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STUDY ON DNA POLYMORPHISM OF THE THREE INDIGENOUS DESI BREEDS IN THE STATE OF MAHARASHTRA BY RAPD-PCR METHOD

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ABSTRACT :

The genetic diversity among three indigenous cow breeds was explored by random amplified polymorphic DNA (RAPD) analysis. The DNA samples were pooled from three breeds of Desi cow viz, Red Kandhari, Khillari and Deoni and twenty random primerswere screened. All the three desi cow breeds were successfully typed for RAPD analysis. The five selected primers showed most repeatable pattern in amplification. The UPGMA dendrogram constructed by computer program for clustering these three breeds revealed Red Kandhari breed exhibit separate cluster and had maximum genetic distance compare to other two breeds. The study revealed enables the determination of genetic relations andfingerprints of the three cow breeds

INTRODUCTION

The relationship between human and bovine population in India has religious doctrine and also play important role in the rural economy by providing meat, milk, leather, dung and use in draft purposes[1].The world cattle inventory in 2017 is at *998.3 million* head and India ranks first with 303.3 (30.39 %)million head. India has 37 pure cattle breeds of which Red Kandhari , Deoni and Khillari are most common in the Maharashtra, India. All these indigenous cattle breeds are known by local term'Desi Cow'.The Deoni (Dongari) breed was evolved through the crossbreeding of the Gir cattle with the Dangi breeds and local desi cattle of Nizam [2]. Based on their body colour pattern Deoni breed has evolved into three morphological types viz Balankya (complete white body coat and without any spot on the body), Wannera, (white body and black shades on sides of the face) and Waghya (white and black shades/spot/patches scattered all over the body)[3]**.** Red Kandhari is purest Indian breed with almost universal deep red coloured skin.The Khillari cattle breed is characterised by presence of long and pointed horns follow thebackward curve of the forehead. The Red Kandhari and Khillari breed is raise for draft purpose while the Deoni is for dual purpose cattle.

RFLP, RAPD, AFLP and VNTR are routinely used genetic markers for taxonomiccharacterisation of organismat nuclear level,[4]. RAPD is a PCR based technique for identifying geneticvariation by using single arbitrary primer in a PCR reaction, resulting in amplification of many discrete DNA. It is rapid and efficient method used to screen for DNA sequence based polymorphism at a very large number of loci without use of any sequencing technique[5]**.**RAPD has been routinely used for breed characterization in cattle [3**,** 6]**.** Present study aimed to determinethe breed-specific primers and RAPD fingerprints and genetic diversities in Khillari,Deoni and Red Kandhari cattle breed**.**

MATERIALS AND METHODS

Selection of Animal and Collection of Blood Samples:

The three breeds of Desi cow viz Red Kandhari, Deoni and Khillari were selected for RAPD analysis, based on their morphmetric features. About 15 blood samples of each breed were collected from different locations of Maharashtra. The blood samples of Deoni cow were kindly provided by College of Veterinary and Animal Sciences (COVAS), Udgir, Dist. Latur. The blood samples of Red Kandhari and Khillari cow were collected from the local veterinary practioners of Parbhani, Nanded, Hingoli and Kolhapur district. Before the collection of blood samples these regions were surveyed for the availability of cattle breed.

3 mL of blood was aseptically collected from jugular vein of each animal in a 5 ml EDTA vacutaine in November-December 2016. The blood samples were gently mixed with EDTA (present in the vacutaine) and carried to the laboratory. The blood samples were further spotted on Whatman filter paper and were dried for 24 hours at room temperature and stored at 4^0C until further use.

