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Genetic Polymorphism of Some BBRI Developed Rice Cultivars by SSR Markers

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ABSTRACT

Genetic diversity and relationships among seven modern rice cultivars were evaluated using 299 SSR markers that covered the rice genome. A total of 405 alleles were detected at 165 polymorphic SSR loci and the number of alleles per marker ranged from 2 to 5, averaging of 2.45 alleles per locus. The band size for a given microsatellite locus variation between 73 bp (RM9) to 335 bp (RM539). Polymorphism information content (PIC) values ranged from 0.21 to 0.70, with an average of 0.36. Total 43 markers were found as highly informative on 12 chromosomes. Among them, marker RM437 showed the highest polymorphism followed by RM9, RM472, RM85, RM548, RM334, RM336, RM464, RM222 and RM229 which might be effectively used for genetic diversity and relationship study of rice. Comparatively higher genetic distance was observed between BRRI dhan47 and BRRI dhan50 genotypes pair than the other combinations. Cluster analyses were used to group cultivars by constructing dendrogram based on SSR marker analysis which grouped the ice cultivars into three groups, where cluster 1 contained only one cultivar, and cluster 2 and cluster 3 consists of three cultivars each. The results of the genetic diversity will be useful for the selection of the parents for developing a rice breeding program and will construct a genetic fingerprint for varietal identification and background selection during breeding program.

Keywords: Genetic diversity, Cluster analysis, Microsatellite marker, Molecular diversity

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops supporting more than half of the world population [1]. South Asia, one of the major centers for rice domestication, has been described as the “food basket” and “food bowl” of Asia [2]. Compared with other crop species, the genetic diversity in the world rice germplasm is quite large. Three subspecies, i.e., indica, japonica and javanica, compose a large reservoir of rice germplasm including a variety of local landraces and cultivars [3-5]. Despite the richness of genetic resources, only a small proportion of the world rice germplasm collections have been used in breeding programs. As a consequence a high genetic similarity is found within several commercial rice germplasms around the world [6].

Genetic similarity and diversity could be assessed by plant morphology, physiology, isozymes, storage protein profiles, DNA markers, etc. [7]. Among different DNA markers, SSR markers have been extensively used as a powerful tool in variety protection [8], molecular diversity studies [9], QTL analysis, pedigree analysis and marker assisted breeding [10]. SSRs have been developed for many crop species, including wheat [11], maize [12], sorghum [13], tomato [14], etc. In rice, SSRs have been used to assess the genetic diversity of both wild and cultivated species [15-18]. SSR markers are particularly suitable for evaluating genetic diversity and relationships among closely related plant

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species, populations or individuals [19]. Recently a total of 18,828 Class I (di, tri, tetra nucleotide) SSRs, representing 47 distinctive motif families have been released after completion of the Nipponbare genome sequence [20]. This is the largest numbers of SSR markers publicly available for any crop species. SSR markers are more popular in rice because they are highly informative, mostly monolocus, co-dominant, easily analyzed and cost effective [12].

Using SSRs genetic diversity and polymorphisms of land races, local cultivars and diverse genetic stocks are frequently studied. The objectives of the present study were to use SSR markers: (1) to estimate the genetic diversity; (2) to evaluate of the degree of polymorphism; and (3) to reveal genetic relationships among seven modern rice cultivars released by Bangladesh Rice Research Institute (BRRI) between 1994 and 2011.

MATERIALS AND METHODS

The experiment was conducted at Marker Assisted Selection (MAS) laboratory of Plant Breeding Division of BRRI.

Plant materials and markers

Seven rice cultivars were used in this investigation **(Table 1)**. These cultivars were used in this study to evaluate the use of SSR markers for identification of Arsenic (As) tolerant quantitative Trait Loci (QTL) and for the selection of As tolerant rice genotypes during breeding program. It was found that BRRI dhan47 showed high tolerance to As and BRRI dhan54 and BRRI dhan55 showed medium tolerance (data not published). On the contrary BRRI dhan45 is the most susceptible cultivars followed by the other three varieties used in the present study (unpublished data). A total of 299 SSR markers were used in this study. Markers were selected based on their location to cover all the chromosomes uniformly maintaining more or less similar distance considering distances in base pair (bp).

Table 1. List of rice cultivars used in present investigation.

S. No.	Name of Cultivar	Pedigree	Progenitors	Year of release
1	BRRI dhan28	BR601-3-3-4-2-5	BR6(IR28)/Purbachi	1994
2	BRRI dhan29	BR802-118-4-2	BG90-2/BR51-46-5	1994
3	BRRI dhan45	BR5778-21-2-3	BR2/TETEP	2005
4	BRRI dhan47	IR63307-4B-4-3	IR51511-B-B-34-B/TCCP266-2-49-B-B-3	2007
5	BRRI dhan50	BR6902-16-5-1-1	BR30/IR67684B	2008
6	BRRI dhan54	BR5999-82-3-2-HR1	BR1185-2B-16-1/BR548-128-1-3	2010
7	BRRI dhan55	IR73678-6-9-B	IR64/*Oryza rufipogon*	2011

Isolation of genomic DNA

Seeds were germinated in a germination chamber at 30°C temperature. Three days after germination, germinated seeds were sown in earthen pots. The pots were then kept in a net house. Genomic DNA was isolated from young leaves of 30 days old seedlings following modified Miniscale method as described by Rahman et al. [21]. DNA samples were evaluated both quantitatively and qualitatively using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and the concentration was determined using module Nucleic Acid, Software: 3.3.1.

