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Genome Identity and Diversity Study in Gir and Kankrej (*Bos indicus*) Cattle Breeds using RAPD Fingerprints

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Abstract: RAPD-PCR techniques were applied to study the genetic identity and diversity in two Indian cattle breeds Kankrej and Gir with a battery of 16 decamer primers. The analysis was carried out by using Band Frequency (BF), Band Sharing Frequency (BSF), Genetic Identity (GI), Genetic Distance (GD) and Mean Average Percentage Deference (MAPD). RAPD pattern revealed polymorphism for 163 loci out of 196 loci (83.16%) in Gir and Kankrej breeds. The present study showed that within breed similarity in both the breeds was observed to be greater as compared to similarity between breeds. Within breed genetic similarity was higher in Gir cattle than Kankrej cattle. Primer KMS6 was able to resolve a product of 388 bp that was seen in 11 out of 12 Gir animals. Similarly primer KMS2 revealed Kankrej specific amplicon of 2274 bp that was seen in 11 out of 12 Kankrej animals. The estimate of band sharing frequency between breeds was highest (0.900) with the primer KMS6 and the lowest (0.557) with the primer KMS11. The band sharing frequency pooled over the primer was 0.738±0.2008 between these two breeds. The highest genetic identity estimate (0.943) between the two breeds was obtained with primer KMS3 and the lowest (0.696) with primer KMS10. Similarly the highest genetic distance estimate (0.20) between the two breeds was obtained with KMS2 and the lowest (0.000) with primer KMS12. The highest (44.2) Mean Average Percentage Difference (MAPD) was observed with primer KMS11 and lowest (9.98) with primer KMS6. The phylogenetic tree based on Nei's formula revealed that the primers used for the study were able to show the genetic similarity and diversity within and between breeds.

Key words: DNA, PCR, RAPD, MAPD, Kankrej, Gir, Genetic identity, genetic distance, polymorphism

INTRODUCTION

India has 30 recognized Zebu cattle breeds that are adapted to the tropical environments (Nivsarkar, 2000). Among them Gir and Kankrej are from Gujarat state. Both the breeds are of great importance in India. Molecular biology virtually gains access to the entire genome of cattle. Whereas the Polymerase Chain Reaction (PCR), which induce a methodological revolution, have been applied for genetic variation studies. Application of randomly amplified polymorphic DNA (RAPD) techniques has greatly increased the ability to understand the genetic relationship within species at molecular level. This technique has achieved a great deal of acceptance due to its simplicity, readability directly on gel, low cost involvement, requirement of little amount of DNA as compared to other methods (Williams *et al*., 1993) and is highly informative without prior knowledge of sequence information (Crowhurst *et al*., 1991; Bostock *et al*., 1993; Kemp and Teale, 1992). RAPD has been used for population analysis in detecting genetic differences in humans and in estimation of inbreeding in cattle (Bhattacharya *et al*., 2003). The present study was undertaken to determine the genetic identity and diversity within and between Gir and Kankrej (*Bos indicus*) cattle breeds using RAPD fingerprints.

MATERIALS AND METHODS

The study was carried out on 12 randomly selected male animals of Gir and Kankrej breeds of cattle each. Blood samples were collected into the heparinized blood collecting tubes from the organized farms and sperm stations across the Gujarat State. Genomic DNA was

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isolated from blood cells by lysis under the condition that degrades protein and preserve the integrity of DNA molecules as described by Shambrook *et al.* (1989). The quality and quantity of DNA was analyzed by agarose gel electrophoresis and spectrophotometer.

