



Agro-Morphological, Physico-Chemical and Molecular Characterization of Rice Germplasm with Similar Names of Bangladesh

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Abstract: Thirty-one duplicate and similar named rice germplasms of Bangladesh were studied to assess the genetic variation for the agro-morphological and physico-chemical traits and simple sequence repeat banding patterns during 2009–2012 at Bangladesh Rice Research Institute. The range of variations within the cultivar groups showed higher degree. The principal component analysis showed that the first five components with vector values > 1 contributed 82.90% of the total variations. The cluster analysis grouped the genotypes into four clusters, where no duplicate germplasm was found. The highest number (11) of genotypes was constellated in cluster I and the lowest (3) in cluster II. The intra- and inter-cluster distances were the maximum in cluster I (0.93) and between clusters I and IV (24.61), respectively, and the minimum in cluster IV (0.62) and between clusters I and III (5.07), respectively. The cluster mean revealed that the crosses between the genotypes of cluster I with those of clusters II and IV would exhibit high heterosis for maximum good characters. A total of 350 alleles varied from 3 (RM277) to 14 (RM21) with an average of 7.8 per locus were detected at 45 microsatellite loci across the 31 rice accessions. The gene diversity ranged from 0.48 to 0.90 with an average of 0.77, and the polymorphism information content values from 0.44 (RM133) to 0.89 (RM206) with an average of 0.74. RM206, RM21, RM55, RM258 and RM433 were considered as the best markers on the basis of their higher polymorphism information content values. The dendrogram from unweighted pair-group method with arithmetic average clustering also classified the genotypes into four groups, where group IV comprised of 20 genotypes and group III of one genotype, but no duplicate was found. Finally, similar and duplicate named rice germplasms need to be conserved in gene bank as are distinct from each other.

Key words: diversity; simple sequence repeat; similar name; landrace rice; agro-morphological and physico-chemical trait

Bangladesh has abundant diversified rice landraces from time immemorial, since rice plays an important role in the livelihood, cultures and socio-economic aspects of the people, and is also the main cereal food in Bangladesh. International Rice Research Institute (IRRI) gene bank contains more than 8 000 traditional

rice varieties collected from Bangladesh (Hossain et al, 2013). However, now rice diversity in Bangladesh is threatened due to extensive cultivation of modern varieties all over the country along with various intervention of rice habitat (Ahmed et al, 2010).

Exploring diversity in the landrace collection is

Received: 8 June 2015; **Accepted:** 14 December 2015

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Peer review under responsibility of China National Rice Research Institute

<http://dx.doi.org/10.1016/j.rsci.2016.06.004>

very important for identifying new genes and further improvement of the germplasm (Thomson et al, 2007). However, it was identified that duplicate(s) named rice germplasms were cultivated all over Bangladesh (Hamid et al, 1982). As a result, a particular cultivar got many slightly deviated names or even different cultivars got the same name given by different farmers. Hence, similar and duplicate named rice germplasms need to be studied whether they are same or different.

Molecular characterized data are the legal evidence for the DUS (distinctness, uniformity and stability) test and is being practiced worldwide. Among PCR based markers, microsatellite marker (simple sequence repeat, SSR) is highly polymorphic, more reproducible, co-dominant and well distributed throughout the rice genome (Chen et al, 2002). Their map positions on the rice genome are well known. SSRs have been used to identify duplicates on germplasm banks and populations of different species (Irish et al, 2010) and to study individuals with close relations (Song et al, 2003). They are user friendly, suitable for purity control (Nandakumar et al, 2004) and elimination of duplicates (Lund et al, 2003). The present study was, therefore, undertaken to assess the genetic variation in 31 duplicate and similar named rice germplasms of Bangladesh by studying the agro-morphological and physico-chemical traits and SSR banding patterns.

