

RAPD MARKERS FOR CHARACTERIZATION OF MURRAH AND NILI-RAVI BREEDS OF BUFFALO

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ABSTRACT

Random amplified polymorphic DNA technique was used to characterize Murrah and Nili-Ravi breeds of buffalo. From 11 random primers, a total of 110 bands were amplified and 78 of these (about 71%) were found to be polymorphic in Murrah breed, while a total of 57 bands were amplified and 34 of these (about 60%) were found to be polymorphic in Nili-Ravi breed. Low overall genetic distance of 0.26 and 0.18 was observed within Murrah and Nili-Ravi breeds, respectively, as compared to genetic distance of 0.53 between breeds. Mean average percentage difference in Murrah and in Nili-Ravi breed was 21.29 and 14.57, respectively. Four primers viz. OPB-06, OPI-01, OPI-04 and OPI-07 resolved Murrah breed specific amplicons.

Key words: RAPD, buffalo, Murrah, Nili-Ravi

Awareness of the value of genetic resources has stimulated the study on genetic diversity in native breeds. Knowledge of genetic variation within and among breed is very important for better understanding, improving the economic traits and breed characterization. Various types of markers including morphological, cytological, biochemical and molecular are used for this purpose. Morphological markers such as coat colour, horn types etc. usually exhibit low level of variation and are not of much help in characterization of buffalo breeds. Biochemical markers have been tried extensively but are not found encouraging, as they often express low level of polymorphism and are sex limited and age dependent. Further these markers reflect variability in the coding sequences, which constitute less than 3 per cent of the total genome.

Recent developments in DNA technologies have made it possible to uncover a large number of genetic polymorphisms at the DNA sequence level and to use them as markers for evaluation of the genetic basis for the observed phenotypic variability. Random amplification of polymorphic DNA (RAPD) by the polymerase chain reaction (PCR) is a means of detecting polymorphisms for genetic mapping and strain identification

(Welsh and McClelland, 1990, Williams *et al.*, 1990). RAPD method applies the PCR with a single short oligonucleotide primer, short fragments of genomic DNA, which are size-fractionated by agarose gel electrophoresis. The fact that RAPD surveys multiple loci in the genome makes the method attractive for analysis of genetic distance and phylogeny reconstruction (Clark and Lanigan, 1993). It is a powerful tool in DNA fingerprint analysis of various animal species, gene mapping studies, population analysis and identification of breeds. The present study is aimed at searching of markers, which may help in differentiating the animals so that pure germplasm of the breeds could be conserved, propagated and improved and to study the genetic variability between and within breeds.

MATERIALS AND METHODS

Genomic DNA was isolated from blood of 20 animals each of Murrah and Nili-Ravi breeds using the protocol of Sambrook and Russel (2001) with slight modifications. The quality of isolated genomic DNA was evaluated by agarose gel electrophoresis. The concentration of DNA was also checked by UV spectrophotometer taking optical density (O.D.) at 260 and 280 nm. The genomic DNA was diluted to the concentration

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of 25 ng/μl. Forty random primers were used for screening polymorphism. Only 11 primers successfully amplified the genomic DNA from most of the animals. The amplifications were reproducible and distinct.

PCR reaction mixture consisting of 2 mM MgCl₂, 100 μM dNTPs, 1.0 U *Taq* polymerase, 40 ng of random primer with 50 ng of genomic DNA. The amplification was carried out for forty five cycles with initial denaturation at 95°C for 5 minutes, second denaturation for one minute at 94°C, annealing at 36°C for one minute and extension for 2 min at 72°C and final extension at 72°C for 5 min. All the amplified products were separated by electrophoresis in 1.4% agarose gels containing 0.05% ethidium bromide and scored by gel documentation system. Only clear bands of RAPD-PCR products on agarose gels were scored. Genetic similarity index was calculated on the basis of band frequencies (Lynch, 1990). Mean average percentage difference (MAPD) was determined by formula of Gilbert *et al.*, (1990). Band sharing frequency (BSF) was estimated as reported by Jeffreys and Morton, (1987). The genetic distance was calculated by the method of Nei's (1972).

RESULTS AND DISCUSSION

RAPD-PCR Analysis: The characteristics of the amplification profiles of Murrah breed using 11 random primers have been presented in the Table. The bands amplified from these primers also varied in size ranging from 150 bp to 3000 bp. All 11

primers detected polymorphism in Murrah breed. From the 11 random primers, a total of 110 bands were amplified and 78 of these (about 71%) were polymorphic. Number of polymorphic loci ranged from 2 to 13. A maximum of 13 polymorphic loci were with primer OPAM-07, while OPB-06 and OPI-07 could amplify only 2 polymorphic loci. OPI-09 and OPA-07 generated 100 % polymorphism. The average number of bands amplified per primer ranged from 3.82 to 7.10 and their size ranged from 150 to 3000 bp.