RAPD- PCR technique and analysis of DNA fingerprints: A battery of 16 decamer primers synthesized from Bangalore Genei (P) Ltd. India, were employed in the representative DNA samples of Gir and Kankrej breeds of cattle. The sequence and Guanine and Cytosine (GC) contents are mentioned in Table 1. The standard condition required for PCR amplification was determined empirically to get the reproducible bands. The amplification reaction was carried in 0.2 mL microfuse tubes using programmable thermal cycler (BIOMETRA). Each 25 μL reaction mixture comprised of 50 ng genomic DNA, 400 μM each of dNTPs, 40 ng primers, 1 U Taq DNA polymerase, 2.5 μL of 10X Taq DNA polymerase buffer (500 mM KCl, 100 mM Tris KCl, 1.5 mM $MgCl_2$ and 1% Triton X-100) and 1 mM Magnesium acetate. Amplification of DNA was carried out at 94°C for 3 min, followed by 35 cycles 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 using programmable thermal cycler (BIOMETRA). The randomly amplified PCR products were electrophoresed on 2% agarose gel along with DNA molecular size marker (Phi x 174 DNA/ Hae III digested). The image was captured by DNR imaging system. Using Total lab software from DNR Imaging System, all amplified and reproducible bands were scored and the presence and absence of a band was recorded as 1 and 0, respectively. Comparison of all RAPD fingerprints of the breed was done on the samples run on the same gel.

Band frequency (BF): Band frequency of RAPD fingerprints were calculated by using the formula:

$BF = n/N$

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

Band sharing frequency (BSF) Band Sharing frequency were calculated in pair wise comparisons as described by Gwakisa *et al.* (1994)

$$BSF_{(\text{between breeds})} = 2\,B_{GK}/[\,B_G + B_K]$$

Where B_{GK} is the number of bands common to Gir and Kankrej for a primer. B_G is the total number of bands for Gir for a particular primer. Similarly B_K is the total number of bands for Kankrej for a primer. Average of band sharing frequency was calculated by dividing the sum of

Table 1: The sequence and guanosine-cytosine contents of the oligoes

Oligoes	Sequence 5'- - -3'	Length	GC(%)
KMS1	CAGGCCCTTC	10-mer	70
KMS2	TGCCGAGTCG	10-mer	70
KMS3	AATCGGGCTG	10-mer	60
KMS4	GGTCCCTGAC	10-mer	70
KMS5	GGGTAACGCC	10-mer	70
KMS6	TCTGTGCTGG	10-mer	60
KMS7	GGTGACGCAG	10-mer	70
KMS8	CTGAGACGGA	10-mer	60
KMS9	GAACCTGCGG	10-mer	70
KMS10	ACGGCCGTCT	10-mer	70
KMS11	TGCCCGTCGT	10-mer	70
KMS12	CTCTCCGCCA	10-mer	70
KMS13	AGCGTCCTCC	10-mer	70
KMS14	GCCGTCCGAG	10-mer	80
KMS15	CAGCCTGGGA	10-mer	70
KMS16	CGGTGGCGAA	10-mer	70

BSF value of pair wise comparison by the total number of pairs compared. Within breed band sharing frequency was calculated as the average of band sharing frequencies within the breeds pair wise using the formula:

$$BSF_{(\text{within Gir breeds})} = 2\,B_{Gi.\,Gii}/[B_{Gi} + B_{Gii}] \text{ and}$$
$$BSF_{(\text{within Kankrej breeds})} = 2B_{Ki\,Kii}/[B_{Ki} + B_{kii}]$$

Where $B_{Gi.Gii}$ is the band sharing frequency within a pair of Gir and $B_{Ki\,Kii}$ is the band sharing frequency within a pair of Kankrej. B_{Gi}, B_{Gii}, B_{Ki} and B_{Kii} are the total bands observed in the individual animal of Gir and Kankrej breeds, respectively.

Genetic identity (GI): Genetic identity between two breeds was obtained as follows:

$$GI_{(\text{between breeds})} = 1/N\sum_{i=1}^{N} 2BFi_G.\,BFi_K/[\,BFi_G^{\,2} + BFi_K^{\,2}]$$

Where BFi_G and BFi_K are the frequency of occurrence of i^{th} band in two different breeds respectively and N is the total number of bands scored as per Apuya *et al.* (1988)

Genetic distance (D): The genetic distances were obtained by the formula of Lynch (1991).

$$D_{GK} = -\ln BSF_{GK}/\sqrt{(BSF_G \times BSF_K)}$$

Where BSF_{GK} is the Band sharing frequency between Gir and Kankrej breeds of cattle. BSF_G is the Band sharing frequency within Gir breeds. BSF_K is the Band sharing frequency within Kankrej breeds.

