Genetic Identity between Bhadawari and Murrah Breeds of Indian Buffaloes (*Bubalus bubalis*) Using RAPD-PCR

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ABSTRACT : Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analysis was carried out with a battery of 11 random decamer primers to study band frequency (BF), genetic identity index (I) and mean average percentage difference (MAPD) between Bhadawari and Murrah breeds of buffalo. The primers OPA04 and BG15 resolved a band of 460 bp, which was present only in animals of Bhadawari breed. Whereas, the primers OPA14, BG27 and BG28 produced Murrah specific fragments of sizes 730 bp and 1,230 bp, respectively. The estimate of genetic identity index was highest (0.845) with the primer OPA01 and the lowest (0.479) with the primer BG27. The genetic identity index pooled over the primers was 0.596±0.037 between these two breeds. The highest MAPD estimate (53.9) between the two breeds was obtained with the primer BG27 and the lowest (14.3) with the primer OPA01. It might be concluded that the genetic identity index between these two breeds calculated on the basis of BF showed moderate level of genetic identity with the primers employed. MAPD calculated on the basis of uncommon bands also demonstrated lower to medium level of genetic difference between Bhadawari and Murrah breeds of buffalo. (*Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 5 : 603-607*)

Key Words : Bhadawari and Murrah Buffaloes, Band Frequency, Genetic Identity Index, Mean Average Percentage Difference, RAPD-PCR

INTRODUCTION

Bhadawari buffaloes are well known for high content of butter fat in their milk (6 to 12.5%). This breed is also an efficient converter of coarse feed into butter fat. Better marketing facilities for liquid milk have tempted the farmer to cross these buffaloes with Murrah. All the same time, the Murrah is considered to be the best milch and meat breed of buffaloes with increasing popularity among farmers. Bulls of this breed have also been used for increasing milk production of other low productive breeds. In fact, this breed has even found an important place in livestock industry of many developing countries and animals of this breed have been exported to various countries like Bulgaria, The Philippines, Malaysia, Thailand, China, Indonesia, Bangladesh, Nepal, former USSR, Myanmar, Vietnam, Brazil and Sri Lanka etc. for maintaining in pure form as well as for improving the local buffaloes. Because of their tremendous importance in dairy sector it is essential to characterize these breeds at molecular level. *Ex-Situ* conservation and maintenance of precious germplasm of certain buffalo breeds in pure form warrants the precise identification of superior germplasm which could be effectively used in the genetic improvement programmes. Therefore, identification and characterization of breeds at genomic level is of utmost importance. The trend of molecular genetics has changed gear since the invention of

polymerase chain reaction (PCR) by Mullis 1987. A novel technique, one among the many offshoots of PCR, is the random amplified polymorphic DNA (RAPD) product analysis, proposed by Williams et al. (1990) as a DNA based technique for evaluating genetic variation and relatedness within and among species/breeds. This technique has been used widely in many species for genetic characterization of breeds as well as individuals (Gwakisa et al., 1994; Sivakumar, 1997; Kumar, 2001; Ramesha et al., 2002). Beside these this technique has also been successfully applied for differentiating geographically isolated populations or breeds (Bowditch et al., 1993). As the reports on RAPD-PCR analysis in buffaloes are scanty, the present study was undertaken with the objective to evaluate the genetic identity between Bhadawari and Murrah breeds of buffalo using RAPD-PCR.

MATERIALS AND METHODS

Two breeds of buffalo, Bhadawari and Murrah were used in the present study. These buffaloes were maintained at Buffalo and Jamunapari Goat Breeding Farm, Etawah, Uttar Pradesh, and Cattle and Buffalo farm, LPM Section, Indian Veterinary Research Institute, Izatnagar, respectively.