Molecular analysis through SSR

Polymerase chain reaction (PCR) amplification was carried out in a 10 µl reaction mixture, each reaction containing 25 ng of template DNA, 0.5 µM of each forward and reverse primer, 3 mM $MgCl_2$, 0.2 mM of dNTP mix, 1x PCR buffer and 1 unit of *Taq* DNA polymerase (Bio basic, Canada). Amplifications were performed at G-strom DNA Thermal Cycler (Gene Technology Ltd., England). The temperature cycles were programmed as initial denaturation at 94°C for 5 min, 35 cycles of 45 s denaturation at 94°C, 45 s annealing at 55°C, 1 min and 30 s extention at 72°C and additional temperature of 72°C for 7 min for final extension and finally cooled at 4°C temperature. 10x Loading dye was added to each well of the PCR plate and the amplified PCR products were subjected to electrophoresis in 8% polyacrylamide gel in 0.5x TBE buffer at 100 V for 1.30 to 2.15 h (depending on product size) along with a known DNA ladder. The gels were stained with ethidium bromide for 25-30 min and DNA fragments were visualised under UV light and gels were photographed using gel documentation system (BIO-RAD).

DATA ANALYSIS AND CLUSTERING

The molecular weights for each band were measured in base pairs using Alpha Ease FC 5.0 software (Alpha Innotech Corporation). 165 polymorphic SSR markers **(Table 2)** distributed across 12 chromosomes were used for diversity analysis. Data of non-amplified and monomorphic SSR markers were not used for diversity study. Summary

statistics, including the number of alleles per locus, major allele frequency and Polymorphic information content (PIC) values were determined using Power Marker version 3.25 [22]. PIC values were calculated for each of the SSR loci with the following formula proposed by Anderson et al. [23].

$$PICi = 1 - \sum_{j=1}^{n} (Pij)^2$$

Where, n is the number of marker alleles for marker i and Pij is the frequency of the j^{th} allele for marker i

Table 2. Nei's genetic distant (below diagonal) values among studied rice genotypes.

Cultivar	BRRI dhan28	BRRI dhan29	BRRI dhan45	BRRI dhan47	BRRI dhan50	BRRI dhan54	BRRI dhan55
BRRI dhan28	0.0000						
BRRI dhan29	0.4483	0.0000					
BRRI dhan45	0.3419	0.5043	0.0000				
BRRI dhan47	0.7059	0.7436	0.7458	0.0000			
BRRI dhan50	0.5565	0.4956	0.6261	0.7672	0.0000		
BRRI dhan54	0.4528	0.4857	0.5283	0.7009	0.4906	0.0000	
BRRI dhan55	0.5045	0.4818	0.5727	0.7321	0.4771	0.0571	0.0000

The genetic distance was calculated using the Nei distance [24]. The allele frequency data from Power Marker version 3.25 was used to export the data in binary format (allele presence=1; allele absence=0) for analysis with Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) version 2.2 [25]. The similarity matrix was calculated with the Simqual subprogram using the Dice coefficient [26] and subjected to cluster analysis by unweighted pair group method for arithmetic mean (UPGMA) and a dendrogram was generated using the programme NTSYS-pc.

RESULTS

Allelic diversity

The molecular marker is a useful tool for assessing genetic variations and resolving cultivar identities. Keeping this in mind, a total of 299 SSR markers was used across the tested cultivars. Among them, 27 markers did not amplify at all and 107 markers showed monomorphic nature. Therefore, these 134 markers do not have importance of diversity and genetic relationship study of the cultivars. The rest 165 markers showed polymorphism among the tested cultivars. A total of 405 alleles were detected at these SSR markers among the tested rice genotypes **(Table 3)**. The average number of alleles per locus was 2.45, ranging from 2 to 5 across 165 SSR markers, with 2 alleles for 108 markers, 3 alleles for 40 markers, 4 alleles for 16 markers and 5 alleles for 1 marker. The overall size of PCR products amplified using 165 markers ranged from 73 bp (RM9) to 335 bp (RM539). The molecular size difference between the smallest and the largest allele for a given locus varied widely from 3 to 109 bp **(Table 3)**. There was a considerable range in allele frequency (42.86-85.71%); on an average 68.46% of the rice varieties shared a common allele. No relationships were found among the number of alleles and nucleotide motif composition or the number of repetitions in the motifs.

Table 3. Allelic variation and polymorphic information content (PIC) values of different rice cultivars for 165 SSR polymorphic markers.

