MOLECULAR CHARACTERIZATION OF SLECTED LOCAL CATTLE USING RAPD MARKERS

A Thesis

By

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Examination Roll No: MS-080712
Session: 2007-2008

Submitted To

Biotechnology and Genetic Engineering Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh
In partial fulfillment of the requirement for the degree of Masters of Science (MS) in Biotechnology and Genetic Engineering

BIOTECHNOLOGY AND GENETIC ENGINEERING DISCIPLINE
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KHULNA-9208
BANGLADESH

JULY 16, 2011

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DECLARATION

This thesis report is the original research work of the author, except as otherwise stared. The material presented has not submitted either in whole or in part for a degree for this or any other university. The findings of the research will not be/ has not been published anywhere without the concurrence of the concerned supervisor and co-supervisor.

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This thesis is dedicated to my beloved parent

Acknowledgement

At first, I would like to express my greatest and deepest gratitude to the almighty Allah, to whom all praises go for enabling me to complete this work. Guidance, help and co-operation have been received from several persons and authority during the tenure of the study, the author is immensely grateful to all of them. Although it is not possible to mention everyone by name, it will be an act of ungratefulness if some names are not mentioned here.

I would like to express my deepest and sincere gratitude, heartfelt respect, profound regards and indebtedness to my respected thesis supervisor **Dr. Khondoker Moazzem Hossain,** Professor and Head, Biotechnology and Genetic Engineering Discipline, Khulna University for his efficient supervision, invaluable advice and excellent support, scholastic guidance, untiring assistance, constructive criticism, valuable suggestions and continuous encouragement in every aspect from the very beginning to the completion of this research work.

I would like to express my sincere appreciation, gratefulness and deep indebtedness to my reverend teacher and co-supervisor **Dr. S.M. Mahbubur Rahman,** Professor, Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208 for his kind co-operation, excellent advice, affection, constructive comments, valuable suggestions and encouragement throughout this research work.

I am grateful of Ministry of Science Information and Communication Technology (MOSICT) through my honorable Supervisor, Professor, **Dr. Khondoker Moazzem Hossain,** honorable co-supervisor Professor, **Dr. S.M. Mahbubur Rahman** and honorable teacher **Dr. Md. Morsaline Billa** (Associate Professor) for providing financial support to conduct the research work.

Sincere appreciations and thanks are due to **Saifur, Sayeem, Mitul, Arif and Salim** of BGE discipline of Khulna University for their encouragement, sincere co-operation and suggestion regarding conduction of research work.

Finally, all my hearty thanks and gratefulness are due to my parents for all their immeasurable blessing, continuous inspiration and financial support throughout this study.

