

Genetic Diversity of Red Chittagong Cattle Using Randomly Amplified Polymorphic DNA Markers

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Abstract: Problem statement: Red Chittagong Cattle (RCC) is a potential genetic resource in Bangladesh having dairy potentialities, high resistance to disease and survive harsh environmental condition while require low input supply. This resource is now disappearing due to indiscriminate crossing with other local and exotic cattle. To conserve this resource, find out the genetic diversity of RCC is prime concern. **Approach:** To determine the genetic variation of Red Chittagong Cattle and to find out the genetic present status for their future improvement and conservation program. The experiment was conducted in the Molecular Genetics Laboratory under Animal Production Research Division, Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh. DNA was extracted from 100 RCC blood samples and the extracted DNA was observed by gel electrophoresis. Randomly 16 primers were tested among them three primers were matched and found polymorphic. Random Amplification of Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analysis was carried out using DNA samples of RCC. Only unambiguous, reproducible and scorable polymorphic fragments were taken into consideration for analysis. Data were analyzed by using a computer program POPGENE (Version 1.31). **Results:** The highest and the lowest level of gene frequency value (0.9590 and 0.0440) were observed in allele 0 and 1 with BMC1222-2 locus and only one monomorphic locus (OPB07-1) was found. The Nei's gene diversity and Shannon's information indicated that the highest and the lowest diversity were found in Anwara (0.2925) and Chandonish (0.2147) respectively. The pair-wise mean genetic distance value ranged (below diagonal) from 0.0092-0.0279. The minimum distance was found between Satkania and Chandonish. On the other hand maximum distance was found between RCC BLRI and Potia. The dendrogram segregated the five population of RCC into two clusters: RCC BLRI and Anwara is one cluster whereas Satkania, Chandonish and Potia made another cluster. **Conclusion:** The genetic diversity of RCC is relatively higher for a prescribed breed and therefore, have an opportunities to improve them using selective breeding like ONBS.

Key words: Red chittagong cattle, RAPD, PCR, genetic variation, genetic distance

INTRODUCTION

Red Chittagong Cattle (RCC) is a potential and promising animal genetic resource in Bangladesh. It is a dwarf type, red color, having dairy potentialities, high resistance to disease and survive harsh environmental condition while require low input supply. The productive and reproductive performance of RCC comprises; daily milk yield, lactation length, age at puberty, service per conception and post partum heat period are 2.46 ± 0.40 kg^[1], 283 ± 58.7 ^[1], 33.41 ± 4.23 months^[2], 1.47 ± 0.61 ^[2] and 54.28 ± 9.64 days^[2]

respectively. The milk fat % is 6, which is higher than other dairy breed.

Like other developing countries, breed substitution and crossbreeding program has been practiced indiscriminately in Bangladesh for many years with exotic breed and as a result valuable genetic resources is being lost or diluted day by day. Assessment of genetic variation is a crucial element in determining breeding strategies and for effective and meaningful conservation program^[3].

Genetic diversity or similarity may be measured through genetic markers. These have been used to

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determine evolutionary relationship within and between species, genera or higher taxonomic categories^[4] 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 programs in order to introduce agro-ecological 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 cattle 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.

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] RAPDs^[6] storage proteins like hordeins^[7]. However, DNA markers seem to be the best candidates for efficient evaluation and selection of animal population. Unlike protein markers, DNA markers segregate as single genes and they are not affected by the environment as morphological markers. Presently, the PCR-based RAPD fingerprinting assays are being used for characterization of zebu cattle breeds^[8], for detection of genetic variations in cattle and sheep^[9].

Estimation of genetic variation increasingly is being based upon information at the DNA level by various molecular markers such as, RAPD, AFLP, RFLP, SSR or Microsatellite. 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^[10,11]. This procedure detects nucleotide sequence polymorphisms in a DNA amplification-based assay using only a single primer of arbitrary nucleotide sequence. The polymorphism between individuals result from sequence differences in one or both of the primer binding sites and are visible as the presence or absence of a particular RAPD band. The aim of this work is to determine the genetic variation and relatedness of Red Chittagong Cattle and to find out the present status for their future improvement and conservation programme.

MATERIALS AND METHODS

This experiment was conducted in the Molecular Genetics Laboratory under Animal 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.

Collection of blood: One hundred blood samples were collected from RCC; 40 from Bangladesh Livestock Research Institute (BLRI) RCC Farm and 60 from four areas (Anwara, Potia, Chandonish, Satkania) of Chittagong district. Blood were taken from the jugular vein using specialized vacuum blood collection vial in which EDTA was used as an anticoagulant. The samples were kept at -20°C until the study start.

