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Assessment of genetic variability in Murrah, Bhadawari and Nili-Ravi buffalo breeds of India by RAPD markers

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Abstract

The present study was conducted for breed characterization and genetic diversity within and between Murrah, Bhadawari and Nili-Ravi breeds by RAPD-PCR. Genomic DNA was isolated from 20 unrelated animals of each breed. Out of 40 random primers of operon series OPU and OPV, only ten were found informative and were used further for amplification of genomic DNA. From the amplification profile of these primers values of band frequency, genetic distance, genetic similarity, band sharing frequency, average percentage difference and mean average percentage difference was calculated from ten random primers. A total of 188 bands were amplified between as in three breeds and out of these 112 were polymorphic (59.57%). In Murrah, Bhadawari and Nili-Ravi overall polymorphism of 65.51, 63.49 and 53.44 percent were observed respectively. Higher genetic similarities of 0.84, 0.77 and 0.70 in Murrah, Bhadawari and Nili-Ravi were observed respectively. Mean Average Percentage Difference (MAPD) values of 11.41, 13.62 and 18.53 were observed in Murrah, Bhadawari and Nili-Ravi. MAPD values of 69.0, 67.0 and 40.0 were observed between Bhadawari and Nili-Ravi, Bhadawari and Murrah and Murrah and Nili-Ravi respectively. These values indicated the higher genetic diversity between breeds. Therefore, RAPD-PCR plays an important tool in species identification, speciation and breed identification as well as phylogeny analysis.

Keywords: RAPD, buffalo, PCR, fingerprinting, genetic variation

Introduction

India emerged as one of the largest milk producing country and the major portion of total milk production in India is from buffaloes. Buffalo (*Bubalus bubalis*) is the only livestock species which has an exclusive domain limited to just 40 countries in the world. India is the world leader in buffaloes as it possesses nearly half (78 million) of the world buffalo population (FAO, 2004). It is popularly called 'Asian Animal' as 97% of total buffalo stock is present in Asia alone. India is a harbinger of some of the best riverine breed of buffaloes. Murrah and Nili-Ravi enjoy a pre-eminent position among the high milk yielding breeds. Murrah is one of the best dairy buffaloes also known as black gold. Its home tract lies in Rohtak, Hisar, Jind and Gurgaon districts of Haryana. Bhadawari buffaloes are known for their high fat content. They are found in the ravines of Chambal and spread over Agra and Etawah districts of Uttar Pradesh, and Bhind and Morena districts of Madhya Pradesh.

Despite its leading role in country's milk and meat production, sheer negligence is observed regarding germplasm conservation of various breeds of buffalo. The number of purebred animals of most indigenous breeds is about 20 to 25 percent. Rests of the buffaloes are non-descript. There is a general concern that the genetic variation within domestic animal species is disappearing through breed substitution and interbreed crossing. Any reduction in the diversity of genetic resources narrows the scope to respond to selective breeding (Sethi *et al.*, 2005) [9]. Awareness of the value of genetic resources has stimulated the study of the genetic diversity of native breeds. Detailed knowledge of genetic variation within and among breeds is very important for understanding and improving the economic traits and for breed characterization. There are several ways to characterize the germplasm. Various types of markers such as phenotypic, chromosomal, biochemical and molecular markers are used for this purpose. Randomly amplified polymorphic DNA is a polymerase chain reaction based technique, using arbitrary primers to detect changes in the DNA sequences at sites in the genome, which anneal

by primer. The RAPD method described by William *et al.* (1990) [11] provides a faster and less expensive alternative to RFLP analysis. This technique has been used in the characterization of breeds/ animals. Despite the advantages of using traditional PCR assays for detecting DNA alteration, there are numbers of potential difficulties, first, the nucleotide sequences flanking upstream and downstream the target DNA needs to be determined. Second the size of the PCR product plays a crucial role in the detection of DNA damage because the amplification of short fragments (less than 300bp) may slightly reduce and possibly not inhibited at all even in case of extensive DNA damage. Third, it is conceivable that any reduction in the intensity of PCR amplicons could be due to partial inhibition of the PCR reaction caused by factors other than DNA damage such as residuals phenol remaining after DNA extraction. Alternatively, if an amplicon entirely disappears, it could be argued that the PCR did not work at all, irrespective of the presence of DNA damage, such disadvantage as described can be eliminated by using the random amplified polymorphic DNA initially established and still used for genetic mapping and taxonomic identification (Williams *et al.* 1990) [11]. This study was undertaken to investigate the usefulness of RAPD analyzed in the characterization of Buffaloes breed's samples.

