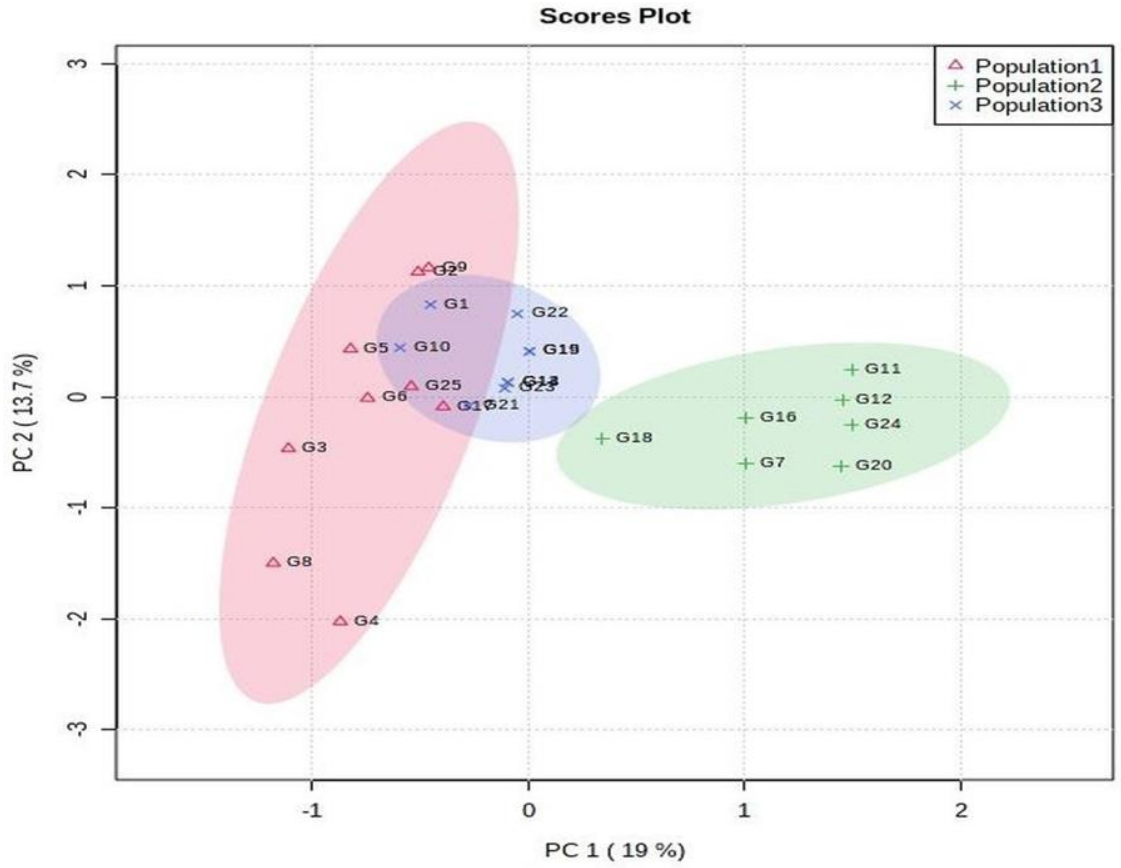

Figure 5: Two-dimensional principal component analysis (PCA) based on SSR polymorphisms in the 25 T. aman genotypes.

4. Discussions

Molecular characterization and genetic diversity study of rice genotypes are one of the major determinates for characterization, conservation and breeding programs. Molecular marker-based identification and characterization of T. aman rice may help to the concise classification of genotypes, identifying collections, screen its genotypes or genetic diversity and also helpful to observe the taxonomic relationships (Jain et al., 2017). Again, molecular markers are used in the genetic diversity assessment for their hyper variability, greater reproducibility, having neutral and free from environmental fluctuations (Bhandari et al., 2017).