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

$$\text{Percentage Difference (PD)} = N_{GK}/N_G+N_K] \times 100$$

$$\text{Average Percentage Difference (APD)} = 1/c \sum_{i=1}^{N} Pdi$$

$$\text{Mean Average Percentage Difference (MAPD)} = 1/R \sum_{i=1}^{N} APDi$$

Where N_{GK} are the number of fragment that differed between two animals, for a single primer. N_G and N_K are the number of bands observed in individual breeds. C is the number of interbreeds pair wise comparison and R is the number of random primers used.

RESULTS AND DISCUSSION

DNA analyzed by agarose gel electrophoresis followed by observation on UV transilluminator revealed sharp high molecular weight bands. The visual estimation revealed good concentration of DNA. The OD 260 nm : OD 280 nm ratio ranged between 1.7-2.0 indicating a good quality and purity of DNA.

Analysis of randomly amplified polymorphic DNA (RAPD) fingerprints: Sixteen primers were screened to amplify DNA samples of Gir and Kankrej. The numbers of RAPD bands observed in the range of 7-17 with molecular size ranging from 241-2941 bp. 163 out of 196 loci (83.16%), were polymorphic. The amplification patterns of representative samples of Gir and Kankrej breeds with two primers have been shown in Fig. 1-2, respectively.

Band frequency: Most of the primers showed polymorphic bands in both the breeds. KMS1, KMS2, KMS7 and KMS8 primers exhibited polymorphic bands of 1537, 2274, 691 and 345 bp with a band frequency of 1.0, 0.92, 0.75 and 0.75, respectively only in Kankrej breed. Similarly KMS2, KMS4, KMS6, KMS7, KMS9, KMS10, KMS11, KMS13 and KMS14 showed polymorphic bands of 431, 704, 388, 753, 2226, 1990, 2096, 928 and 644 bp with a band frequency of 1.0, 0.58, 0.92 0.67, 0.75, 0.75, 0.92, 0.75 and 0.58, respectively only in Gir breed. Similar findings in buffalo were reported by Arvndakshan and Nainar (1998) and Singru (1998). A few publications also demonstrated breed specific RAPD fingerprints (Gwakisa *et al.*, 1994; Shivakumar, 1997; Ahn *et al.*, 1999). Primers KMS1 and KMS2 produced breed specific bands in 92-100% animals of Kankrej breed. Similarly, primers KMS2, KMS6 and KMS11 produced breed specific bands in 92-100 % animals of Gir breed. Such amplicons could be used to develop breed specific Sequence Characterised Amplified Region (SCAR).

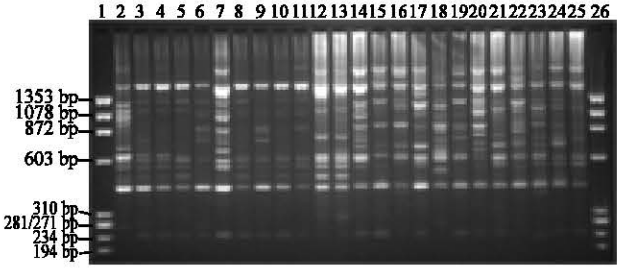

Fig. 1: RAPD fingerprints of genomic DNA using primer KMS-2. Lane # 1 : Phi X 174 DNA/*Hae* 7III digested marker, lane # 2-13: Gir DNA samples, lane# 14-25 : Kankrej DNA samples and lane # 26: Phi X 174 DNA/*Hae* III digested marker