Material and Method

Blood samples were collected from 20 unrelated animals each of Murrah, Nili Ravi and Bhadawari breeds, maintained at Govt Livestock Farm, Hisar and Uttar Pradesh Pandit Deen Dyal Uppadhaye Pashu Chikitsa Vigyan Avam Gau-Anusandhan Sansthan, Mathura (U.P), respectively. Approximately 10 ml of venous blood was collected in 15 ml sterile polypropylene centrifuge tubes containing 0.5 ml of 0.5M EDTA as an anticoagulant. The blood was gently mixed with anticoagulant, and kept on ice to prevent cell lysis. Subsequently, the blood samples were transported to the laboratory and stored at 4°C till the isolation of DNA. The extraction of DNA was from 10 ml of blood using the protocol by Sambrook and Russel (2001) [8] with slight modifications.

Randomly Amplified Polymorphic DNA (RAPD) markers were used to identify the genetic relatedness within and between breeds. Initially, 40 random primers (10 bp) of series OPU and OPV were employed on representative samples for amplification. The screening revealed 10 random primers (Table-1) to be informative in detecting polymorphism within and between breeds. All the 10 primers successfully amplified the genomic DNA from most of the animals and produced reproducible and distinct bands.

The RAPD-PCR technique is highly sensitive to minor alterations in the reaction conditions. The number, intensity of amplification and reproducibility of amplified DNA fragments depend on a variety of variables such as PCR cycling conditions (Yu and Pauls, 1992; Don *et al.*, 1991) [12, 3], template and primer concentrations (Muralidharan and Wakeland, 1993) [4], and the components of the PCR buffer (Ponce and Micol, 1992) [6]. It is important to provide optimum conditions for primers to ensure efficient and reproducible amplification from reaction to reaction. 2.5 mM MgCl₂, 100 μM dNTPs, 1.25 U Taq polymerase, and 100 μM primers with 50 ng of genomic DNA were finalized to be the components of the PCR mix.

Different PCR programs were tried for obtaining reproducible results. The reactions were carried out in a programmable

thermocycler (GstromTM). The following program was found to produce satisfactory results and hence used in subsequent amplifications.

Table 1: PCR cycling conditions

S. No.	Steps	Temp	Time
1.	Initial denaturation	95 °C	5 min
2.	40 cycles of		
a)	Denaturation	94 °C	1 min
b)	Annealing	37 °C	1 min
c)	Primer extension	72 °C	2 min
3.	Final extension	72 °C	7 min
4.	Store	4 °C	∞

The amplified products were analysed by running in a 1.5 % w/v agarose gel at 1-2 V/ cm for approx 2 hrs in 1X TAE buffer. The gel was stained with ethidium bromide, visualized under UV transilluminator (Biovis Gel Documentation system) and photographed for documentation. Molecular sizes of various fragments were estimated by running standard size markers (100 bp DNA marker, MBI Fermentas), to determine the size of the amplicons. Only prominent and distinct bands were scored. The presence or absence of the band was recorded as '1' and '0', respectively and a binary data matrix was made. Following statistical analyses were carried out for assessing the genetic variability and relatedness in three breeds of buffalo. Band frequencies of RAPD fingerprints were computed using the binary data matrix.