Extraction of DNA

A method described by Nguyen [7] was used here for the extraction of DNA from dried blood samples. The blood cells were lysed by Lysis Buffer I and Proteinase K. Lysis buffer I was prepared by using Tris 10mM HCl, 5mM MgCl2 , 1% Triton X100(v/v), 1% SDS (w/v) , 10mM

EDTA, adjusted with pH 8.0. The dried blood spot on filter paper was vortexed with300µl Lysis buffer Ifor 30s and followe by incubation at 85^0C for 20 min., The lysates were cooled down to room temperature for 10min and mixed with 0.02mg Proteinase K was added with proper vortex for 30s and incubated at 65^0C for 1hr. The same amount of buffer phenol: chloroform: isoamyl-alcohol (25:24:1) was added in the sample and mixed well for 30s. The mixture was then centrifuged at at 10,000 rpm for 4min at room temperature and the upper phase was taken out and treated with Sodium Acetate (3M, pH 5.2), and Iso-Propanol. Finally the sample was washed by70% Ethanol and DNA was collected by elution with 50µl dH2O. The concentration of DNA was determined using UV- 1800 spectro -photometer (SchimadzuCorporation). The DNA was stored at -20^0C for further use.

PCR amplification:

Initially 20 RAPD primers were used for the PCR amplification and out of these 20 primers, only 5 primers were taken further for final analysis. The list of the selected primers used is given in table 1 . PCR amplification was performed using Biometra thermal cycler. The PCR mixture contained 2.5µl of 10X buffer, 1µl of primer, 2.5µl of 2.5mM of each dNTP, 2.5 Units of Taq DNA polymerase and 1µl Template DNA. The PCR amplification cycle consisted of, a cycle of 5 min at 94 °C; 35 cycles of 45 sec at 94 °C, 45 sec at 36 °C, 1 min and 30 sec at 72 °C; and 1 cycle of 5 min at 72 °C.

Gel electrophoresis

Gel electrophoresis was performed using 1.4% agarose to analyse the size of amplified PCR product. SYBR DNA staining dye was used for staining of DNA bands. The gel was observed under Green view illuminator.

Analysis of RAPD Data:

For RAPD analysis of electrophoretograms obtained with five selected primers of three breeds were displayed on a computer screen and transformed manually into a binary data matrix, i.e. the presence or absence of bands scored as either 1 or 0 respectively. For the phylogenic analysis of data software PAST(Version-3.14) were used .The genetic similarity (GS) between two breeds were calculated by the Nei and Li equation[8]formula GSij=2Nij/(Ni + Nj).Where

Nij indicates the number of common bands between breed i and j;Ni denote total number of bands in breed i whereas Nj represents total number of bands scored for breed j. The genetic distance(GD) between two breeds were obtained from Lynch equation [9]**:** GDij = 1- GSij. Two assumptions (same position

on the gel were not occupied by markers from different loci and presence of band represents dominant genotype in Hardy-Weinbergequilibrium) were made for the analysis of RAPD data. The phylogenetic cluster tree was constructed by using unweighted pair group method of analysis (UPGMA)with the matrices of Nei coefficient.

RESULTS AND DISCUSSION

RAPD assay is most easy and cost effective assay for finding the bias in genetic characters of an organism. This was the first study carried out for comparison of genetic diversity in three breeds of indigenous cow from Maharashtra,India

The RAPD analysis in three breeds of cow showed variability in genetictraits. The concentration of DNA obtained in three breeds Red Kandhari, Deoni and Khillari after extraction from representative blood samples ere 40.0; 36.4 and 53.1 µg/ml respectively. The absorbance ratio of extracted DNA 260/280 nm was fall in the range of 1.8-2.0 indicates highest degree of purity. The molecular weightladder used for this analysis was ranged from100-10,000 bp.

Figure-1: Electrophoretic bands of DNA obtained from dried bloodsamplesof three breeds-A) Red KandhariB)Deoni and C) Khillari. Electrophoretic band pattern L indicates molecular weight marker.

Each breed produced breed specific fingerprint. Sample A-C produced the fingerprints of three breeds Red Kandhari, Deoni and Khillari respectively. Out of 20 random primers tested on the DNASamples 15 were discarded based on reproducibility,thickness, size and,expected segregation observed in a mapping sample. The sequence of remaining 5 primers selected for final analysis to evaluate genome variabilityis presented in table I.

Table-I: Selected primers used for PCR amplificationOf three breed samples.

