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GENETIC DIVERSITY OF *BALAM* AND *JESSO-BALAM* RICE (*Oryza sativa* L.) GERMPLASM OF BANGLADESH REVEALED BY SSR MARKERS

M.S. Ahmed[1], M. Khalequzzaman[1], M.K. Bashar[2] and A.K.M. Shamsuddin[3]

[1] Genetic Resources and Seed Division, Bangladesh Rice Research Institute, Gazipur 1701, Bangladesh
[2] Harvest-Plus, CIAT, Banani, Dhaka 1213, Bangladesh;
[3] Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.
*Corresponding author: sharfuddin.brri@gmail.com

ABSTRACT

A total of 72 rice genotypes including *Balam* (40), *Jesso-Balam* (27) rice land races and popular varieties (5) from Bangladesh were characterized using 45 SSR markers for studying genetic diversity and identification of duplicate germplasm. Altogether 430 alleles were detected which varied from 4 to 18 per locus with an average of 9.6. The highest number of alleles (18) was found in the locus RM302. The highest gene diversity (0.91) was found in RM337 and RM224, while the lowest (0.52) was found in RM237. The PIC values ranged from 0.90 to 0.46 with an average of 0.78. The highest PIC value was observed in loci RM337 and RM224 followed by 0.89 in RM55, RM258, RM21 and RM206, respectively. The gene diversity and PIC values revealed that RM337, RM224, RM55, RM258, RM21 and RM206 were the best markers to identify and distinguish the genotypes. Besides, the UPGMA clustering method generated seven clusters, where no duplicate genotype was found. It also showed that *Balam* and *Jesso-Balam* groups of germplasm were constellated into separate clusters. The Nei's genetic distance ranged from 0.3556 to 1.0. Conserved, characterization and utilization of the unique and distinct variability of all the similar or duplicate named land races of *Balam* and *Jesso-Balam* rice is suggested.

Key word: *Balam*; *Jesso-Balam*; duplicate germplasm; rice; SSR markers.

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INTRODUCTION

Bangladesh is self-sufficient in rice production, however with the increasing population; the land under cultivation in the country is gradually decreasing due to urbanization. Moreover, abiotic and biotic stresses are also responsible for limiting rice production. Therefore, development of new rice varieties utilizing diverse adaptive genes with traditional, cultural, medicinal and nutritional values is urgently required to increase rice production in areas with limited land and water resources.

Molecular characterization is more frequently used these days than morphological study due to its accuracy and reproducibility. Utilization of PCR-based microsatellite or simple sequence repeat (SSR) DNA marker technologies are technically simple, efficient, quicker and cheaper (Tabkhkar et al., 2012). Microsatellite markers are highly

polymorphic, more reproducible, co-dominant and well distributed throughout the rice genome (Temnykh et al., 2001; McCouch et al., 2002).

A total of 12,487 rice germplasm of Bangladesh with their local names were listed after a country wide survey (Hamid et al., 1982). It was then observed that multiple rice germplasm bearing the same or similar name were existed across the country.

The objectives of the present study were to ascertain genetic diversity among 72 rice genotypes of Bangladesh using SSR markers and to identify duplicate ones for efficient conservation and utilization of these plant genetic resources (PGR) for rice improvement program.

MATERIALS AND METHODS

Plant material: A total of 67 accessions of rice land races of Bangladesh named *Balam* (40) and *Jesso-Balam* (27) along with five popular varieties *viz.* BR7, BR16, BRRI dhan 50, Nizersail and Basmati-375 (Table 1) were characterized during the year 2012. Seeds of these genotypes were collected from the Genebank of Bangladesh Rice Research Institute (BRRI), Gazipur.

Genomic DNA extraction: Total genomic DNA was extracted from 10 days-old seedlings using standard Miniscale method as described by Zheng et al. (1995) with some modifications (Collard et al., 2007).

