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Genetic Differentiation of Indian Zebu Cattle (*Bos tauraus*) Breeds Using Random Oligonucleotide Primers (RAPD-PCR) in Amravati Region, Maharashtra, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In India, insufficient characterization skills and extensive crossbreeding of cattle have led to the loss of several breeds. The purpose of this work is to find polymorphic primers for identifying Zebu cattle breeds. Random crossbreeding has resulted in genetic losses among the Gaolao, Krishna Valley, and Hallikar breeds. Genetic characterization and cryopreserving semen are critical

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components of conservation and breeding initiatives. The study highlights the urgent need for effective genetic characterization of Zebu cattle breeds in India, identifying 8 polymorphic and 4 monomorphic primers through RAPD-PCR. These findings are crucial for accurate breed identification, which is essential for conservation efforts and informed breeding strategies to preserve genetic diversity and prevent the decline of indigenous breeds like Gaolao, Krishna Valley, and Hallikar. Genomic DNA was isolated from whole blood using a DNA extraction kit and amplified with RAPD-PCR. A total of 12 primers were evaluated, and agarose gel electrophoresis revealed that eight (OPA-09, OPA-15, OPB-13, OPB-07, OPB- 05, OPA-13, OPA-18, and OPA-01) were polymorphic and four (OPB-06, OPA-04, OPB-03, and OPA-02) were monomorphic. Polymorphic primers demonstrated relatedness between some of the available breeds. Primers OPA-09 and OPA-15 showed a higher degree of polymorphism than primers OPB-13, OPB-07, OPB-05, OPA-13, OPA-18, and OPA-01, indicating that primers OPA-09 and OPA-15 may successfully identify various cattle breeds.

Keywords: Indian Zebu; polymorphic; characterization; RAPD-PCR; Gaolao; Krishna Valley; Hallikar.

1. INTRODUCTION

India has many different species of indigenous cattle. The primary indigenous breeds include Gaolao, Krishna Valley and Hallikar, in addition to various alien breeds. Since 2000, India has experienced indiscriminate cow breeding as a result of random livestock movements across farms. As a result, it is critical to screen Randomly Amplified Polymorphic Deoxyribonucleic Acid (RAPD) primers for polymorphisms that can be utilized to determine cattle genotypes. Indiscriminate crossbreeding has resulted in the loss of breeds [1], such as the Gaolao cattle breed [2], Krishna Valley [3], and Hallikar [4].

Molecular markers based on DNA sequence differences can successfully discriminate between closely related genotypes and can accurately identify closely related genotypes due to their resistance to selection [5,6]. For effective breeding and conservation programs to be carried out, it is critical to screen RAPD primers to determine the genotypes in cattle. The polymerase chain reaction (PCR) approach uses amplified DNA sequences as molecular markers with minimal template DNA [7,8].

“Random Amplified Polymorphic Deoxyribonucleic Acid-Polymerase Chain Reaction (RAPD- PCR) annealing occurs when a random primer of any sequence binds to specific related priming sites of the template genomic DNA, producing amplicons if the priming sites are within an amplifiable distance of each other” [1,9]. Williams et al. [10] developed “the RAPD analysis method, which employs short oligonucleotide primers to amplify a wide range of fragments from a template DNA in PCR processes with a lower annealing temperature. The RAPD PCR detects polymorphisms used in genetic mapping and strain identification. It is the superior method because it is (a) less expensive than previous methods for finding genetic variants, such as protein markers; (b) faster; and (c) without the need for previous sequencing details. The ability of RAPDs to survey many loci has been used to compute genetic distance and reconstruct phylogenies”.

“Awareness of genetic variation is required for breed characterization in order to undertake effective breeding efforts across and within individuals. The RAPD technique has been successfully utilized to analyze bovine species” [1,11,12]. “Other studies that have used RAPD markers include Japanese black cattle (Wagyu), Zebu cattle, German native cattle, and Korean native cattle” (Yeo et al., 2000). Hwang et al. [13] state that RAPD markers are an important tool for correlation studies in cattle.

RAPD and microsatellite markers are both used for genetic characterization, but each has unique advantages and limits. The advantages of RAPD over microsatellite markers include its simplicity and cost-effectiveness. It requires no prior knowledge of the DNA sequence, making it suitable for studies with few resources. RAPD is a rapid procedure that uses a single PCR reaction with arbitrary primers. RAPD may discover polymorphisms across the genome without requiring precise sequence information.

