Genetic Distance among South Indian Breeds of Zebu Cattle Using Random Amplified DNA Markers

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ABSTRACT : Random Amplified Polymorphic DNA (RAPD) assay was conducted to identify polymorphic markers in Amrithmahal, Krishna Valley, Hallikar, Deoni, Khillari, Ongole and Malnad Gidda breeds of South Indian cattle using twenty six primers. Of the 93 RAPD markers obtained, 53 were present in all breeds, 22 were individual specific and 18 were polymorphic for different breeds. Dual purpose breeds viz., Krishna Valley and Ongole showed less genetic divergence between them as compared to their genetic divergence from draft breeds viz., Amrithmahal, Hallikar and Khillari. Malnad Gidda was found to be a distinctly different from others studied. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 3 : 309-314)***

Key Words : Zebu Cattle, Genome, RAPD, DNA Fingerprints

INTRODUCTION

Zebu breeds of cattle from Southern India are well known for their draft efficiency and resistance to diseases. Crossbreeding of these breeds with high milk producing *Bos taurus* breeds was initiated in the early 1950's for rapid increase in the milk production. Though a substantial improvement in the milk production performance due to intensive crossbreeding is observed, there is deterioration in other valuable traits like disease resistance, heat tolerance and adaptability to the specific local agro-climatic conditions. At present breed characters in many of these breeds have been diluted. Further many of them are threatened with extinction. There is an urgent need for their conservation. Characterization and evaluation of genetic differences among these breeds is necessary for effective and meaningful conservation of these breeds.

DNA fingerprints are considered to be a primary source of informative marker loci and have the advantage of detecting several loci. During the last decade, several techniques such as restriction fragment length polymorphism (RFLP), microsatellites, minisatellites, polymerase chain reaction-RFLP (PCR-RFLP) and random amplified polymorphic DNA (RAPD) have been extensively adapted to identify breed specific genetic markers. Further, attempts were also made to associate these markers with quantitative traits and disease resistance.

Random amplified polymorphic DNA markers are based on amplification of genomic DNA by PCR using random sequence oligonucleotide primers (Williams et al., 1990). Short oligonucleotide primers in PCR reactions with lowered annealing temperature will generally amplify a spectrum of fragments from almost any template DNA. One or more of them are often polymorphic and this polymorphism (RAPD) can be genetically mapped. Breed characterization requires knowledge of genetic variation that can be effectively measured within and between populations (Hetzel and Drinkwater, 1992). Hwang et al. (2001) reported that RAPD markers are valuable in linkage analysis in poultry. RAPD technique has been successfully applied for characterization of bovine populations (Kemp and Teale, 1992, 1994; Gwakisa et al., 1994; Bardin et al., 1995; Nagaraja, 1998; Thiyagarajan, 2000) and sheep breeds (Kantanen et al., 1995).

This study was undertaken with the objectives of determining the genetic distances between zebu breeds of Southern India and to identify suitable RAPD markers unique to these breeds.

MATERIALS AND METHODS

Experimental animals

Animals belonging to seven zebu breeds of Southern India (Amrithmahal, Krishna Valley, Hallikar, Deoni, Khillari, Ongole and Malnad Gidda) with their home tract (figure 1), were sampled from different herds. The Hallikar breed has a history of more than 600 years. This breed is acclaimed as a progenitor of most of the zebu breeds of Southern India including Amrithmahal, Khillari and Kangayam. Amrithmahal breed was evolved from Hallikar and other closely related Mysore type of cattle. Similarly Khillari breed owes it origin from Hallikar breed. Ongole breed of cattle owes it origin to the cattle brought from South-East of Sindhu river. While Krishnavalley breed is admixture of four distinct breeds, having derived inheritance from Gir, Ongole, Kankerj and Hallikar. Deoni breed of cattle was also evolved from Gir cattle by crossing with local breeds like Dangi and Dongari breeds found in Maharashtra.

