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RESEARCH ARTICLE

DNA BARCOADING OF LOCAL CATTLE SPECIES FOUND IN KERALA USING RAPD MARKERS AND COMPARISON OF ITS GENETIC PROFILE WITH OTHER HYBRID VARIETIES

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ABSTRACT

Indigenous cattle like Vechur, Vatakara animals and Kasaragod cattle have many adaptations to survive in our climatic conditions. For increased production of milk, we used to cross bred our native cattle with imported cattle like H.F, Jersy, Brownswiss, etc**.** Past breeding strategies for dairy cattle have been very effective in producing rapid genetic gain to achieve industry targets and raise profitability. Such gains have been largely facilitated by intense selection of sires combined with the use of artificial insemination. However, this practice can potentially limit the level of genetic diversity through inbreeding and selection plateaus. Cattle genetic diversity is currently under threat mainly due to extensive planned as well as indiscriminate cross breeding among local populations. . Genetic diversity is the basis for present day diversified living systems and future genetic improvement needs. This diversity should be properly utilized, improved and conserved. The calculation of pair wise genetic similarities by indices between individuals by phylogenic analysis after *RAPD-PCR* technique may help to select animals possessing the least genetic similarity and might be a valuable tool for selection in the future germplasm collections.

INTRODUCTION

Cattle of Kerala are mainly classified as cross-bred and indigenous. Cross-breeds constitute about 82% of the total cattle population available in Kerala and indigenous cattle constitute the remaining 18%. The indigenous breeds of the tropics in general are well known for their heat tolerance, resistance to various parasites and diseases, adaptability to atmospheric changes and high returns even under poor feeding conditions. During the time of white revolution many cattle breeds were imported from various countries and among them Jersy, Brown Swiss and Holstein Friesian are the major ones. HF is famous for its milk quantity and Jersy is famous for the quality of milk. We have cross bred these cattle with our indigenous cattle for improving our milk storage or for the satisfactions of our high yield of milk. Kerala witnessed a rapid increase in milk production which has been attributed to increased productivity of milk animals brought about through genetic improvements. The evolution of organized development of animal husbandry in the State was achieved by facilitating the spread of cross breeding. In the process over the generations, the increasing population of cross-bred animals had more and more of genetic commonness and less and less of genetic variety. Due to the disordered and uncontrolled cross breeding, the cross bred cattle lost most of the good qualities (milk quantity and quality of the imported cattle) along with the good specific features of our local cattle species.

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So an idea about the genetic relationships between indigenous and cross-bred cattle in Kerala is essential for the proper future breeding programs

MATERIALS AND METHODS

Collection of Sample

Blood was collected from varieties of cattle from various farms of Kerala.

Genomic DNA Isolation

Using Lysisbuffer, Chloroform/Isoamylalcohol 24:1,SE-Buffer,Sodium acetate, Proteinase K(10 mg/ml) and SDS genomic DNA isolation has been done.

Agarose Gel Electrophoresis

10µl of DNA sample and marker DNA was mixed with 8 microlitre of 1X loading dye and loaded in to the wells of 1% Agarose gel and run at 70-75 volt for one hour.DNA in the Agarose gel was viewed by placing the Agarose gel in UV trans illuminator with UV of wavelength 312nm.

Random Amplified Polymorphic DNA (RAPD)

In the present case study two RAPD reactions were done using two different primers. Two reactions were carried out in a final volume of 25 micro litre mixture consisting of 2 µl of template DNA,3U of sigma Taq DNA polymerase,10mM of dNTP mix, 25mM $MgCl_2$ in 10x PCR buffer and 1 micro litre of

primer1(5^1GTTTCGCTCC3^1) for first reaction and 1 µl of primer2(5^1AAGAGCCCGT3^1)for second reaction. PCR reactions were conducted simultaneously on all isolates in *PCR* tubes in an Eppendorf Master cycler gradient thermo cycler with following protocol: initial denaturation for 4 min at 94°C, followed by 42 cycles of 30 second at 94^0C,1 min at 34^0C,1min at 72^0C with a final extension step of 5 min at 72^0C.Estimate run time was given as 3 hours 22 minutes 55 seconds. The temperature rises up to 100 degrees & then reduced to 94 degrees before it started run.