The Author

CONTENTS

ABSTRACT

The study was conducted at Animal Biotechnology Cell Culture and Molecular Biotechnology laboratory of Biotechnology and Genetic Engineering Discipline, Khulna University, Bangladesh. DNA was extracted from 20 local cattle blood samples and the extracted DNA was observed by gel electrophoresis. Random Amplification of Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analysis was carried out using DNA samples of local cattle. Only distinct, reproducible and scorable polymorphic fragments were taken into consideration for analysis. Two primers those used in RAPD analysis of 20 cattle amplified 95 different reproducible bands of which 72 (75.79%) were found to be polymorphic and 23 bands (24.21%) were found to be monomorphic in nature. The frequencies of polymorphic bands varied from primer to primer. Though no sample-specific marker was identified, the high level of polymorphism revealed by the proportion of polymorphic loci (83.33%) indicated that RAPD markers could be considered as effective tools for estimating genetic diversity in different samples of cattle. The dendrogram shows that all the animals were grouped into two major clusters I and II. Cluster I sub divided into I A and I B. Under cluster –II, Madaripur-1, 6, 7 and Sariatpur-18, 20 were genetically similar. Therefore, they grouped into same cluster. Under cluster -1, Madaripur-2, 3, 4, 5, 8, 9 Faridpur-10, 11, 12, 13, 14, 15, 16 and Sariatpur-17, 19 showed same characteristics but different from cluster-1. Similarly sub-cluster-1A Madaripur- 2, 4, 5, 8, 9; Faridpur-10, 11, 12, 13, 14, 15, 16; Sariatpur-17were similar but were dissimilar from sub-cluster -1B-Madaripur-3, 9. There was a high level of genetic variation among the studied samples of cattle as indicated by the proportion of polymorphic loci. Estimation of higher level of genetic variation in the breeds might be consistent with the fact that it is a highly polymorphic animal. The values of pair-wise comparisons of Nei's (1979) genetic distance among 20 cattle genotypes were computed from combined data sets for the 2 primers ranging from 0.00 to 1.00. Comparatively, higher genetic distance (1.00) was found among 14 (Madaripur-3, 9), (Faridpur- 11, 15), (Sariatpur-18, 20) versus Madaripur-2, 5, 7, 9, Faridpur- 11, 12, 15 and Sariatpur-17, 18. The lowest genetic distance (0.00) was observed between Faridpur-15 and Madaripur-5. The range of genetic distance of 20 genotypes is (1.00±0.00) and the difference between the highest and lowest genetic distances indicated the presence of variability among the 20 genotypes of cattle. The genetic diversity of local cattle is relatively higher for a prescribed group of animal and therefore, have an opportunities to improve them using crossbreeding programme.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIARTION

%	:	Percent
ml	:	Micro liter
mM	:	Micro molar
0 C	:	Degree Celsius
A260	:	Spectrophotometer Absorbance Reading at 260 nm
A280	:	Spectrophotometer Absorbance Reading at 280 nm
AFLP	:	Amplified Fragment Length Polymorphism
Contd.	:	Continued
d H2O	:	Distilled water
DNA	:	Deoxyribonucleic acid
dNTPS	:	Deoxy nucleotide triphosphates
DW	:	Distilled water
e.g.	:	Exempli gratia = for example
EDTA	:	Ethylene Diaminetetra Acetic Acid
et al.	:	Et alia = and others
EtBr	:	Ethidium Bromide
etc.	:	et. cet. Era = and the others
gm	:	Gram
HCl	:	Hydrochloric acid
i.e.	:	id est = that is
M	:	Molar
mg	:	Milligram
mg/l	:	Milligram per litre

ml.	Milliliter	
mM	:	Milli mole
N	:	Normal solution
Na2EDTA:		Sodium salt of ferric ethylene diamine tetraacetate
NaCl	:	Sodium chloride
NaOH	:	Sodium hydroxide
OD	:	Optical density
PCR	:	Polymerase Chain Reaction
pH	:	Negative logarithm of hydrogen ion (H+) concentration
RAPD	:	Random Amplified Polymorphic DNA
RFLP	:	Restriction Fragment Length Polymorphism
SDS	:	Sodium Dodecyl Sulphate
SSR	:	Simple Sequence Repeats
UV	:	Ultra violet
viz.	:	Videlicet (= namely)
w/v	:	Weight per volume

Introduction

Cattle are one of the most important domestic animals in Bangladesh like many other developing countries. It is raised mainly for milk and meat production with skin as a secondary product.

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, (Botstein *et al.* 1980).

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, isozymes storage proteins like glutenins, gliadins, hordeins 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 markers, (Erlich, 1989).

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. 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 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, (Baumung *et al.* 2004).

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 intra-specific genetic variation at nuclear level, (Goldstein *et al.* 1995)

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 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, (Erlich, 1989).

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, identification of breeds, genetic diversity analysis and predicting quantitative variation within germless. 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, (Williams *et al.* 1990).

Objectives Of the study:

This thesis work has been undertaken the following objectives:

- To determine the molecular characterization of selected experimental cattle and compared the relationship among selected cattle of selected regions by RAPD markers.
- To find out the genetic distance for determining the genetic variability of selected experimental cattle.