Colour of Amrithmahal cattle varies from white to light

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Received May 10, 2001; Accepted October 8, 2001

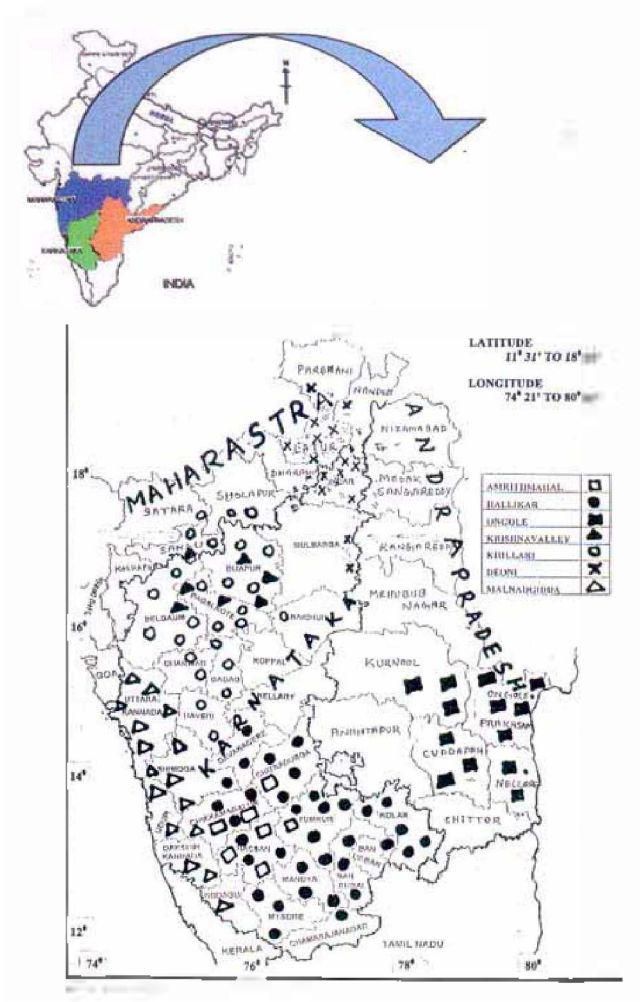

Figure 1. Map of Karnataka and bordering districts of Maharastra and Andra pradesh showing distribution of zebu cattle under study

gray while Hallikar cattle are gray to dark gray in colour with deep shading on fore and hind quarters and light gray/white markings on face especially around eyes. Amrithmahal animals are larger in size compared to Hallikar cattle. Khillari breed in general has compact, cylindrical and long body with strong set of legs. The muzzle in Khillari is mottled is colour where as Krishnavalley cattle has black muzzle. The Ongole breed of cattle is heavy having long body and long and muscular limbs. The colour is white with light gray markings on head, neck and hump in males. Deoni breed of cattle is medium sized and has prominent forehead, well-developed dewlap and sheath. The ears are pendulous and drooping. Malnad Gidda cattle are phenotypically very distinct from other breeds studied. They are small in size (height 80.67 to 82.71 cm) as compared to all other breeds studied. They have compact body frame and weigh around 80-120 kgs. Malnad Gidda cattle are distributed predominantly in hilly Malnad and adjacent coastal districts of Karnataka.

The Hallikar, Amrithmahal and khillari breeds are purely draft purpose breeds exclusively used for agricultural operations and for carrying loads and transportation. While, Ongole, Krishnavalley and Deoni are dual purpose breeds which are not only used for agricultural operations but also for milk production.

Blood samples from 150 animals of Amrithmahal, 150 of Krishna Valley, 45 of Deoni, 39 of Hallikar, 19 of Khillari, 9 of Ongole and 27 of Malnad Gidda were obtained.

DNA samples

High molecular weight genomic DNA was extracted from peripheral blood as described by Miller et al. (1988). Samples were adjusted to a concentration of 20 µg/ml before PCR amplification. The PCR amplification was performed on individual animal DNA and also on breed specific pools. The breed specific DNA pools were prepared by mixing equal quantities of DNA from 16 individuals of the same breed, except for Ongole. In the case of Ongole breed, DNA pool was prepared by mixing equal quantity of DNA from 9 individuals.

PCR primers

Based on earlier reports on RAPD studies in zebu breeds, 26 arbitrary oligonucleotide primers obtained from Bangalore Genei (P) Ltd., Bangalore, India were used for amplification of the breed specific DNA pools. Out of the 26 primers used, 19 either failed to produce polymorphic fragments or failed to amplify the DNA and hence, were not used for further analysis. In all, seven primers OPAA 13, ILO 868, ILO 876, ILO 1127, ILO 526, OPAA 17 and ILO 1065, were used to study the polymorphism within and among different cattle breeds. These seven primers produced consistent polymorphic banding patterns.