Extraction of genomic DNA: Genomic DNA was extracted from blood following the method described by^[12] with some modifications. 500 µl of blood was taken in a microcentrifuge tube and breaks the cell membrane with 1X Standard Saline Citrate (SSC) buffer. Then protein were precipitated by sodium acetate and digested with proteinase K at 55°C for 1 h. After that, aqueous layer was removed by Phenol: Chloroform: Isoamyle alcohol (25: 24:1) and precipitated with 100% ethanol. DNA was reprecipitated and washes with Na acetate and 70% ethanol. Finally DNA samples were dried and dissolved in TE buffer and stored at -20°C until use.

Gel documentation: Extracted DNA was confirmed by electrophoresis separation on 1% agarose gel staining with ethidium bromide.

DNA quantification: Ultraviolet (UV) spectrophotometer was used for the quantification of DNA concentration. For the quantification of DNA, 260nm absorbance is recommended^[13]. Double-stranded DNA was calculated by using the following formula:

$$\text{DNA concentration (ng } \mu\text{L}^{-1}) = \frac{\text{Absorbance} \times \text{volume of distilled water} (\mu\text{L})}{\text{Amount of DNA sample} (\mu\text{L})} \times \text{Conversion factor (0.05)} \times 1000$$

Primer selection: Randomly 16 primers were tested among them three primers were matched and found polymorphic. These primer sequences are shown in Table 1.

Table 1: Primer matched

Primer code	Primer sequence 5'-3'	GC content (%)
OPA 02	TGCCGAGTCG	70
OPB 07	GGTGACGCAG	70
BMC1222	CCTGAGTGTTCCTCCTGAGT	55

Table 2: Estimation of gene frequency

Allele/ Locus	OPA02-1	OPA02-2	OPA02-3	OPA02-4	OPB07-1	OPB07-2	OPB07-3	OPB07-4	OPB07-5	BMC122-1	BMC122-2	BMC122-3	BMC122-4
Allele 0	0.572	0.563	0.749	0.569	-	0.343	0.649	0.624	0.436	0.704	0.956	0.853	0.093
Allele 1	0.428	0.437	0.251	0.431	1.000	0.657	0.351	0.376	0.564	0.296	0.044	0.147	0.907

Marker 1 2 3 4 5 6 7 8 9 10

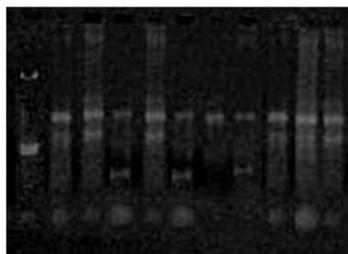


Fig. 1: RAPD Profiles of RCC using primer OPA-02

PCR amplification: PCR reactions were performed on each DNA sample in a 25 μ L reaction mix containing 4.0 μ L dNTPs of 2.5 mM, 2.5 μ L of 10 X Ampli Taq Polymerase buffer, 1.25 μ L of 50 mM MgCl₂, 1 μ L of 100 p.mol μ L⁻¹ primer, 0.20 μ L of Taq polymerase (5U μ L⁻¹), 4.0 μ L of DNA template and suitable amount of distilled water. PCR cycling conditions were: 3 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at annealing temperature (32-38°C) of each primer, 2 min elongation at 72°C and final extension of 10 min at 72°C.

Agarose gel electrophoresis: The amplified product from each sample was separated electrophoretically on 2% agarose gel containing ethidium bromide in 1X TAE buffer. DNA bands were observed under UV light on a UV transilluminator and photographed by a polarized camera. A molecular weight marker DNA (100 bp DNA Ladder) was electrophoresed alongside the RAPD reactions.

Analysis of molecular data: Different measures of within breed genetic variations, namely number of alleles, allele frequencies^[14], gene diversity, Shannon's information index, genetic distance were estimated using the POPGENE software package^[15] with 1000 simulated samples to evaluate variability at DNA level.

RESULTS

Estimation of genetic similarity within and between breeds and genetic distance among different breeds of livestock is an important application of the

DNA based genetic markers. The above information is of immense importance in breed characterization and conservation studies as well as in selection programs as they are essential for efficient sampling and utilization of germ plasm resources and for making decisions regarding choice of parents^[16]. In present investigation, genetic diversity was determined by the estimation of gene frequency, genetic variation, pair wise genetic distance and dendrogram illustration.

Gene frequency: Gene frequency measures the frequency in the population of a particular gene relative to other genes at its locus. In population genetics, gene or allele frequencies show the genetic diversity of a species population or equivalently the richness of its gene pool. The gene frequencies of RCC in different locations are shown in Table 2.

Genetic variation: Genetic variation is important because it provides the "raw material" for natural selection. Genetic variation is caused by variation in the order of bases in the nucleotides in genes. Genetic variation among individuals within a population can be identified at a variety of levels. It is possible to identify genetic variation from observations of phenotypic variation in either quantitative traits or discrete traits^[17]. The genetic variation in RCC is shown as Nei's gene diversity, Shannon's information and proportion of polymorphic bands in Table 3. Table 3 shows that the Nei's gene diversity was more or less similar for all populations and it ranges from 0.2147-0.2925. The proportion of polymorphic band was higher in Potia population (76.92%) than others.