REVIEW 0F LITERATURE

In the past decade there have been tremendous efforts globally to study genetic diversity in livestock species. This, in part, reflects a measurable indicator of an output to the Food and Agriculture Organization (FAO) global whose programme addresses the needs of both development and conservation of animal genetic resources in the different parts of the world. The efforts also reflect global concern that a surprisingly narrow range of animal species is used for agriculture (sheep, goats, cattle, pigs, buffaloes, chickens and others that have local importance to certain areas) and this pool is threatened by genetic dilution. Shrinkage of the animal genetic resource pool is, to a great extent, a result of the radical shift of livestock development from subsistence to commercial farming systems. The world is witnessing increased specialisation of animal production objectives and competitive pressures arising from the specialisation. In Bangladesh, where the role of the livestock sector in gross agricultural output is increasing, specialisation has resulted in breed replacement, where indigenous cattle are silently upgraded via artificial insemination with semen of *Bos taurus* breeds to achieve productivity gains. Although indigenous zebu cattle account for as much as 90% of the total cattle populations in Asian countries (Teale *et al.* 1993), crossbreeding with taurine breeds constitutes a significant threat to the local genetic resource. Cattle breeds of Bangladesh are of particular importance because of their adaptation to a wide range of environmental conditions, such as heat, drought and tropical diseases. It is important to know the diversity and relationship between the breeds and strains of livestock. Moreover, it is important because, quite often, the same breed may be known by different names or two breeds may be known by the same name, based on geographical locations of such populations (Gwakisa *et al.* 1994). The need to curb the threats of dilution and extinction of the African animal genetic resources, by strategic development and conservation, has never been more justifiable and is now, with the available technology, timely. Efficient strategies for conservation require sets of genetic markers, which characterise distinct populations (Kemp and Teale, 1994).

2.1. Origin and Distribution

There are many species belonging to the family are found throughout the world. The existing main groups of bovidae (domestic cattle) e.g., *Bos indicus* (India and Africa) and the other 2.Bos taurus (European), are descended from *Bos primigenius*, the original wild cattle of Europe, now no longer in existence. *Bos taurus* found in Europe and North America are all non humped cattle which were first domesticated nearly 10,000 years ago somewhere in middle East. Our indigenous zebu cattle (*Bos indicus*) or humped cattle might have been produced by selection from non-humped cattle either in South or South West Asia.The local cattle originated in Africa, Asia. The original stocks were the black, red, gray and white animals. After the New World was settled and markets began to develop for milk in America, Europe. The breed is widely distributed in Western Europe, India, China, Pakistan, Bangladesh, South America and South Africa (www.FAO.com).

2.2 Morphological characteristics

Local cattle are an important part of Bangladesh. The physical and morphological characteristics and the productive and reproductive performances of local cattle were studied. Morphometric measurements (body length, height at wither and heart girth) were taken from local cattle of different categories. In all categories of the animals, heart girth was highest followed by height at wither and body length giving the appearance of a small, compact body. Males had greater body measurements when compared to females. It is observed that the body length, body height and heart girth of adult Red Chittagon females were 114.38±1.56 cm, 107.71±0.93 cm and 139.85±1.63 cm respectively. For adult male the same measurements were 134, 125 and 168 cm respectively. The coat color of local cows was mostly black, white, gray and red brown. The tail switch was black in all animals. The horns were small to medium and curved inward with pointed tips. The ears were small and erect with a sideways orientation and had pointed tips. The face was small and narrow with a flat forehead. The body was small, compact and less fleshy. The skin was tight, the dewlap was medium and the hump was small in females and developed in males. The tail was long and reached to below the hock. Cows had small udders, teats and milk veins. The animals of this breed were usually quiet but adult males were of aggressive temperament, (Habib *et al.* 2003).