Reasons why microsatellite markers might not be used: Microsatellite markers necessitate the use of unique primers for each locus, which necessitates more complicated and costly procedures, such as sequence information and

primer design. The use of microsatellite markers necessitates greater technical skill and more advanced equipment, which may not be available. in all research locations. Microsatellite markers take longer to develop and analyze than RAPD markers do.

In this Zebu cattle breed study, RAPD was most likely chosen because of its low cost, simplicity, and ability to generate polymorphic markers fast without prior sequence information. This makes it an appropriate choice for initial genetic characterization and conservation efforts, particularly when resources and time are limited.

This study verified RAPD primers to find polymorphic primers for Indian Zebu Cattle breeds. This study is relevant to India's conservation and breeding programs. As a result, identifying RAPD primers capable of determining genotypes in cattle is significant.

2. MATERIALS AND METHODS

2.1 Blood Collection

A syringe and 18G needle were used to collect blood from three cows after the region was sterilized with 70% ethanol. To prevent clotting, the blood was promptly transferred into ethylene diamine tetra acetic acid (EDTA) containing blood tubes and gently inverted ten times. Blood tubes were transported to the laboratory in ice bags and stored in a freezer at - 20ºC.

2.2 DNA Extraction

High-molecular-weight genomic DNA was extracted from whole blood by using the Qiagen DNeasy® Blood & Tissue Kit (50), Cat. No. 69504. By taking a 2-ml microcentrifuge tube, add 20 µl of proteinase K and added 100 µl of anticoagulant blood sample, adjusted the volume to 220 µl with PBS (Potassium Buffer Saline, pH 7.2). Added 200 µl of lysis buffer AL (without ethanol addition), mixed thoroughly by vertexing and incubated at 56 °C for 10 minutes. Added 200 µl of ethanol (96–100%) to the sample and mixed thoroughly by vertexing to yield a homogeneous solution. Pipetted the mixture from step 3 into the DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuged at ≥ 6000 g (8000 rpm) for 1 minute. Discard the supernatant through the collection tube. Placed the DNeasy Mini spin column in a new 2 ml collection tube, added in it 500 µl Buffer AW1 and centrifuged for 1 minute at ≥6000 g (8000 rpm). Discard the flow through the collection tube. Place the DNeasy Mini spin column in a new 2 ml collection tube, added 500 µl Buffer AW2 and centrifuged for 3 minutes at 20,000g (14,000 rpm) to dry the DNeasy membrane. Placed the DNeasy Mini spin column in a clean 2ml microcentrifuge tube and pipetted 200µl buffer AE directly onto the DNeasy membrane. Incubated at room temperature for 1 minute and then centrifuged for 1 minute at ≥6000 g (8000 rpm) to elute. The eluted genomic DNA from Zebu cattle blood was stored at -20 °C.

2.3 RAPD-PCR Reaction

RAPD-PCR was performed in a 25 µl reaction using 1.5 µl of Taq polymerase 7.5 units (Thermo, HSN). 1.5 µl taq buffer (Thermo, HSN), 2 µl dNTP's 0.4 mM (Thermo, HSN) 3 µl Tris and HCl buffer (pH 8.0) 3 µl primer (10 mM. Operon technology) and 1 µl (50 to 100 ng) diluted genomic DNA mix with 12 µl nuclease-free water in a 0.2 ml PCR tube. The BioEra's thermocycler was used to perform the polymerase chain reaction (PCR). The cycling conditions were as follows: pre-denaturation: 95°C for 3 min; denaturation: 95°C for 1 min. annealing: 36°C for 1 min. extension: 72°C for 1 min. final extension: 72°C for 8 min. a total of 41 cycles. Electrophoresis of amplification products on 1.5% agarose gels in 1X Tris base, acetic acid, and EDTA (TAE) buffer with ethidium bromide at 150V was performed for 45 minutes.