Genomic DNA isolation

Thirty animals were chosen randomly from each of the two breeds for the collection of blood samples. Genomic DNA was isolated from 20 ml of venous blood as per Anderson et al. (1986) with some modifications. DNA samples were checked for quality by running them in 0.7%

Table 1. Primer sequence with length and GC contents

Primer	Sequence (5′--------3′)	Length	GC %	Screened/ Employed
OPA01	5′CAGGCCCTTC3′	10- mer	70	Employed
OPA02	5′TGCCGAGTCG3′	10- mer	70	Employed
OPA04	5′AATCGGGCTG3′	10- mer	60	Employed
OPA06	5′GGTCCCTGAC3′	10- mer	70	Screened
OPA09	5′GGGTAACGCC3′	10- mer	70	Screened
OPA13	5CAGCACCCAC3′	10- mer	70	Screened
OPA14	5′TCTGTGCTGG3′	10- mer	60	Employed
OPB07	5′GGTGACGCAG3′	10- mer	70	Employed
OPG05	5′CTGAGACGGA3′	10- mer	60	Employed
OPG07	5′GAACCTGCGG3′	10- mer	70	Screened
OPG10	5′AGGGCCGTCT3′	10- mer	70	Screened
OPG11	5′TGCCCGTCGT3′	10- mer	70	Employed
OPG13	5′CTCTCCGCCA3′	10- mer	70	Employed
OPG16	5′AGCGTCCTCC3′	10- mer	70	Screened
BG15	5′GCCGTCCGAG3′	10- mer	80	Employed
BG16	5′CAGCCTGGGA3′	10- mer	80	Screened
BG27	5′CGGTGGCGAA3′	10- mer	70	Employed
BG28	5′CGCCCCACGT3′	10- mer	80	Employed

Agarose gel. Only intact DNA samples devoid of smearing were used for further analysis. The DNA concentration was calculated by measuring OD at 260 nm (1 OD_{260}=50 μg of double stranded DNA/ml).

Primers

Initially a battery of 18 random decamer primers was employed in representative samples of Bhadawari and Murrah breeds of buffaloes. Out of which, 11 primers yielded polymorphic pattern and hence they were used in this study. The sequence and Guanine Cytosine (GC) contents of the 18 primers are presented in Table 1.

PCR amplification

The reaction mix was prepared in 25 μl reaction volume having 50 μg of genomic DNA, 100 μm each of dNTPs, 1 μm of tetra methyl ammonium chloride (TMAC), 40 ng of primers, 0.5-1.0 U of *Taq* DNA polymerase and 2.5 μl of 10×*Taq* DNA polymerase buffer (500 mM Kcl, 100 mM Tris Hcl, 1.5 mM $MgCl_2$, 1% Triton X-100). Cycling conditions were used as per Plotsky et al. (1995) i.e. 36°C for 5 min followed by 45 cycle of 94°C for 1 min, 36°C for 1 min, 72°C for 2 min and final extension at 72°C for 5 min. 25 μl of PCR product was electrophoressed in 8% polyacrylamide gel and the gel was stained with ethidium bromide. The gel was viewed under UV light and photographed. DNA ladder (100 bp) or λ-DNA- *HindIII* /*EcoRI* double digests was used as molecular size marker.

Analysis of RAPD fingerprints

Only prominent and distinct bands were scored. The absence and presence of a band in the RAPD patterns was scored as zero or one, respectively. The band frequencies, genetic identity index and mean average percentage difference were computed as follows:

Band frequency (BF)

Band frequencies of RAPD fingerprints were determined as the ratio of number of animals carrying a particular band to the total number of animals screened using the following formula:

$$BF=n/N$$

Where, n is the number of animal carrying a particular band and N is the total number of animal screened.

Genetic identity index (I)

Genetic identity index was estimated as per Lynch, (1990):

$$I=1/N \sum_{i=1}^{n} [2 (f_{ix})(f_{iy})/\{f_{ix}\}^2+(fiy)^2\}]$$

Where, N is total number of bands and f_{ix} and f_{iy} are the frequencies of band 'i' in Bhadawari and Murrah breeds, respectively.