2..3 Reproductive characteristics

In Madaripur, Faridpur and Sariatpur district of Bangladesh, there are two cattle production systems: a draught-oriented system with local Deshi cattle and more milk-oriented production. Deshi animals are small. The calving interval in Deshi cows is one year long. The great majority of the bullocks and the bulls are used as draught animals. A local cow can produce 3-5 liter of milk per day (www. NCBI. Com). Indigenous cattle in Bangladesh are adapted to harsh local environmental conditions like high temperatures, drought, and tropical diseases these cattle are important resources. They also have important roles in socio-economic development. The origins of these breeds form the basis for understanding and improving them. Milk production in Bangladesh is reported to have increased from 14.9 thousand tonnes in the year 1993–94 to 16.2 thousand tonnes in the year 1997–98. This increase was due to recent government policy and to NGOs involvement (e.g. subsidies to establish small dairy farms, soft fund loans from the government as well as NGOs and improved veterinary health care) in dairy development activities, (Ahmed, 2000).

2. 4. Need for the Genetic Characterization of Livestock Population

The 'World Watch List for Domestic Animal Diversity Report documents more than 6300 breeds of breeds of livestock belonging to 30 domesticated species. These breeds were developed following domestication and natural and human selection over the past 12,000 years. The current number of breeds is likely an underestimation as a large proportion of indigenous livestock populations of the developing world, where most animal genetic resources are today found, have yet to be described at phenotypic and genetic levels. Livestock populations have evolved unique adaptation to their agricultural production system and agro-ecological environments. Their genetic diversity has provided the material for the very successful breeding improvement programs of the developed world in the 19th and 20th century, (Schearf, 2003)

However, livestock diversity is shrinking rapidly. With the exception of the wild boar - the ancestor of the domestic pig - and wild red junglefowl - the ancestor of the domestic chicken the putative wild ancestors of our major livestock species, the reservoir of genetic diversity, are now either extinct (e.g. the auroch the wild ancestor of cattle or the ancestral species of the Old World camelids) or low in numbers and threatened by extinction (e.g. wild goat populations of the Near

East, vicuña from Andean plateau, wild donkey in Africa). Among the domesticated populations, it is estimated than 1 to 2 breeds are lost every week. However, the impact of these losses on the global or the local diversity remains undocumented. While it is already too late for many breeds in Europe, the situation is also particularly worrying in the developing world where rapid changes in production systems are leading to the replacement of breeds or at best crossbreeding. There is therefore an urgent need to document the diversity of our livestock genetic resources and to design strategies for their sustainable conservation. The task is enormous. It has prompted the Food and Agricultural Organization (FAO) and other international organizations to develop domestic animal diversity information systems and databases (DAGRIS and DAD-IS 2.0). More recently, FAO has initiated a major country-driven documentation exercise, the 'State of the World's Animal of Animal Genetic Resources' (AnGR). Molecular markers provide different types of information such as individual genetic identification, parentage analysis, population diversity estimations, differentiation of populations, and calculation of genetic distances, genetic relationships, population genetic admixture estimation and inbreeding estimation. Genetic characterization through the use of molecular marker methods associated to powerful statistical approaches is providing new avenues for decision-making choices for the conservation and rational management of AnGR, (Schearf, 2003).

2.5. Molecular techniques in the study of animal genetic diversity

A variety of different molecular techniques are being used in various laboratories for the study of inter- and intra-specific genetic variation at the DNA level. The most widely used techniques are restriction fragment length polymorphisms of nuclear DNA and mitochondrial DNA, minisatellites, microsatellites, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism and sequencing of mitochondrial DNA. These techniques differ in the type of data they generate, in the way that they resolve genetic variations and in the taxonomic levels at which they may be most appropriate. As a rich selection of molecular techniques is available, choice of method to use for genetic diversity is quite often dictated by the power of the method to generate reproducible polymorphism that can either be tracked in a Mendelian fashion or can segregate a phenotypic trait in a predictable manner. However, aspects such as lack of specialised equipment, high running costs and lack of technical competence continue to impede many laboratories in research institutions. Many researchers in Africa would

benefit if laboratory technology development was paralleled by (1) simplification in technology and ultimately reduction in expense and (2) increase in analytical power per unit effort. The discovery of the polymerase chain reaction (PCR)-based RAPD technique has made a substantial contribution as a simple technique, which is affordable to a broad community working in molecular genetics with limited resources.