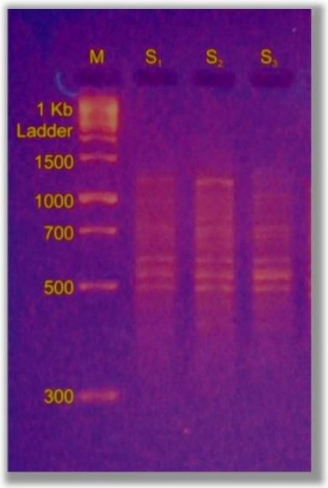
2.4 Gel Electrophoresis

To prepare a 1.5% agarose gel, dissolved 3.0 g of agarose powder in 200 ml of TAE buffer (1X). A hot magnetic stirrer was used to dissolve the gel in a conical flask covered with aluminium foil. When the gel was still hot, added ethidium bromide (10 mg/ml) and stirred into the agarose solution to create a homogenous solution. After cooling until the conical flask could be grasped; the solution was placed on a gel box (Galileo Biosystems). After solidification, the gel was suspended in 1X TAE buffer. The RAPD-PCR products (10 µl PCR amplification products along with 2 µl of DNA loading dye) were separated on 1 percent Agarose gel containing Ethidium bromide (0.5 µl 1/10 ml of gel) at 100 volts for three hours using 1 x TBE buffer. A UV-transilluminator was used to visualize the bands, which were then photographed with a Canon T5 EOS Rebel DSLR digital camera.

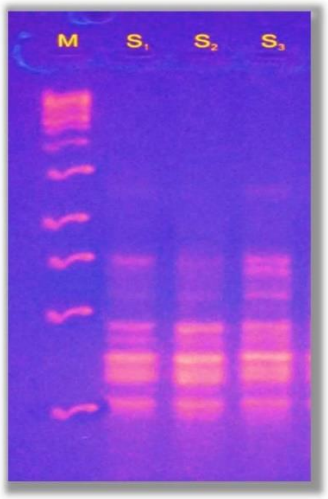
3. RESULTS

Out of 25 random primers tested on DNA samples, 13 were eliminated due to the absence of distinct amplified bands. The remaining 12 primers were used based on the number and strength of bands to assess genetic variability. We scored fingerprints based on the assumption that each band represented a single locus. Based on the quantity and strength of the bands, they were utilized to assess the variability of the genome. To obtain fingerprint scores, one band was thought to correspond to one locus. RAPD-PCR was carried out with 12 primers: OPA- 01, OPA-02, OPA-04, OPA-09, OPA-13, OPA-15, OPA-18, OPB-03, OPB-05, OPB-06, OPB-07 and OPB-13. There are 2 primers that showed 100% polymorphism (OPA-9 and OPA-15), and 10 primers were found to have different polymorphisms in nature (OPA-1, OPA-02, OPA-13, OPA-18, OPB-03, OPB-05, OPB-06, OPB-07, and OPB-13).

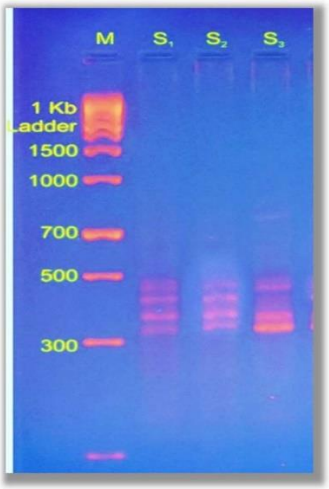
These primers amplified 2–36 bands with sizes ranging from 250 to 1500 bp. Among the 248 loci amplified, 131 (52.82%) showed polymorphisms. Table 1 shows the average number and size of bands obtained from the different random primers. Primers OPB-07 and OPB-13 showed a higher degree of polymorphism. Fig. 1 displays the RAPD fingerprints of primer- amplified bands. Data from Table 1 was used to create a dendrogram indicating genetic relationships among the selected breeds (Fig. 2). The genetic links among the breeds showed two separate groups: Gaolao is a separate group, and the other one includes Krishna Valley and Hallikar.



