Molecular Characterization of Rathi and Tharparkar Indigenous Cattle (*Bos indicus*) Breeds by RAPD-PCR

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ABSTRACT : Random amplification of polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analysis was carried out using DNA samples of 30 animals of Rathi cattle and 42 animals of Tharparkar cattle. Genomic DNA was isolated as per standard protocol and evaluated for its quality, purity and concentration. Twenty three random primers were screened out of which 15 primers yielded satisfactory amplifications and were used for further analysis. Average numbers of polymorphic fragments per primer were 7.07±0.86 in Rathi and 6.80±0.61 in Tharparkar cattle. The percentage of polymorphic bands in these two cattle breeds were 86 and 87%, respectively. Within breed genetic similarities for pooled over primers in the animals of Rathi and Tharparkar breeds were 0.577±0.30 and 0.531±0.02, respectively on the basis of band frequency (BF) and 0.645±0.04 and 0.534±0.04, respectively on the basis of band sharing (BS). Averages of between breed genetic similarities for pooled over primers were 0.97 and 0.92 according to BF and BS, respectively, which reflect higher degree of genetic similarity between Rathi and Tharparkar cattle breeds. Index of genetic distance based on BF and BS for pooled over primers was 0.030±0.011 and 0.088±0.031, respectively. Percentage of polymorphic bands and within-breed genetic similarities on the basis of band frequency (BF) and band sharing (BS) for pooled over primers revealed higher genetic similarity in Rathi than Tharparkar cattle population. High estimates of between breed genetic similarities for pooled over primers indicated that either Rathi is having decent from Tharparkar or both the cattle breeds are having common descent. Low value of Index of genetic distances between these two cattle breeds may be due to the fact that Rathi and Tharparkar cattle breeds are the native of Thar Desert in Northwest India. The results of between breed genetic distances also confirm the existence of high degree of genetic similarity between these two breeds of cattle. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 9 : 1204-1209)***

Key Words : RAPD-PCR, Molecular Characterization, Rathi and Tharparkar Cattle

INTRODUCTION

Characterization of Indian zebu cattle has attempted by biometrical methods, which are based exclusively on quantitative traits. As these traits are controlled by multiple loci and are affected by polygene and environmental factors, genetic improvement in these traits relatively slow. Efforts were also made to explore polymorphisms on the basis of biochemical, immunological and cytogenetic markers. However, since these polymorphisms could not exploit the genetic variation at DNA level, hence they were of little importance and could not be used in precise identification of an individual. Molecular genetic techniques are efficient for evolutionary, ecological and population genetics studies. Among these a technique termed as Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) has been successfully applied in genetic studies of various animals and plant species (Michelmore et al., 1991; Welsh and McClelland, 1991; Baird et al., 1992; Chapaco et al., 1992) as well as for molecular characterization of bovine populations (Bardin et al., 1992; Kemp and Teale, 1992). RAPD-PCR is based on amplification of DNA in the polymerase chain reaction (PCR) by short random primers. This technique has several advantages over others as it is non-radioactive, easy to perform, involves low cost, readable directly on the gel, does not require knowledge of prior sequence and also requires very little amount of DNA. India has 26 recognized cattle breeds among these Rathi is a dual-purpose cattle breed having home tract in Alwar and Rajputana region of Rajasthan and spread up to Bikaner district. Milk yield of Rathi cow is about 4.5 kg per day. Bullocks of this breed are used for draft purpose. Selective breeding of Rathi cattle in closed herd system at Livestock Research Station (LRS), Nohar of Rajasthan Agricultural University, Bikaner resulted in production of germ plasma having dam's yield 2.233 kg per lactation in elite herd. Tharparkar is also dual-purpose cattle breed and the preferred synonyms for this breed are Thari, Grey Sindhi and White Sindhi. Cows of this breed are considered to be temperamental and having average milk yield of 1,000 to 7,500 lb. (Wahid, 1971). Bullocks of this breed are of medium size and steers and having a reputation of willing workers. This breed is known for their high disease resistance. Very few reports are available on molecular characterization of indigenous breeds; therefore present investigation was conducted to analyze the genetic variability and similarity between and within Rathi and Tharparkar cattle populations by RAPD-PCR.