4.1 Molecular polymorphism

Twelve (12) polymorphic SSR markers across twenty-five (25) T. aman rice genotypes revealed a higher number of alleles (50) (Table 2). It was ranged from 2 to 7 with an average of 4 alleles per locus. A group researcher observed 2 to 8 alleles per locus with an average 3.8 in BRRI released varieties (Ali et al., 2010). This finding that is almost similar to our study. A study observed 2 to 6 number of alleles per locus with an average 3.63 across of 11 polymorphic SSRs in 24 rice genotypes (Sooganna et al., 2019). In contrast, some researcher observed 13 to 34 alleles per locus of eight SSRs with an average 19.88 over 96 Aman rice genotypes (Siddique et al., 2016).

In SSRs, detection of unique alleles is imperative in breeding purposes for identifying specific genotypes and a potential novel allele's bank (Thudi et al., 2011; Behera et al., 2012, Singh et al., 2013). In our study, a total of 11 unique alleles were detected by SSRs (Table 3), and each genotype revealed unique allele for at least one microsatellite locus. However, the frequency of alleles ranged from 48% (RM252) to 92% (RM320) with an average of 69% alleles (Table 3), that is higher than (Islam et al., 2019; Siddique et al., 2014; Siddique et al., 2016; Jayabalan et al., 2019).

The expected heterozygosity in the individuals can be estimated through the gene diversity. In our study, the gene diversity ranged from 0.15 to 0.70 giving a mean of 0.47. This medium gene diversity exhibited a close genetic makeup among genotypes. One of the possibilities of close genetic makeup may be the number of genotypes (25). This mean gene diversity value was lower compared to (Islam et al., (2019), 0.56, and (Ahmed et al., 2016) 0.77, respectively.

The PIC indicates the level of polymorphism by determining the allele diversity at a particular locus and served as suitable index for evaluation of genetic diversity. Botstein et al., (1980) categorized the PIC index in to three groups, namely high diversity (PIC>0.5), intermediate (PIC = 0.25-0.50) and low diversity (PIC<0.25). Out of the 12, only 2 markers (RM252 and RM206) exhibited PIC values greater than 0.5. These markers are considered as highly informative or polymorphic and could be utilized in future marker assisted breeding. The intermediate PIC value was recorded in RM208 (0.49), RM228 (0.48) and RM190 (0.46). This result suggested that the mentioned markers might be utilized for characterization of T. aman rice genotypes from a diverge origin due to considerable PIC values.

4.2 Genetic dissimilarity matrix

The knowledge of genetic background study is important to design a novel breeding program and expecting higher heterosis values. We studied

genetic dissimilarity through the coefficient of genetic distance. The greater range (0-92%) of Nei's (1983) genetic distance revealed among genotypes. Among these, the lowest genetic distance existed between Aman Dhan-2 × Aman Dhan-1, Bansha Pur × Aman Dhan-3 (0%) exhibited a very close relationship. The observed genetic dissimilarity revealed a common ancestral origin, or higher rate of interbreeding due to similar alleles present in their genome. The highest genetic distance was found between Bohi Trimota × Ranga Aman, Bohi Trimota × Purple Rice-2, Bohi Trimota × Purple Rice-3 (92%) showing the diverse genetic background. The genotypes Bohi Trimota, Ranga Aman, Purple Rice-2 and Purple Rice-3 were collected from four different districts Pirojpur, Khulna, Cummila and Gazipur, respectively. The high level of similarity could be due to the intra specific variation in the germplasm used. A group researcher observed lowest Nei's pairwise distance (0.25) between Kalizira and Khirsha and highest genetic distance (1.0) between BRRI Dhan34 and ChiniSagor, Kalizira, Khirsha, LR-189, LR-42 etc (Haque et al., 2018). A group researcher observed highest genetic diversity of 62.3% between Pipanfary Red1 and FL478 and lowest between NBGS3 and NBGS2 (4.1%) (Gasim et al., 2019).

4.3 Cluster analysis

The cluster analysis was performed with a view to identify and classify diversity of rice genotypes based on UPGMA method. The studied rice genotypes were grouped into six major clusters namely clusters I, II, III, IV, V and VI (Figure 3). A group researchers classified 63 rice cultivars into 5 clusters based on Dice similarity coefficient and UPGMA method (Tarang et al., 2020; Jackson et al., 1989).