Marker ID	Chr. No.	Position (Mb)	Repeat Motif	Total allele	Allele size (bp)		Highest frequency allele		PIC value
					Range	Difference	Size (bp)	Frequency (%)	
RM495	1	0.21	(CTG)7	2	152-165	13	165	57.14	0.37
RM490	1	6.67	(CT)13	3	98-104	6	101	66.67	0.45
RM493	1	12.28	(CTT)9	4	216-243	27	238	57.14	0.57
RM9	1	23.32	(GA)15GT(GA)2	4	73-166	93	113	42.86	0.64
RM5	1	23.97	(GA)14	2	202-205	3	202	71.43	0.32
RM306	1	24.45	(GT)18(AT)8CT(GT)6	2	166-169	3	166	85.71	0.21
RM488	1	24.81	(GA)17	2	180-198	18	180	85.71	0.21
RM237	1	26.81	(CT)18	2	141-146	5	146	85.71	0.21
RM297	1	32.09	(GA)13	2	155-198	43	198	57.14	0.37
RM543	1	32.78	(GCG)10	2	101-106	5	106	85.71	0.21
RM212	1	33.05	(CT)24	3	124-157	33	128	57.14	0.50
RM486	1	34.95	(CT)14	3	116-127	11	116	71.43	0.41
RM315	1	36.73	(AT)4(GT)10	2	128-134	6	128	85.71	0.21
RM472	1	37.88	(GA)21	4	306-329	23	329	42.86	0.64
RM431	1	38.89	(AG)16	2	278-281	3	278	85.71	0.21
RM104	1	40.16	(GA)9	2	246-249	3	246	71.43	0.32
RM529	1	40.67	(CT)12	3	283-289	6	283	42.86	0.58
RM485	2	0.93	(TA)18	2	306-363	57	363	85.71	0.21
RM154	2	1.08	(GA)21	4	168-190	22	168	57.14	0.57
RM211	2	2.02	(TC)3A(TC)18	3	153-174	21	174	71.43	0.41
RM279	2	2.88	(GA)16	3	131-179	48	131	57.14	0.50
RM423	2	3.83	(TTC)9	3	271-286	15	280	50.00	0.54
RM555	2	4.30	(AG)11	2	233-242	9	242	85.71	0.21
RM492	2	7.28	(GA)11	2	236-242	6	242	57.14	0.37
RM324	2	11.38	(CAT)21	2	168-190	22	168	71.43	0.32
RM561	2	18.76	(GA)11	2	197-200	3	200	60.00	0.36
RM341	2	19.33	(CTT)20	2	163-176	13	176	71.43	0.32
RM327	2	20.05	(CAT)11(CTT)	2	214-219	5	214	85.71	0.21

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RM475	2	20.40	(TATC)8	2	202-212	10	212	57.14	0.37
RM262	2	20.79	(CT)16	2	150-161	11	161	57.14	0.37
RM263	2	25.86	(CT)34	3	161-201	40	184	42.86	0.53
RM526	2	26.66	(TAAT)5	3	246-256	10	246	50.00	0.55
RM530	2	30.53	(GA)23	2	156-168	12	168	85.71	0.21
RM250	2	32.77	(CT)17	3	152-161	9	158	57.14	0.50
RM208	2	35.13	(CT)17	3	174-148	10	184	57.14	0.50
RM138	2	35.67	(GT)14	2	215-218	3	218	57.14	0.37
RM231	3	2.45	(CT)16	2	167-184	17	184	85.71	0.21
RM489	3	4.33	(ATA)8	2	237-242	5	237	71.43	0.32
RM545	3	4.94	(GA)30	2	227-230	3	227	71.43	0.32
RM517	3	6.16	(CT)15	2	268-285	17	285	71.43	0.32
RM218	3	8.41	(TC)24ACT5(GT)11	2	140-143	3	140	85.71	0.21
RM232	3	9.75	(CT)24	4	151-172	21	159	57.14	0.57
RM7	3	9.82	(GA)19	2	176-190	14	176	66.67	0.35
RM563	3	11.07	(CCT)6	2	192-196	4	196	85.71	0.21
RM411	3	21.42	(GTT)7	2	99-108	9	99	85.71	0.21
RM347	3	26.74	(GGC)5(AT)7	2	220-257	37	257	80.00	0.27
RM520	3	30.91	(AG)10	2	253-273	20	273	57.14	0.37
RM293	3	31.64	(GT)20	2	215-219	4	215	71.43	0.32
RM468	3	32.67	(TAT)8	2	264-273	9	273	85.71	0.21
RM571	3	33.15	(GT)11(AG)13	2	192-197	5	197	57.14	0.37
RM565	3	35.24	(GA)11	2	164-187	13	164	57.14	0.37
RM570	3	35.58	(AG)15	3	253-280	27	272	50.00	0.54
RM442	3	35.78	(AAG)10	3	249-267	18	260	60.00	0.50
RM114	3	36.10	(GA)7	2	180-192	12	180	85.71	0.21
RM85	3	36.34	(TGG)5(TCT)12	4	82-110	28	82	42.86	0.64
RM551	4	0.17	(AG)18	2	197-200	3	197	85.71	0.21
RM261	4	6.57	C9(CT)8	2	121-132	11	132	85.71	0.21
RM471	4	18.82	(GA)12	2	102-113	11	113	66.67	0.35
RM417	4	19.42	(GA)7	2	256-269	13	256	85.71	0.21
RM119	4	21.24	(GTC)6	3	170-177	7	177	71.43	0.41