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MATERIALS AND METHODS

Experimental animals

Randomly selected 30 Rathi and 40 Tharparkar animals maintained at Livestock Research Station (LRS) of Rajasthan Agricultural University, Nohar, District-Hanumangarh, Rajasthan and Central Cattle Breeding Farm (CCBF), Suratgarh, District-Hanumangarh, Rajasthan, respectively were used in the present investigation.

DNA samples

Blood samples were collected in sterile polypropylene centrifuge tubes containing anticoagulant. The blood was gently mixed with anticoagulant, and kept on ice to maintain low temperature in order to prevent cell lysis. Subsequently the blood samples were transported to the laboratory and stored at 4°C until the isolation of genomic DNA. The genomic DNA was isolated by phenol extraction method (Anderson et al., 1986) with some modifications.

Amplification of genomic DNA by PCR

The amplifications were carried out in 0.2 ml of PCR reaction tubes using a programmable thermal cycler (M. J. Research, USA). The reaction mix was prepared in 25 µl reaction volume having 50 ng of genomic DNA, 100 µl each of dNTPs, 1 µM of Tetra Methyl Ammonium Chloride (TMAC), 40 ng of primer, 0.5-1.0 U of *Taq* DNA Polymerase and 2.5 µl of 10 X *Taq* DNA Polymerase buffer (500 mM KCl, 100 mM Tris HCl, 1.5 mM $MgCl_2$, 1% Triton X-100). Various PCR programmes were tested for obtaining reproducible results and ultimately amplification of DNA was carried out by initial denaturation at 95°C for 5 min, 45 cycles of denaturation, annealing and primer extension at 94, 36 and 72°C for 1 min each, respectively followed by final extension at 72°C for 5 min. The amplified products were electrophoresed by running in a 1.4% w/v agarose gel at 1-2 V/cm for approximately 4-6 h in 1×TAE buffer. The gels were stained with Ethidium Bromide, viewed under UV light and documented for further analysis.

Recording of data and statistical analysis

Only distinct and prominent bands were scored and the presence and absence of a band was recorded as `1' and `0', respectively. RAPD patterns of Rathi and Tharparkar animals were compared within as well as between breeds. Genetic similarity within and between the populations was calculated using two measures of genetic similarity i.e. band sharing and band frequency.

Genetic similarity based on band sharing

The band sharing (BS) frequency between two animals was calculated as an expression of similarity of RAPD fingerprints of animals from either the same or different breeds (Jeffrey and Morton, 1987; Wetton et al., 1987; Dunnington et al., 1990) using the formula.

$$BS=2\ N_{ab}/(N_a+N_b)$$

Where, N_{ab} is a number of common fragments observed in a and b animals and N_a and N_b are the total number of fragments scored in animals a and b, respectively. Within breed similarity (WS_i) was calculated as the average of B_{ab} across all the possible comparisons between animals within i^{th} breed. Between breed similarity (BS_{ij}), corrected for within breed similarity were estimated (Lynch, 1990) as.

$$BS_{ij}=1+BS'_{ij}-(WS_i+WS_j)/2$$

Where, WS_i and WS_j are the values of within breed similarity in i^{th} and j^{th} breeds, respectively and BS'_{ij} is the average similarity between all possible comparisons between animals of i^{th} and j^{th} breeds. The value of BS'ij may exceed 1. The genetic distance (Dij) was estimated as.

$$D'ij=-\ln [BS'_{ij}/\sqrt{(WS_i \cdot WS_j)}]$$

Genetic similarity based on band frequency

The genetic similarity within the breed was estimated as.

$$U=1/N\sum_{i=1}^{N} V_i$$

Where, V_i is the frequency of occurrence on the i^{th} band and N is the total number of bands scored. The genetic similarity between two breeds was obtained as follows.