Result and Discussion

Ex-situ conservation and maintenance of precious germplasms of dairy buffalo breed need precise identification of superior germplasm hence the identification and characterization of breeds at the genetic level are very important. The present study was under taken to find out the randomly amplified polymorphic DNA marker in characterization of Murrah, Bhadawari and Nili-Ravi breeds of buffalo. Genomic DNA was isolated from 20 randomly selected unrelated individuals of Murrah, Bhadawari and Nili-Ravi. Quality and purity of DNA were checked by 0.7% agarose gel purity. Ratio of absorbance at 260 nm and 280 nm was taken for all the DNA samples and samples having a ratio between 1.7 to 1.9 were considered good and were used subsequently. Samples were screened using 40 random primers of operan series OPU and OPV. Out of 40 Random primers screened, only 10 primers were found to be informative and were used in the subsequent study. Representative RAPD pattern by one of the Primer OPU-01 in Bhadawari, Murrah and Nili-Ravi are presented in Figure.1, 2 and 3).

In Murrah breed, a total number of bands amplified 58 out of these 38 are polymorphic (65.51%). A number of bands amplified 2 to 9 while number of polymorphic loci ranged from 5.5 to 6.0 with band size of 200 – 1700 bp. In Bhadawari breed, a total number of bands amplified 63 out of these 40 were polymorphic (63.49 %). Number of band amplified 1 to 11 while number of polymorphic loci of 4.0 to 5.5 with band size size of 300 bp to 2000 bp. In Nili-Ravi breed, total number of band amplified 64 out of these 34 were polymorphic (53.44 %). A number of bands amplified 5 to 11 while number of polymorphic loci of 4.5 to 5.0 with band size of 300 -3000 bp. With breed overall genetic similarity was 0.84, a 0.77 and 0.70 in Murrah, Bhadawari and Nili-Ravi, respectively. Overall genetic similarity 0.20, 0.14 and 0.20 in

Bhadawari and Murrah, Bhadawari and Nili-Ravi and Murrah and Nili-Ravi, respectively. These values indicated that the genetic similarity within the breed is more than between breeds.

In Murrah, the value of band sharing frequency (BSF) ranged from 0.72 to 0.99, with primers OPV-02 and OPV-20, respectively. In the case of Bhadawari value of BSF ranged from 0.54 to 0.99 with primer OPV-01 and OPV-14, respectively and in Nili-Ravi value of BSF ranged from 0.39 to 0.95 with primer OPV-01 and OPV-20, respectively. BSF was 0.56, 0.53 and 0.67 between Murrah and Bhadawari, Bhadawari and Nili-Ravi and Murrah and Nili-Ravi, respectively. The genetic distance was calculated using within and between breed BSF and the value of genetic distance was observed 0.164, 0.245 and 0.860 between Bhadawari and Murrah, Bhadawari and Nili-Ravi and Murrah and Nili-Ravi, respectively. The genetic distance was lower between Murrah and Nili-Ravi as compared to between Bhadawari and Murrah and between Bhadawari and Nili-Ravi breeds. The present results of Murrah and Bhadawari band frequency are very comparable to the reports of Barwar, 2007^[2] who observed that average band sharing frequency between breeds varied from 0.08 to 0.72. The overall average band frequency between breeds was 0.30 ± 0.06 . And he also observed the within breed BSF in Murrah as 0.89 and Bhadawari 0.84 and the between breed BSF 0.30. Singru (1998)^[11] 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 employed in that study were different. Saifi *et al.* (2004)^[7] found the genetic identity index between Murrah and Bhadawari breeds to be 0.596 ± 0.037 . No report on this aspect could be found in Nili-Ravi.

Mean average percentage different (MAPD) was calculated as a measure of genetic diversity within and between breeds. In Murrah, APD value ranged from 0.73 to 18.40 with primer OPV-20 and OPU-05, respectively and MAPD was 11.41 in Bhadawari, APD ranged from 0.04 to 34.31 with primer OPV-14 and OPU-02, respectively, and MAPD was 13.62, however in Nili-Ravi, APD ranged from 5.48 to 40.10 with primer OPV-14 and OPV-20, respectively and MAPD 18.53. Between breed APD values between Bhadawari and Murrah ranged from 46.17 to 95.35 with primer OPU-02 and OPU-01,