RM252	4	25.17	(CT)19	3	223-229	6	223	42.86	0.53
RM470	4	28.09	(CTT)14	3	102-131	29	131	57.14	0.50
RM317	4	29.06	(GC)4(GT)18	3	166-174	8	174	71.43	0.41
RM349	4	32.49	(GA)16	2	128-131	3	128	71.43	0.32
RM348	4	32.65	(CAG)7	2	136-139	3	136	85.71	0.21
RM131	4	34.42	(CT)9	2	205-216	11	216	85.71	0.21
RM127	4	34.52	(AGG)8	2	204.213	9	204	85.71	0.21
RM567	4	34.53	(GA)21	3	247-261	14	256	60.00	0.50
RM280	4	34.98	(GA)16	2	164-194	30	164	85.71	0.21
RM559	4	35.15	(AACA)6	2	166-182	16	166	83.33	0.24
RM159	5	0.48	(GA)19	3	250-258	8	253	71.43	0.41
RM548	5	2.81	(CT)12	4	271-280	9	271	42.86	0.64
RM574	5	3.45	(GA)11	2	153-157	4	157	66.67	0.35
RM437	5	3.87	(AG)13	5	247-268	21	247	42.86	0.70
RM289	5	7.81	G11(GA)16	2	105-152	47	105	85.71	0.21
RM509	5	16.32	(TC)11	2	146-149	3	149	85.71	0.21
RM598	5	16.75	(GCA)9	3	166-173	7	170	71.43	0.41
RM440	5	19.91	(CTT)22	2	215-221	6	215	57.14	0.37
RM305	5	20.94	(GT)4+degener	2	215-220	5	215	71.43	0.32
RM538	5	26.03	(GA)14	3	296-310	14	304	57.14	0.50
RM274	5	26.84	(GA)15-7-(CGG)5	2	153-167	14	167	85.71	0.21
RM480	5	27.31	(AC)30	2	209-212	3	209	71.43	0.32
RM334	5	28.48	(CTT)20	4	191-212	21	212	42.86	0.64
RM31	5	28.61	(GA)15	3	137-160	23	140	42.86	0.58
RM540	6	0.38	(AG)16	2	171-189	18	189	85.71	0.21
RM589	6	1.38	(GT)24	3	177-186	9	186	42.86	0.53
RM204	6	3.16	(GT)24	3	97-120	23	97	71.43	0.41
RM314	6	4.84	(GT)8(CG)3(GT)5	2	101-113	11	101	71.43	0.32
RM402	6	6.39	(ATA)7	2	136-141	5	141	66.67	0.35
RM549	6	6.97	(CCG)9	3	149-162	13	162	57.14	0.50
RM539	6	8.16	(TAT)21	2	314-335	21	314	71.43	0.32
RM136	6	8.75	(AGG)7	2	96-103	7	103	57.14	0.37
RM527	6	9.86	(GA)17	2	234-245	11	245	57.14	0.37

RM541	6	19.51	(TC)16	3	194-205	11	197	66.67	0.45
RM275	6	24.32	(GA)15	2	116-120	4	120	75.00	0.30
RM528	6	26.55	(AGAT)9	2	239-242	3	239	85.71	0.21
RM30	6	27.25	(AG)9A(GA)12	2	91-99	8	91	57.14	0.37
RM400	6	28.43	(ATA)63	2	194-201	7	194	83.33	0.24
RM494	6	31.08	(AGA)16	2	181-184	3	184	85.71	0.21
RM295	7	0.41	(GA)2A(GA)3 G2(GA)9	2	187-192	5	187	71.43	0.32
RM481	7	2.87	(CAA)12	4	147-171	24	147	57.14	0.57
RM125	7	5.47	(GCT)8	3	121-148	27	121	71.43	0.41
RM180	7	5.73	(ATT)10	4	106-215	109	106	57.14	0.57
RM432	7	18.95	(CATC)9	2	168-181	13	181	57.14	0.37
RM560	7	19.58	(CT)12	2	250-278	38	250	85.71	0.21
RM336	7	21.87	(CTT)18	4	148-208	60	148	42.86	0.64
RM455	7	22.35	(TTCT)5	2	131-135	4	135	57.14	0.37
RM505	7	24.52	(CT)12	2	189-213	24	189	85.71	0.21
RM234	7	25.47	(CT)25	3	140-159	19	159	71.43	0.41
RM18	7	25.65	(GA)4AA(GA)(AG)16	3	161-177	16	167	42.86	0.53
RM478	7	25.94	(AG)12	2	205-211	6	205	85.71	0.21
RM248	7	29.33	(CT)25	4	88-105	17	100	50.00	0.62
RM172	7	29.56	(AGG)6	2	161-168	7	168	85.71	0.21
RM337	8	0.15	(CTT)4-19-(CTT)8	3	160-194	34	194	42.86	0.53
RM407	8	0.52	(AG)13	2	167-176	9	176	57.14	0.37
RM152	8	0.68	(GGC)10	3	145-161	16	161	42.86	0.53
RM25	8	4.37	(GA)18	2	145-148	3	145	85.71	0.21
RM5556	8	4.58	(TG)15	2	101-129	28	129	57.14	0.37
RM126	8	5.22	(GA)7	2	179-185	6	179	85.71	0.21
RM6208	8	5.78	(CGG)8	2	134-141	7	141	85.71	0.21
RM210	8	22.47	(CT)23	3	156-169	13	169	57.14	0.50
RM256	8	24.27	(CT)21	2	114-147	33	114	71.43	0.32
RM447	8	26.54	(CTT)8	2	101-113	12	101	85.71	0.21
RM458	8	27.35	(TAG)8	2	184-196	12	184	80.00	0.27
RM264	8	27.92	(GA)27	2	181-185	4	181	57.14	0.37