PCR amplification

The amplifications were carried out in 0.2 ml PCR reaction tubes using a programmable thermal cycler (Eppendorf, USA). The 30 µl reaction mix comprised of 50 ng template DNA, 40 pM primer, 200 µM each of the four d-NTPs, 1 unit of *Taq* DNA polymerase (Bangalore Genei, Bangalore), 1.5 mM $MgCl_2$ and 1X PCR buffer (Bangalore Genei, Bangalore). The 1X PCR buffer consisted of 10 mM Tris Hcl pH 8.3, 50 mM Kcl and 0.1% TritonX-100. The content was mixed thoroughly and centrifuged for 10 sec. at 5,000 rpm. A PCR programme with an initial denaturation at 95°C for 2 min., second denaturation for 45 sec. at 94°C, annealing at 35°C for 45 sec. and an extension duration of 2 min. at 72°C was used. Temperature cycling included 39 cycles of 10 sec. at 94°C, 10 sec. at 35°C, 2 min. at 72°C and final extension at 72°C for 3 min. The PCR products were electrophoresed at 100

V in 2-3% agarose gel in 1X TBE buffer containing 0.5 µg/ml ethidium bromide along with a DNA molecular size marker. RAPD fingerprints were visualized and documented using Gel documentation system (Gel doc 2000, Bio-Rad, USA).

Scoring of bands and statistical analysis

Only distinct bands representing RAPD fingerprints were scored and compared among samples run on the same gel. A total of 21 interbreed pair wise comparisons were made.

Band Sharing (BS) : Band sharing was calculated as a measure of similarity of RAPD fingerprints of animals from either the same or different breeds (Gwakisa et al., 1994) as follows:-

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

Where, B_{ab} is the number of bands shared by individuals 'a' and 'b', and B_a and B_b are the total number of bands for individuals 'a' and 'b' respectively.

Mean Average Percentage Difference (MAPD) : The dissimilarities between different breeds were expressed in the form of MAPD as described by Gwakisa et al., (1994) by using the following method.

1. Percentage Difference $(PD)=[(N_{ab})/(N_a+N_b)]\times100$

2. Average Percentage Difference $(APD)=(\sum_{i=1}^{c} PD_i)/C$

3. Mean Average Percentage Difference (MAPD)=

$(\sum_{i=1}^{R} APD_i)/R$

Where,

N_{ab} is the number of fragments that differed between individuals 'a' and 'b' for a primer.

N_a is the number of fragments resolved in individual 'a',

N_b is the number of fragments resolved in individual 'b',

C is the number of interbreed pair wise comparisons. (i=1, ...16), and

R is the number of random primers used (R=1, ...7).

Construction of Dendrograms : The Dendrogram of relationship among seven breeds of zebu cattle studied was constructed using the genetic Distances as estimated by the Distance Wagner procedure (Farris, 1972). The genetic distances were estimated from the MAPD calculated as above.

RESULTS AND DISCUSSION

It is well established that DNA fingerprints can be successfully used to compare genetic distances between human beings (Gill et al., 1985), sheep (Cushwa et al., 1996, Dodgson et al., 1996), dogs and cats (Jefferys and Morton, 1987) and *Bos indicus* and *Bos taurus* cattle (Kemp and Teale, 1994). The present study aimed at determining the genetic distances between different breeds of cattle of Southern India. 25 out of the 26 RAPD primers amplified at least one discrete reaction product (range 1 to 18). The sizes of the products ranged from 60 bp to 3,250 bp. The reproducibility of RAPD analysis was determined using different brands of *Taq* DNA polymerase enzyme and at intervals of time on the same DNA samples with identical assay parameters. Except for few minor bands, all major bands appeared consistently. The intensity of RAPD bands varied between individuals for the same primer. This could be due to variations in the number of loci amplified by the primer.

Screening of RAPD primers

Out of 93 RAPD markers obtained from 7 primers, 53 were common to all breeds, 22 were individual specific and 18 were polymorphic for different breeds. The number and frequency of RAPD bands per primer in different breeds are presented in table 1.

The utility of primer ILO 1127 for identifying breed specific locus, if any, was evaluated. A high percentage of polymorphism was observed in different breeds. A distinct band of about 1.4 kb size was amplified only in Malnad Gidda, Hallikar and Amrithmahal pools. Another fragment of 0.65 kb was uniquely observed in Malnad Gidda. When individual samples of Malnad Gidda were amplified, only 18 out of 27 animals showed the specific fragments.