SSR markers and PCR amplification: Forty-five well distributed SSR primers pairs were selected for their high polymorphic nature in previous studies on rice (Junjian et al., 2002; Thomson et al., 2007; Hossain 2008; Masuduzzaman 2010). The source, repeat motifs, primer sequences and chromosomal positions for these markers can be found in the rice genome database (http: //www. gramene.org). The PCR reaction volume was 10 µl, and comprised of 3.0 µl of genomic DNA (20-25 ng/µl), 1.0 µl of 10X PCR buffer ($MgCl_2$ free), 1.35 µl of 25 mM $MgCl_2$, 0.20 µl of 10 mM dNTPs, 0.5 µl of 10 µM forward primer, 0.5 µl of 10 µM reverse primer, 0.02 µl of 5 U/µl Taq DNA polymerase and 3.37 µl sterile deionized water. The temperature profile was an initial denaturation step for 5 min at 94°C, followed by 35 cycles of denaturation (94°C) for 0.45 min, annealing (55/61°C) for 0.45 min and primer elongation (72°C) for 1.30 min and then a final extension at 72°C for 7 min. Finally, the PCR product was analyzed using 8% PAGE gel in 1X TBE buffer at 75 volts for about 2-2.5 hours depending upon the allele size and documented as JPEG file.

Data analysis: The size of the band for each marker was determined with the help of Alpha EaseFC 4.0. The summary statistics including the number of alleles, major allele size and frequency, gene diversity and polymorphism information content (PIC) values were determined using Power Marker version 3.25 (Liu and Muse, 2005). Allele molecular weight data as calculated in Power Marker for determining frequency and genetic distance

Table 1. List of 72 rice genotypes including *Balam* and *Jesso-Balam* germplasm

Sl. no.	Name	Code name	Acc. No.	Collection		Season	Sl. no.	Name	Code name	Acc. No.	Collection		Season
				Thana	District						Thana	District	
1	Balam	B1	1430	Dhaka	Dhaka	T. Aman	**37**	MogaiBalam	B37	965	Fakirhat	Khulna	Aus
2	Balam	B2	995	Phultala	Khulna	Aus	**38**	MurkiBalam	B38	771	Bansakhali	Chittagong	T. Aman
3	Balam	B3	516	Kurigram	Rangpur	T. Aman	**39**	PatniBalam	B39	4838	Shympur	Satkhira	T. Aman