RM23679	9	0.86	(AGAA)10	2	251-254	3	251	85.71	0.21
RM316	9	1.07	(GT)8(TG)9(TTTG)4(TG)4	3	194-202	8	200	71.43	0.41
RM23778	9	3.96	(ATAA)20	2	286-304	18	286	85.71	0.21
RM464	9	6.57	(AT)21	4	268-279	11	279	42.86	0.64
RM219	9	7.88	(CT)17	2	201-220	19	220	85.71	0.21
RM23958	9	7.99	(CT)15	3	104-113	9	104	57.14	0.50
RM566	9	14.70	(AG)15	2	254-280	26	254	85.71	0.21
RM434	9	15.66	(TC)12	2	154-160	6	154	85.71	0.21
RM257	9	17.71	(CT)24	2	147-156	9	156	85.71	0.21
RM242	9	18.81	(CT)26	2	236-244	8	244	71.43	0.32
RM107	9	20.06	(GA)7	2	246-252	6	246	85.71	0.21
RM201	9	20.17	(CT)17	2	141-148	7	148	85.71	0.21
RM215	9	21.18	(CT)16	2	150-153	3	150	85.71	0.21
RM205	9	22.72	(CT)25	2	119-123	4	119	85.71	0.21
RM474	10	1.81	(AT)13	3	236-293	57	293	57.14	0.50
RM222	10	2.61	(CT)18	4	220-238	18	220	42.86	0.64
RM244	10	-	(CT)4(CG)3C(CT)6	2	164-167	3	167	85.71	0.21
RM216	10	5.35	(CT)18	3	136-153	17	136	57.14	0.50
RM239	10	9.62	(AG)5TG(AG)2	2	145-148	3	145	57.14	0.37
RM258	10	18.01	(GA)21(GGA)3	2	148-155	7	155	57.14	0.37
RM304	10	18.65	(GT)2(AT)10(GT)33	3	161-172	11	161	57.14	0.50
RM147	10	20.94	(TTCC)5(GGT)5	2	87-92	5	87	85.71	0.21
RM228	10	22.24	(CA)6(GA)36	2	108-111	3	108,111	50.00	0.38
RM333	10	22.37	(TAT)19(CTT)19	2	197-200	3	200	57.14	0.37
RM590	10	23.04	(TCT)10	2	153-157	4	153	71.43	0.32
RM332	11	2.84	(CTT)5-12-(CTT)14	2	184-197	13	184	85.71	0.21

RM167	11	4.07	(GA)16	2	124-152	28	152	71.43	0.32
RM209	11	17.80	(CT)18	4	131-159	28	156	57.14	0.57
RM229	11	18.40	(TC)11(CT)5C3 (CT)5	4	114-124	10	116	42.86	0.64
RM457	11	19.06	(TTAA)5	2	238-244	6	244	71.43	0.32
RM254	11	20.90	(TC)6ATT(CT) 11	2	166-172	6	166	71.43	0.32
RM224	11	21.84	(AAG)8(AG)13	3	140-167	27	167	71.43	0.41
RM206	11	22.01	(CT)21	3	155-179	24	179	57.14	0.50
RM144	11	28.28	(ATT)11	3	232-259	27	232	71.43	0.41
RM19	12	2.43	(ATC)10	2	176-180	4	180	83.33	0.24
RM277	12	18.29	(GA)11	2	123-128	5	123	71.43	0.32
RM519	12	19.90	(AAG)8	2	141-145	4	145	71.43	0.32
RM313	12	20.87	(GT)6CA(CG)5 -6-(GT)8	2	115-118	3	115	85.71	0.21
RM235	12	26.10	(CT)24	2	110-140	30	140	57.14	0.37
RM12	12	26.95	(GA)21	2	170-202	32	202	66.67	0.35
Total				**405**					
Average				**2.45**				**68.46**	**0.36**