$$I=1/N\sum_{i=1}^{N}(2.V_i^{(1)} \cdot V_i^{(2)})/[\{V_i^{(1)}\}^2+\{Vi^{(2)}\}^2]$$

Where, $V_i^{(1)}$ and $V_i^{(2)}$ are the frequency of occurrence of i^{th} band in breeds 1 and 2 respectively and N is total number of bands scored. The index of genetic distance was estimated as -In (I) (Apuya et al., 1988)

RESULTS AND DISCUSSION

Random amplification of polymorphic DNA-Polymerase Chain Reaction RAPD-PCR is a powerful molecular genetic technique for detection of genetic variability and similarity in the different breeds/population of the livestock (Kemp and Taele, 1992; Cushwa et al., 1996). This technique is highly sensitive to minor alterations in the reaction conditions (Yu and Pauls, 1992; Bowditch et al., 1993) hence; optimization of reaction

Table 1. Average number and size of bands obtained from different random primers

Primer	Sequence (5'----------3')	GC%	Average number of bands		Size range (bp)	
			Rathi	Tharparkar	Rathi	Tharparkar
OPA01	5' CAG GCC CTT C3'	70	5.33±0.67	3.50±0 87	400-1,800	400-1,800
OPA02	5' TGC CGA GCT G3'	70	6.43±0.65	3.50±0.67	600-1,200	600-1,200
OPA04	5'AAT CGG GCT G3'	60	3.00±0.01	2.75±0.75	564-1,800	350-1,800
OPA14	5'TCT GTG CTG G3'	60	4 67±0 33	4.00±0 45	250-1,280	250-1,280
OPB07	5'GGT GAG GCA G3'	70	3 00±0 45	2.50±0 75	500-1,800	500-1,600
OPG05	5'CTG AGA CGC A3'	60	5.50±0.34	5.00±0.45	210-1,260	210-1,500
OPG07	5'GAA CCT GCG G3'	70	3 33±0 33	4.00±0 41	475-1,475	400-1,300
OPG10	5'AGG GCC GTC T3'	70	3 50±0 56	3.83±0 60	280-1,680	280-1,680
OPG11	5'TGC CCG TCG T3'	70	4.67±0.33	4.60±0.87	310-3,000	310-2,100
OPG13	5'CTC TCC GCC A3'	70	5.00±0.52	5.00±0.52	260-2,300	260-2,600
OPG16	5'AGC GTC CTC C3'	70	6 43±0 65	3.50±0 67	250-3,000	250-2,600
BG15	5'GCC GTC CGA G3'	80	5 33±0 36	4.17±0 70	240-2,750	240-1,400
BG16	5'CAG CCT GGC G3'	80	5.00±0.45	5.17±0.48	280-1,510	280-2,100
BG27	5'CGG TGG GGA A3'	70	6.50±0.56	6.25±0.48	245-2,100	245-1,000
BG28	5'CGC CCC ACG T3'	80	2 80±0 80	4.60±0 24	564-1,800	380-1,800

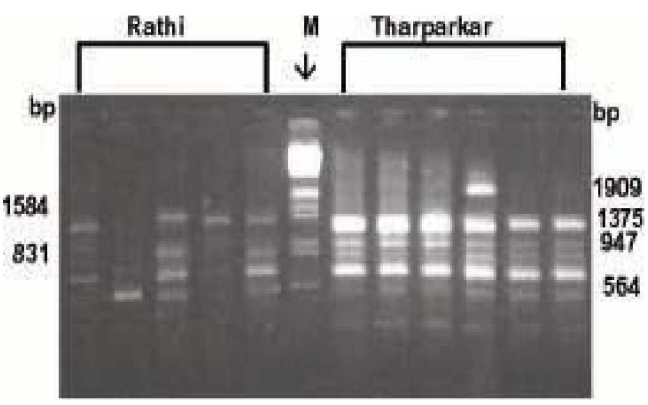

Figure 1. RAPD fingerprints of genomic DNA derived from two breeds of cattle using primer BG 28. Lane M: λ-DNA EcoRI and HindIII double digest marker.

conditions was carried out for each of the primers by varying certain components of PCR. Cycling conditions were also optimized to get the good and reproducible results. Annealing temperature was the most important variable, which was optimized using the value T_m=4 (G+C)+2 (A+T).

