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Research Article

MOLECULAR CHARACTERIZATION OF KANGAYAM CATTLE BY IDENTIFYING DNA MARKERS USING RANDOM AMPLIFIED POLYMORPHIC DNA

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ABSTRACT
Twenty DNA samples of Kangayam cattle were taken into consideration for RAPD analysis. Out of nine random primers tested, only five random primers gave amplified product with the genomic DNA samples. The five random primers were ILO 1127, ILO 526, ILO 868, ILO 876 and BG 85. Kangayam DNA gave breed specific fragments at 1kb with ILO 1127, at 1.1kb with ILO 526,at 1.6 kb with ILO 868, at 0.88 kb with ILO 876 and at 1.3kb with BG 85. This Band Sharing values of individuals ranged from 0 to 0.75 (ILO 1127), 0 to 0.4 (ILO 526), 0 to 0.6667 (ILO 868), 0 to 0.8 (ILO 876) and 0 to 1.0 (BG 85). The mean BS values were 0.1382 ± 0.04 (ILO 1127), 0.0738 ± 0.03 (ILO 526), 0.2665 ± 0.04 (ILO 868), 0.2638 ± 0.06 (ILO 876) and 0.1392 ± 0.06 (BG 85). The intra-breed polymorphism among the individuals of Kangayam cattle with respect to five primers were found to be 86.18 per cent (ILO 1127), 92.62 per cent (ILO 526), 73.35 per cent (ILO 868), 73.62 per cent (ILO 876) and 86.08 per cent(BG 85). This study represents the application of RAPD-PCR technology for authentication and differentiation of Kangayam cattle by deducing breed specific fragments.

***Key Words*:** *Kangayam Cattle, Random Amplified Polymorphic DNA, Breed Specific Fragments, Polymorphism, Band Sharing, Intra Breed Polymorphism*

INTRODUCTION
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). As per the survey carried out in the breeding tract of Kangayam in the year 1996, the total estimated population was around 4,79,000. Recently, a decline in Kangayam cattle population has been observed as a result of reduction in the need for animal draught power for agricultural operations. The present population size does not warrant any immediate measures for conservation. However, consolidated efforts should be made for genetic improvement of draught capacity in bullocks and milk yield in cows. Indian breeds of cattle are specifically adapted to different agroclimatic conditions and are known for resistance against many diseases. However, these traits of indigenous breeds could not be exploited in the crossbreeding programme due to inadequate information on polymorphic markers.

Eventhough studies on biochemical polymorphism and blood grouping yielded some results, the levels of polymorphism in these loci are not sufficient to mark the trait to be useful in breeding programmes. The establishment of such markers will pave the way for Marker Assisted Selection (MAS) which is not yet practised in indigenous breed development programmes.

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.The primary aim of characterization programme is to study differentiation of population within species. Information on polymorphic loci can be employed to detect population of specific alleles, to measure the extent of genetic diversity in each species and to evaluate the change in variation in species over a period

(Kantanen *et.al.*1995). Breed characterization requires knowledge of genetic variation that can be effectively measured within and between populations

RAPD method is advantageous because of its extreme simplicity and requirement of minimal amount of genomic DNA . The basic strategy involves the PCR amplification of random fragments of genomic DNA with single or multiple primers of arbitrary sequence. Our concern in this study is with characterization of Kangayam cattle. In an attempt to identify DNA markers, we have applied RAPD-PCR analysis (Williams *et.al.* (1990) to individual Kangayam DNA samples as a means to achieve rapid screening of nine primers for their capacity to reveal population specific polymorphisms.

MATERIALS AND METHODS

Primers

Nine random primers were obtained from Bangalore Genei Pvt.Ltd. with the G-C content in the range of 60-80 per cent. Nucleotide sequences of RAPD primers used are depicted in Table-1

Genomic DNA

High molecular weight template was prepared from peripheral blood mononuclear cells collected from 22 Kangayam cattle maintained at District Livestock Farm,Hosur, Tamil Nadu. 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 centrifuged for 10 sec. At 5000 rpm.

The PCR amplifications were performed with the 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 ml of PCR product was subjected to electrophoresis at 100 volts in two per cent 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.; 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

Nine random primers were used. Only the following primers gave amplified products viz. ILO 1127, ILO 526, ILO 868, ILO 876 and BG 85.

Breed specific fragments
Kangayam DNA gave breed specific fragments at 1kb with ILO 1127, at 1.1kb with ILO 526,at 1.6 kb with ILO 868, at 0.88 kb with ILO 876 and at 1.3kb with BG 85.

Table 1 : Nucleotide sequence of RAPD primers

Sl.No	Code	Nucleotide sequence
1	ILO 1127	5' CCGGTGTGGG 3'
2	ILO 526	5' GCCGTCCGAG 3'
3	ILO 868	5' CAGCCTCGGC 3'
4	ILO 876	5' GGGACGTCTC 3'
5	BG 85	5' TACGCAGACT 3'
6	BG 86	5' TGGACTAGAG 3'
7	BG 87	5' GCATGCGATC 3'
8	BG 88	5' ACGTCGAGCA 3'
9	BG 89	5' ACGCCGTACG 3'

The results of band sharing analysis among individuals of Kangayam cattle with each primer are given in Table -2. This Band Sharing values of individuals ranged from 0 to 0.75 (ILO 1127), 0 to 0.4 (ILO 526), 0 to 0.6667 (ILO 868), 0 to 0.8 (ILO 876) and 0 to 1.0 (BG 85). The mean BS values were 0.1382 ± 0.04 (ILO 1127), 0.0738 ± 0.03 (ILO 526), 0.2665 ± 0.04 (ILO 868), 0.2638 ± 0.06 (ILO 876) and 0.1392 ± 0.06 (BG 85). The intra-breed polymorphism among the individuals of Kangayam cattle with respect to five primers were found to be 86.18 per cent (ILO 1127), 92.62 per cent (ILO 526), 73.35 per cent (ILO 868), 73.62 per cent (ILO 876) and 86.08 per cent(BG 85).