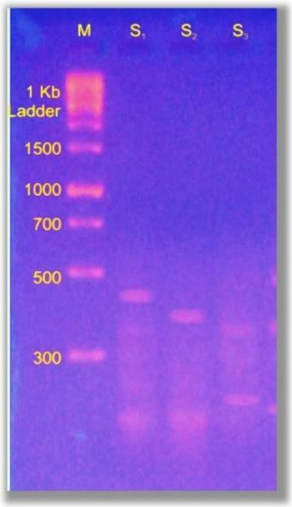
OPA-01



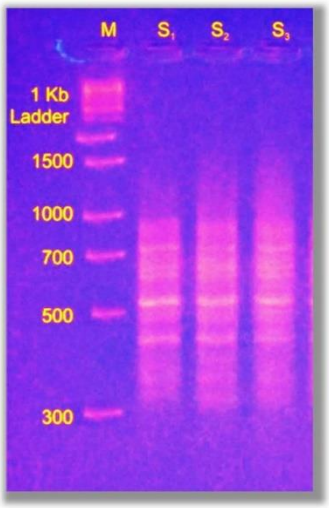
OPA-02



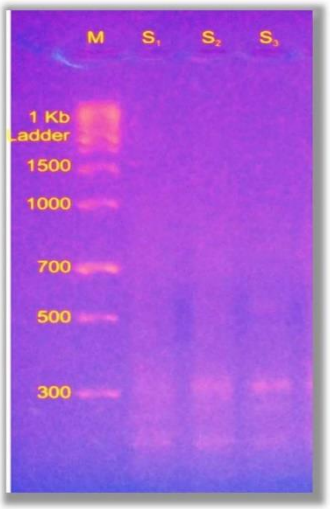
OPA-04



OPA-09



OPA-13



OPA -15

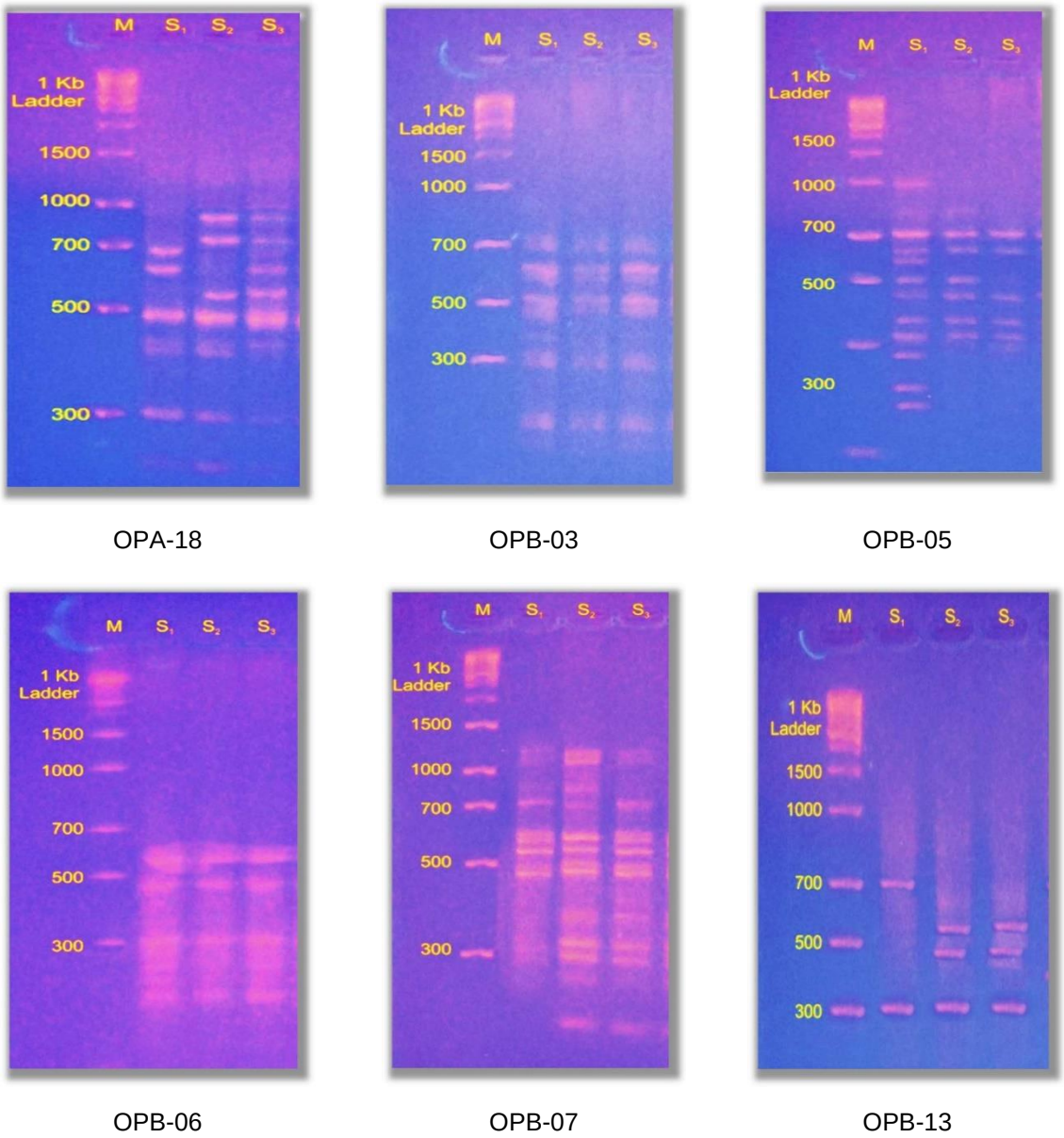


Fig. 1. Agarose gels showing polymorphic and monomorphic primers (OPA-01, OPA-02, OPA-04, OPA-09, OPA-13, OPA -15, OPA-18, OPB-03, OPB-05, OPB-06, OPB-07, 0OPB- 13)

Table 1. Average number and size of bands obtained from different random primers

Primers	Sequence (5'---------- 3')	GC%	Average No. of Bands	Polymorphic Range (bp) Bands & %	
OPA-01	CAGGCCCTTC	70	19	10 (52.63)	400-1200
OPA-02	TGCCGAGCTG	70	25	10 (40)	300-1500
OPA-04	AATCGGGCTG	60	21	06 (28.51)	300-700
OPA- 09	GGGTAACGCC	70	15	15 (100)	250-500
OPA-13	CAGCACCCAC	70	27	15 (55.56)	300-1000
OPA-15	TTCCGAACCC	60	06	06 (100)	250-300
OPA-18	AGGTGACCGT	60	27	15 (55.56)	300-1000
OPB-03	CATCCCCCTG	70	18	03(16.67)	250-700
OPB-05	TGCGCCCTTC	70	36	21 (58.34)	300-1000

Primers	Sequence (5'---------- 3')	GC%	Average No. of Bands	Polymorphic Range (bp) Bands & %	
OPB-06	TGCTCTGCCC	70	12	00 (0)	250-600
OPB-07	GGTGACGCAG	70	30	21 (70)	250-1200
OPB-13	TTCCCCCGCT	70	12	09 (75)	300-1200

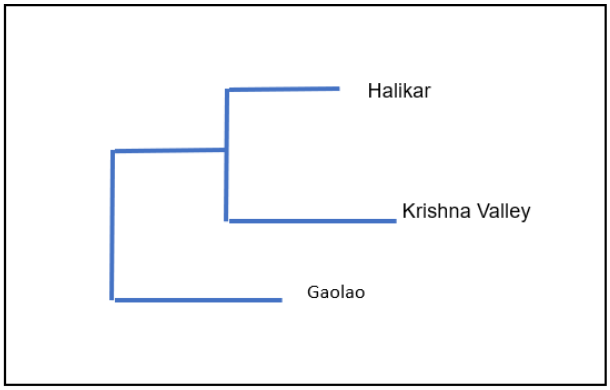


Fig. 2. Dendrogram constructed using Nei coefficient of similarity between the breeds

4. DISCUSSION

