Molecular Characterization of Black Bengal and Jamuna Pari Goat Breeds By RAPD Markers

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Abstract: This experiment was conducted in the Genetics, Breeding and Reproductive Biotechnology Laboratory under Goat and Sheep Production Research Division, Bangladesh Livestock Research Institute (BLRI), Saver, Dhaka, Bangladesh. DNA was extracted from 14 goat breeds and the extracted DNA was observed by gel electrophoresis. Eight goat specific primers were synthesized by ASM-800 DNA synthesizer and screened in the study and all these primers were capable of priming polymorphic amplification pattern in both the breeds. Random amplification of polymorphic DNA – Polymerase Chain Reaction (RAPD-PCR) analysis was carried out using DNA samples of 14 black bengal goat and Jamuna pari goat breeds. Only unambiguous, reproducible and scorable polymorphic fragments were taken into consideration for analysis. Data were analyzed by using a computer programme POPGENE (Version 1.31). Highest level of Nei’s[1] gene diversity value (0.4898) was observed in BMS 1494 locus and the mean genetic diversity was obtained 0.3724 among the 14 goat breeds. The highest number of polymorphism obserbed in primer BM1818. The pair-wise genetic distance value ranged from 0.2500 to 1.000. Dendrogram based on Nei’s[1] genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the 14 goat breeds. Within Jamuna pari goat genetic similarity is low as well as black Bengal goat.

Key words: RAPD, Black Bengal goat, Jamuna Pari goat, Molecular Characterization, finger printings, genetic distance

INTRODUCTION

Black Bengal goat is the heritage and pride of Bangladesh. It is a dwarf breed found almost in all villages of Bangladesh. Like other domestic goat breeds in South Asia, Bengal goat believed to be derived from wild bezoar of Pasang (Capra aegagrus)[2] with infiltrated blood from markhor (Capra falconeri). Considering the geographical and cultural connection between south China and the Bengal area from old times across the South-Eastern off-shot of Tibetan plateau, Nozawa[3] suggested that Bengal goat inherit some genetic material from the South Chinese goats. Jamuna pari (Nubian type) is another important goat breed in Bangladesh.

A molecular marker is a DNA sequence which is readily detected and whose inheritance can easily be monitored. The use of molecular marker is based on naturally occurring DNA polymorphism, which forms the basis for designing strategies to exploit for applied purpose. A marker must be polymorphic, that is, it must exist in different forms so that chromosome carrying the mutant gene can be distinguished from the chromosome with the normal gene by a marker it also carries[4].

Molecular markers are the molecules that could be used to trace a desired gene(s) in examined genotypes. In fact, a piece of DNA or a protein can be used as a marker. Earlier approaches that made selection of specific traits easier were based on the evaluation of morphological traits[5], isozymes[6] storage proteins like glutenins, gliadins, hordeins[7] etc. However, DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA markers segregate as single genes and they are not affected by the environment as morphological makers.

Genetic diversity may be measured through genetic markers. These have been used to determine evolutionary relationship within and between species, genera or higher taxonomic categories[8]. However, breeders tend to concentrate on specific genotypes for determination of genetic diversity which combine traits of interest and may be used as progenitors in several breeding programmes in order to introduce agronomical important traits. In an attempt to solve the problem of maintaining pure breeds using the observed morphological characteristics that require a lot of time and effort, the use of molecular markers in maintaining goat breeds is more suitable and less time consuming. Moreover, molecular markers are important tools in
tagging desirable loci underlying the traits which have breeding importance.

Estimation of genetic variation increasingly are being based upon information at the DNA level by various molecular markers such as, Randomly amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeat (SSR) or Microsatellite etc. Among them, RAPD markers, generated by the polymerase chain reaction (PCR) is widely used since 1990's to assess infra-specific genetic variation at nuclear level\(^9,10\).

RAPD is a PCR based technique for identifying genetic variation. It involves the use of a single arbitrary primer in a PCR reaction, resulting in the amplification of many discrete DNA. The technique was developed independently by two different laboratories\(^9,10\) and called as RAPD and AP-PCR (Arbitrary Primed PCR) respectively. This procedure detects nucleotide sequence polymorphisms in a DNA amplification-based assay using only a single primer of arbitrary nucleotide sequence.