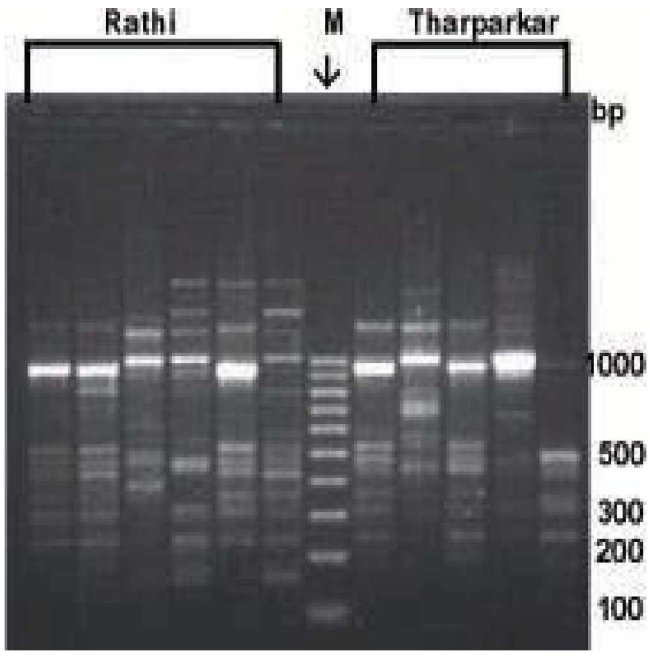

Figure 2. RAPD fingerprints of genomic DNA derived from two breeds of cattle using primer BG 27. Lane M: 100 bp DNA ladder.

Screening of RAPD primers

Initially, 23 random primers were used in representative samples of Rathi and Tharparkar cattle breeds for the study of amplification patterns. 15 out of 23 primers were capable of exhibiting polymorphic amplification patterns and hence, they were used in the subsequent analysis with more number of DNA samples of the animals of these two breeds. The sequence and GC contents of the 15 primers are given in Table 1.

RAPD fingerprints

The individual samples from Rathi and Tharparkar cattle breeds were analysed with 15 primers screened in the study and all these primers were capable of priming polymorphic amplification pattern in both the breeds. Only distinct, reproducible and scorable amplified products were taken into consideration for analysis. The molecular sizes of bands which were differing by ±5% on the different gels were considered as same band. The amplification patterns of representative samples of Rathi and Tharparkar cattle breeds with different primers have been shown in Figure 1, 2 and 3. The primers OPA01, OPA02, OPA04, OPA14, OPB07, OPG05, OPG07, OPG10, OPG11, OPG13, OPG16, BG15, BG16, BG27 and BG28 produced total number of polymorphic bands as 6, 7, 7, 8, 8, 9, 8, 8, 13, 9, 11, 14, 8.

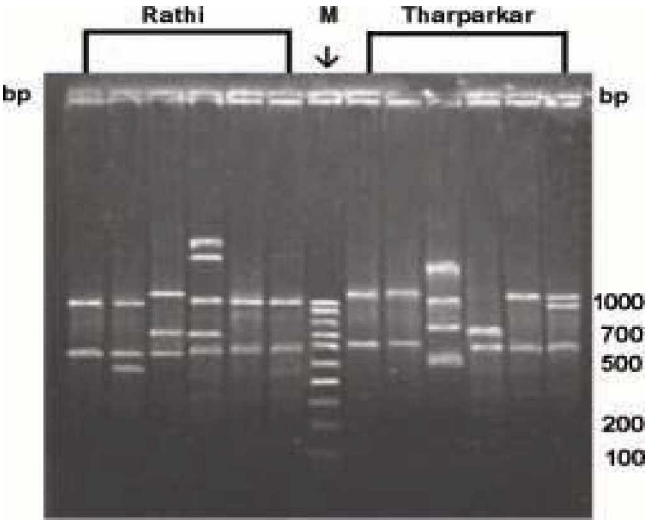

Figure 3. RAPD fingerprints of genomic DNA derived from two breeds of cattle primer OPB 07. Lane M: 100 bp DNA ladder.