Table 1. Number and frequency of RAPD bands per primer in different breeds

Primers	OPAA 13	OPAA 17	ILO 1127	ILO 1065	ILO 876	ILO 868	ILO 526
Breed	Number of bands (average)						
Amrith-Mahal	4-8 (6)	4-8 (6)	8-11 (9.5)	10-14 (12)	13-17 (15)	4-7 (5)	4-8 (6)
Hallikar	4-8 (6)	4-8 (6)	7-10 (8.6)	13-17 (15)	8-10 (9)	4-7 (5.5)	4-8 (6)
Khillari	4-14 (11.2)	2-3 (2.5)	8-11 (10)	5-8 (6.5)	9-15 (12)	4-7 (5.5)	3-7 (4.4)
Krishna Valley	7-16 (13)	6-8 (7)	7-10 (8.6)	8-10 (9)	7-14 (11)	3-6 (5)	8-10 (9)
Ongole	14-18 (16.5)	7-11 (8.7)	8-10 (9)	12-16 (14)	10-18 (15)	4-8 (6)	8-12 (10)
Deoni	5-7 (5.8)	7-10 (8.6)	13-16 (14)	5-8 (6.5)	7.14 (11)	5-8 (7)	8-10 (9)
Malnad Gidda	4-6 (4.7)	6-10 (8)	8-11 (9.5)	7-12 (10)	5-8 (6.5)	1-3 (2)	3-7 (4.4)

Another breed specific band of about 0.93 kb was observed in Amrithmahal breed, but not in all animals of the breed.

The RAPD primer ILO 1127 successfully amplified cattle DNA and could be used to differentiate *Bos taurus* cattle from *Bos indicus* cattle (Gwakisa et al., 1994). They observed a RAPD fragment of 0.97 kb exclusively in the pooled sample of Tanzanian short horned zebu animals. Interestingly, no such RAPD product specific to Indian zebu animals could be amplified in the present study.

ILO 526 produced moderate to high polymorphism. The amplified products of Krishna Valley and Ongole breeds gave specific fragment of 0.77 kb, which was absent in other breeds studied. The ILO 526 amplified fingerprints appeared with a frequency of 88% among Ongole cattle and less than 29% in Krishna Valley cattle.

ILO 868 produced the moderate polymorphic pattern. Most of the prominent bands were common to all the breed pools studied. This primer produced breed specific fragment of 0.37 kb in Khillari (figure 2) breed but not in other breeds studied.

Primer ILO 876 produced a 0.95 kb product similar to the one reported in African zebu breeds. Though breed specific bands could not be detected using this primer, it exhibited highly polymorphic fingerprints. Similar findings were reported by Nagaraja (1998) in their study with different breeds of zebu cattle in India. The results of the present study, where 0.97 kb fragment was amplified in all the breed pools, considered in conjunction with the reports of earlier workers that few *Bos taurus* animals also carried this fragment renders this primer (ILO 876) non-suitable for breed characterization by RAPD analysis. On the other hand Gwakisa et al. (1994) reported that ILO 876 produced a 0.9 kb sized fragment only in *Bos indicus*.

Figure 2. Amplification of breed wise pooled cattle DNA by random primer ILO 868

Lane No.	Details
1	Amrithmahal
2	Krishna Valley
3	Ongole
4	Khillari
5	Hallikar
6	Deoni
7	Malnad Gidda
8	100 bp ladder DNA marker

Primers ILO 1065, OPAA 13 and OPAA 17 produced moderate to high polymorphism. OPAA 13 produced 1.8 kb product in Amrithmahal breed pool and OPAA 17 produced a fragment of 1.3 kb in Ongole and Krishna Valley pools. However, these bands were not observed in all the animals included in the breed pool. This OPAA 13 primed RAPD was revealed in 61% of Amrithmahal, but less than 5% in Hallikar and Khillari breeds.

The band sharing within the breeds for different primers (table 2) indicated that the average band sharing ranged from 58% (in Malnad Gidda cattle for OPAA 17 and in Krishna Valley cattle for OPAA 13) to 98% (in Amrithmahal cattle for ILO 1065 and ILO 868). The magnitude of intrabreed RAPD variation in different cattle breeds was found to be lower compared to interbreed variation. The interbreed dissimilarities expressed in the form of mean average percentage difference provides a measure of genetic divergence in terms of genomic DNA fingerprints between breeds. The values for MAPD between different breeds using the RAPD fingerprints (table 3) ranged from 16.20±4.06 (between Krishna Valley and Ongole cattle) to 36.90±0.05 (between Deoni and Malnad Gidda). Ongole and Krishna Valley breeds had the least variation (16.20±4.06), followed by Hallikar and Khillari (16.80±1.82) and Amrithmahal and Hallikar breeds (19.46±3.77). The highest genetic divergence was observed between Malnad Gidda and Deoni (36.90±0.05).