4	Balam	B4	841	Balaganj	Sylhet	Aus	**40**	SamritiBalam	B40	3670	Jamalpur	Jamalpur	T. Aman
5	Balam	B5	4050	Srimongol	M.Bazar	T. Aman	**41**	BR7	BR7	6868	Genebank	Gazipur	T. Aman
6	Balam	B6	692	B.Baria	Comilla	T. Aman	**42**	BR16	BR16	6874	,,	,,	,,
7	Balam	B7	853	Phultala	Khulna	Aus	**43**	BRRI dhan50	BR50	6882	,,	,,	,,
8	Balam	B8	3643	Sherpur	Sherpur	T. Aman	**44**	Naizersail	NS	49	,,	,,	,,
9	Balam	B9	843	Tajpur	Sylhet	T. Aman	**45**	Jesso-Balam TAPL-1	JBPL1	2470	,,	,,	,,
10	Balam	B10	3516	Baraigran	Rajshahi	Aus	**46**	Jesso-Balam TAPL-2	JBPL2	2468	,,	,,	,,
11	Balam	B11	683	B.Baria	Comilla	T. Aman	**47**	Jesso-Balam TAPL-3	JBPL3	2461	,,	,,	,,
12	Balam	B12	720	Hajiganj	Comilla	B. Aman	**48**	Jesso-Balam TAPL-4	JBPL4	2457	,,	,,	,,
13	Balam	B13	855	Biswanath	Sylhet	Aus	**49**	Jesso-Balam TAPL-5	JBPL5	2460	,,	,,	,,
14	Balam	B14	4045	-	Kustia	Aus	**50**	Jesso-Balam TAPL-6	JBPL6	2467	,,	,,	,,
15	Balam	B15	842	CH. Ghat	Sylhet	T. Aman	**51**	Jesso-Balam TAPL-7	JBPL7	2465	,,	,,	,,
16	Balam	B16	823	Daulatpur	Sylhet	T. Aman	**52**	Jesso-Balam TAPL-8	JBPL8	2458	,,	,,	,,
17	Balam	B17	1013	Kochoa	Khulna	T. Aman	**53**	Jesso-Balam TAPL-9	JBPL9	2475	,,	,,	,,
18	Balam	B18	839	Beaurbazar	Sylhet	Aus	**54**	Jesso-Balam TAPL-10	JBPL10	2469	,,	,,	,,
19	BanafulBal	B19	4164	Sadar	Jessor	T. Aman	**55**	Jesso-Balam TAPL-11	JBPL11	2462	,,	,,	,,
20	Baulam	B20	3565	Nalchiti	Barisal	Aus	**56**	Jesso-Balam TAPL-12	JBPL12	2471	,,	,,	,,
21	Baulam	B21	3730	Najirpur	Barisal	Aus	**57**	Jesso-Balam TAPL-13	JBPL13	2479	,,	,,	,,
22	BetiBalam	B22	1011	Kochoa	Khulna	T. Aman	**58**	Jesso-Balam TAPL-14	JBPL14	2464	,,	,,	,,
23	BhuaBalam	B23	878	Gol Pgorw	Sylhet	T. Aman	**59**	Jesso-Balam TAPL-15	JBPL15	2480	,,	,,	,,
24	Boilam	B24	3538	Sonagazi	Noakhali	Aus	**60**	Jesso-Balam TAPL-16	JBPL16	2474	,,	,,	,,
25	Boilam	B25	4608	Sadar	Noakhali	Aus	**61**	Jesso-Balam TAPL-17	JBPL17	2455	,,	,,	,,
26	Boislam	B26	3201	Tejgoan	Dhaka	Aus	**62**	Jesso-Balam TAPL-18	JBPL18	2463	,,	,,	,,
27	Boislam	B27	3497	Shitakundu	Chittagong	Aus	**63**	Jesso-Balam TAPL-19	JBPL19	2453	,,	,,	,,
28	Bola Balam	B28	4836	Shympur	Satkhira	T. Aman	**64**	Jesso-Balam TAPL-20	JBPL20	2476	,,	,,	,,
29	JB TAPL*	B29	2456	BRRI	Gazipur	T. Aman	**65**	Jesso-Balam TAPL-21	JBPL21	2472	,,	,,	,,
30	KabraBalar	B30	240	Trisal	Mymensingh	T. Aman	**66**	Jesso-Balam TAPL-22	JBPL22	2477	,,	,,	,,
31	KartikBala	B31	696	Faridganj	Comilla	B. Aman	**67**	Jesso-Balam TAPL-23	JBPL23	2473	,,	,,	,,
32	KhudBalam	B32	3668	Jhenagali	Sherpur	T. Aman	**68**	Jesso-Balam TAPL-24	JBPL24	2466	,,	,,	,,
33	KhudBalam	B33	2089	Jamalpur	Jamalpur	Aus	**69**	Jesso-Balam TAPL-25	JBPL25	2454	,,	,,	,,
34	Lal Balam	B34	2115	Narshindi	Dhaka	Aus	**70**	Jesso-Balam TAPL-26	JBPL26	2459	,,	,,	,,
35	LonaBalam	B35	4789	Shympur	Satkhira	T. Aman	**71**	Jesso-Balam TAPL-27	JBPL27	2478	,,	,,	,,
36	MakaiBala	B36	4158	Kulawra	M. Bazar	T. Aman	**72**	Basmati-375	BM	--	,,	,,	,,

by using 'Nei 1983' distance (Nei and Takezaki, 1983), were also used to construct UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) dendrogram using NTSYS-pc version 2.2 (Rohlf, 2002) software.