The RAPD technology has provided a quick and efficient screen for DNA sequence-based polymorphisms at a very large number of loci. The major advantage is that no prior DNA sequence information is required. The vast range of potential primers that can be used gives the technique great diagnostic power. Reproducible RAPD bands can be found by a careful selection of primers, optimization of PCR conditions for the target species and replication to ensure that only the reproducible bands are scored.

RAPD analysis has been used extensively for various purposes which include identification and classification of accessions\(^11,12\), identification of breeds\(^13\), genetic diversity analysis\(^14-16\) and predicting quantitative variation within germless\(^17\). RAPD markers have been successfully used for genetic variability analysis in a number of Animal species. These include cattle, goat, horse, buffalo, ostriches, chicken, broilers, quails, doves, pigs, emus etc.

The aim of this work is to evaluate genetic variation and relatedness of goat breeds by Random Amplified Polymorphic DNA (RAPD) technique. The study was conducted to determine the genetic variation between the breeds, to determine the genetic distance between two popular goat species, to select the suitable parent for crossing that can be used in hybrid production and to classify the goat breeds in Bangladesh.

**MATERIALS AND METHODS**

This experiment was conducted in the Genetics, Breeding and Reproductive Biotechnology Laboratory under Goat and Sheep Production Research Division, Bangladesh Livestock Research Institute (BLRI), Saver, Dhaka, Bangladesh. In this chapter details of different materials used and methodologies followed for the study have been described.

We used RAPD markers for genetic characterization of those goat breeds. 19 blood samples were collected from already selected pure Black Bengal goat and Jamuna pari goat from different areas. At least 5mL blood sample were collected in EDTA Vacutainer (Becton Dickinson) in the morning and after collection of the sample then kept into ice box. The samples were kept at \(-20^\circ C\) upto the work. Before take the sample it is required to thaw.

**Extraction of DNA from blood**: Blood samples were collected in sterile polypropylene centrifuge tubes containing anticoagulant. The blood was gently mixed with anticoagulant and kept on ice to maintain low temperature in order to prevent cell lysis. Subsequently the blood samples were transported to the laboratory and stored at 4°C until the isolation of genomic DNA. The genomic DNA was isolated by Roe et al\(^18\). The isolated DNA was checked by mini-gel method\(^19\).

**Preparation of agarose gel**: At first, 1.5g agarose powder (Invitrogen\(^TM\) life technologies) was taken in a 500 mL Erlenmeyer flask containing 7.5mL electrophoretic buffer (20X TAE buffer) and 142.5mL distilled water. The flask was enclosed with aluminum foil. Then the agarose was dissolved using microwave oven with occasional swirling until complete disappearance of agarose particles. A agarose solution was cooled to about 50°C. 25μL (5 mg mL\(^{-1}\)) ethidium bromide was added and mixed well by gentle shaking. The molten gel was poured immediately on to a clean gel bed (18x13x0.5 cm\(^3\) in size) and comb was inserted. Air bubbles were removed by pushing away to the side using a disposable tip. After one hour, gel became completely cooled at room temperature and solidified. The comb was removed gently and kept aside.

**Primer selection**: Eight goat specific primers were synthesized by ASM-800 DNA synthesizer and screened on a sub sample of two randomly chosen individuals from two different breeds to evaluate their suitability for amplification of the DNA sequences, which could be scored accurately. Primers were evaluated based on intensity of bands, consistency within individual, presence of smearing and potential for population discrimination. The details of the primers are given in Table 1. A final subset of four primers exhibiting good quality banding patterns and sufficient variability were selected for further analysis.

**PCR amplification and agarose gel electrophoresis**: The amplification conditions were based on Williams et al\(^10\) with some modifications. PCR reactions were performed on each DNA sample in a 10 μL reaction mix containing 2.5
RESULTS AND DISCUSSION

RAPD amplification of polymorphic DNA-Polymerase Chain Reaction RAPD-PCR is a powerful molecular genetic technique for detection of genetic variability and similarity in the different breeds/population of the livestock\textsuperscript{21,22}. This technique is highly sensitive to minor alterations in the reaction conditions\textsuperscript{23,24} hence; optimization of reaction conditions was carried out for each of the primers by varying certain components of PCR. Cycling conditions were also optimized to get the good and reproducible results.