The highest numbers of genotypes (7) were included in Cluster V with five sub-clusters. Surprisingly, these genotypes originated from different districts *viz*. Tangail, Barisal, Madaripur, Faridpur, Mymensingh, Khulna, and Gazipur. Due to the diverse origin, the more cluster sub-groups (5) exhibited in this cluster. The second largest cluster was Cluster IV and VI those contained 6 genotypes in each. Both of the clusters were divided into three sub-clusters where the sub-clustering genotypes share common gene pools. The cluster IV and Cluster VI were diverse with a genetic distance of 0.25 and 0.08, respectively. We found genotypes were originated from different districts *viz*. Cluster IV genotypes from Dhaka, Tangail and Kishorganj and Cluster VI genotypes from Gazipur, Mymensingh, and Tangail districts. Cluster I, II and III consisted with two genotypes in each. Here, the genotypes in cluster I and II were originated from different districts *viz.* Khulna, Patuakali and Faridpur but the genotypes in cluster III were originated from Dhaka.

Overall, the genotypes Molla Diga, Chini Sagar, Gabura, Telot and Pura Binni were characterized as monogenetic in nature i.e. they are not sharing any genetic similarity between themselves and between the entire twenty-five genotypes studied. Based on the cluster distance scale in Fig. 3, the maximum inter-cluster distance was revealed between cluster I (>0.3) and cluster VI (0.16). Therefore, the genotypes from these clusters might be chosen for potential parents / donors in the future breeding programs. Pathak *et al*., (2020) classified 29 genotypes into two main clusters with a dissimilarity coefficient of 0.36 and classified aus rice genotypes into five distinct groups with a coefficient value of 0.49 in UPGMA dendrogram (Khalequzzaman et al., 2017).

4.4 Population structure

Population structure analysis grouped tested genotypes into three populations namely population 1, 2 and 3. The maximum numbers (10) of genotypes were grouped in population 2, with lower (6) in population 3. These three populations were significantly different from each other. This was established by pairwise FST that ranged from 0.022 to 0.579. a group researcher observed four populations with FST, ranging from 0.108 to 0.207 (Islam et al., 2018). In addition, the population structure displayed whether the pigmented rice genotypes belonging to the pure or admixture type. The genotypes with a probability value greater than 0.80 are considered as pure. Following this, a significant proportion of pure population was revealed by (>50%) pure in all populations. The remaining portions in each sub-population were admixture type. Some researchers exhibited three populations where population 2 had maximum 282 pure (86 %) and minimum 47 (14 %) (Singh et al., 2016). Again, the maximum genetic (net nucleotide) distance (0.0792) was observed between population 1 and 3 that depicted the suitability of the genotypes in hybridization program.

4.5 Principal Component Analysis

Principal Component Analysis (PCA) analysis showed that all 25 T. aman rice genotypes formed three subgroups of points in a 2D PCA plot. Here, the PC1 and PC2 explained higher variation (>25 %). According to a study, the higher variation (>25 %) could be utilized together with cluster analysis to found suitable and related genotypes (Ahmad et al., 2015). In this study, most individuals were grouped more closely according to their origin compared to the UPGMA dendrogram. The population three groups significantly differed from population 1 and 2. In some study, author grouped the Indian rice cultivars and wild genotypes into three major groups those distributed across the principal component quadrants (Surapaneni et al., 2016).

5. Conclusion

In summary, the markers RM252 and RM206 were most effective markers in the detection of polymorphism over the entire 25 T. aman rice genotypes studied. The hybridization could be design between the two distant populations cluster I and cluster VI. The highest genetic dissimilarities between Bohi Trimota and Ranga Aman, Bohi Trimota and Purple Rice-2, Bohi Trimota and Purple Rice-3 make them suitable as parents to produce new varieties by plant breeders or geneticist for the benefit of the farmers. The population structure displayed three distinct populations 1, 2 and 3. These populations were confirmed by further clustering in PCA 2D plot. Here, population 1 and 3 were more diverse. Overall, the major outcome of the research is the one step forward for the construction of sovereignty of Bangladeshi rice gene pool. The diverse parents identified in this study also helpful in parental selection during backcross breeding and will assist in broadening genetic makeup of rice genotypes in future.

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