RESULTS AND DISCUSSION

Allelic diversity: A total of 430 alleles were detected at the loci of 45 microsatellite markers across the 72 genotypes (Table 2). The number of alleles varied from 4 to 18 per locus, with

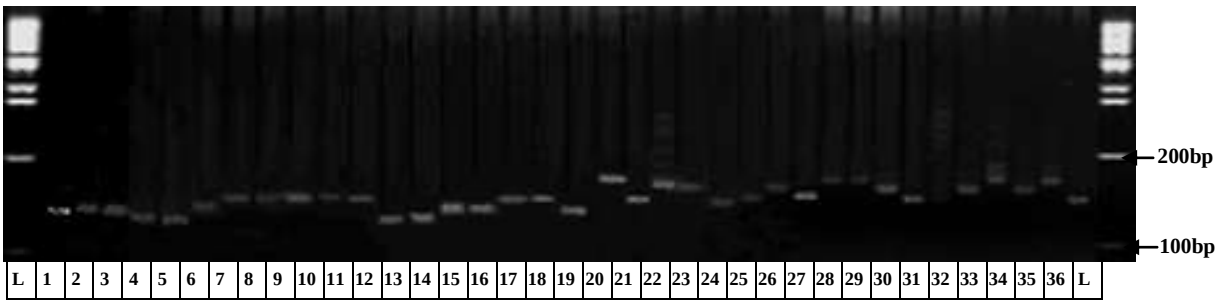
Table 2 Allele variation, gene diversity and PIC values of 45 SSR markers across 72 genotypes of rice