Pair wise genetic distance and dendrogram: Pair wise genetic distance and dendrogram indicates the genetic relationship among different groups. Nei's pair-wise genetic distance, calculated between all pairs of 5 populations, is shown in Table 4. The pair-wise mean genetic distance value ranged (below diagonal) from 0.0092- 0.0279. The average pair wise genetic distance, 0.0155 was found among 5 populations.

Dendrogram stability is an important aspect to be considered in genetic variability studies^[18]. A dendrogram based on genetic distance using the Unweighted Pair-Group Method of Analysis (UPGMA) is shown in Fig. 2.

Table 3: Estimates of genetic variation

Population	Nei's gene diversity	Shannon's Information	Proportion of polymorphic bands (%)
RCC BLRI	0.2259	0.3278	53.85
Satkania	0.2750	0.4010	69.23
Potia	0.2706	0.4046	76.92
Chandonish	0.2147	0.3160	53.85
Anwara	0.2925	0.4228	69.23

Table 4: Pari-wise genetic distance among RCC population in different locations

	RCC				
	BLRI	Satkania	Potia	Chandonish	Anwara
RCC BLRI	****	0.9603	0.9568	0.9683	0.9895
Satkania	0.0229	****	0.9805	0.9872	0.9814
Potia	0.0279	0.0120	****	0.9820	0.9725
Chandonish	0.0235	0.0092	0.0102	****	0.9827
Anwara	0.0107	0.0112	0.0203	0.0173	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

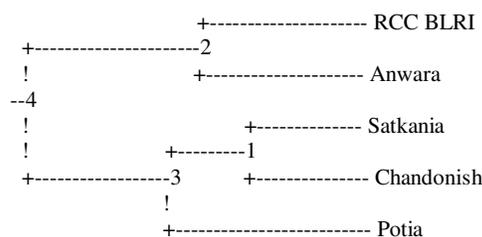


Fig. 2: UPGMA dendrogram based on Nei^[18] genetic distance summarizing the data on genetic differentiation in five populations of RCC using RAPD

Discussion

Among the 16 primer initially tested, three primers OPA-02, OPB-07 and BMC122 yielded polymorphic bands. They generated 13 distinct bands or RAPD markers, of which 12 (93.31%) were considered as polymorphic. The DNA polymorphisms are detected by band presence versus band absence. Primer OPB-07 gave DNA profiles with more bands than OPA-02 and BMC122. Figure 1 shown the RAPD profile of Red Chittagong cattle using primer OPA-02.

Although only three primers were used the present study, a total of 12 (92.31%) polymorphic loci detected in the present study indicate the effectiveness of RAPD techniques to study polymorphism and genetic relatedness in the five different Red Chittagong population.

Gene frequency: The highest and the lowest level of gene frequency value (0.9590 and 0.0440) was observed in allele 0 and 1 with BMC1222-2 locus and only one monomorphic locus (OPB07-1) was found (Table 2). The RAPD method only score the

homozygous and heterozygous dominant allele but unable to detect homozygous recessive one^[19].

Genetic variation: Seven to ten polymorphic (53.85-76.92%) loci present in all the five populations (Table 3) indicated that sufficient level of genetic variation was observed among different individuals of each population. The Nei's gene diversity and Shannon's information indicated that the highest diversity found in Anwara (0.2925) (Table 3). The highest gene diversity indicated that the Anwara RCC population is alarming to deteriorate with native or exotic blood than other populations. On the other hand, the Chandonish showed lowest genetic diversity (0.2147).

Pair Wise genetic distance: The minimum distance was found between Satkania and Chandonish (0.0092) (Table 4). It's indicated that the genetic relationship between these populations was closer than others. On the other hand, maximum distance was found between RCC BLRI and Potia. Over all, the pair wise genetic distance values were low for all populations and it indicated that the RCC population was closer related.

Dendrogram: Dendrogram based^[20] genetic distance using UPGMA depicted the relationship among five population of RCC (Fig. 2). The dendrogram segregated the five population of RCC into two clusters: RCC BLRI and Anwara is one cluster whereas Satkania, Chandonish and Potia made another cluster. These cluster indicated that RCC BLRI was belongs to Anwara population.

CONCLUSION

It can be concluded that overall genetic variation in each population from^[20] gene diversity, Shannon's Information index and proportion of polymorphic loci points of views is relatively higher for a prescribed breed.

The differences of between population genetic variations in the five studied populations were low and therefore, there is scope of selective breeding (like, ONBS) for future improvement of Red Chittagong Cattle.

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