Polymorphism in SSR markers

PIC values, a reflection of allele diversity and frequency among the varieties, were calculated for all the markers. The PIC values for the microsatellite loci varied from 0.21 to 0.70 with a mean of 0.36. Among the markers used in the present study, RM 437 on chromosome 5 is highly informative since it recorded high PIC value (0.70). Total 43 primers, 5 primers (RM493, RM9, RM212, RM472, RM519) on chromosome 1, 7 primers (RM154, RM279, RM423, RM263, RM526, RM250, RM208) on chromosome 2, 4 primers (RM232, RM570, RM442, RM85) on chromosome 3, 3 primer (RM252, RM470, RM567) on chromosome 4, 5 primers (RM548, RM437, RM538, RM334, RM31) on chromosome 5, 2 primer (RM589, RM549) on chromosome 6, 5 primers (RM481, RM180, RM336, RM18, RM248) on chromosome 7, 3 primer (RM337, RM152, RM210) on chromosome 8, 2 primer (RM464, RM23958) on chromosome 9, 4 primer (RM474, RM222, RM216, RM304) on chromosome 10, 3 primers (RM209, RM229, RM206) on chromosome 11, were found as highly informative because only PIC values 0.50 or more indicate high polymorphism. The Highest informative markers were found on chromosome 2. The second highest informative markers were obtained at chromosome 1, chromosome 5, chromosome 7 and no one found on chromosome 12.

Genetic distance-based analysis

The values of pair-wise comparisons of Nei's genetic distances (D) between genotypes were computed from combined data for the 165 primers, ranged from 0.0571 to 0.7672, whereas average genetic distance was 0.51 **(Table 3)**. Comparatively higher genetic distance was observed between BRRI dhan47 and BRRI dhan50 genotypes pair (0.7672) followed by BRRI dhan47 and BRRI dhan45 genotypes pair (0.7456) than the other combinations **(Table 2)**. On the other hand, BRRI dhan54 and BRRI dhan55 cultivar pair showed the maximum similarity (0.0571).

The dendrogram based on Dice similarity index and UPGMA method **(Figure 1)** was obtained from the binary data that was deduced from the DNA profiles of the samples analyzed. Three distinct clusters were created from the analysis of the pooled SSR marker data at the similarity coefficient of 0.49. The cluster analysis showed high genetic variation among the rice cultivars studied, with similarity coefficient value ranging from 0.26 to 0.90. Cluster 1, Cluster 2 and Cluster 3 comprised one, three and three cultivars, respectively. Cluster 1 included only BRRI

dhan47. While the second cluster was divided into two sub-clusters; the first one comprised two cultivars (BRRI dhan54 and BRRI dhan55) and the second sub-cluster included only BRRI dhan50. Cluster 3 was also divided into two sub-clusters; BRRI dhan29 alone grouped in a sub-cluster and remaining two cultivars (BRRI dhan28 and BRRI dhan45) had been grouped in another sub-cluster.

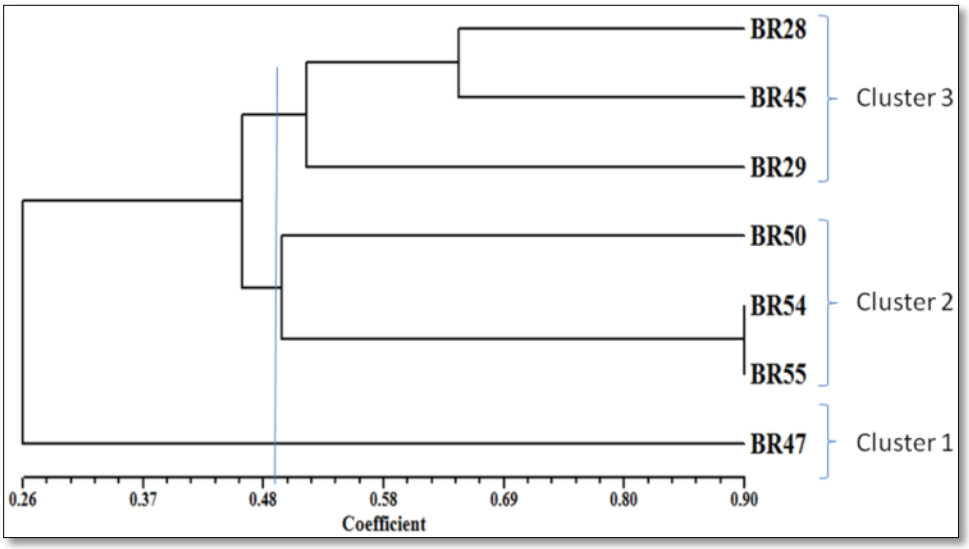

Figure 1. Dendrogram showing the genetic relationships among 7 rice cultivars using UPGMA cluster analysis of Dice genetic similarity coefficients generated from 120 SSR markers.
Legend: BR28=BRRI dhan28; BR29=BRRI dhan29; BR45=BRRI dhan45; BR47=BRRI dhan47; BR50=BRRI dhan50; BR54=BRRI dhan54; BR55=BRRI dhan55

DISCUSSION

Polymorphism in SSR markers

SSRs were chosen for the analysis of genetic diversity of BRRI rice cultivars due to the fact that SSR markers are very powerful for differentiating individual germplasm accessions, particularly when they are closely related [21,27-29]. In this study, a microsatellite fingerprint database was generated for the tested rice genotypes using 299 SSRs. Among these markers, 27 markers did not amplify which might be due to the selection of these markers based on published japonica rice base sequences that might differ from indica type rice cultivars studied here [20]. These markers might be useful for japonica type rice genotypes rather than be indica rice. In addition, 107 marker showed monomorphic allele among the cultivars which means that these markers are not useful for the study of genetic diversity and genetic relationship of the studied cultivars. In case of closely related cultivars these phenomenon have been common and are reported in many previous studies [5,15,17].