Chrom.	Marker	Position (cM)	Repeat motif	Allele(s)		Major allele(s)		Rare alleles	Null alleles	Gene diversity	PIC values
				No.	Ranges (bp)	Size (bp)	Freq (%)				
	RM283	31.4	**(GA)18**	8	141-164	147	30.56	4	1	0.80	0.77
1	**RM259**	54.2	**(CT)17**	13	151-203	170	19.44	7	0	0.88	0.86
	RM237	115.2	**(CT)18**	5	124-135	133	66.67	1	1	0.52	0.49
	RM302	147.8	**(GT)30(AT)8**	18	107-222	131	20.83	14	0	0.88	0.87
	RM154	4.8	**(GA)21**	9	157-183	163	30.56	5	0	0.79	0.76
2	**RM279**	17.3	**(GA)16**	7	144-173	156/158	22.22	2	4	0.83	0.81
	RM324	66.0	**(CAT)21**	10	124-172	163	33.33	5	0	0.82	0.80
	RM250	170.1	**(CT)17**	8	142-156	152	51.39	5	3	0.70	0.67
	RM60	0.0	**(ATTA)5AATCT(AATT)**	9	153-182	175	31.94	4	0	0.81	0.78
3	**RM218**	67.8	**(TC)24ACT5(GT)11**	12	114-152	126	16.67	5	1	0.89	0.88
	RM55	168.2	**(GA)17**	13	134-140	212/229	13.89	6	3	0.90	0.89
	RM227	214.7	**(CT)10**	7	95-109	97	30.56	2	3	0.78	0.75
	RM307	0.0	**(AT)14(GT)21**	11	126-180	131	19.44	5	1	0.88	0.87
4	**RM273**	94.4	**(GA)11**	5	200-209	202	61.11	2	0	0.55	0.49
	RM241	106.2	**(CT)31**	10	100-134	126	22.22	5	0	0.86	0.84
	RM127	150.1	**(AGG)8**	7	207-224	222	33.33	3	1	0.79	0.76
	RM413	26.7	**(AG)11**	11	68-109	88	25.00	6	1	0.86	0.85
5	**RM267**	28.6	**(GA)21**	11	130-177	169	16.67	5	0	0.87	0.86
	RM161	96.9	**(AG)20**	7	166-188	171	52.78	3	2	0.66	0.63
	RM133	0.0	**(CT)8**	4	225-233	230	52.78	2	0	0.56	0.46
6	**RM584**	26.2	**(CT)14**	12	150-214	194	19.44	5	0	0.89	0.87
	RM541	75.5	**(TC)16**	8	142-174	147	19.44	4	1	0.80	0.77
	RM162	108.3	**(AC)20**	6	226-236	231	43.06	2	0	0.72	0.68
	RM125	24.8	**(GCT)8**	6	124-149	137	37.50	3	0	0.75	0.71
7	**RM214**	34.7	**(CT)14**	11	107-139	115	36.11	6	0	0.80	0.78
	RM18	90.4	**(GA)4AA (GA)(AG)16**	7	149-169	163	25.00	2	0	0.82	0.79
	RM337	1.1	**(CTT)4-19-(CTT)8**	12	156-211	193	13.89	7	3	0.91	0.90
8	**RM223**	80.5	**(CT)25**	10	138-175	163	22.22	6	2	0.84	0.82
	RM256	101.5	**(CT)21**	10	103-138	108	29.17	5	0	0.83	0.81
	RM433	116	**(AG)13**	11	207-237	215/217/223	15.28	4	2	0.88	0.87
	RM296	0.0	**(GA)10**	9	114-139	136	29.17	4	1	0.82	0.80
9	**RM242**	73.3	**(CT)26**	10	192-224	214	19.44	5	2	0.87	0.86
	RM215	99.4	**(CT)16**	9	146-173	163	25.00	3	1	0.85	0.84
	RM311	25.2	**(GT)3(GTAT)8(GT)5**	9	166-191	187	31.94	4	0	0.81	0.78
10	**RM271**	59.4	**(GA)15**	8	88-105	98	26.39	4	1	0.80	0.77
	RM258	70.8	**(GA)21(GGA)3**	15	130-167	146	18.06	9	0	0.90	0.89
	RM171	92.8	**(GATG)5**	7	323-354	340	41.67	3	1	0.74	0.70
	RM21	85.7	**(GA)18**	15	129-173	129	22.22	12	1	0.90	0.89
11	**RM229**	77.8	**(TC)11(CT)5C3(CT)**	9	109-133	122	18.06	2	0	0.87	0.86
	RM206	102.9	**(CT)21**	14	119-169	127	16.67	8	0	0.90	0.89
	RM224	120.1	**(AAG)8(AG)13**	15	123-160	127	18.06	10	3	0.91	0.90
	RM286	0.0	**(GA)16**	11	96-125	104	18.06	6	0	0.88	0.86
12	**RM19**	20.9	**(ATC)10**	9	220-242	228	29.17	4	0	0.82	0.80
	RM247	32.3	**(CT)16**	8	126-165	132	27.78	3	2	0.81	0.78
	RM277			4	116-126	123	54.17	1	0	0.61	0.55
	Minimum			4.0	68	88	13.9	1	0	0.52	0.46
	Average			9.6	-	157.7	29.1	4.7	0.9	0.81	0.78
	Maximum			18	354	340	66.7	14	4	0.91	0.90
	Sum			430				213	41		

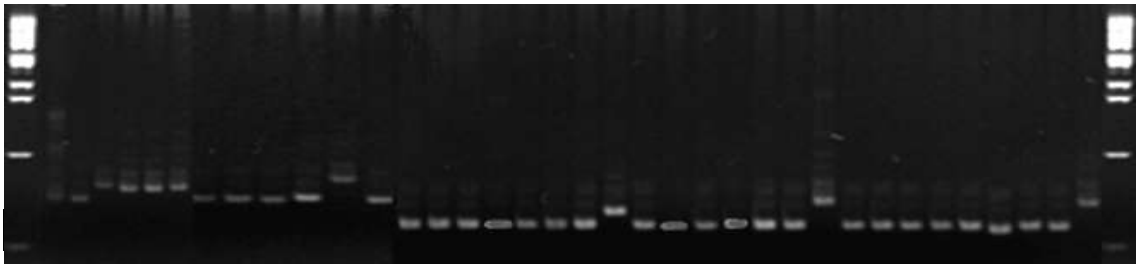
* Marker position (cM), repeat motif, specification and annealing temperature (ºC) can be obtained from
http://www.gramene.org.