MATERIALS AND METHODS

Agro-morphological and physico-chemical characterizations

Thirty-one genotypes in which 21 from Kartiksail and 10 from Dhali boro groups of Bangladesh along with BR23 as standard check were used (Table 1). To study 14 agro-morphological characters, the 35-day-old single seedlings were transplanted per hill for each accession with the space of 20 cm × 25 cm, at Gazipur, Bangladesh Rice Research Institute (BRRI). The chemical fertilizer (N-P-K-S) dose of 60-50-40-10 kg/hm² was applied. Nine plants from each entry were randomly selected for recording data on seedling height, plant height, culm diameter, panicle exertion, effective tillering number per hill, days to maturity, average primary and secondary branch number per panicle, grain yield per panicle, grain length, awn length, 1000-grain weight, grain yield per hill and biological yield. Besides for physico-chemical study, milling rate (Adair, 1952), cooking time (Juliano et al,

1969), amylose content (Juliano, 1971) and protein content (AOAC, 1995) were measured at Grain Quality and Nutrition Division, BRRI during 2011. Genetic diversity was worked out for the principal component analysis (Rao, 1964) and Mahalanobis' generalized distance (D²) analysis (Rao, 1952). All multivariate analyses were performed using the GENSTAT 5.5 program.

Molecular characterization

Plant materials and genomic DNA extraction

The 31 duplicate and similar named rice germplasms along with five popular BRRI varieties viz. BR4, BR14, BR23, BRRI dhan 28 and BRRI dhan 29 were studied in the Molecular Laboratory of Genetic Resources and Seed Division of BRRI during 2011–2012. Total genomic DNA was extracted from leaves of 10–12 day-old seedlings (Collard et al, 2007).

SSR markers and PCR amplification

Forty-five well distributed SSRs were selected from

Table 1. List of 31 duplicate and similar named rice germplasms of Bangladesh.

Serial No.	Accession name	Accession No. ^a	Origin
KS1	Kartik Sail	3243	Sylhet
KS2	Kartik Sail	776	Chittagong
KS3	Katih Shail	438	Rajshahi
KS4	Kartik Sail	539	Rangpur
KS5	Kartik Sail	77	Dhaka
KS6	Kati Shail	170	Tangail
KS7	Kartik Sail	3662	Sherpur
KS8	Kati Shail	3631	Rajshahi
KS9	Kartika	4053	Sylhet
KS10	Kartik Sail	4881	Tangail
KS11	Kartik Sail	76	Dhaka
KS12	Kati Shail	437	Rajshahi
KS13	Kartik Sail	78	Dhaka
KS14	Kartik Sail	1882	Kishorganj
KS15	Kartik sail (2)	689	Comilla
KS16	Kartik sail (2)	846	Sylhet
KS17	Kartik Sail	664	Comilla
KS18	Kartik Sail	1887	Kishorganj
KS19	Kartik Sail	844	Sylhet
KS20	Katih Shail	994	Khulna
KS21	Kartik Sail	845	Sylhet
DB1	Dhali Boro	2250	Sylhet
DB2	Dhali Boro	2247	Sylhet
DB3	Dhali Boro	2249	Sylhet
DB4	Dholi Boro	180	Tangail
DB5	Dhali Boro	2245	Sylhet
DB6	Dholi Boro	4396	Sylhet
DB7	Dhali Boro	2246	Sylhet
DB8	Dhali Boro	2244	Sylhet
DB9	Dhali Boro	2248	Sylhet
DB10	Dhali Boro	2243	Sylhet

^a, BRRI gene bank accession number.

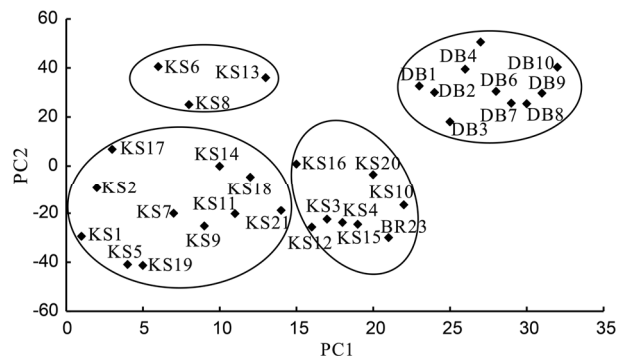


Fig. 1. Scatter diagram of 31 duplicate and similar named rice germplasms and the check BR23.