11 and 5 with molecular size range from 400-1,800 bp, 600-1,200 bp, 350-1,800 bp, 250-1,280 bp, 500-1,600 bp, 210-1,500 bp, 400-1,475 bp, 280-1,680 bp, 310-3,000 bp, 260-2,600 bp, 250-3,000 bp, 240-2,750 bp, 280-2,100 bp, 245-2,100 bp and 380 to 1,800 bp, respectively.

Maximum number of polymorphic bands was 14 with primer BG15 and minimum 5 with primer BG28. Average number bands observed in Rathi ranged from 2.80±0.80 (BG28) to 6.50±0.56 (BG27), whereas in Tharparkar these were ranging from 2.50±0.75 (OPB07) to 6.25±0.48 (BG27) (Table 1). Molecular sizes of these bands ranged between 210 to 3,000 bp and 210 to 2,600 bp in the two cattle breeds, respectively. Average number of polymorphic fragments per primer in Rathi and Tharparkar cattle were 7.07±0.86 and 6.80±0.61 with the molecular size range as 210-3,000 bp and 210-2,600 bp, respectively. The percentage of polymorphic bands in Rathi and Tharparkar cattle were 86 and 87%, respectively (Table 2).

Within breed genetic similarity

Within breed genetic similarity differed with the random primers used in both the cattle breeds. Within breed genetic similarities on the basis of band frequency (BF) ranged from 0.403 (OPG11)-0.762 (OPG16) and 0.339 (OPA02) -0.920 (BG28) in Rathi and Tharparkar cattle breeds, respectively. Whereas, within breed genetic similarities on the basis of BF for pooled primers were 0.577±0.30 in Rathi cattle and 0.531±0.02 in Tharparkar cattle. Similarly, within breed genetic similarities on the basis of band sharing (BS) in Rathi and Tharparkar cattle ranged from 0.353 (OPG10) -0.914 (BG28) and 0.254 (OPA04) -0.779 (OPA 01), respectively. Whereas, within breed genetic similarities on the basis of BS for pooled primers were 0.645±0.04 and 0.534±0.04, respectively in the animals of both breeds. These results indicated that the Rathi cattle were genetically more similar than Tharparkar on the basis of Band sharing (BS) as well as Band frequency (BF). This may be due to fact that the blood samples of Rathi cattle were collected from the animals maintained in a close herd and there was no exchange of germplasm from another herd of Rathi cattle. However, the blood samples of Tharparkar cattle were collected from the animals maintained in an open herd, where breeding bulls were exchanged from other farms of Tharparkar cattle. Because of these reasons the Rathi cattle population was found genetically more similar as compared to the Tharparkar cattle.

In this study, primer BG28 revealed Rathi specific amplicons which were seen in all the animals studied. Similarly primer OPA04 revealed Tharparkar specific product that too were seen in all the animals of this breed. Similar findings of breed/species specific amplicons have been reported by Gwakisa et al. (1994) and Kantanen et al. (1995) although these workers have used different breeds and primers. Bhattacharjya (1999) characterize three cattle breeds namely Hariana, Tharparkar and Red Sindhi by RAPD-PCR. He reported that the highest magnitude of band sharing frequency within breeds was observed in Red Sindhi cattle indicating greater homogeneity within the population. The highest mean average percentage difference was obtained between Hariana and Red Sindhi. These divergence analyses suggested that Hariana cattle are more distant from Red Sindhi than Tharparkar whereas, Red Sindhi cattle are closer to Tharparkar as compared to Hariana with respect to band sharing frequency. There was no report available in the literature on the molecular characterization of Rathi cattle to compare or contrast the results of the present investigation; therefore, these findings may be considered as base line information on molecular characterization of Rathi cattle.