Mean average percentage difference (MAPD)

Mean average percentage difference (MAPD) was calculated using the following formula (Gilbert et al., 1990 and Yukhi and O'Brien, 1990)

$$Percentage\ difference\ (PD)=\{N_{ab}/(N_a+N_b)\}\times100$$

$$Average\ percentage\ difference\ (APD)=I/C \sum_{i=1}^{C}PDI$$

$$Mean\ average\ percentage\ difference\ (MAPD)=I/R \sum_{i=1}^{R}APDi$$

Where, N_{ab} are the number of fragment that differed between two animals, a and b for a single primer, N_a and N_b are the number of fragments resolved by a and b animals, C is the number of inter-breed pair-wise comparison and R is the number of random primers used.

RESULTS AND DISCUSSION

Band frequency

The band frequencies of the RAPD-PCR products with all the 11 primers employed in the present investigation are presented in Table 2. Primer, OPA01 resolved only three bands, out of which two bands were present in all animals of both the breeds. One band of 780 bp was present in all animals of Bhadawari breed but in only 29% animals of

Table 2. Band frequencies (BF) of various amplified products in Bhadawari (B) and Murrah (M) buffaloes with different primers

Primers	Band size (bp)	B	M	Primers	Band size (bp)	B	M
OPA04	1,385	0.00	0.50	BG27	1,025	0.20	0.80
	1,115	0.33	0.50		705	0.00	1.00
	855	0.33	1.00		560	0.80	1.00
	520	0.67	0.50		525	0.80	0.20
	460	1.00	0.00				
	370	0.33	1.00				
OPA02	3,615	0.00	0.13	OPG05	995	0.00	0.07
	905	0.33	0.50		780	0.89	0.40
	795	0.50	0.75		700	0.67	0.60
	645	0.33	0.50		645	1.00	0.73
	600	1.00	1.00		510	0.89	1.00
	575	0.50	0.00		480	0.89	1.00
OPB07	925	0.20	0.20	OPG13	950	0.33	0.33
	845	0.80	0.20		665	0.67	0.00
	735	1.00	1.00		600	0.00	0.33
	585	0.20	0.00		470	1.00	0.83
	525	0.40	0.20		445	1.00	0.67
	455	0.40	0.20		380	0.33	0.33
	400	0.20	0.40		220	0.33	0.17
BG15	1,585	1.00	0.00	BG28	770	0.33	0.67
	1,320	1.00	0.00		695	0.00	0.33
	980	1.00	0.00		620	1.00	0.67
	925	1.00	0.60		595	0.00	0.33
	835	0.80	0.20		555	0.00	1.00
	750	1.00	0.60		515	0.67	0.67
	705	1.00	0.00		480	1.00	0.33
	650	1.00	0.60		455	1.00	0.33
	415	1.00	1.00				
	365	1.00	1.00				
OPA14	1,870	0.80	1.00	OPG11	1,710	0.20	0.00
	1,775	0.60	0.00		1,540	0.20	0.50
	1,585	0.60	1.00		1,395	0.00	0.50
	1,395	0.60	1.00		1,270	0.80	0.50
	1,300	0.00	1.00		1,080	0.00	0.50
	1,230	0.00	0.60		1,060	0.40	0.50
	1,100	1.00	0.40		995	1.00	1.00
	1,015	0.20	0.60		940	0.20	0.50
	925	0.80	0.40		825	0.80	1.00
	835	0.80	1.00		785	0.00	0.50
	730	0.00	0.40		715	0.60	0.25
	695	0.80	0.20		595	0.60	0.00
	640	1.00	1.00				
OPA01	780	1.00	0.29				
	505	1.00	1.00				
	435	1.00	1.00				

Murrah breed. OPA02 primer revealed one out of six bands of 600 bp, which was monomorphic in both the breeds, whereas all other bands were polymorphic.