the previous studies on rice (Junjian et al, 2002; Joshi and Behera, 2006; 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 total PCR reaction volume was 10 μ L, composed of 3.0 μ L genomic DNA, 1.0 μ L of 10 \times PCR buffer (MgCl₂ free), 1.35 μ L of 25 mmol/L MgCl₂, 0.2 μ L of 10 mmol/L dNTPs, 0.5 μ L of 10 μ mol/L forward and reverse primers, 0.02 μ L of 5 U/ μ L *Taq* DNA polymerase and 3.43 μ L 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 45 s, annealing (55 °C or 61 °C) for 45 s and primer elongation (72 °C) for 1.3 min and then a final extension at 72 °C for 7 min.

Electrophoresis and visualization of amplified products

The 10 μ L of PCR product with 2 μ L of a loading dye was analyzed using 8% polyacrylamide gel electrophoresis in 1 \times TBE buffer at 75 V for about 2.0–2.5 h depending upon the allele size. The gels were stained with ethidium bromide solution (0.5 mg/mL) for 25 min and exposed to UV light using the gel documentation system.

Allele scoring and data analysis

The size of the band for each marker was scored by AlphaEaseFC 4.0 software. The summary statistics, including the number of alleles, major allele size and frequency, gene diversity and polymorphism information content (PIC) value, were determined using PowerMarker version 3.25 (Liu and Muse, 2005). Allele molecular weight data as implemented in PowerMarker for determining allelic frequency and genetic distance by using Nei's distance (Nei and Takezaki, 1994) were also used to construct UPGMA

Table 2. Distribution of 31 duplicate and similar named rice germplasms into 4 clusters for 18 morpho-physicochemical characters.

Cluster	No. of genotypes	Serial No.
I	11	KS1, KS2, KS5, KS7, KS9, KS11, KS14, KS17, KS18, KS19, KS21
II	3	KS6, KS8, KS13
III	7	KS3, KS4, KS10, KS12, KS15, KS16, KS20
IV	10	DB1, DB2, DB3, DB4, DB5, DB6, DB7, DB8, DB9, DB10

(unweighted pair-group method with arithmetic average) dendrogram in NTSYS-pc ver 2.2 (Rohlf, 2002) for showing the genetic inter-relationship among the genotypes. Besides, the Spearman's rank correlation coefficient (r_s) was calculated and then tested by using the criterion given by Steel and Torrie (1980).

RESULTS

Agro-morphological and physico-chemical characterizations

Analysis of variance showed highly significant differences ($P < 0.01$) among the 32 genotypes for all the 18 morpho-physicochemical characters. The principle component analysis (PCA) showed that the first five components with vector values > 1 , accounted 82.90% of the total variations in which components I, II, III, IV and V contributed 47.76%, 13.29%, 8.62%, 6.89% and 6.34%, respectively. Fig. 1 showed a scatter diagram of the first two principle components, which apparently distributed the 31 genotypes into four clusters.

The 31 genotypes of rice germplasm were also grouped into four clusters on the basis of non-hierarchical clustering method using Mahalanobis' D^2 statistic (Table 2). The clustering pattern also revealed that the genotypes constellated in the same cluster were not originated from the same geographic region.

The distribution pattern of cluster indicated that the highest number of genotypes (11) clubbed into cluster I, followed by clusters IV and III, while the lowest number (3) included in cluster II. The average intra- and inter-cluster distances (D^2) are presented in Table 3. The intra-cluster distance was maximum in cluster I (0.93) and minimum in cluster IV (0.62), denoting that the genotypes of cluster I were the most diverse and those of cluster IV were most similar or less diverse. Regarding the inter-cluster distance, the maximum genetic distance was observed between the clusters I and IV (24.61), while the minimum was observed

Table 3. Average intra- (bold) and inter-cluster distances (D^2) for 18 morpho-physicochemical characters of 31 duplicate and similar named rice germplasms.

Cluster	I	II	III	IV
I	0.93			
II	6.91	0.69		
III	5.07	10.21	0.74	
IV	24.61	22.17	22.06	0.62

between the clusters I and III (5.07).