Electrophoretic Separation of PCR Product

With 2% Agarosegel,Electrophoretic separation carried out at 70-75 volt for 1 hour. The DNA present in the samples was viewed by placing Agarose gel in TU1002 UV trans illuminator at 312nm.

Phylogenetic Analysis

From the results of RAPD, adendrogram was constructed using software's 'FREE TREE' and 'TREE VIEW' by Nei&Li/Dice method and UPGMA method. The phylogenetic relationships between the strains were compared.PCR products were scored across the lanes as variables. The presence of a band of amplified DNA was scored as 1 and absence as 0.The genetic dissimilarity matrix among genotypes was estimated according to Nei and Leis'(1979).The similarity coefficient was used to construct a dendrogram by the un-weighted pair group method with arithmetic averages (UPGMA) (Rohlf,1993).

RESULTS AND DISCUSSION

Genomic DNA Isolation: DNA was extracted from the collected samples and the results obtained are discussed hereunder.

Agarose Gel Electrophoresis: DNA in Agarose gel was viewed by placing the Agarose gel in a UV trans illuminator. In UV-transilluminator, the DNA was observed as bright bands. The result is shown in Fig. 1.

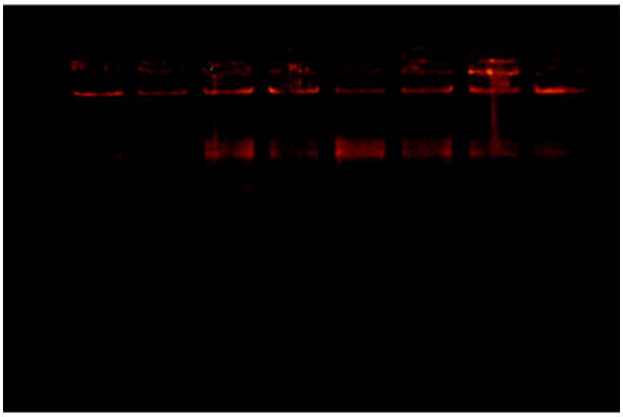


Fig. 1 Agarose Gel Electrophoresis of DNA

Rapd-Pcr

Primer-1(5^1GTTTCGCTCC3^1) which was used in the first RAPD reaction is more suitable for our study. It gave more information about the diversity (up to 8 bands were observed in RAPD with primer-1) compared to primer2 (5^1AAGAGCCCGT3^1) (Maximum six bands were observed in RAPD with primer-2). The results are shown in Fig. 2 and 3.