The total number of bands amplified per primer ranged from 1 to 9 in Nili-Ravi using 11 primers (Table). Only 8 primers (72%) detected polymorphism. A total of 57 bands were amplified and 34 of these (about 60%) were polymorphic. A maximum of 10 polymorphic loci were amplified with primer OPI-09. The average numbers of total bands amplified per primer ranged from 2.13 to 4.25 and these bands ranged in the size of 150 to 1510 bp.

The total number of bands amplified per primer ranged from 5 to 14 in both Murrah and Nili-Ravi breeds. The band size ranged from 150 bp to 3000 bp. A total of 114 bands were amplified and 56 of these (about 49%) were polymorphic. Number of polymorphic loci ranged from 2 to 11. A Maximum of 11 polymorphic loci were amplified with OPAM-07 and OPB-20, whereas OPB-03, OPB-06 and OPAA-16 could amplify only 2 polymorphic loci. With OPB-03 only 22% polymorphic loci could be detected while OPI-01 generated 83% polymorphic loci.

Band frequency: It is the ratio of number of

Table
Amplification profile for different primers in two breeds of buffalo

Primer code	Murrah				Nili-Ravi			
	Loci amplified	Polymorphic loci	Size range (bp)	% Polymorphism	Loci amplified	Polymorphic loci	Size range (bp)	% Polymorphism
OPI-09	12	12	275-1325	100.00	10	10	345-1325	100.00
OPB-20	06	03	425-1245	50.00	04	01	425-680	25.00
OPAA-16	14	12	225-1510	85.70	08	06	225-1510	75.00
OPA-07	12	12	225-3000	100.00	04	03	575-1460	100.00
OPAM-07	14	13	310-1400	92.85	03	02	385-1280	66.66
OPI-01	09	06	615-1390	66.66	05	03	350-680	60.00
OPB-06	06	02	375-1000	33.33	04	00	375-710	00.00
OPB-05	05	03	290-940	60.00	02	00	575-940	00.00
OPI-07	10	02	410-2500	20.00	03	00	410-1500	00.00
OPB-03	09	05	225-1150	55.55	06	02	225-1150	33.33
OPI-04	13	08	150-1500	61.53	08	07	150-800	87.50

animals carrying a particular band to the total number of animals screened. OPI-09 revealed 13 bands out of which 12 were present in Murrah and 10 in Nili-Ravi. Primer OPB-20 resolved 2 monomorphic bands out of total 6 bands amplified in both breeds. A band of 680 bp was present in all the individuals of Nili-Ravi breed but only in 55 % animals of Murrah breed. Similarly a band of 425 bp was present in all the animals of Murrah breed and 65 % individuals of Nili-Ravi breed. Primer OPA-07 amplified 12 bands out of which only 4 bands were present with variable frequency in Nili-Ravi breed. In Murrah individuals all the 12 products had variable frequency. OPAM-07 resolved 14 bands out of which a band of 385 bp was monomorphic to both breeds. Rest of the bands were polymorphic. OPI-01 produced 12 bands. Out of which three bands 1930, 1710 and 885 bp were unique to Murrah breed. A band of 440 bp was present in 85% individuals of Nili-Ravi breed but not in Murrah breed.

Primer OPB-06 revealed 2 monomorphic out of 6 bands amplified in both breeds. Two bands of 1000 bp and 510 bp were detected in Murrah breed only. OPB-05 revealed 2 monomorphic out of 5 bands to both breeds. OPI-07 produced 10 bands out of which 5 were unique to Murrah breed (Fig). Three bands of 1500, 605 and 410 bp were present in all individuals of both breeds. Primer OPB-03 revealed 3 monomorphic out of 9 bands. Two bands of 515 and 440 bp were present in 85% individuals of Murrah breed but not seen in Nili-Ravi breed. Primer OPI-04 resolved 13 bands where a band of 950 bp was unique to Murrah breed and was found in all the animals.

Genetic similarity: The genetic similarity represents the relatedness of the two breeds or populations with respect to the sequences amplified in PCR. In Murrah breed, the estimates of genetic similarity differed not only for the magnitude, but also in the trend with different random primers. With OPB-20, estimates varied from 0.67 to 1.00, while with OPAM-07 a much wider range of estimates (0.14 to 1.00) was observed. Maximum genetic similarity of 0.89 was observed with OPB-03 and OPI-04. Minimum genetic similarity of 0.64 was with OPAA-16, followed by 0.66 with OPAM-07. In general within Murrah breed overall genetic

similarity in breed was 0.79. The present results are quite comparable to the reports of Aravindakshan and Nainar (1998) who observed within breed genetic similarity in Murrah as 0.81 ± 0.02 . Slight variation in the value could be due to difference in the primers used.

With OPB-20 within Nili-Ravi breed, estimates varied in a narrow range from 0.86 to 1.00, much wider range of estimates i.e. 0.22 to 1.00 was observed with OPI-04. Maximum genetic similarity of 0.93 was observed with OPB-20 followed by genetic similarity of 0.92 with OPI-01. Minimum genetic similarity 0.74 was observed with OPA-07, followed by 0.81 with OPAA-16. Within breed overall genetic similarity was more (0.85) in Nili-Ravi than the Murrah (0.79).