Again, the highest inter-genotype distance (D^2) was observed between KS15 and DB7 (4.2840) and the lowest between DB2 and DB9 (0.2750), while among the Kartiksail group, the highest was between KS8 and KS16 (3.4110) and the lowest was between KS2 and KS7 (0.3050), whereas among Dhali boro rice, the highest was between DB4 and DB7 (1.4470).

The cluster mean values revealed that none of the cluster showed the highest or lowest mean values in all the characters (Table 4). The genotypes of cluster I produced the highest means for culm diameter, primary and secondary branch number per panicle, grain yield per panicle, grain length, grain yield per hill and biological yield. Similarly, the genotypes of cluster II produced the highest means for seedling height plant height and 1000-grain weight, while the genotypes of cluster IV produced the highest means for protein content and the shortest plant. Therefore, the crosses between the genotypes of cluster I with those of clusters II and IV would exhibit high heterosis as well as transgressive segregation and could club the maximum good characters.

The canonical variate analysis revealed that 96.83% of the total variation accounted by the first two canonical roots in which 91.01% accounted by canonical root 1 and 5.82% by canonical root 2. The result also revealed that protein content, primary branch number per panicle, effective tiller number per hill, 1000-grain weight, biological yield and secondary branch number per panicle contributed maximum to the genetic divergence (Table 4).

Molecular characterization

A total of 350 alleles varied from 3 (RM277) to 14 (RM21) with an average of 7.8 per locus were detected at 45 microsatellite loci across the 31 rice genotypes (Table 5). The major allele size ranged from 68 bp (RM413) to 314 bp (RM171), and the frequency ranged from 17% to 69%. The gene diversity varied from 0.48 (RM133) to 0.90 (RM206) with an average of 0.77 and the PIC value from 0.44 (RM133) to 0.89 (RM206) with an average of 0.74. The result also revealed that SSR markers, RM206 (0.89), RM21 and RM55 (0.88), RM258 (0.87) and RM433 (0.86), were robust enough to distinguish the duplicate and similar named rice germplasms for higher PIC values and should be considered as the best markers. Fig. 2 showed the gel pictures of amplified fragment using RM224.

The dendrogram, constructed using unweighted pair-group method with arithmetic means clustering method, clustered the genotypes into four groups (Fig. 3). Group I comprised of 11 genotypes, namely DB1, DB2, DB3, DB4, DB5, DB6, DB7, DB8, DB9, BR4

Table 4. Means and latent vectors for 18 morpho-physicochemical characters of 31 duplicate and similar named rice germplasms.

Character	Cluster I	Cluster II	Cluster III	Cluster IV	Vector I	Vector II	Combined ranking ^a
Seedling height (cm)	66.0	69.9	56.7	34.8	+0.1093	-0.0729	9
Plant height (cm)	123.9	125.6	111.1	106.1	-0.1082	+0.0332	12
Culm diameter (mm)	5.27	5.16	4.77	4.28	+1.7199	-1.7880	11
Panicle exertion (mm)	0.64	0.53	0.44	6.13	-1.6266	-0.2472	18
Effective tillering number per hill	11.8	11.0	12.1	10.3	+0.2065	+0.2738	3
Days to maturity (d)	143	141	142	160	-0.2783	-0.1692	14
Primary branch number per panicle	10.6	9.0	9.1	7.1	+0.8470	+0.1551	2
Secondary branch number per panicle	33.6	21.7	24.9	16.9	+0.1115	+0.2400	6
Grain yield per panicle (g)	3.2	2.7	2.3	1.9	+0.2634	-1.1171	17
Grain length (mm)	8.55	8.44	8.17	7.62	+0.2474	-0.3449	13
Awn length (mm)	0.6	21.5	0.0	30.5	-0.0198	+0.1587	7
1000-grain weight (g)	23.19	27.18	20.82	20.69	-0.0652	+0.5106	4
Grain yield per hill (g)	22.42	21.28	22.00	17.28	-0.3271	-0.1641	15
Biological yield (g)	40.9	37.0	33.1	27.7	+0.1759	+0.2617	5
Milling rate (%)	68.2	65.3	69.9	68.7	+0.0361	-0.6330	16
Cooking time (min)	17.0	20.0	16.5	19.1	-0.4281	+0.3856	10
Amylose content (%)	25.30	25.27	25.76	21.17	+0.2999	-0.2623	8
Protein content (%)	7.65	9.03	7.92	9.12	+0.8121	+0.6391	1