Traditional cattle molecular characterization approaches, such as protein markers, are time consuming and difficult to understand when compared to molecular genetic analysis like RAPD-PCR [14]. Molecular DNA polymorphisms are recommended for determining genetic variety in animal breeds [15]. The results demonstrate that polymorphic primers (Fig. 1), OPA-09, OPA-15, OPB-13, OPB-07, OPB-05, OPA-13, OPA-18, and OPA-01, are appropriate primers for distinguishing Gaolao, Krishna Valley, and Hallikar cow breeds. RAPD-PCR provides several features that make it the preferred molecular approach in Asia. During the screening of the 12 primers, primers OPA-09, OPA-15, OPB-13, OPB-07, OPB-05, OPA-13, OPA-18, and OPA-01 displayed polymorphisms, indicating potential differences among the three (Gaolao, Krishna Valley, and Hallikar) cow genotypes. This means that when these primers are used on control samples, genotypic variations in purebred animals become clear. The monomorphic primers OPB-06, OPA-04, OPB-03, and OPA-02 revealed no polymorphisms and hence cannot be utilized to distinguish genotypic differences amongst the breeds used in this investigation. Ramesha et al., [16] investigated the genetic distance between South Indian Zebu cattle breeds Krishna Valley and Hallikar by using different random amplified DNA markers.

