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Research Article

MOLECULAR CHARACTERIZATION OF BUFFALO BREEDS BY RANDOM AMPLIFICATION OF POLYMORPHIC DNA MARKERS

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ABSTRACT

Randomly amplified polymorphic DNA technique was used to analyze 20 blood samples each of Murrah, Bhadawari and Nili-Ravi breeds by using random primers. A total of 188 bands were amplified in three breeds and out of which 112 were polymorphic (59.57%). In Murrah, Bhadawari and Nili-Ravi overall percentage polymorphism observed was 65.51, 63.49 and 53.44, respectively. Genetic similarity of 0.84, 0.77 and 0.70 was observed in Murrah, Bhadawari and Nili-Ravi, respectively. Within breed MAPD value of 11 41, 13.62 and 18.53 were observed in Murrah, Bhadawari and Nili-Ravi while MAPD 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 that higher genetic diversity between breeds than within breed. Primers OPU-05, OPU-14, OPU-19, OPV-14, OPV-20 in Murrah, OPU-01, OPU-05, OPU-07, OPU-14, OPV-14 in Bhadawari and OPU-02, OPU-05, OPU-19, OPV-01, OPV-14 in Nili-Ravi resolved breed specific bands.

Key words: RAPD, buffalo, characterization, fingerprinting, genetic variation

India has best riverine breeds of buffalo in the world. Out of the well defined buffalo breeds, Murrah, Bhadawari, and Nili- Ravi are considered to be the best dairy in the Indian subcontinent. Knowledge of genetic variation within and among breed is very important for better understanding, improving the economic traits and breed characterization. To design rational breeding strategies for optimal utilization and conservation of available genetic variability in Indian buffaloes, it is essential to understand their genetic architecture and relationship between them. 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 polymerase chain reaction (RAPD-PCR) is a mean of detecting polymorphism for genetic mapping and breed identification (Welsh and McClelland, 1990, Williams *et al.*, 1990). RAPD-PCR provides a faster and less expensive alternative to RFLP analysis. This technique has been used in the characterization of breeds/animals.

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MATERIALS AND METHODS

Genomic DNA was isolated from blood of 20 animals each of Murrah, Bhadawari and Nili-Ravi breeds using 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 at 260 and 280 nm. The genomic DNA was diluted to the concentration of 25 ng/ul. Forty random primers were used for screening polymorphism. Only ten random primers gave informative polymorphism. The primers are OPU01, OPU02, OPU05, OPU07, OPU14, OPU19, OPV01, OPV02, OPV14 and OPV20 with GC content ranging from 60-70 %.

PCR reaction mixture consisting of 2.0 mM $MgCl_2$, 10.0 mM dNTPs,1.0 U taq polymerase, 40 ng of random primer with 50 ng of genomic DNA. The amplification was carried out for forty cycles with initial denaturation at 95°C for 5 min., second denaturation for 1 min. at 94°C, annealing at 37°C for 1 min. and extension for 2 min. at 72°C and final extension at 72°C for 7 min. All the amplified products were separated by electrophoresis in 1.4% agarose gel containing 0.05%

ethidium bromide and scored by gel documentation (Biovis gel) 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 and genetic distance were calculated as reported by Jeffrey's and Morton (1987) and Nei (1972), respectively. The protocol for PCR 2.5 mM $MgCl_2$, 100 µM dNTPs, 1.25 U Taq polymerase and 100 µM primers with 50 ng of genomic DNA was finalized

RESULTS AND DISCUSSION

RAPD-PCR analysis: In Murrah breed using 10 random primers, total number of amplified bands visualized were 58 and out of these 38 (65.51%) were found to be polymorphic. The average number of amplified bands per primer ranged from 5.5 to 6.0 and band size from 200-1700 bp while in Bhadawari breed, a total of 63 bands were obtained and out of which only 40 were polymorphic giving about 63.49% polymorphism. The average number of amplified band per primer ranged from 4.0 to 5.5 and band size from 300-2000 bp. In Nili-Ravi breed, a total of 64 bands were visualized and out of these only 34 were found to be polymorphic giving about 53.44% polymorphism. The average number of amplified bands per primer ranged from 4.5 to 5.0 and band size from 300-3000 bp. Within breed overall genetic similarity was 0.84, 0.77 and 0.70 in Murrah, Bhadawari and Nili-Ravi, respectively. Overall between breeds genetic similarity observed was 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 breed is more than between breeds.