Primer	Sequences 5'-3'
OPA09	GGGTAACGCC
ABA05	AGGGGTCTTG
ABA07	GAAACGGGTG
ABA16	AGCCAGGCGA
UBC478	CGAGCTGGTC

Primers with ten nucleotides and a (G+C) content of at least 50% are often used in RAPD study. Primers having high (A+T) content may frequently results in DNA primer hybrid melting during polymerization [2]. All the five primers used in the RAPD analysis were 10-mer long with GC content of 60-70%.The amplified products obtained after electrophoresis with selected primers ranged from 200 bp to 1200 bp in size.

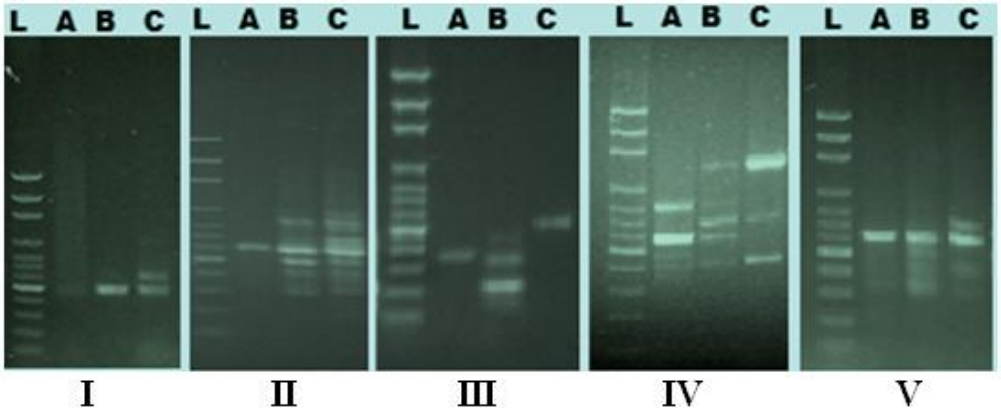

Figure-2:RAPD pattern generated using fiveprimers on three experimental breeds. The alphabetsA,B,C corresponds to,Red Kandhari,Deoni and Khillari respectively.The roman numerals I,II,III,IV and V at the base of each plate corresponds the RAPD primers OPA09, ABA05, ABA07, ABA16 and UBC478 respectively

The highest degree of polymorphim was observed in the Red Kandhari breeds (58%) compare to Deoni (31%) and Khillari (47%).The highest degree of polymorphism in Red Kandhari breeds may be due to their distribution in large geographical area for the draught purpose.It was found that the highest value of Nei genetic distance coefficient (0.74) was between Red Kandhari and Deoni breeds. Lower values of genetic similarities indicated a high degree of genetic diversity between these two cattle. On the other hand, the lowest distance coefficient (0.69) observed between Deoni and Khillari indicates, the genetic relationship between local cattle population was closer than others. Red Kandhari and Deoni localised within similar area and they had less significant distance coefficient values, this may be due to differences in their breed development programme[10]. The highest genetic diversity is disquieting as it indicates that the population may depreciate due to crossing with other populations of either native or exotic breed.

Table2. Estimation of pair wise genetic similarity and distancecoefficient between experimental Indigenousbreeds.

	Red Kandhari	Deoni	Khillari
Red Kandhari	0.00	0.74	0.73
Deoni	0.26	0.00	0.69
Khillari	0.27	0.31	0.00

Genetic similarities were studied through analysis of RAPD data from the three breed's shows genetic relatedness. Using the Nei's genetic distance matrix values, a dendrogram was constructed to obtain the clustering of three breeds. The UPGMA dendogram based on genetic distance was differentiated into two main clusters viz, clusters-I and II. Cluster I is further subdivided into two sub-clusters, I (A) and I(B) respectively.

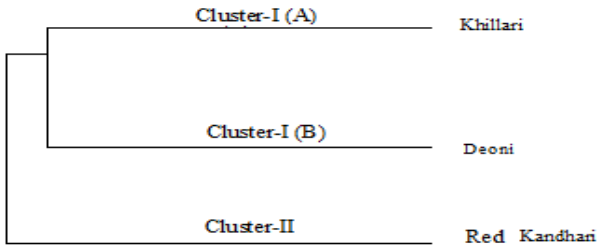

Figure-3: UPGMA dendogram based on Nei's genetic distance data regarding clustering of three experimental breeds.

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