Screening of RAPD primers: Eight random primers were used in representative 14 samples of Black Bengal Goat and Jamuna pari goat breeds in the study of amplification patterns. All of 8 primers were capable of exhibiting polymorphic amplification patterns and hence, they were used in the subsequent analysis with more number of DNA samples of the animals of these two breeds. Out of 8 primers 4 primers produced higher numbers of polymorphisms. Reproducibility of primer also checked and similar type of polymorphisms were obtained.

RAPD fingerprints: The individual samples from Black Bengal goats and Jamuna pari goat breeds were analysed with 8 primers screened in the study and all these primers were capable of priming polymorphic amplification pattern in both the breeds. Only unambiguous, reproducible and scorable polymorphic fragments were taken into consideration for analysis. The amplification patterns of representative samples of Black Bengal goats and Jamuna pari goat breeds were analysed with 8 primers screened in the study and all the primers were capable of priming polymorphic amplification pattern in both the breeds. Only unambiguous, reproducible and scorable polymorphic fragments were taken into consideration for analysis. The amplification patterns of representative samples of Black Bengal goats and Jamuna pari goat breeds were analysed with 8 primers screened in the study and all the primers were capable of priming polymorphic amplification pattern in both the breeds. Only unambiguous, reproducible and scorable polymorphic fragments were taken into consideration for analysis. The amplification patterns of representative samples of Black Bengal goats and Jamuna pari goat breeds were analysed with 8 primers screened in the study and all the primers were capable of priming polymorphic amplification pattern in both the breeds. Only unambiguous, reproducible and scorable polymorphic fragments were taken into consideration for analysis. The amplification patterns of representative samples of Black Bengal goats and Jamuna pari goat breeds were analysed with 8 primers screened in the study and all the primers were capable of priming polymorphic amplification pattern in both the breeds. Only unambiguous, reproducible and scorable polymorphic fragments were taken into consideration for analysis. The amplification patterns of representative samples of Black Bengal goats and Jamuna pari goat breeds were analysed with 8 primers screened in the study and all the primers were capable of priming polymorphic amplification pattern in both the breeds. Only unambiguous, reproducible and scorable polymorphic fragments were taken into consideration for analysis.
Table 4: Pair-wise mean genetic distance of 14 goat breeds

|   | B1     | B2     | B3     | B4     | B5     | B6     | B7     | B8     | B9     | J10    | J11    | J12    | B13    | B14    |
|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|   | ****   | 1.0000 | 0.7500 | 0.5000 | 0.7500 | 1.0000 | 1.0000 | 0.2500 | 1.0000 | 0.2500 | 1.0000 | 0.2500 | 0.5000 |
| B2 | ****   | 0.7500 | 0.5000 | 0.7500 | 1.0000 | 1.0000 | 0.2500 | 1.0000 | 0.7500 | 0.2500 | 1.0000 | 0.2500 | 0.5000 |
| B3 | ****   | 0.7500 | 1.0000 | 0.5000 | 0.7500 | 0.5000 | 0.5000 | 0.7500 | 0.5000 | 0.5000 | 0.7500 | 0.5000 | 0.5000 |
| B4 | ****   | 0.7500 | 0.5000 | 0.7500 | 0.7500 | 0.5000 | 0.7500 | 0.7500 | 0.7500 | 0.5000 | 0.7500 | 0.5000 | 0.7500 |
| B5 | ****   | 0.7500 | 0.5000 | 0.7500 | 0.7500 | 0.5000 | 0.5000 | 0.7500 | 0.5000 | 0.7500 | 0.5000 | 0.7500 | 0.5000 |
| B6 | ****   | 0.7500 | 0.5000 | 0.7500 | 0.7500 | 0.5000 | 0.7500 | 0.5000 | 0.7500 | 0.5000 | 0.7500 | 0.5000 | 0.7500 |
| B7 | ****   | 0.7500 | 0.5000 | 0.7500 | 0.7500 | 0.5000 | 0.7500 | 0.5000 | 0.7500 | 0.5000 | 0.7500 | 0.5000 | 0.7500 |
| B8 | ****   | 0.7500 | 0.5000 | 0.7500 | 0.7500 | 0.5000 | 0.7500 | 0.5000 | 0.7500 | 0.5000 | 0.7500 | 0.5000 | 0.7500 |