In this study 165 SSR markers showed polymorphism among the tested modern rice cultivars that are commercially cultivated in Bangladesh. The variations of allele number per locus are within the range 2 to 5 with an average 2.58 which is quite comparable to the values reported by Prabakaran et al. [30] (range 2 to 3, mean 2.2) and Joshi [17] (average 2.2 alleles) but quite low compared with those reported Siwach et al. [31] (range 1-8, mean 4.58) and Matin et al. [32] (range 3-7, mean 4.4). Comparatively low allele per locus might be due to the use of lower number of genotypes in the present study. Close genetic make-up would be another cause of low allele number.

In our study, lower PIC value (0.40) indicated that the genotypes are not much diverged and are closely related. Besides that, identified highly informative 40 SSR markers may be useful tools in diversity studies as well as future genetic studies of rice germplasm, especially, in marker assisted breeding and QTL identification. The PIC value observed in our study are comparable to many of the previous reports, for example, 0.28 to 0.57 with a mean of 0.43 [30], 0.16 to 0.84 with an average 0.49 [21] and 0.20 to 0.90 with an average of 0.56 [33]. High PIC values can be attributed to the use of more informative markers [21]. PIC values indicated that RM437 (PIC value of 0.70) might be the best marker for diversity analysis of rice genotypes followed by RM9, RM472, RM85, RM548, RM334, RM336, RM464, RM222 and RM229 (PIC value of 0.64) **(Table 3)**.

Genetic relationships among the rice cultivars

The UPGMA cluster analysis showed that all the seven rice cultivars could be easily distinguished based on the information generated by the 165 SSR markers. BRRI cultivars used in the present study have not been examined previously in terms of genetic relatedness using high number

of SSR molecular markers covering all the 12 chromosomes of rice genome. In this study, three clusters were obtained with similarity coefficients of 0.49. Matin et al. [32] studied cluster analysis of the 12 deep water rice germplasms of Bangladesh and showed four major groups. Though the genetic divergence was not very high, but our studied cultivars showed considerable genetic diversity. Narrow genetic base of modern high yielding rice cultivars is available from several countries, including Latin America [7,16], Japan [34], USA [28], Korea [35] and Taiwan [36]. This might be due to the selection pressure and adaptation capability of the specific genotype in the specific climatic conditions. However, among the studied genotypes, the most As tolerant cultivar, BRRI dhan47 showed considerable high genetic distance with the most As susceptible cultivar BRRI dhan45. Therefore, the studied polymorphic SSRs could be effectively used for QTL identification for As tolerance in rice by crossing between these two cultivars.

CONCLUSION

In this study, a microsatellite fingerprint database was generated for 7 rice genotypes using 299 SSRs and genetic divergence was estimated. This study demonstrated that the tested samples possessed a considerable level of microsatellite variation. The markers used here were of value for DNA fingerprinting of rice varieties and constructing a database for breeding programs, especially in background selections during backcross breeding. In addition, the data could be used for As tolerant QTL identification.

REFERENCES

1. Ramkumar G, Biswal AK, Mohan KM, Sakthivel K, Sivaranjani AKP, et al. (2010) Identifying novel alleles of rice blast resistance genes Pikh and Pita through allele mining. Int Rice Res Notes 117: 4185.
2. Pachauri V, Taneja N, Vikram P, Singh NK, Singh S (2013) Molecular and morphological characterization of Indian farmers rice varieties (*Oryza sativa* L.). Austr J Crop Sci 7: 923-932.
3. Khush GS (1997) Origin, dispersal cultivation and variation of rice. Plant Mol Biol 35: 25-34.
4. Lu HM, Redus A, Coburn JR, Rutger JN, Mccouch SR, et al. (2005) Population structure and breeding patterns of 145 US rice cultivars base don SSR marker analysis. Crop Sci 45: 66-76.
5. Garris AJ, Tai TH, Coburn JR, Kresovich S, McCouch SR (2005) Genetic structure and diversity in *Oryza sativa* L. Genetics 169: 1631-1638.
6. Herrera TG, Duque DP, Almeida IP, Núñez GT, Pieters AJ, et al. (2008) Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. Electron J Biotechnol 11: 1-15.
7. Fuentes JL, Escobar F, Alvarez A, Gallego G, Duque MC, et al. (1999) Analyses of genetic diversity in Cuban rice varieties using isozyme, RAPD and AFLP markers. Euphytica 109: 107-115.
8. Rongwen J, Akakya MS, Bhagwat AA, Lavi U, Cregan PB (1995) The use of microsatellite DNA markers for soybean genotype identification. Theor Appl Genet 90: 43-48.
9. Sebastian LS, Hipolito LR, Tabanao DA, Maramara GV, Caldo RA (1998) Molecular diversity of Philippines-based rice cultivars. SABRAO J Breed Genet 30: 83-90.
10. Chen X, Temnykh S, Xu Y, Choand YG, McCouch SR (1997) Development of a microsatellite framework map providing genome wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet 95: 553-567.
11. Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK (2000) The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor Appl Genet 100: 584-592.
12. Gracia AAF, Benchimol LL, Antonica MM, Geraldi IO, Deuza AP (2004) Comparison of RAPD, RFLP, AFLP and SSR marker for diversity studies in tropical maize inbred lines. Euphytica 108: 53-63.
13. Smith JSC, Kresovich S, Hopkins MS, Mitchell S, Dean RE, et al. (2000) Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. Crop Science. 40: 226-232.
14. Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: A method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92: 191-203.
15. Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, et al. (2000) Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100: 713-722.
16. Cuevas-Perez EF, Guimaraes PE, Berrio LE, Gonzales DI (1992) Genetic base of the irrigated rice in Latin America and the Caribbean. Crop Sci 32: 1054-1059.
17. Joshi RK, Behera L (2006) Identification and differentiation of indigenous non-Basmati aromatic rice genotypes of India using microsatellite markers. Afr J Biotechnol 6: 348-354.
18. Tu M, Lu BR, Zhu Y, Wang Y (2007) Abundant within-varietal genetic diversity in rice germplasm from Yunnan province of China revealed by SSR fingerprints. Biochem Genet 45: 789-801.
19. Kostova A, Todorovska E, Christov N, Hristov K, Atanassov A (2006) Assessment of genetic variability