^a, Combined rankings were estimated according to positive values of both vector I and vector II for the character..

and BR23. Group II consisted of 4 genotypes viz. BR14, BRR1 dhan 28, BRR1 dhan 29 and DB10. However, Group III consisted with only KS7. Group IV had the other 20 genotypes of Kartiksail group except KS7. The Nei's genetic distance ranged from 0.3111 (DB1 and DB2) to 0.9778 (KS8 and DB1) among all the genotypes, and it varied from 0.4444 (between KS13 and KS14) to 0.9333 (between KS7 and KS19) and from 0.3111 (between DB1 and DB2)

to 0.8667 (between DB5 and DB10) among the two groups, respectively.

Separate rankings were done on the basis of D^2 and Nei's genetic distances, and correlation was estimated through Spearman's rank correlation coefficient (r_s). The calculated rank correlation coefficient and t value at $(n - 2)$ degree of freedom were 0.16 and 0.889, respectively, indicating statistical association ($P < 0.4$) between groups.

Table 5. Allele variation, gene diversity and polymorphism information content of 45 simple sequence repeat markers across 31 duplicate and similar named rice germplasms.

Chromosome	Marker	Position (cM)	No. of alleles	Size (bp)	Major allele		No. of rare alleles	No. of null alleles	Gene diversity	Polymorphism information content
					Size (bp)	Frequency (%)				
1	RM283	31.4	5	141–150	150	44	4	1	0.62	0.55
	RM259	54.2	6	166–187	170	39	4	0	0.76	0.73
	RM237	115.2	6	116–133	130	33	3	1	0.78	0.75
	RM302	147.8	9	107–214	178	31	7	2	0.82	0.80
	RM154	4.8	8	157–183	163/167	22	6	0	0.84	0.82
2	RM279	17.3	8	135–173	166	33	6	3	0.82	0.80
	RM324	66.0	9	132–166	166	28	7	0	0.82	0.80
	RM250	170.1	5	149–155	152	61	4	0	0.59	0.56
	RM60	0.0	10	147–182	162	25	8	1	0.86	0.85
3	RM218	67.8	10	126–152	136	22	8	3	0.86	0.84
	RM55	168.2	10	215–240	229	17	9	3	0.89	0.88
	RM227	214.7	4	97–105	97	47	1	1	0.66	0.60
	RM307	0.0	6	123–177	131	69	5	3	0.50	0.48
4	RM273	94.4	7	200–217	202	39	4	0	0.77	0.74
	RM241	106.2	7	100–130	126	44	5	0	0.72	0.69
	RM127	150.1	4	212–224	218	56	1	0	0.61	0.56
	RM413	26.7	9	68–105	68	22	7	2	0.86	0.85
5	RM267	28.6	8	150–185	166	36	6	0	0.75	0.71
	RM161	96.9	6	166–182	171	36	3	0	0.77	0.73
	RM133	0.0	4	225–232	227	69	2	0	0.48	0.44
6	RM584	26.2	10	160–214	163	33	8	0	0.83	0.81
	RM541	75.5	11	142–194	160	25	9	0	0.84	0.83
	RM162	108.3	6	223–233	223	33	3	0	0.77	0.74
	RM125	24.8	4	130–145	141	61	1	0	0.56	0.51
7	RM214	34.7	8	110–137	117	33	5	0	0.78	0.75
	RM18	90.4	8	160–180	169	22	5	1	0.85	0.84
	RM337	1.1	6	162–202	168	42	3	0	0.71	0.67
8	RM223	80.5	8	138–163	155	31	5	0	0.82	0.80
	RM256	101.5	8	103–138	108	36	5	0	0.78	0.76
	RM433	116.0	13	207–248	237	28	12	1	0.87	0.86
	RM296	0.0	8	121–139	125	39	6	0	0.72	0.68
9	RM242	73.3	8	190–214	203	28	6	0	0.83	0.81
	RM215	99.4	7	159–176	163	31	6	1	0.83	0.81
	RM311	25.2	10	170–201	176/182	19	7	0	0.86	0.84
10	RM271	59.4	8	81–98	93	28	6	1	0.82	0.80
	RM258	70.8	13	139–167	146	22	11	1	0.88	0.87
	RM171	92.8	6	314–340	314	36	4	0	0.75	0.72
	RM21	85.7	14	129–173	166	22	12	0	0.89	0.88
11	RM229	77.8	8	105–129	112	25	5	0	0.82	0.80
	RM206	102.9	13	116–171	157	17	12	0	0.90	0.89
	RM224	120.1	9	123–155	147	36	7	0	0.80	0.78
	RM286	0.0	6	96–121	96	31	4	0	0.79	0.75
12	RM19	20.9	8	223–239	228	28	5	1	0.83	0.81
	RM247	32.3	6	126–144	136	31	3	0	0.78	0.75
	RM277	57.2	3	116–123	116/119	47	1	0	0.55	0.45
	Minimum		3		68	17	1	0	0.48	0.44
Average		7.8		161.2	35	5.8	0.6	0.77	0.74	
Maximum		14		314	69	12	3	0.90	0.89	