between Bhadawari and Nili-Ravi ranged from 37.03 to 96.36 with primer OPU-02 and OPV-02, respectively and MAPD was 69.0. APD between Murrah and Nili-Ravi ranged from 20.0 to 64.97 with primer OPV-20 to OPV-01, respectively, and with MAPD was 40.0. These values indicate that high between breed genetic diversity as compared to within breeds. The present results of Murrah and Bhadawari genetic similarities are very comparable to the reports of Barwar (2007)^[2] who observed that genetic similarity between breeds ranged from 0.12 to 0.69 with an overall genetic similarity of 0.31 ± 0.06 . Within breed genetic similarity was higher in comparison to between breed genetic similarities, indicating that genetic divergence was higher between breeds than within breeds. This indicates that less genetic variation between Murrah and Nili Ravi. MAPD values were 67.05 between Murrah and Bhadawari breeds using different primer (Barwar, 2007)^[2]. This indicates Bhadawari and Murrah and Bhadawari Nili-Ravi are more distant as compare to Murrah and Nili-Ravi breeds. Saifi *et al.* (2004)^[7] reported MAPD between Murrah and Bhadawari breeds to be 34.70 ± 3.80 . MAPD value of 24.16 ± 3.55 between Murrah and Surti breeds using a different set of primers has been reported by Aravindakshan and Nainar (1998)^[1]. This indicates that Murrah and Nili Ravi breeds are more distant than Murrah and Surti breeds whereas the genetic divergence is comparable to that between Murrah and Bhadawari breeds. However before drawing a final conclusion the breeds should be analyzed with the same set of primers.

In the present study five primers (OPU-05, OPU-14, OPU-19, OPV-14, OPV-20) resolved Murrah specific bands another five primers (OPU-01, OPU-05, OPU-07, OPU-14 and OPV-14) gave Bhadawari specific bands and other five primers (OPU-02, OPU-05, OPU-19, OPV-01, and OPV-14) provided Nili-Ravi specific bands. Similarly Saifi *et al.* (2004)^[7] suggested that primers OPA-04 and BG-15 resolved Bhadawari specific products that were seen in all animals of this breed while primers OPA-14, BG-27 and Bg-28 revealed Murrah specific amplicons. Singru (1998)^[11] also reported that primers P2 and A2 primers amplified specific fragments in Surti and Nagpuri breeds, while primer ILO 14 gave reproducible bands in Jaffarabadi and Murrah breeds. Hence RAPD can be considered an efficient method for generating breed specific markers