Primer OPA04 resolved a band of 460 bp (out of total six bands) present only in Bhadawari buffaloes. Another band of 1,385 bp was present in 50% individual of Murrah breed but totally absent in Bhadawari buffaloes. Other products were having different frequencies in both the breeds. The primer OPA14 revealed 13 bands from which one band was unique in Murrah breed, which was present in all the animals of this breed. Two more bands of 730 and 1,230 bp were seen only in Murrah buffaloes with variable frequencies. Similarly, one band of 1,775 bp was seen in 60% of Bhadawari animals but not in the animals of Murrah breed. However, a band of 640 bp was present in all the animals of both the breeds.

Another Primer OPB07 revealed seven bands out of which one band of 735 bp was present in all animals of both the breeds. The other bands had low frequencies in the animals of both the breeds. OPG05 primer revealed six
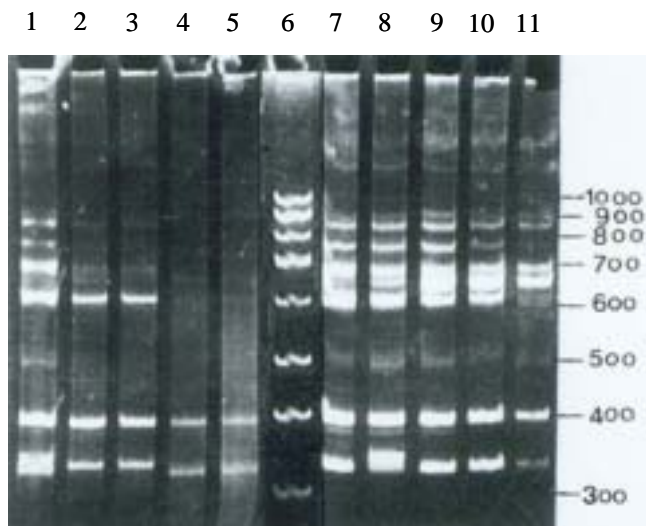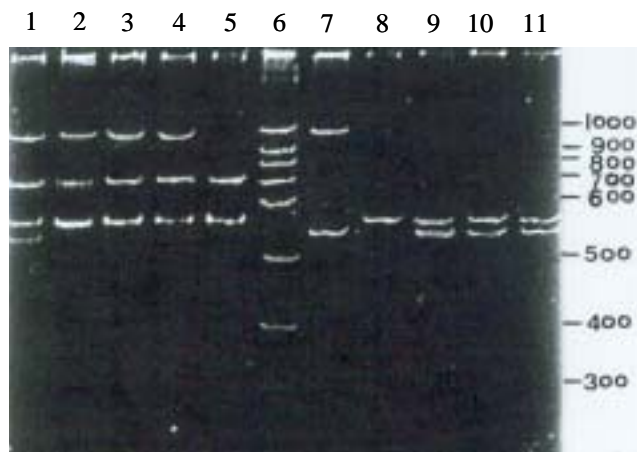
Figure 1. RAPD fingerprints of genomic DNA using primer BG15. Lane 1-5: DNA samples of Murrah, Lane 6: 100bp ladder, Lane 7-11: DNA samples of Bhadawari.

Figure 2. RAPD fingerprints of genomic DNA using primer BG27. Lane 1-5: DNA samples of Murrah, Lane 6: 100bp ladder, Lane 7-11: DNA samples of Bhadawari.

Table 3. Genetic identity index (I) between Bhadawari and Murrah buffaloes with different primers

Sl. No.	Primers	Genetic identity index (I)
1	OPA01	0.845
2	OPA02	0.627
3	OPA04	0.511
4	OPA14	0.560
5	OPB07	0.582
6	OPG05	0.780
7	OPG11	0.495
8	OPG13	0.675
9	BG15	0.512
10	BG27	0.479
11	BG28	0.488
Overall (Pooled over primers)		0.596±0.037

amplicons and two of them (480 and 510 bp) were present in all animals of Murrah breed. These products also had high frequency in Bhadawari. Surprisingly one band of 995 bp was seen only in one animal of Murrah breed. Primer OPG11 revealed a band of 995 bp in all animals of both breeds. Three bands of 785, 1,080, 1,395 bp were seen in 50% of Murrah animals but were completely missing in Bhadawari animals. In contrast to this, two bands of 595 and 1,710 bp were seen only in some animals of Bhadawari breed. Primer OPG13 revealed seven amplicons out of which one (600 bp) was seen only in Murrah animals although its frequency was low (0.33). Similarly a band of 665 bp was seen only in Bhadawari animals, which had a moderate frequency of 0.67.