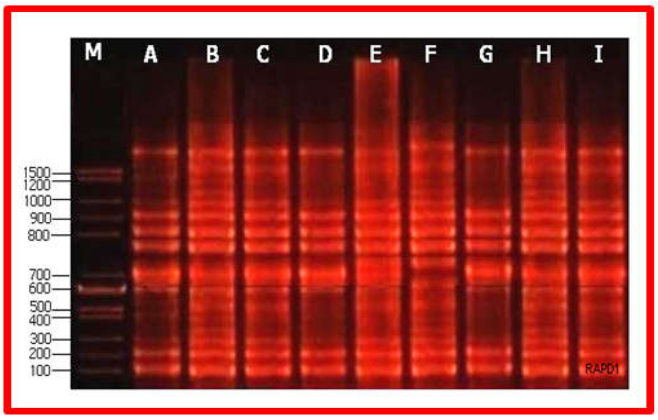


Fig. 2 RAPD with Primer-1

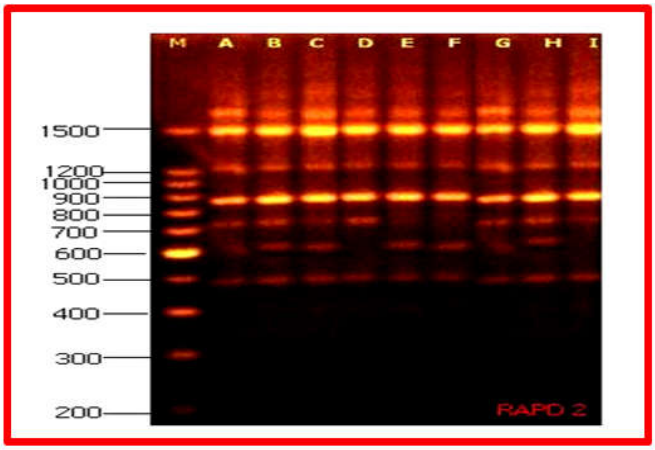


Fig. 3 RAPD with Primer-2

Phylogenetic Analysis

Similarity Index Calculation By Binomial Method

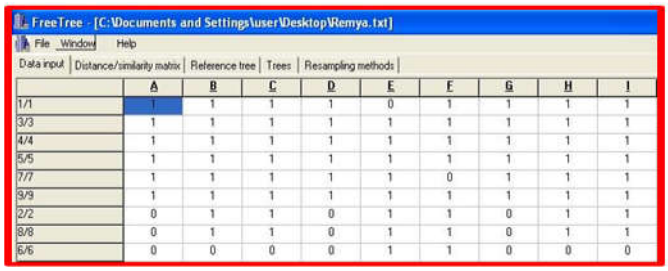
The nine samples of cattle were represented as sample (A-I).The bands obtained by different samples from *RAPD1&RAPD2* result are denoted as (1) and the absence of bands as (0).(Figure4&7)

Distance /Similarity Matrix

Figure 5&Figure 8 showed extend of similarity between the samples with respect to *RAPD1&RAPD2*.The zero value denoted that there is no similarity between the samples and the blank spaces denoted absolute similarity between the samples.

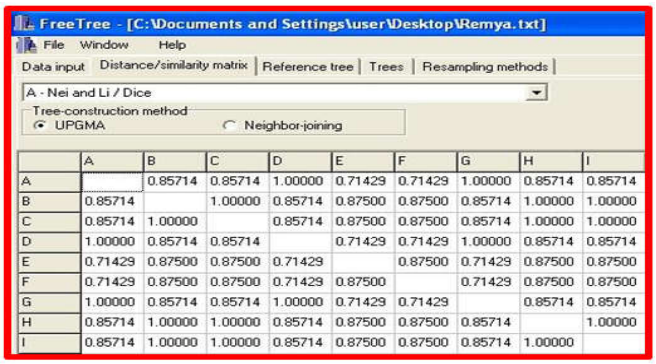
Dendrogram

The dendrogram segregated the nine samples in to two main clusters. First (kasaragode cattle), fourth(303) and seventh(323) samples in one cluster and the remaining are in other cluster.(Figure6&Figure9).The later cluster is further divided in to many sub clusters.Second (Vechur)and third(Vatakara animals) came under same group and eighth sample (291) has some diversity when comparing with second (vechur) and third(vatakara animals)samples and according to the increasing order of diversity,we can align our remaining samples as sample nine(314),sample five(313) and sample five(313) and sample six(298)respectively(figure6)



	A	B	C	D	E	F	G	H	I
1/1	1	1	1	1	0	1	1	1	1
3/3	1	1	1	1	1	1	1	1	1
4/4	1	1	1	1	1	1	1	1	1
5/5	1	1	1	1	1	1	1	1	1
7/7	1	1	1	1	1	0	1	1	1
9/9	1	1	1	1	1	1	1	1	1
2/2	0	1	1	0	1	1	0	1	1
8/8	0	1	1	0	1	1	0	1	1
6/6	0	0	0	0	1	1	0	0	0