an average of 9.6 alleles per loci indicating high allele diversity. The highest number of alleles (18) was found for RM302, followed by RM224, RM21, RM258 (15), RM206 (14) and RM259, RM55 (13) and the lowest (4) for RM277 and RM133. Similarly, Thomson et al. (2007) and Masuduzzaman (2010) detected 394 and 337 alleles with an average of 13 and 11 which varied from 4-21 in 330 and 160 rice accessions using 30 SSR markers, respectively. The band size ranged from 68bp at RM413 to as high as 354bp at RM171. Islam et al. (2008) also reported similar result on 21 stress tolerant rice genotypes using 100 SSR markers. The range of band size for the major allele per single marker varied from 225-233bp at RM133 to 107-222bp at RM302, respectively. The gel picture of amplified fragment using RM224 marker also showed the variations of band size among the 72 genotypes (Fig. 1). The size of major alleles per marker varied from 88 (RM413) to 340bp (RM171) and the frequency (%) ranged from 13.9 (RM55 and RM337) to 66.7 (RM237), with an average of 29.1. On the other hand, the null alleles can arise from point mutation(s) in one or both of the primer sites. The highest (4) number of null alleles was detected at RM279. But when the observation of an allele is less than 5%, it is considered as a rare allele. The highest number (14) of rare alleles was recorded at RM302 and the lowest (1) at RM237.

Marker performance: The gene diversity value was ranged from 0.91 (RM337 and RM224) to 0.52 (RM237)(Table 2) indicating the existence of high degree of gene diversity. Hassan et al. (2012) studied 59 rice genotypes using eight SSR primers and found a mean of 0.87 that ranging from 0.79 to 0.95, which also revealed the high gene diversity of the genotypes. The level of polymorphism among the 72 genotypes was evaluated by calculating polymorphism information content (PIC) values for each of the 45 SSR loci. The PIC values varied widely among loci and ranged from a low of 0.46 (RM133) to a high of 0.90 (RM337 and RM224) per marker, with an average of 0.78.



a) Lane 1 to 36 (from left to right) represent B1 to B36 as mentioned in Table 1



b) Lane 37 to 72 (from left to right) represent B37 to Basmati-375 as mentioned in Table 1

Fig. 1 DNA profile for RM224 across 72 genotypes of rice

The result also revealed that RM337, RM224, RM55, RM258, RM21 and RM206 were the best microsatellite or SSR markers to identify, distinguish and sort out duplicate of studied germplasm due to high gene diversity and PIC values. Kaushik et al. (2011) also demonstrated that SSRs are the best for differentiating closely related Basmati, *indica* or *japonica* rice varieties. Therefore, it can be said that the microsatellite or SSR markers were successfully generate significantly high degree of polymorphism in rice to differentiate effectively the similar and duplicate named or closely related genotypes.

Genetic diversity and relationship: An un-rooted neighbor-joining tree was constructed showing the genetic relationships among the 72 genotypes on the basis of the alleles detected across the 45 microsatellite loci (Fig. 2). The tree revealed that the genotypes were distributed into seven clusters, where *Balam* and *Jesso-balam* rice were grouped into separate clusters.