Band sharing frequency: In Murrah, band sharing frequency (BSF) ranged from 0.72 to 0.99 with primers OPV-02 and OPV-20, respectively. In Bhadawari BSF ranged from 0.54 to 0.99 with primer OPV-01 and OPV-14, respectively while in Nili-Ravi BSF ranged from 0.39 to 0.95 with primer OPV-01 and OPV-20, respectively. The BSF was 0.56, 0.53 and 0.67 between Murrah and Bhadawari, Bhadawari and Nili-Ravi and Murrah and Nili-Ravi, respectively. 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 in between Murrah and Nili-Ravi as compared to between Bhadawari and Murrah and between Bhadawari and Nili-Ravi breeds. 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. Saifi *et al.* (2004) found genetic similarity between Murrah and Bhadawari breeds to be 0.60±0.04.

Genetic similarity: 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 primers OPV-20 and OPU-05, respectively and MAPD was 11.41. In Bhadawari, APD ranged from 0.04 to 34.31 with primers OPV-14 and OPU-02, respectively with MAPPD of 13.62. However, in Nili-Ravi, MAPD was 18.53, and APD ranged from 5.48 to 40.10 with primers OPV-14 and OPV-20, respectively.

Between breeds APD values between Bhadawari and Murrah ranged from 46.17 to 95.35 with primers OPU-02 and OPU-01 with MAPD of 69.00. Between Bhadawari and Nili-Ravi, APD ranged from 37.03 to 96.36 with primers 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 and OPV-01, respectively, and with MAPD value of 40.0. These values indicate high genetic diversity between breed as compared to within breeds. The MAPD value of 24.16±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±3.80%. Ahlawat *et al.* (2007) reported MAPD value of 21.29 and 14.57 in Murrah and Nili-Ravi breeds, respectively and between breeds MAPD was 36.64 which are comparable with the results obtained in the present study.

Breed specific primers: Five random primers were found to be breed specific each for Murrah breed (OPU-05, OPU-14, OPU-19, OPV-14, OPV-20), for

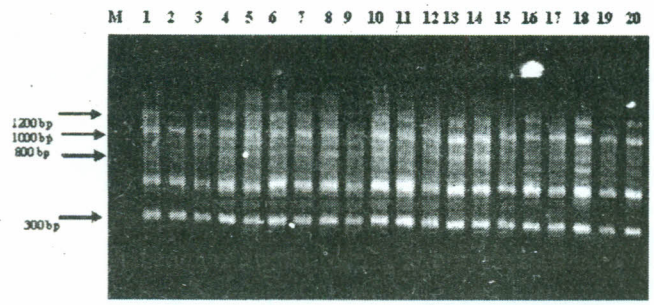

Fig 1. RAPD pattern using primer OPU-19 in Bhadawari.
(M=100 bp ladder)

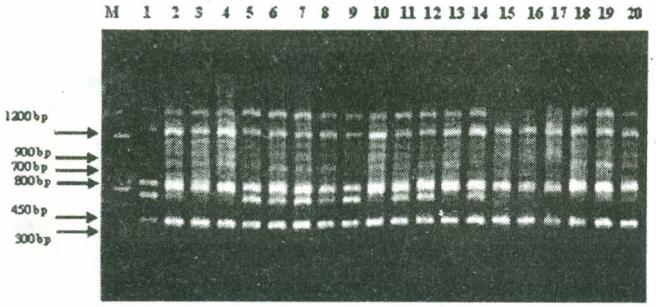

Fig 2. RAPD pattern using primer OPU-19 in Murrah.
(M = 100 bp ladder)

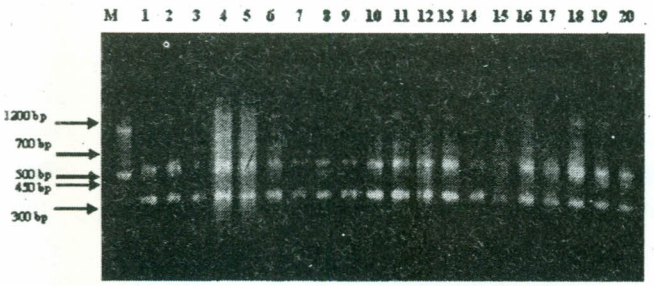

Fig 3. RAPD pattern using primer OPU-19 in Nili-Ravi.
(M = 100 bp ladder)

Bhadawari breed (OPU-01, OPU-05, OPU-07, OPU-14, OPV-14) (Figs 1, 2, 3) and for Nili-Ravi (OPU-02, OPU-05, OPU-19, OPV-01, OPV-14). The present study provided the basic platform for characterization of buffalo breeds at the molecular level with DNA markers. 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 products. In summary, RAPD-PCR is efficient in differentiating between and within breed at molecular level, however, analysis of more number of animals of each breed is required to improve the reliability of the results.

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