Fig. 1: RAPD profiles of fourteen goat breeds using primer BMC1222 Reverse. Lane 1-9 and 13 and 14 are Black Bengal goat and Lane 10-12 are Jamuna pari goat breeds

Fig. 2: RAPD profiles of fourteen goat breeds using primer BMC1222 Forward. Lane 1-9 and 13 and 14 are Black Bengal goat and Lane 10-12 are Jamuna pari goat breeds

Data were analyzed by using a computer programme POPGENE (Version 1.31)[20] and found dendrogram a UPGMA (Unweighted Pair Group Method of Arithmetic Means).

Gene diversity: Nei’s[1] gene diversity values are shown in (Table 2). Highest level of Nei’s[1] gene diversity value (0.4898) was observed in Bms1494 locus and the mean genetic diversity was obtained 0.3724 among the 14 goat breeds (Table 2).

Gene frequency: The highest level of gene frequency value (0.9286) was observed in allele 0 with the primer BM1818 and the lowest frequency value (0.0714) was obtained in allele 1 using same primer (Table 3). The RAPD method only score the homozygous and heterozygous dominant allele but unable to detect homozygous recessive one (H.S.Chawla).

Pair-wise genetic distance: The pair-wise mean genetic distance value ranged from (above diagonal) 0.2500 to 1.000.

Dendrogram without root: Dendrogram based on Nei’s[1] genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the 14 goat breeds. Within Jamuna pari goat genetic similarity is low as well as black Bengal goat. But genetic distance is high between the two breeds. All data were analyzed by a computer programme, POPGENE (Version 1.31) [20]. The UPGMA dendrogram (Fig. 1) shows that the 14 breeds divided in three main clusters. Cluster 1 consisted of B1609, B1853, B1952, B1969, B1990, J22 and J01. The 2nd cluster consisted of three breeds like B0701, B1861 and B1752 and cluster 3 consisted with B080302, B1648, B1972 and J08. Cluster 1 shows Black Bengal goat and Jamuna pari goat breeds of same region. Cluster 2 and 3 is concentrated with all black Bengal goat breeds.

Dendrogram with root: Dendrogram with root means to show the ancestors of the progenies. In the present study 14 goat breeds were studied for their genetic characterization J01, J08 and J22 are Jamuna pari goat breeds and B1609, B1853, B1861, B1648, B1752, B1952, B1969, B1972, B1990, B070104 and B080302 are black Bengal goat breeds. Cluster 1 consisted of B1609, B1853, B1952, B1969, B1972, B1990, B070104 and B080302 are black Bengal goat breeds. Cluster 1 consisted of B1609, B1853, B1952, B1969, B1972, B1990, J22 and J01 that mean those had a common ancestor. The 2nd cluster consisted of three breeds like B0701, B1861 and B1752 and cluster 3 consisted with B080302, B1648, B1972 and J08. Cluster 1 shows Black Bengal goat and Jamuna pari goat breeds of same region. Cluster 2 and 3 is concentrated with all black Bengal goat breeds.
CONCLUSION

It is concluded that the results of the present study indicated that using these markers genetic diversity within black Bengal goat breeds is low and genetic distance within Jamuna pari goat breeds also low. Genetic distance between black Bengal goat and Jamuna pari goat breeds is very high. In fact according to the report of Kantanen et al.\(^{[25]}\), goat breeds have high degree of homogeneity. This RAPD work is the initial work in Bangladesh and has studied only with 14 goat breeds. It is suggested to study with more primers and large samples to analyze genetic characteristics between Black Bengal goat and Jamuna pari goat breeds.

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