In general, overall estimates of genetic similarity between breeds ranged from 0.29 to 0.88 with an overall value of 0.62. Within breed genetic similarity was higher in comparison to between breeds indicating that genetic divergence was higher between breeds than within breeds. Singru (1998) reported overall between breed genetic similarity of Murrah with Surti, Jaffarabadi and Nagpuri breeds in the range of 0.36 ± 0.08 to 0.56 ± 1.07 . The results seem to be comparable although the primers used were different. Saifi *et al.* (2004) found genetic similarity between Murrah and Bhadawari breeds to be 0.60 ± 0.04 .

The estimates of the genetic distance differed widely with the primer, not only for the magnitude, but also in the trend. The estimates varied from 0.12 to 0.37 with OPI-04 and OPAA-16, respectively, in Murrah breed. The overall genetic distance was 0.26. Widest range of distance from zero to 0.97 was observed with OPAM-07 and narrowest from zero to 0.40 with OPB-20. Maximum genetic distance (0.37) was observed with OPA-07 and minimum value (0.07) was for OPB-20. The overall genetic distance within breed was estimated to be 0.18. The estimates varied in maximum range of zero to 1.51 with OPI-04 while the range was narrowest (zero to 0.15) with OPB-20. Between breed highest genetic distance was 0.94 with primer OPI-01 whereas lowest value of 0.13 was observed with primer OPB-03. The overall genetic distance between breeds was 0.53. Genetic distance within breeds was lower in comparison to between breeds.

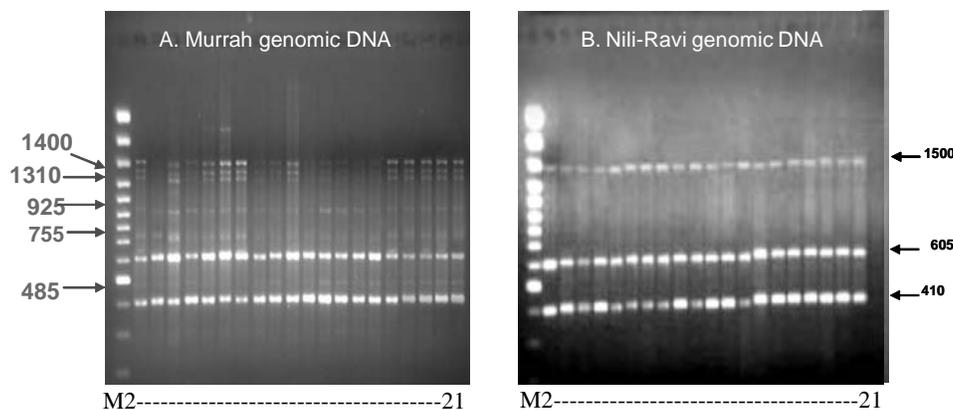


Fig. RAPD banding pattern of Nili-Ravi (left) and Murrah (right) using the random primer OPI-07. The band size of 1500,600 and 400 are common in both breeds whereas the remaining bands are unique to Murrah breed. Lane M indicates 100 bp DNA ladder, lanes 2 to 21 are the DNA sample numbers 1 to 20 of both breeds.

MAPD value was calculated as a measure of inter breed divergence from the RAPD fingerprints obtained with 11 primers. In Murrah breed, APD ranged from 11 to 36% with MAPD of 21.29 % while it ranged from 7 to 26% in Nili-Ravi breed with MAPD of 14.57%. The results indicate that very little genetic diversity can be detected within breeds with these primers or might be due to small sample size. Between breed APD has a range of 12 to 71% with MAPD of 36.64%. The medium value of MAPD between these breeds indicates medium genetic variation between breeds. MAPD value of $24.16 \pm 3.55\%$ between Murrah and Surti breeds using different set of primers has been reported by Aravindakshan and Nainar (1998). Saifi *et al.* (2004) reported MAPD between Murrah and Bhadawari breeds to be $34.70 \pm 3.80\%$. However, before drawing conclusion the breeds should be analysed with same set of primers.

Breed specific primers: Out of the 11 primers studied, only four primers viz. OPB-06, OPI-01, OPI-04 and OPI-07 resolved Murrah breed specific bands. These results need to be confirmed in a large sample size before these primers can be recommended to be breed specific. Singru (1998) reported that primers P2 and A2 amplified specific fragments in Surti and Nagpuri breeds, while primer ILO-14 gave reproducible bands in Jaffarabadi and Murrah breeds. Saifi *et al.* (2004) suggested that primers OPA-04 and BG-15 resolved Bhadawari specific products while primers OPA-14, BG-27 and BG-28 revealed Murrah specific amplicons. RAPD used to study to measure genetic distance between Murrah and

Nili-Ravi breeds can be considered an efficient method for generating breed specific markers.

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