induced by chemical mutagenesis in elite maize germplasm via SSR markers. J Crop Improv 16: 37-48.

20. IRGSP (International Rice Genome Sequencing Project); Matsumoto T (2005) The map-based sequence of the rice genome. Nature 436: 793-800.
21. Rahman MM, Rasaul MG, Hossain MA, Iftekharuddaula KM, Hasegawa H (2012) Molecular characterization and genetic diversity analysis of rice (*Oryza sativa* L.) using SSR markers. J Crop Improv 26: 244-257.
22. Liu K, Muse SV (2005) Power marker: An integrated analysis environment for genetic marker analysis. Bioinformatics 21: 2128-2129.
23. Anderson JA, Churchill GA, Autique JE, Tanksley SD, Sorreils ME (1993) Optimizing parental selection for genetic-linkage maps. Genome 36: 181-186.
24. Nei M (1973) The theory and estimation of genetic distance. In edited by Morton NE, Genetic structure of populations. Honolulu, HI: University of Hawaii Press, pp: 45-54.
25. Rohlf FJ (2002) NTSYS-pc: Numerical taxonomy and multivariate analysis system. Setauket, NY: Exeter Software.
26. Dice LR (1945) Measures of the amount of ecologic association between species. Ecology 26: 297-302.
27. Bligh HFJ, Blackhall NW, Edwards KJ, McClung AM (1999) Using amplified length polymorphisms and simple sequence length polymorphisms to identify cultivars of brown and white milled rice. Crop Sci 39: 1715-1721.
28. Xu Y, Beachell H, McCouch SR (2004) A marker-based approach to broadening the genetic base of rice in USA. Crop Sci 44: 1947-1959.
29. Jeung JU, Hwang HG, Moon HP, Jena KK (2005) Fingerprinting temperate japonica and tropical indica rice genotypes by comparative analysis of DNA markers. Euphytica 146: 239-251.
30. Prabakaran A, Paramasivam K, Rajesh T, Rajarajan D (2010) Molecular characterization of rice land races using SSR markers. Electron J Plant Breed 1: 512-516.
31. Siwach P, Jain S, Saini N, Chowdhury VK, Jain RK (2004) Allelic diversity among Basmati and non-Basmati long-grain indica rice varieties using microsatellite markers. J Plant Biochem Biotechnol 13: 25-32.
32. Matin S, Ashrafuzzaman M, Islam MM, Sikdar SU, Zobayer N (2012) Molecular marker based (SSR) genetic diversity analysis in deep water rice germplasms of Bangladesh. Int J Biosci 2: 64-72.
33. Jain S, Mitchell SE, Jain RK, Kresovich S, McCouch SR (2003). DNA fingerprinting and phylogenetic analysis of Indian aromatic high quality rice germplasm using panels of fluorescent-labeled microsatellite markers. In: Advance in Rice Genetics, Khush GS, Brar DS and Hardy B (Eds), IRRI, Philippine, pp: 162-165.
34. Hashimoto Z, Mori N, Kawamura M, Ishii T, Yoshida S, et al. (2004) Genetic diversity and phylogeny of Japanese sake-brewing rice as revealed by AFLP and nuclear and chloroplast SSR markers. Theor Appl Genet 109: 1586-1596.
35. Song MT, Lee JH, Cho YS, Jeon YH, Lee SB, et al. (2002) Narrow genetic background of Korean rice germplasm as revealed by DNA fingerprinting with SSR markers and their pedigree information. Korean J Genet 24: 397-403.
36. Lin MS (1991) Genetic base of japonica rice varieties released in Taiwan. Euphytica 56: 43-46.