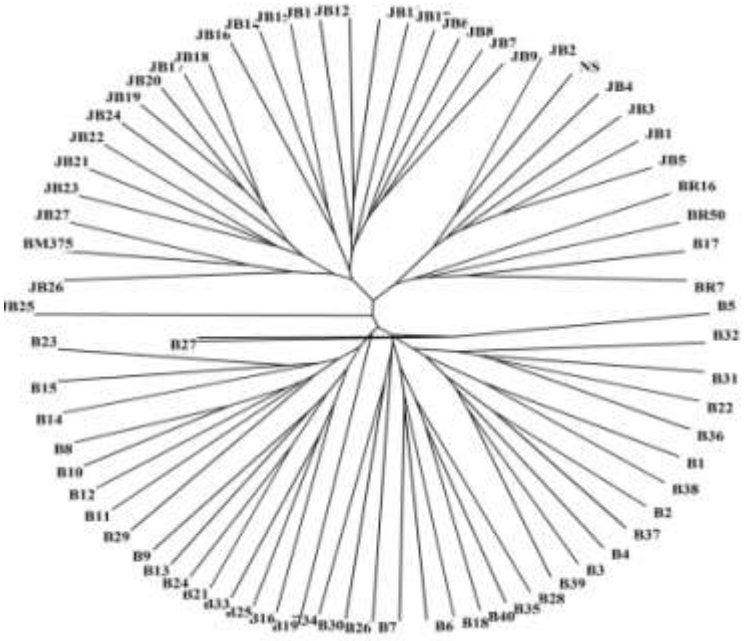


Fig. 2 An un-rooted neighbor-joining tree showing the genetic relationships among 72 rice genotypes based on 430 alleles detected by 45 SSR markers

The 40 *Balam* genotypes were grouped into four clusters (Fig. 3), where cluster 1 contained 20 genotypes (B1, B2, B3, B4, B6, B7, B18, B22, B26, B28, B30, B31, B32, B34, B35, B36, B37, B38, B39 and B40), cluster 2 three genotypes (B5, B20 and B27) and cluster 4 16 genotypes (B8, B9, B10, B11, B12, B13, B14, B15, B16, B17, B21, B23, B24, B25, B29 and B33) with four popular varieties (BR7, BR16, BRRI dhan50 and Nizersail). However, cluster 3 and cluster 5 comprised only of one genotype each (B19 and Basmati-375, respectively).

The 27 *Jesso-Balam* genotypes were grouped into five clusters (Fig. 4), where cluster 1 contained 10 genotypes (JB17, JB18, JB19, JB20, JB21, JB22, JB23, JB24, JB26 and JB27) and one popular variety named Basmati-375, cluster 2 three popular varieties (BR7, BR16 and BRRI dhan50) and cluster 3 five genotypes (JB1, JB2, JB3, JB4 and JB5) and one popular variety named Nizersail. However, cluster 4 comprised only of one genotype (JBPL25).