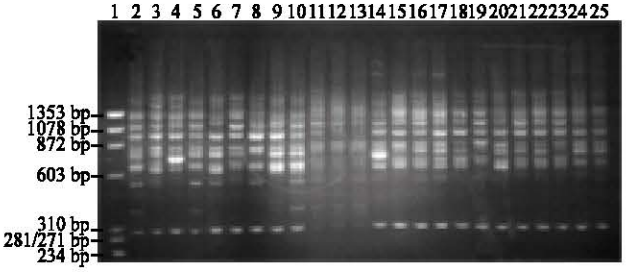

Fig. 2: RAPD fingerprints of genomic DNA using primer KMS-6. Lane # 1 : Phi X 174 DNA/*Hae* III digested marker, lane # 2-13: Gir DNA samples, lane# 14-25: Kankrej DNA samples

Table: 2 Band sharing frequency within Gir and Kankrej breeds

Oligoes	Band sharing frequency within Gir breeds	Band sharing frequency within Kankrej breed
KMS1	0.8685	0.8579
KMS2	0.8401	0.7920
KMS3	0.8338	0.7772
KMS4	0.8277	0.8820
KMS5	0.8622	0.9554
KMS6	0.9146	0.9636
KMS7	0.8492	0.8844
KMS8	0.8059	0.8208
KMS9	0.6841	0.7853
KMS10	0.7821	0.7869
KMS11	0.6803	0.6618
KMS12	0.5911	0.6905
KMS13	0.7183	0.7710
KMS14	0.7725	0.7865
KMS15	0.8351	0.8480
KMS16	0.7333	0.7141
Mean average	0.9217±0.0856	0.8111±0.0854

Band sharing frequency: The average band sharing frequency within and between breeds in Gir and Kankrej are given in Table 2. Most of the primers revealed band sharing within and between breeds. The frequency varied in Gir from 0.91-0.59 with respect to primers KMS6 and KMS12 while in Kankrej it varied from 0.96-0.66 for primers

Table 3: Band sharing frequency and genetic identity between Gir and Kankrei breeds

Oligoes	Band sharing frequency between Gir and Kankrej	Genetic identity between Gir and Kankrej
KMS1	0.7428	0.8085
KMS2	0.6705	0.7250
KMS3	0.7868	0.9434
KMS4	0.8211	0.7579
KMS5	0.8550	0.8509
KMS6	0.9002	0.9058
KMS7	0.8285	0.8085
KMS8	0.7856	0.9140
KMS9	0.7018	0.7160
KMS10	0.6475	0.6930
KMS11	0.5579	0.8483
KMS12	0.6502	0.8500
KMS13	0.6994	0.8418
KMS14	0.7352	0.7257
KMS15	0.8056	0.8804
KMS16	0.6292	0.7646
Mean average	0.7386±0.2008	0.7698±0.1092

Table 4: Percentage difference and genetic distance between Gir and Kankrej breeds

Oligoes	Percentage difference between Gir and Kankrej	Genetic distance between Gir and Kankrej
KMS1	25.7221	0.1503
KMS2	32.9487	0.1960
KMS3	21.3241	0.0229
KMS4	17.8923	0.03978
KMS5	14.4984	0.0597
KMS6	9.9809	0.0420
KMS7	17.1481	0.0449
KMS8	21.4413	0.0347
KMS9	29.8221	0.04344
KMS10	35.2467	0.1919
KMS11	44.2127	0.1847
KMS12	34.9820	-0.0175~0.00
KMS13	30.0596	0.0621
KMS14	26.4823	0.0585
KMS15	19.4419	0.0436
KMS16	37.0781	0.1398
Mean average	26.1426±9.3759	0.0810±0.0675

KMS6 and KMS11, respectively. Similarly band-sharing frequency between Gir and Kankrej varied from 0.90-0.55 for primers KMS6 and KMS11 (Table 3). The pooled average band sharing frequency within Gir breed was 0.92±0.08 and Kankrej breed was 0.81±0.08. The obtained data of band sharing frequency on the basis of the primers revealed that Gir breeds are genetically more similar than Kankrej breeds. Sharma *et al.* (2004) reported similar observation in Rathi and Tharparker cattle. Within breed genetic similarity was higher than between breeds (0.74±0.20). Primers KMS5 and KMS6 generated relatively higher value of band sharing frequencies within and between breeds (Table 2 and 3) showing that these primers could be less informative in differentiating the breeds. Similar finding have also been reported by Kantanen *et al.* (1995).