DISCUSSION

Out of nine random primers used in this study, only five random primers gave amplification. All the five primers gave reproducible finger prints in Kangayam cattle. As the non-reproducibility of RAPD is a major demerit, the problem was overcome by using uniform method of DNA isolation that is 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(Micheli et al.,1994).

Intra breed polymorphism percentage for Kangayam with ILO 1127 was 86.18. In contrast to this result, Aravindakshan (1997) reported intrabreed polymorphism for Ongole as 55.6 per cent. The higher value obtained in this study could be due to the scoring of bands by software aided gel documentation system. Kangayam DNA revealed intra-breed polymorphism with ILO 526 as 92.62 per cent which could be compared with the report by Aravindakshan(1997) who observed intra-breed polymorphism as 62.5 per cent in Ongole DNA.

Ramesha et al. ,(2002) observed low intra breed polymorphism (58 per cent) in Malnad Gidda and Krishna Valley while using the primers OPAA 17 and OPAA 13 and they also observed high intra- breed polymorphisms(98 per cent) in Amrithmahal cattle by using the primers ILO 868 and ILO 1065.

The intra-breed polymorphism values deduced in this study using rest of primers ILO 868, ILO 876 and BG 85 could not be compared because of non-availability of reports on Knagayam cattle using three primers. Out of five primers, ILO 1127,ILO 876 and BG 85 gave band sharing value as 0.75,0.8 and 1. These high frequencies suggested that it is a potential marker for the sampled sub-population of Kangayam breed. Although the other two primers did not give higher frequencies in band sharing, the frequencies could be raised to higher level by increasing the sample size from this population.

Since each RAPD finger print may be representing or linked to a separate allele, any shared finger print may be contemplated as a product of the same allele (Jeffreys and Morton 1987; Dunnigton et.al., 1990)

Table 2: Band sharing values of Kangayam breed for different primers

Lane comparison	ILO 1127	ILO 526	ILO 868	ILO 876	BG 85
1-2	0.1538	0.1667	0.4286	0	0
1-3	0.1818	0	0	0	1.0000
1-4	0	0	0.1428	0	0
1-5	0.2222	0.2222	0.1250	0.2857	0
1-6	0.2222	0	0.1667	0	0
1-7	0	0	0.2000	0	0
2-3	0.1053	0.3076	0.3333	0.4211	0
2-4	0	0.4000	0.6667	0.5333	0
2-5	0	0	0	0	0
2-6	0	0	0	0.8000	0
2-7	0	0	0.1667	0.4000	0
3-4	0.2353	0.1428	0.1667	0.4444	0
3-5	0.2222	0	0.2857	0.3158	0
3-6	0	0	0.4000	0.4444	0
3-7	0	0	0.5000	0.3333	0
4-5	0.4348	0.1667	0.4444	0	0.4000
4-6	0	0	0.2857	0.2857	0.5714
4-7	0	0	0.1667	0.7143	0.2857
5-6	0.1250	0	0.3750	0.1333	0
5-7	0.2500	0	0.1428	0	0
6-7	0.7500	0.1428	0.6000	0.4286	0.6667
Mean BS	0.1382±0.04	0.0738±0.03	0.2665±0.04	0.2638±0.06	0.1392±0.06
Intra-breed Polymorphism in per cent	86.18	92.62	73.35	73.62	86.08

The major advantages of RAPD analysis over microsatellites and RFLP is the availability of informative markers without prior sequence information, even though RAPD markers were applied mapping studies(e.g. Welsh et al., 1991; Reiter et al., 1992;Levin et.al., 1993)

This study represents the application of RAPD-PCR technology for authentication and differentiation of Kangayam cattle by deducing breed specific fragments at 1kb with ILO 1127, at 1.1kb with ILO 526,at 1.6 kb with ILO 868, at 0.88 kb with ILO 876 and at 1.3kb with BG 85.

In support of this findings, Ramesha et al.,(2002) reported that ILO 526 produced breed specific fragment of 0.77 kb with Ongole DNA collected from its home tract. He also reported that ILO 868 produced 0.37 kb breed specific fragment with DNA samples of Khillari cattle and ILO 1127 generated breed specific band of 0.65 kb in Malnad Gidda. A 0.46 kb breed specific fragment was amplified by primer ILO 868 in Deoni cattle and a 0.87 kb breed specific fragment was amplified by primer ILO 876 in Deoni cattle (Appannavar et al. ,2003).

This approach , in comparison with other methods used for breed characterization has several advantages. RAPD markers allow multilocus finger printing in contrast to protein polymorphism. However, comparison of RAPD -PCR to other DNA based methods as mitochondrial sequencing and microsatellites, will require a reliable designation of RAPD allele patterns in order to allow exchange of such markers between laboratories (Gwkisa et al., 1994).

Finally, we could conclude that this approach will pave the way for effective characterization of Kangayam cattle and will make a valuable contribution to phylogenetic study of Kangayam cattle, thereby deducing the evolutionary relationship with a broad range of cattle breeds.

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