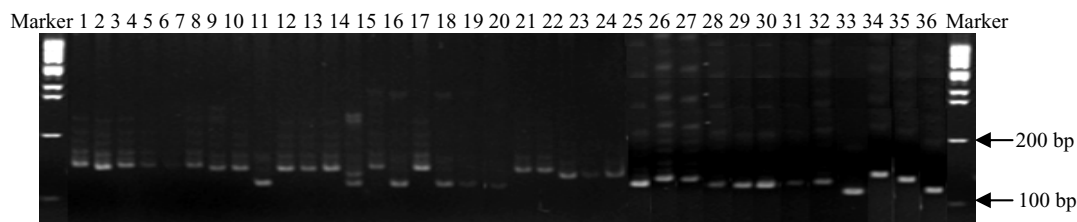


Fig. 2. DNA profile of 36 rice genotypes using RM224 marker.

1, KS1; 2, KS2; 3, KS3; 4, KS4; 5, KS5; 6, KS6; 7, KS7; 8, KS8; 9, KS9; 10, KS10; 11, KS11; 12, KS12; 13, KS13; 14, KS14; 15, KS15; 16, KS16; 17, KS17; 18, KS18; 19, KS19; 20, KS20; 21, KS21; 22, BR4; 23, BR23; 24, DB1; 25, DB2; 26, DB3; 27, DB4; 28, DB5; 29, DB6; 30, DB7; 31, DB8; 32, DB9; 33, DB10; 34, BRR1 dhan 28; 35, BRR1 dhan 29; 36, BR14.

DISCUSSION

Highly significant differences among the genotypes were observed, indicating the presence of wide genetic variations. The result also revealed that no duplicate genotype was existed. Hossain (2008) also observed highly significant differences among the 78 aromatic and fine grain landraces with duplicate names for all the agro-morphological and physico-chemical characters studied. However, Fukuoka et al (2006) studied aromatic landraces for quantitative traits and found significant variation among the genotypes with the same name.

The PCA showed that the first five components accounted 82.9% of the total variations. Sohrabi et al (2012) and Chakravorty et al (2013) observed the contribution of 76.7% and 75.9% of the first six and four components to the total variation in rice, respectively.

The genotypes were grouped into four clusters using Mahalanobis' D^2 statistic. Datt and Mani (2003) and Roy et al (2004) also found five and four clusters from 35 Aman and 61 elite Basmati rice cultivars by using D^2 statistics. The clustering pattern revealed that the genotypes constellated in the same cluster were not originated from the same geographic region.

Chakravorty et al (2013) evaluated 51 rice landraces and also found no parallel relationship between genetic and geographical divergence.