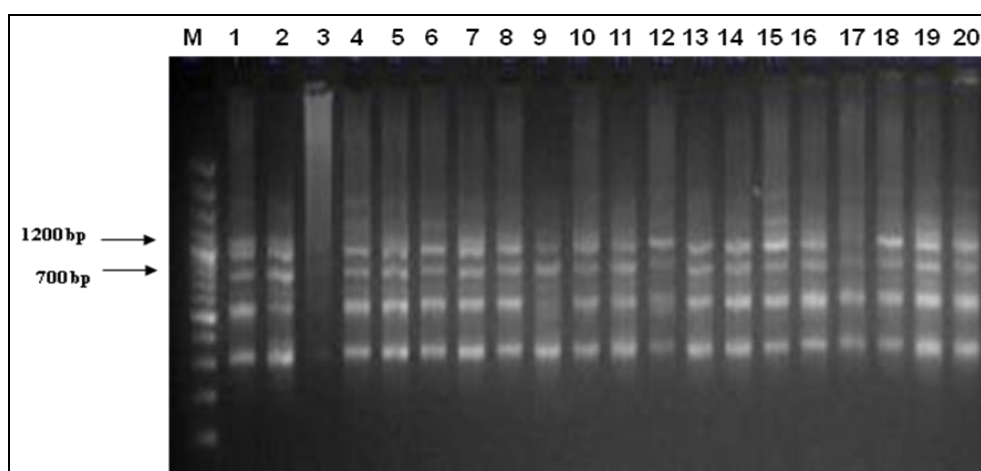


Fig 1: RAPD pattern by Primer OPU-01 in Bhadawari

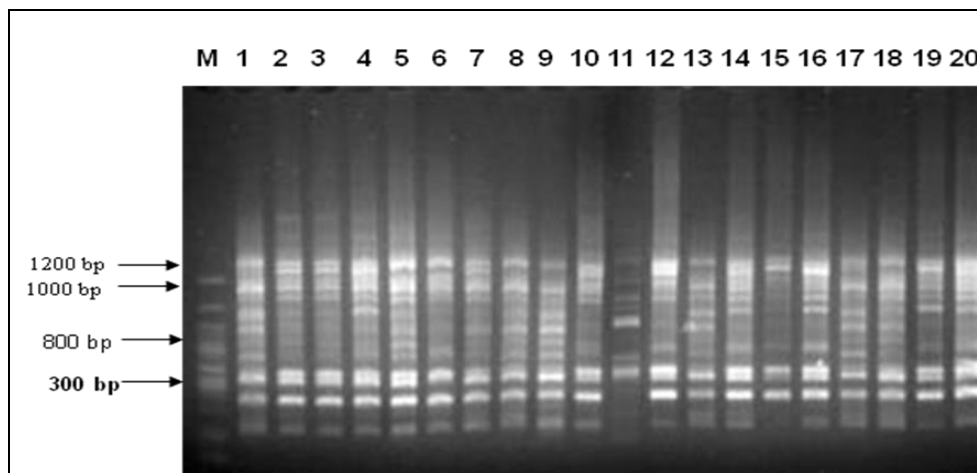


Fig 2: RAPD pattern by Primer OPU-01 in Murrah

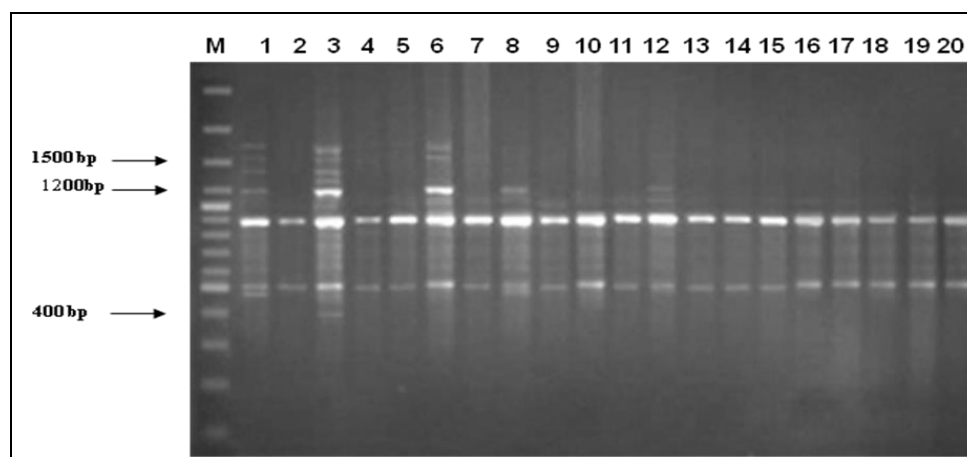


Fig 3: RAPD pattern by Primer OPU-01 in Nili Ravi

Conclusion

In our study we found that a simple RAPD-PCR is efficient in differentiating between and within breed at the molecular level, however, RAPD techniques unravels polymorphic pattern in DNA of individuals. This approach is cheaper and easier as compared to other methods of DNA fingerprinting. These polymorphisms are further utilized in characterization of different breeds. Breeds of Buffalo (Murrah and Bhadawari) belonging to different geographical region show more genetic diversity or polymorphism than Nili Ravi and Murrah breeds belonging same geographical region. Inter and intra breed variation at the DNA sequence level can be useful in planning strategies for breeding and conservation of different breeds of buffalo. Therefore, RAPD-PCR plays an important tool in species identification, speciation and breed identification as well as phylogeny analysis. Moreover it may be also helpful in cataloging and germplasm characterization of Buffalo.

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