The high degree of resemblance of DNA bands between Ongole and Krishna Valley breeds observed in this study is not surprising since one of the breeds used for synthesizing the Krishna Valley was Ongole. Similarly, Hallikar was closely related to Khillari and Amrithmahal breeds (figure 3). There is overlapping in the breeding tracts of Amrithmahal and Hallikar breed (figure 1). Hallikar breed was extensively used for developing many of the present day South Indian breeds. Hence, close relationship of Hallikar with Amrithmahal and Khillari is expected. Though Krishna Valley and Khillari breeds belong to same area, the genetic divergence is 24.5±3.15. Even according to the history of breed development, Krishna Valley breed was developed by using Ongole, Gir, Kankrej and Hallikar type animals but not Khillari. Dual purpose breeds Krishna Valley and Ongole showed less genetic divergence between them as compared to their genetic divergence from draft breeds viz., Amrithmahal, Hallikar and Khillari breeds.

Table 2. Band Sharing (BS) within the breeds

Breed	Amrith-mahal	Hallikar	Khillari	Krishna Valley	Ongole	Deoni	Malnad Gidda
Primers							
OPAA 13	0.86	0.88	0.88	0.58	0.66	0.77	0.61
OPAA 17	0.88	0.77	0.73	0.61	0.76	0.74	0.58
ILO 1127	0.92	0.88	0.91	0.73	0.91	0.93	0.73
ILO 1065	0.98	0.77	0.67	0.88	0.88	0.88	0.61
ILO 876	0.97	0.96	0.96	0.77	0.88	0.93	0.73
ILO 868	0.98	0.81	0.88	0.97	0.88	0.93	0.73
ILO 526	0.96	0.96	0.89	0.96	0.76	0.96	0.81
Average for 7 primers	0.94±0.05	0.86±0.08	0.84±0.11	0.79±0.16	0.82±0.09	0.88±0.09	0.68±0.09

Table 3. Overall "MAPD" values between breeds (for 7 primers)

Breed	Hallikar	Khillari	Krishna Valley	Ongole	Deoni	Malnad Gidda
Amrith-Mahal	19.46±3.77	23.53±4.56	35.06±9.05	31.66±3.25	25.97±2.69	35.47±13.67
Hallikar	-	16.80±1.82	29.66±4.06	25.78±2.67	26.50±2.96	23.53±2.43
Khillari	-	-	24.50±3.15	29.67±3.23	21.67±2.41	26.97±6.05
Krishna Valley	-	-	-	16.20±4.06	29.63±3.23	29.63±4.77
Ongole	-	-	-	-	20.06±3.33	29.67±2.43
Deoni	-	-	-	-	-	36.90±0.05

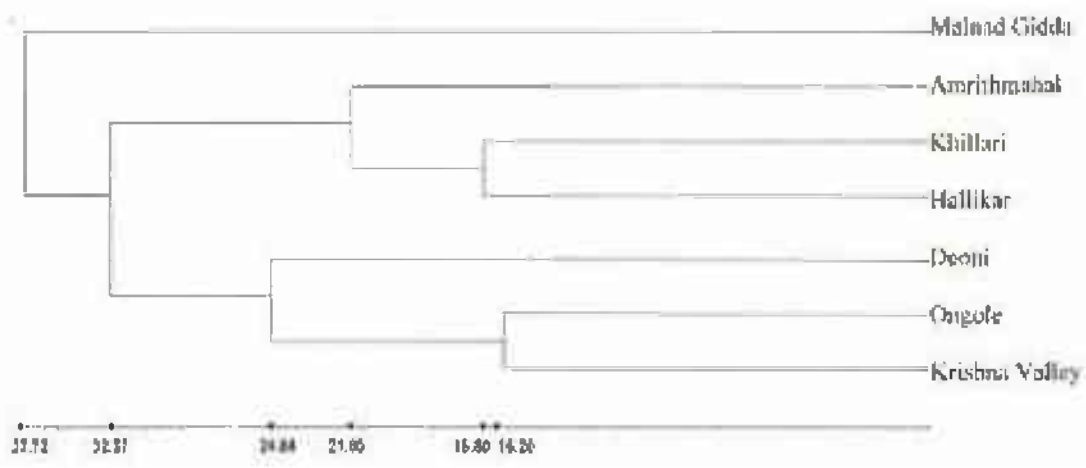

Figure 3. Dendrogram of relationship among seven breeds of zebu cattle using the genetic distances as estimated by the Distance Wagner procedure

ACKNOWLEDGEMENTS

We thank the Department. of Biotechnology. Government of India for financial assistance for carrying out the work. We thank Mr. L. Krishnamurthy and Mr. Charan Singh for their technical assistance in this work.

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