The intra- (0.62–0.93) and inter- (5.07–24.61) cluster distances revealed wide range of diversity among the genotypes (Table 3). Hossain (2008) also reported intra- and inter-cluster distances ranged from 0.0 to 1.02 and 2.21 to 21.59 in rice, respectively.

The clustering result also revealed that no single duplicate genotype was existed among genotypes. Earlier, Fukuoka et al (2006) and Nascimento et al (2011) also found no duplicates from cluster analysis

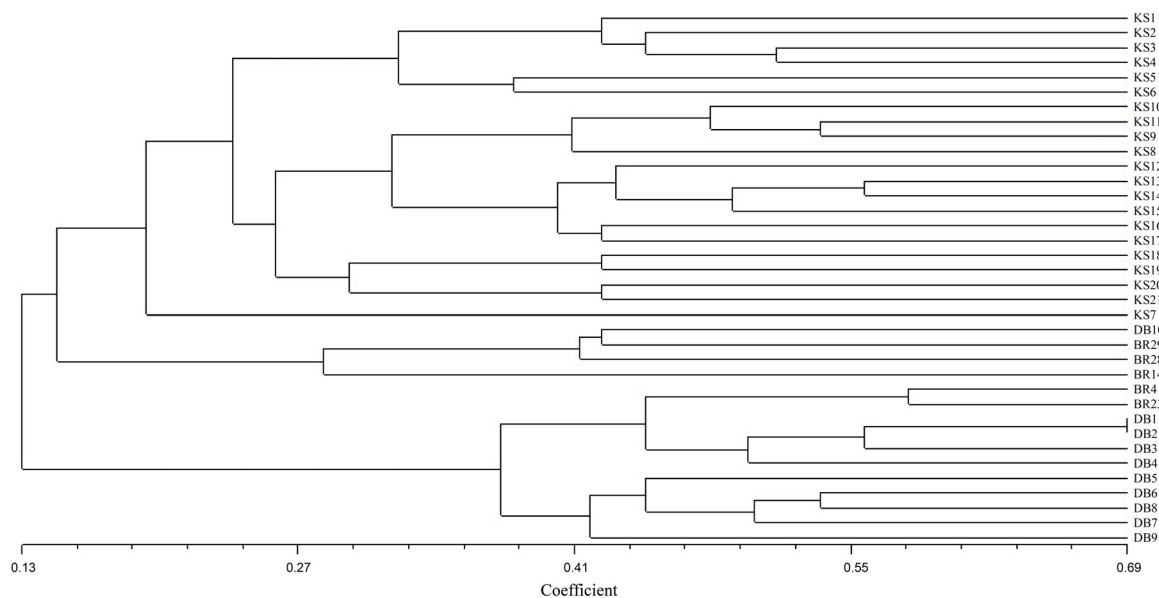


Fig. 3. Dendrogram of 31 duplicate and similar named rice germplasm derived from unweighted pair-group method with arithmetic means cluster analysis based on Nei's genetic distance across 45 simple sequence repeat markers.

BR28, BRR1 dhan 28; BR29, BRR1 dhan 29.

using D^2 statistics in rice.

The cluster mean values for studied morpho-physicochemical characters revealed that the crosses between the genotypes/parents of cluster I with those of clusters II and IV would exhibit high heterosis as well as transgressive segregation and could club the maximum good characters. Sohrabi et al (2012) reported similar trend of conclusions on rice using D^2 statistics.

High numbers of alleles (350) were detected at microsatellite loci across the studied rice genotypes. Thomson et al (2007) and Masduzzaman (2010) also detected 394 and 337 alleles with an average of 13 and 11 among 330 and 160 rice accessions by using 30 SSRs, respectively.

In the present study, the SSRs (RM206, RM21, RM55, RM258 and RM433) were found robust enough to distinguish the duplicate and similar named rice germplasms for their higher PIC values (> 0.86). Kaushik et al (2011) also demonstrated that SSRs are the best for differentiating the closely related rice varieties.

The traditional rice germplasms can offer a valuable gene pool which can be utilized in different varietal improvement/development program in future. It can be concluded that duplicate and similar named rice germplasms need to be conserved in gene bank though having similar or duplicate names.

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(Managing Editor: WANG Caihong)