Genetic identity: Genetic identity varied with the random primers used in both the cattle breeds as presented in Table 3. All primers used showed similarity in (medium to high) genetic identity between the two breeds. Highest genetic identity (0.9434) was observed in primer KMS3 and the lowest genetic identity (0.6930) with primer KMS10. The genetic identity (pooled of all the 16 primers) was 0.77±0.11 between Gir and Kankrej cattle. Shende and Yadav (2004) reported similar observations in Nagpuri and Murrah buffaloes. The higher estimates of genetic identity between Gir and Kankrej cattle in the present study indicated that both are having common descent as reported by Sharma *et al.* (2004) on his work with Rathi and Tharparker breeds. Ramesha *et al.* (2002) observed high degree of resemblance of DNA bands between Ongole and Krishnavelly of cattle breed.

Mean average percentage difference: The MAPD value was calculated from all the averages of two breeds. The average Percentage Difference (APD) estimated for all the primers is presented in Table 4. The highest APD value between these two breeds obtained was 44.21 with primer KMS11 and lowest value of 9.98 with primer KMS6. The MAPD between these two breeds was estimated to be 26.14±9.37% indicating these breeds differed at 26.14% loci amplified by a battery of 16 primers.

Genetic distance: The genetic distance calculated for various primers are given in Table 4. All the primers used showed genetic distance. Primer KMS2 showed highest genetic distance (0.20) between these two breeds while the primer KMS12 indicated lowest genetic distance (0.00). The over all average genetic distance was 0.08±0.06 between Gir and Kankrej breeds. No significant genetic distance was found between the breeds with respect to the primers used. The phylogenetic tree based on Nei's (Nie, 1978) genetic distance (Fig. 3) revealed that the primers used for the study were quite enough to show the genetic similarity and diversity. The animals were separated into two clads. First cluster containing nine Gir animals and second cluster containing all twelve Kankrej animals. Remaining three animals of Gir were out grouped from the cluster that indicates genetic distance within breed. However, with the inclusion of larger sample of animals and more number of primers RAPD would correctly group these samples to the respective breeds.

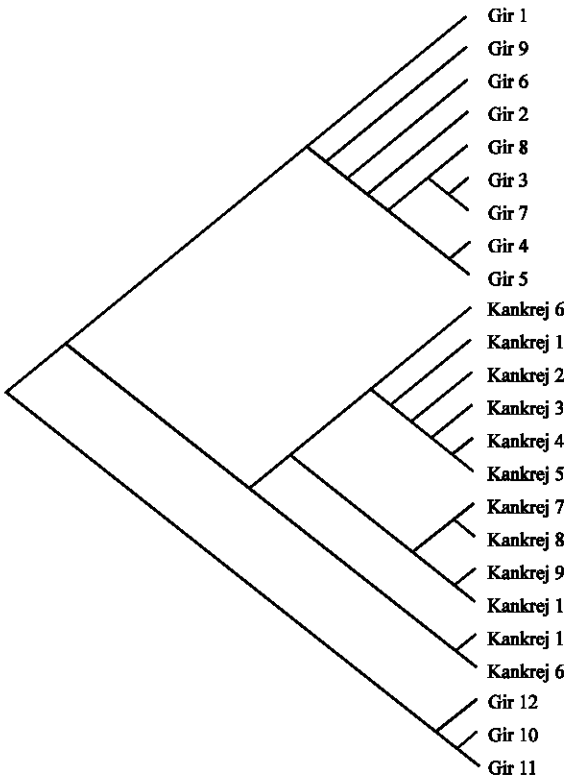

Fig. 3: Phylogenetic tree showing genetic similarity and diversity within and between two cattle breeds (Nei's, 1978)

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