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Research Article

GENETIC DIVERGENCE STUDY BETWEEN UMBLACHERY AND KANGAYAM BREED OF CATTLE USING RANDOM AMPLIFIED POLYMORPHIC DNA

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ABSTRACT
Fifty randomly selected Umblachery and Kangayam cattle were used. Out of nine random primers tested five random primers ILO 1127, ILO 526, ILO 868, ILO 876 and BG 85 yielded amplification with genomic DNA samples. In Umblachery, primers ILO 1127, ILO 526, ILO 876 have the ability to amplify more bands such as 9, 8 and 10 where as ILO 868 and BG 85 gave only 4 bands. All the primers except BG 85 produced polymorphic bands. In the same way, in Kangayam breed, all primer except BG 85 produced more bands (6 to 12) and the numbers of polymorphic bands are two in ILO 1127, three in ILO 526 and one in all other three primers. All the five primers revealed band sharing within and between breeds. The frequency varied in Umblachery from 0.06 to 0.118 with respect to primers ILO 526 and ILO 876 whereas in Kangayam it varied from 0.07 to 0.2665 with respect to primers ILO 526 and ILO 876 respectively. The highest APD value between these two breeds obtained was 88.00 with ILO 868 and the lowest value of 50 with ILO 876.The MAPD between these two breeds was estimated to be 74.93 indicating these two breeds differed at 74.9% of loci amplified by a group of five random primers.

Key Words: *Umblachery, Kangayam, Cattle Breed, RAPD, BSF, MAPD*

INTRODUCTION
Indian breeds of cattle are specifically adapted to different agro-climatic conditions and are known for resistance against many diseases. However, these traits of indigenous breeds could not be exploited in the crossbreeding programme. Further unplanned breeding practices have resulted in large population of crossbreds whose level of inheritance is unknown. Genetic traits like disease resistance and heat tolerance have been substantially reduced. At present, the recognised cattle breeds are facing the threats of genetic dilution, which may further results in genetic extinction. Genetic characterization of this germ plasm and their subsequent conservation is of vital importance. The primary aim of characterization strategy is to estimate differentiation of population within species, information on genetic polymorphism, and genetic diversity in each species and to evaluate the change in variation in species over a period (Kantanen *et al.*, 1995). Breed characterization requires information on genetic variation that can be effectively measured within as well as between populations.

The DNA markers are very effective in estimating within as well as between population genetic variability in view of detecting high polymorphism as compared to other genetic markers such as biochemical and /or cytogenetics markers. Among DNA markers, Random Amplified Polymorphic DNA (RAPD) markers are receiving more attention because of simple, time and cost effective assay and ability to detect high polymorphism. The basic strategy involves the PCR amplification of random fragments of genomic DNA with single or multiple primers of arbitrary sequences.

Umblachery and Kangayam cattle are draught purpose breed. Umblachery is a well known draught breed of Thanjavur, Thiruvarur and Nagapattinam districts in eastern parts of Tamilnadu state in South India. This breed is a outcome of selection for short stature, suitable for work in marshy rice fields of Cauvery delta region (Thangaraju *et al.*, 2001). Umblachery is a medium sized cattle and has compact body and short legs; calves are red or brown in colour at birth. Red colour begins to change to grey at 3 to 4 months

of age. Total grey colour attained even at the age of one year. Bulls are dark grey with black patches on head, back and pelvis. Bullocks are in grey in colour with dark extremities; white star at forehead, white tail twitch and white socks marks in the legs are specific characteristics of this breed (ICAR-NBAGR Network Project Survey Unit, 1999). The Kangayam breed of cattle of Tamil Nadu is best known for its superior draught qualities, adaptation to poor nutrition and longevity (Kandasamy, 2001). Kangayam bullocks are mainly used for transport of agricultural produce and for agricultural operations. The bullocks have been identified as high power animals with maximum power availability of 0.8 hp per pair of bullocks (Surendrakumar, 1988). We have made an attempt to study the genetic divergence between these two breeds using RAPD-PCR markers.

MATERIALS AND METHODS

Primers

Nine random primers were obtained from Bangalore Genie Pvt. Ltd. with the G-C content in the range of 60-80 per cent.