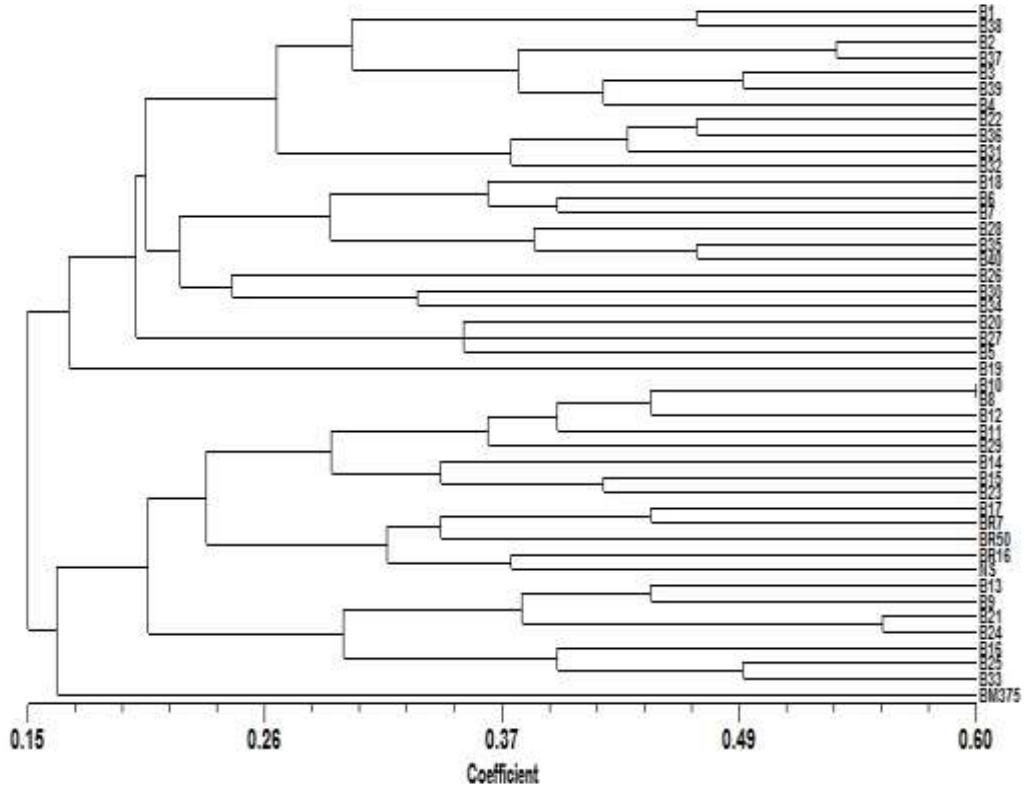


Fig. 3 Dendrogram of 40 *Balam* rice germplasm derived from UPGMA cluster analysis using Nei's genetic distance across 45 SSR markers

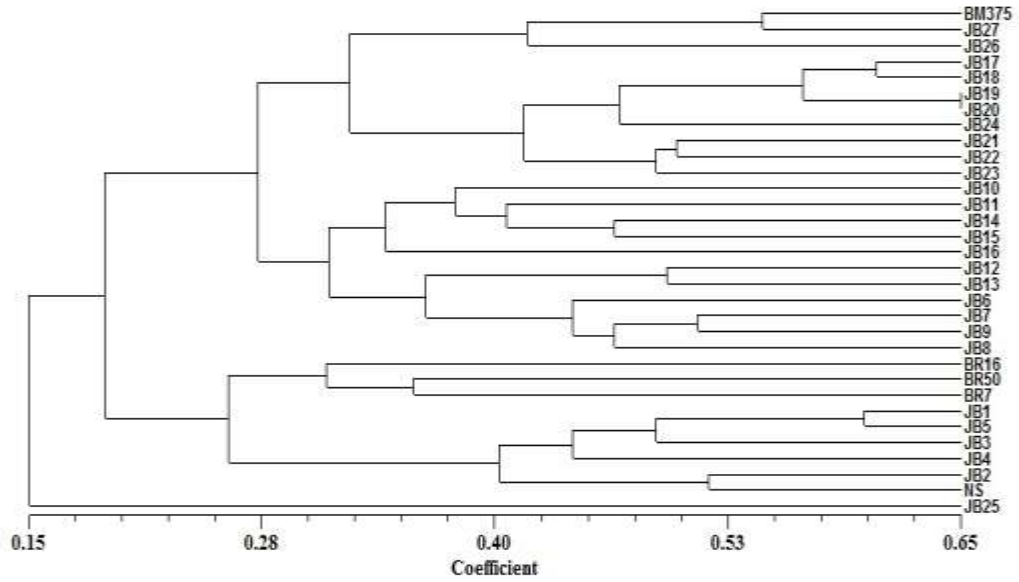


Fig. 4 Dendrogram of 27 *Jesso-Balam* rice germplasm derived from UPGMA cluster analysis using Nei's genetic distance across 45 SSR markers

Genetic distance: The values of pair-wise comparisons of Nei's genetic distance between genotypes, resulting from the mean of different combinations of 72 rice varieties across the 45 microsatellite loci, ranged from 0.3556 to 1.0. The highest genetic distance (1.0) was found between B11 and JBPL17, B24 and JBPL17, B25 and JBPL17, respectively while the lowest (0.3556) between JB19 and JB20. But, Ashfaq and Khan (2012) studied 15 *indica* Basmati advance lines and five Basmati improved varieties across 28 SSR markers and found Nei's genetic distance ranging from 0.07 to 0.95. Finally, it can be said that high genetic diversity was existed among the studied genotypes.

CONCLUSIONS

The assumed similar or duplicate named accessions of *Balam* and *Jesso-Balam* rice land races were not found to be as such and rather those are distinct genotypes. The RM337, RM224, RM55, RM258, RM21 and RM206 were the best markers to identify and distinguish the studied genotypes. The unique and distinct variability discovered in *Balam* and *Jesso-Balam* germplasm of rice emphasizes the need for their proper conservation.

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