Strucken et al., [17] observed genetic diversity and effective population sizes of thirteen Indian cattle breeds including Krishna Valley and Hallikar cattle. This is the first time tostudy the genetic diversity and population structure of Gaolao cow breeds which is presented in this paper.

In a study of Rathi and Tharparkar cattle breeds, Sharma et al., [18] discovered that primers OPA-01, OPA-02, OPA-04, and OPB-07 yield polymorphic bands. Primers OPA-02 and OPA- 04 produced monomorphic bands while primers OPA-01 and OPB-07 produced polymorphic bands. This could have been the result of genotypic variances among the breeds included in the study.

According to Strucken et al., [17], "the phenotypic traits of the breeds employed in their study suggested that the animals were crossbreds between Krishna Valley and Hallikar". Ramesha et al., [16] discovered "a high degree of similarity in DNA bands between Ongole and Krishna Valley cattle breeds. They indicated that the Krishna Valley breed is a blend of four unique breeds i.e., the Gir, Ongole, Kankrej, and Hallikar. The Hallikar cattle breed was found to have a lower genetic distance from Amritmahal due to breeding tract overlap. They also found that dual-purpose breeds Krishna Valley and Ongole have less genetic divergence than draft breeds Amritmahal, Hallikar and Khillari. The polymorphism revealed by the primers OPA-09 and OPA-15 showed that all three breeds were

differentiated by 100% polymorphism. All breeds shared a band size of 250 to 300 bp. This indicated that the breeds are connected or that the band size is peculiar to these cattle".

In the present work, polymorphisms of OPA-18 and OPB-13 are shown by three distinct breeds Gaolao, Krishna Valley, Hallikar. Primer OPB-05 had four different banding patterns. This demonstrates the existence of three distinct breeds or genotypes. A present study found that primer OPB-05 produced 21 polymorphic bands ranging in size from 300 to 1000 bp. Sharma et al., [18] studied in Rathi and Tharparkar indigenous (*Bos indicus*) cattle breeds that primer OPG-07 yielded a total of 8 polymorphic bands ranging in size from 400 to 1475 bp. Similarly, primer OPB-05 can be efficiently utilized to define various cow breeds based on genotypic differences.

According to Mhuka et al., [19] RAPD-PCR is successful in finding polymorphisms in bovine species. Polymorphic primers can identify genetic variants in crossbreed animals across multiple breeds using RAPD-PCR to identify breed genotype. The OPB-03 polymorphic pattern identified three distinct breeds based on their banding patterns. Lanes 2 and 3 had similar band sizes; however, lane 1 representing Gaolao only had one 400-bp band, which was not shared by the remaining, indicating genetic variation or species specificity in a Gaolao cow. The common band size indicated that, despite being crosses of two different breeds, the two animals shared genetic material from a common ancestral lineage. OPB-13 revealed amazing polymorphisms, yet certain lanes did not show a single band. This could be due to nonspecific binding during RAPD-PCR or if the template DNA did not match the existing breeds have the priming locations for OPB-15. This study provides baseline information on the primers suitable for genetic variability analysis of Gaolao, Krishna Valley and Hallikar cows in India. "Likewise, with the polymorphisms acquired with OPX-15, some lanes failed to exhibit even one band. This could have been caused by non-specific binding during RAPDPCR or the template DNA of the breeds present did not include the OPX-15 priming sites" [19].

5. CONCLUSION

RAPD-PCR is found to be successful in detecting polymorphisms within the bovine species. Polymorphic primers (OPA-09, OPA-15, OPB-13, OPB-07, OPB-05, OPA-13, OPA-18, and OPA-01) may detect genotype differences in animals, while monomorphic primers (OPB-06, OPA-04, OPB-03, and OPA-02) cannot do so. Primer OPA-04, which was used by Shrama et al., [18] shown polymorphism in Rathi and Tharparkar breeds, and monomorphic in this study. Polymorphic primers demonstrated relatedness between some of the available breeds. Primers OPA-09 and OPA-15 showed a higher degree of polymorphism than primers OPB- 13, OPB-07, OPB-05, OPA-13, OPA-18, and OPA-01, indicating that primers OPA-09 and OPA-15 may successfully identify various cattle breeds [20].