Genomic DNA

High molecular weight genomic DNA was isolated from peripheral blood mononuclear cells collected from 50 randomly selected Umblachery and Kangayam cattle in their respective breeding tract. The purity and concentration of DNA samples were estimated by spectrophotometer. Then the samples were dissolved in TE buffer (pH 8.0) to make uniform concentration of 50 µg/ml.

RAPD-PCR Amplification

The amplification reactions were carried out in 0.2 ml microfuge tubes using a programmable thermal cycler (MJ Research). Each 20 µl reaction mix comprised of 50 ng of template DNA, one µl of primer (40p.mol/ml), 150 mM of each dNTPs, one unit of Taq DNA Polymerase (Gibco BRL) and 10XPCR buffer (Gibco BRL).The PCR buffer consisted of 10mM Tris pH 8.3, 50mM KCl, 0.0001 per cent gelatine, 0.025 per cent Tween 20, 0.025 per cent Nonidet P 40 and 1.5mM $MgCl_2$. The contents were mixed thoroughly and short spinned

The PCR amplifications were performed using following temperature cycles: an initial 30 seconds at 96˚C for 10 seconds, 35˚C for 10 seconds and 72˚C for 1 minute. After completion of PCR, 10 µl of PCR product was subjected to electrophoresis at 100 volts in 2 % agarose gel in 1XTAE buffer containing 0.5 mg /ml of ethidium bromide along with DNA molecular weight marker (1X174 *Hae* III digested fragment). RAPD finger prints were visualised by UV illumination and documented by photography. The molecular weight of each band was scored by software aided gel documentation system comprised of Ultralum-image scanner, scion image capturing system and Sigma gel package.

Analysis of Data

Scoring of bands and statistical analysis were carried out according to Gwakisa *et al.* (1994). Only distinct and prominent bands were scored. Comparison of RAPD finger prints were made only on samples run on the same gel. The statistical analysis was carried out as follows.

i) Band Sharing (BS)

Band sharing was calculated as an expression of similarity of RAPD finger prints among individuals(Dunnington *et al.*,1990 and Gwakisa *et al.*, 1994) using the formula

$BS = 2(B_{ab})/ (B_a+B_b)$ Where,

B_{ab} is the number of bands shared by individual 'a' and 'b'

B_a is the total number of bands for individual 'a'

$B_{b\ is}$ the total number of bands for individual 'b'

ii) Intrabreed polymorphism = (1-BS) X *100*

RESULTS AND DISCUSSION

Out of 9 random primers were used five primers gave amplified products. (Table-1)

Table 1: Total bands amplified, Number of polymorphic bands and size range of RAPD bands for different primers in Umblachery and Kangayam cattle

Umblachery:

Name of Primer	Total bands amplified	No. of polymorphic bands	Size range of RAPD bands
ILO 1127	9	3	0.3 to 1.3 Kb
ILO 526	8	3	0.3 to 1.35 Kb
ILO 868	4	2	0.6 to 1.35 Kb
ILO 876	10	3	0.28 to 1.35 Kb
BG 85	4	0	0.7 to 1.4 Kb
Overall	35	11	0.28 to 1.4 Kb

Kangayam:

Name of Primer	Total bands amplified	No. of polymorphic bands	Size range of RAPD bands
ILO 1127	12	2	0.3 to 1.35 Kb
ILO 526	7	3	0.5 to 1.078 Kb
ILO 868	6	1	0.6 to 1.6 Kb
ILO 876	6	1	0.5 to 1.078 Kb
BG 85	3	1	0.6 to 1.3 Kb
Overall	34	8	0.3 to 1.6 Kb

All the five primers gave reproducible RAPD bands in Umblachery and Kangayam cattle.Even though the non-reproducibility of RAPD patterns is a major hurdle in RAPD finger printing, the problem overcome by using uniform method of DNA isolation i.e high salt method of DNA isolation throughout the study because differences between DNA preparations affect primer annealing and that is the major cause of non-reproducibility of RAPD patterns.