Between breed genetic similarity

Between breed genetic similarity is the indicator of

Table 2. Average number and size of fragments per primer in Rathi and Tharparkar cattle

Breeds	Average number of fragments per primer			Within breed genetic similarity for pooled over primer		Size Range (bp)
	Total number of bands	Number of polymorphic bands	Polymorphic bands (%)	On the basis of BF	On the basis of BS	
Rathi	8.20±0.68	7.07±0.86	86	0.577±0.30	0.645±0.04	210-3,000
Tharpar-kar	7.86±0.40	6.80±0.61	87	0.531±0.02	0.534±0.04	210-2,600

Table 3. Between breed genetic similarity and index of genetic distance for pooled primers between Rathi and Tharparkar cattle breeds

On the basis of	Between breed genetic similarity	Index of genetic distance
Band frequency (BF)	0.972±0.010	0.030±0.011
Band sharing (BS)	0.923±0.026	0.088±0.031

relatedness between two breeds/population/strains with respect to the sequences amplified in PCR. The genetic similarity between Rathi and Tharparkar breeds ranged from 0.869 (OPA02)-0.999 (OPB07, OPG13 and BG15) and 0.668 (OPA02)-0.999 (OPG05) as estimated on the basis of BF and BS, respectively. These results suggested that all the primers used in the present study showed higher genetic similarities between these two cattle breeds. Average between breed genetic similarities for pooled primers were 0.972±0.01 and 0.923±0.026 (Table 3) according to BF and BS, respectively, which also reflect the higher degree of genetic similarity between Rathi and Tharparkar cattle breeds.

Ramesha et al. (2002) observed high degree of resemblance of DNA bands between Ongole and Krishna valley cattle breeds. They explained that Krishna valley breed is the admixture of four distinct breeds viz. Gir, Ongole, Kankrej and Hallikar. Hallikar cattle breed was observed to have less genetic distance from Amritmahal due to overlapping of the breeding tracts between Hallikar and Amritmahal. They also reported that dual purpose breeds Krishna valley and Ongole showed less genetic divergence between them as compared to their genetic divergence from draft breeds viz., Amritmahal, Hallikar and Khillari breeds.

Index of genetic distance (D)

Index of genetic distance between breeds is the measures of inter breed divergence from RAPD fingerprints. Highest estimates of index of genetic distance on the basis of BF (0.139) and BS (0.404) between Rathi and Tharparkar cattle breeds were observed with the primer OPA02. However, the estimates of index of genetic distance with pooled over primers on the basis of BF and BS were 0.030±0.011 and 0.088±0.031, respectively (Table 3). These results revealed that the trend observed from between breed genetic distances confirm the high degree of genetic similarity between Rathi and Tharparkar cattle.

Similarly, higher estimates of between breed genetic similarities for pooled over primers between Rathi and Tharparkar cattle in present study indicated that either Rathi is having decent from Tharparkar or both Rathi and Tharparkar are having common descent. Lower estimates of index of genetic distances between these two cattle breeds may be because of the fact that these breeds are the native of the Thar Desert in North-West India (Mittal et al., 1989).

CONCLUSION

RAPD-PCR was found effective in detecting the polymorphism within as well as between Rathi and Tharparkar cattle breeds. Within breed genetic similarity was higher in close herd of Rathi cattle as compare to Tharparkar cattle. Genetic similarity between these two breeds of cattle was also observed high according to between breed genetic similarity and index of genetic distances, which may be due to common descent of Rathi and Tharparkar cattle or their common native place. As there was no report available on the molecular characterization of Rathi cattle in literature to compare or contrast, the present findings may be used as base line information for genetic polymorphism study and molecular characterization of Rathi cattle.

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