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

We hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gwakisa P, Kemp S, Teale AJ. Characterization of zebu cattle breeds in Tanzania using random amplified polymorphic markers. Animal Genetics. 1994;25: 89–94.

2. More M, Kuralkar S, Bankar P, Kuralkar P, Deshmukh S. Study of Genetic Polymorphism of DGAT1 Gene in Gaolao Cattle. Ruminant Science. 2022;11(2) :361–364.
3. Karthickeyan SMK, Saravanan R, Thangaraju P. Krishna Valley cattle in India: status, characteristics and utility. Animal Genetic Resources Information. 2006;39:25–37.
4. Kumar S Naveen, Jayashankar MR, Nagaraja CS, Govindaiah MG, Saravanan R, Karthickeyan SMK. Molecular characterization of hallikar breed of cattle using microsatellite markers. asian-Australasian Journal of Animal Sciences. 2006;19(5):622–626.
5. Hassen F, Bekele E, Ayalew W, Dessie T. Genetic variability of five indigenous Ethiopian cattle breeds using RAPD markers. African Journal of Biotechnology. 2007;6(19):2274–2279.
6. Matthew S. Molecular profiling of bauhinia accessions using RAPD markers. International Journal of Pharma and Bioscience. 2010;1(4):199-207.
7. Munthali M, Ford-Loyd B, Newbury J. The random amplification of polymorphic DNA for fingerprinting plants. School of biological sciences, University of Birmingham. Cold Spring Harbour Laboratory Press. Birmingham. UK; 1992.
8. Weber J, May P. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction., 44 The American Journal of Human Genetics. 1989;388–396.
9. Hardys H, Balick M, Schierwater B. Applications of random amplified polymorphic DNA(RAPD) in molecular ecology. Molecular Ecology. 1992; 1:55–63.
10. Williams JGK, Kubelik AR, Livak KJ, Rafalski J Antoni, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 1990;18(22): 6531–6535.
11. Kemp SJ, Teale AJ. Randomly primed PCR amplification of pooled DNA reveals polymorphism in a ruminant repetitive DNA sequence which differentiates Bos indicus and B. taurus. Animal Genetics. 2009; 25:83–88.
12. Kemp S, Teale A. Randomly primed PCR amplification of pooled DNA reveals polymorphism in a ruminant repetitive DNA sequence which differentiates Bos indicus and B. taurus. Animal Genetics. 1994; 25: 83–88.
13. Hwang KC, Song KD, Kim TH, Jeong DK, Sohn SH, Lillehoj HS, Han JY. Genetic linkage mapping of RAPD markers segregating in Korean Ogol chicken - White leghorn backcross population. Asian-Australasian Journal of Animal Sciences. 2001;14(3): 302–306.
14. Sarkar A, Saha M, Patra A, Roy P. Characterization of aeromonas hydrophila through rapd-pcr and sds-page analysis. Open Journal of Medical Microbiology. 2012;02(02): 37–40.
15. Zulu D. Genetic characterization of zambian native cattle breeds. Virginia Polytechnic Institute and State University. 2008;1–61.
16. Ramesha KP, Saravanan T, Rao MK, Appannavar MM, Reddy A. Genetic distance among South Indian breeds of Zebu cattle using random amplified DNA markers. Asian-Australasian Journal of Animal Sciences. 2002;15(3):309–314.
17. Strucken E, Gebrehiwot NZ, Swaminathan M, Joshi S, Mohammad Al Kalaldeh, Gibson JP. Genetic diversity and effective population sizes of thirteen Indian cattle breeds. Genetics Selection Evolution. 2021;53(1).
18. Sharma A, Bhushan B, Kumar S, Kumar P. Molecular characterization of rathi and thaparkar indigenous cattle *(Bos Indicus*) breeds by RAPD-PCR. Animal Genetics Division, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India. 2004;17(9): 1204-1209.
19. Mhuka C, Chatiza FP, Chidzwondo F, Sithole-Niang I, Makuza S, Mlambo SS. Use of RAPD-PCR for breed/genotype identification in Zimbabwean cattle. Journal of Cellular Biotechnology. 2017;2(2):131–137.
20. Hetzel D. Drinkwater RD. the Use of DNA technologies for the conservation and improvement of animal genetic resources. In: Hodges J (Ed) the

management of global animal genetic resources. Proceedings of an FAO Expert Consultation, Rome, Italy. 1992;309.

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