Fig. 4 Similarity Index Calculation by Binomial Method *(RAPD* with Primer-1)



	A	B	C	D	E	F	G	H	I
A		0.85714	0.85714	1.00000	0.71429	0.71429	1.00000	0.85714	0.85714
B	0.85714		1.00000	0.85714	0.87500	0.87500	0.85714	1.00000	1.00000
C	0.85714	1.00000		0.85714	0.87500	0.87500	0.85714	1.00000	1.00000
D	1.00000	0.85714	0.85714		0.71429	0.71429	1.00000	0.85714	0.85714
E	0.71429	0.87500	0.87500	0.71429		0.87500	0.71429	0.87500	0.87500
F	0.71429	0.87500	0.87500	0.71429	0.87500		0.71429	0.87500	0.87500
G	1.00000	0.85714	0.85714	1.00000	0.71429	0.71429		0.85714	0.85714
H	0.85714	1.00000	1.00000	0.85714	0.87500	0.87500	0.85714		1.00000
I	0.85714	1.00000	1.00000	0.85714	0.87500	0.87500	0.85714	1.00000	

Fig. 5 Distance Similarity Matrix of *RAPD* Data with Primer-1

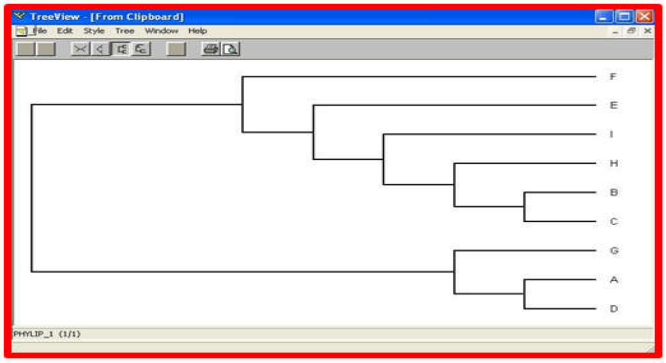
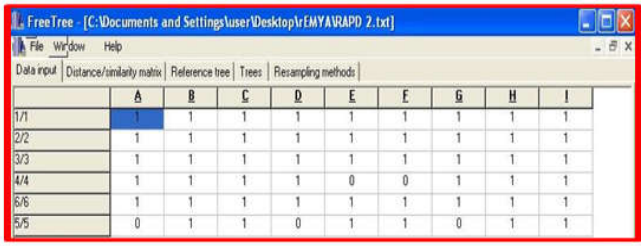
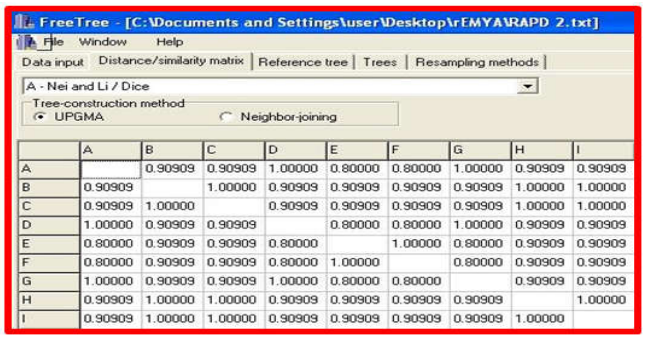


Fig. 6 Dendrogram of RAPD Data with Primer-1



	A	B	C	D	E	F	G	H	I
1/1	1	1	1	1	1	1	1	1	1
2/2	1	1	1	1	1	1	1	1	1
3/3	1	1	1	1	1	1	1	1	1
4/4	1	1	1	1	0	0	1	1	1
6/6	1	1	1	1	1	1	1	1	1
5/5	0	1	1	0	1	1	0	1	1