In Umblachery, primers ILO 1127, ILO 526, ILO 876 have the ability to amplify more bands such as 9, 8 and 10 where as ILO 868 and BG 85 gave only 4 bands. All the primers except BG 85 produced polymorphic bands.

In the same way, in Kangayam breed, all primer except BG 85 produced more bands (6 to 12) and the numbers of polymorphic bands are two in ILO 1127, three in ILO 526 and one in all other three primers.

Table 2: Band sharing frequency within Umblachery and Kangayam

Random Primers	Band sharing frequency within Umblachery breed	Band sharing frequency within Kangayam breed
ILO 1127	0.0877 ± 0.03	0.1382 ± 0.04
ILO 526	0.0617 ± 0.02	0.0738 ± 0.03
ILO 868	0.0660 ± 0.01	0.2665 ± 0.04
ILO 876	0.1180 ± 0.03	0.2638 ± 0.06
BG 85	0.1040 ± 0.04	0.1392 ± 0.06
Mean	0.0875	0.1485

In support of this result, Ramesha *et al.* (2002) reported polymorphic bands in Ongole cattle using ILO 1127 (8 to 10 bands), ILO 526 (8 to 12 bands), ILO 868 (4 to 8 bands) and ILO 876 (10 to 18 bands)

Band sharing frequency:

The average band sharing frequency within and between breeds in Umblachery and Kangayam are given in Table-2

All the five primers revealed band sharing within and between breeds. The frequency varied in Umblachery from 0.06 to 0.118 with respect to primers ILO 526 and ILO 876 whereas in Kangayam it varied from 0.07 to 0.2665 with respect to primers ILO 526 and ILO 876 respectively. This suggests that there were more variations among individual samples in Umblachery and less in Kangayam. Similar findings were reported by Yadav and Yadav (2008) in Black Bengal and Marwari goats.

The lower Band Sharing Frequency within Umblachery might be due to random mating among the population and the blood samples were collected from wider area in the field. These factors contributed more genetic variability or less similarity within the breed.

Similarly, band sharing frequency between Umblachery and Kangayam varied from 0.12 to 0.5 for primers ILO 868 and ILO 876 (Table-3).

Table 3: Band sharing frequency and Average Percentage Difference between Umblachery and Kangayam

Random Primers	Band sharing frequency between Umblachery and Kangayam breed	Average Percentage Difference between Umblachery and Kangayam breed
ILO 1127	0.2540	74.60
ILO 526	0.2258	77.42
ILO 868	0.1200	88.00
ILO 876	0.5000	50.00
BG 85	0.1538	84.62
Mean	0.2507	74.93

The pooled average band sharing frequency within Umblachery breed was 0.15. The Band sharing frequency data of the above random primers revealed that Umblachery breed is more dissimilar than Kangayam breed. The less Band Sharing Frequency value between Umblachery and Kangayam might be due to different geographical locations they belong to. All the primers generated relatively lesser value of band sharing frequencies between breeds (Table 3) showing that these primers could be more informative in differentiating the breeds. Similar reports of low band sharing values among south Indian cattle breeds were reported by Ramesha *et al.*, (2002) and Nagaraju (1998).

Mean Average Percentage Difference:

The MAPD value was calculated from all the averages of two breeds. The APD estimated for all the primers is presented in Table 3. The highest APD value between these two breeds obtained was 88.00 with ILO 868 and the lowest value of 50 with ILO 876.The MAPD between these two breeds was estimated to be 74.93 indicating these two breeds differed at 74.9% of loci amplified by a group of five random primers.

MAPD provides a measure of genetic divergence in terms of genomic RAPD finger prints between breeds. The high genetic divergence between Umblachery and Kangayam coincides with their different geographical locations. Even though both breeds native of Tamil Nadu, Umblachery breed is being reared in Cauvery delta districts such as Thanjavur, Thiruvarur and Nagapattinam districts where as Kangayam breed is reared in Coimbatore, Thirupur, Erode and Dindigul districts. Reports are available on MAPD, which show genetic divergence between different breeds (Gwakisa, 1994; Jha, 2004; Clamp *et al.*, 1993 and Aravindakshan and Nainar, 1998).

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