Primer, BG15 revealed 10 amplified products of which four bands (705, 980, 1,320 and 1,585 bp) were seen in all animals of Bhadawari breed (Figure 1). Two fragments of 365 and 415 bp were seen in all animals of both the breeds. Although the primer BG27 revealed only four distinct bands, one band of 705 bp was present only in Murrah animals (Figure 2). Primer BG28 revealed eight products, one of them was seen in all animals of Murrah breed and two more products (595 and 695 bp) although with low frequencies were seen in Murrah animals only.

It was observed that the primers, OPA04 and BG15 revealed Bhadawari specific products, which were seen in the animals studied. Similarly, OPA14, BG27 and BG28 revealed Murrah specific amplicons that were seen in all animals of this breed. Similar findings of breed/species specific amplicons in buffaloes were reported by Aravindakshan and Nainar (1998) and Singru (1998), although they employed different sets of primers in their study.

However, there is no report available in the literature on this aspect in Bhadawari buffaloes to compare. Present findings may be considered as base line information on molecular genetic characterization of Bhadawari buffaloes. A few reports in cattle also demonstrated breed specific RAPD fingerprints with different primers (Gwakisa et al., 1994; Shivakumar, 1997; Ahn et al., 1999)

Genetic identity index (I)

The genetic identity index represents the relatedness of two breeds/ populations with respect to the sequences amplified in PCR. The primer-wise genetic identity index between these two breeds is given in Table 3. Most of the primers used in the present study showed medium to high genetic identity between the two breeds. Highest genetic identity index (0.845) was observed with the primer OPA01 and the lowest (0.479) with primer BG27. The genetic identity index (pooled over all 11 primers) was 0.596±0.037 between Murrah and Bhadawari buffaloes. There is no report available in the literature to compare on this aspect in buffaloes.

Table 4. Average percentage difference (APD) and mean average percentage difference (MAPD) between Bhadawari and Murrah buffaloes

Sl. No.	Primers	APD
1	OPA01	14.3
2	OPA02	22.8
3	OPA04	48.7
4	OPA14	41.6
5	OPB07	27.2
6	OPG05	20.8
7	OPG11	35.5
8	OPG13	31.3
9	BG15	44.7
10	BG27	53.9
11	BG28	41.4
MAPD		34.70±3.80

Mean average percentage difference (MAPD)

The highest APD value (53.9) between these two breeds was obtained with primer BG27 and the lowest value (14.3) with OPA01 (Table 4). The mean average percentage difference (MAPD) was calculated as measures of inter breed divergence from RAPD fingerprints obtained with 11 primers. The MAPD between these two breeds was 34.70±3.80. Aravindakshan and Nainar (1998) also reported MAPD value 24.16±3.55 between Murrah and Surti breeds using different set of primers. This indicated that Murrah and Bhadawari breeds are probably more distant than Murrah and Surti breeds.

The results of the present study suggested that some of the primers viz. OPA04 and BG 15 resolved Bhadawari specific products that were seen in all animals of this breed. Similarly, primers OPA14, BG27 and BG28 revealed Murrah specific amplicons, which were seen in all the animals studied. APD analysis revealed similar results as observed with genetic identity index analysis, although the basis of analysis was different. The genetic identity index between the two breeds, calculated on the basis of band frequency, indicated moderate level of genetic identity with the primers employed. MAPD calculated on the basis of uncommon bands demonstrated lower to medium level of genetic difference between Bhadawari and Murrah breeds of buffaloes.

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