Fig. 7 Similarity Index Calculation by Binomial Method (RAPD with Primer-2)



	A	B	C	D	E	F	G	H	I
A		0.90909	0.90909	1.00000	0.80000	0.80000	1.00000	0.90909	0.90909
B	0.90909		1.00000	0.90909	0.90909	0.90909	0.90909	1.00000	1.00000
C	0.90909	1.00000		0.90909	0.90909	0.90909	0.90909	1.00000	1.00000
D	1.00000	0.90909	0.90909		0.80000	0.80000	1.00000	0.90909	0.90909
E	0.80000	0.90909	0.90909	0.80000		1.00000	0.80000	0.90909	0.90909
F	0.80000	0.90909	0.90909	0.80000	1.00000		0.80000	0.90909	0.90909
G	1.00000	0.90909	0.90909	1.00000	0.80000	0.80000		0.90909	0.90909
H	0.90909	1.00000	1.00000	0.90909	0.90909	0.90909	0.90909		1.00000
I	0.90909	1.00000	1.00000	0.90909	0.90909	0.90909	0.90909	1.00000	

Fig. 8. Distance Similarity Matrix of RAPD Data with Primer-2

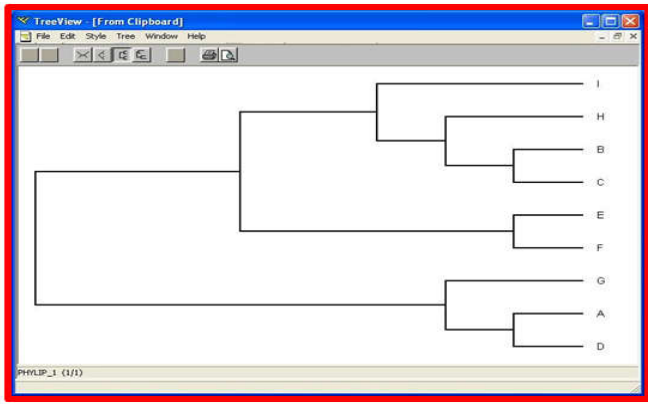


Fig. 9. Dendrogram of RAPD Data with Primer-2

Conclusion

To determine the genetic variation of cattle species in Kerala and to find out the present genetic status for their future improvement and conservation program, DNA was extracted from the blood samples of three indigenous (Vechur cattle, Vatakara animals and Kasaragod cattle) and six cross-bred cattle varieties (cross-bred cattle named 303, 313, 298, 323, 291 and 314) of Kerala. Blood samples were collected and the extracted DNA was observed among them, two primers were found polymorphic.RAPD-PCR analysis was carried out using DNA samples of cattle species in Kerala. From the results of RAPD, phylogenetic analysis was carried out. Two primers viz. primer-1 and primer-2 were used for the genetic variability detection using RAPD and primer-1(5^{1}GTTTCGCTCC3^{1}), which gave more polymorphism was chosen for the comparative study. Vechur and Vatakara animals were found to be closely related in their banding pattern and these native cattle differ from Kasaragod cattle, other native cattle of Kerala. Some hybrid varieties were found to be closely related to indigenous cattle varieties in their banding pattern (eg. cross-bred cattle 303 and 323 are closely related to Kasaragod cattle and cross-bred cattle 291 is closely related to Vechur and Vatakara animals). Some hybrid varieties exhibited more distinct banding pattern when compared to Vechur cattle, Kasaragod cattle and Vatakara animals. (eg: cross-bred cattle 313 and 298). The calculation of pair wise genetic similarities indices between individuals may help to select animals possessing the least genetic similarity and might be a valuable tool for selection in the future germplasm collections. The data reported here provide a valuable insight into genetic diversity and genetic relationships between indigenous and cross-bred cattle in Kerala which can be used to improve the breeding strategies.

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