2. 5. 1. Principle of RAPD

The PCR-based RAPD technique is an attractive complement to conventional DNA fingerprinting. RAPD analysis is conceptually simple. Nanogram amounts of total genomic DNA are subjected to PCR using short synthetic oligonucleotides of random sequence. The amplification protocol differs from the standard PCR conditions in that only a single random oligonucleotide primer is employed and no prior knowledge of the genome subjected to analysis is required. When the primer is short (e.g. 10-mer), there is a high probability that the genome contains several priming sites close to one another that are in an inverted orientation. The technique essentially scans a genome for these small inverted repeats and amplifies intervening DNA segments of variable length. The profile of amplification products depends on the template-primer combination and is reproducible for any given combination. The amplification products are resolved on agarose gels and polymorphisms serve as dominant genetic markers, which are inherited in a Mendelian fashion (Williams *et al.* 1990). Amplification of non-nuclear RAPD markers is negligible because of the relatively small non-nuclear genome sizes. Two modifications of detection of RAPD markers have been described as DNA amplification fingerprinting (DAF) and arbitrarily primed polymerase chain reaction (AP-PCR). DAF uses short random primers of 5-8 base pairs (bp) and visualises a relatively greater number of amplification products by polyacrylamide gel electrophoresis and silver staining (Caetano-Anolles *et al.* 1991). AP-PCR uses slightly longer primers (such as universal M13) and amplification products are radioactively labelled and are also resolved by polyacrylamide gel electrophoresis (Welsh and McClelland 1990). Standard RAPD analysis is performed according to the original method (Williams *et al.* 1990) using short oligonucleotide primers of random sequence which can either be locally synthesised or are commercially available (Operon Technologies Inc., Alameda, California, USA). Only high-molecular weight, i.e. non-degraded,

DNA should be subjected to RAPD analyses. Amplified products can be resolved by gel electrophoresis on 1-2% agarose gels.

2.6. Genetic Characterization of Cattle

Gwakisa *et al.,* (1994) carried out the characterization of Zebu cattle breeds in Tanzania using random amplified polymorphic DNA markers. A total of 141 short primers, of arbitrary nucleotide sequence, were used singly in polymerase chain reactions to amplify DNA fingerprints in pools of DNA representing three Zebu cattle breeds. Two primers, which discriminated between the breed-specific DNA pools, were used further to amplify individual pool components in order to establish band frequencies of the amplified fingerprints. One of the primers (ILO 1127) amplified a RAPD fingerprint in 61% of TSZ animals but less than 6% in the other breeds, while another primer (ILO 1065) revealed a DNA sequence common to 89% of the Boran animals and less than 30% in the other two breeds. Bandsharing and mean average percentage difference calculated within and between the three breeds using RAPD fingerprint data showed a higher degree of homogeneity within than across the breeds and indicated measurable divergence between the three breeds. It is concluded that RAPD polymorphisms are useful as genetic markers for cattle breed differentiation.

Kantanen *et al.,* (1995) application for detecting genetic variation of cattle by Random amplified polymorphic DNA (RAPD).The present study investigated the use of the random amplified polymorphic DNA (RAPD) method to detect genetic variation in cattle. The animals studied consisted of samples from five Finnish cattle breeds: native Eastern (18 animals), Northern (24), Western Finncattle (24), Finnish Ayrshire (24), and Finnish Friesian (18). The cattle populations were analysed with 11 and 13 RAPD primers demonstrating the most repeatable amplification pattern. Two out of ten RAPD fragments tested by cross hybridization showed homology between the two species. The RAPD method did not prove efficient for finding new polymorphisms in either species, because we found only three polymorphic RAPD markers for cattle with different allele frequencies between the breeds. However, the interbreed and intrabreed similarity indices for cattle did not suggest any significant differentiation of the Finnish breeds, contrary to earlier results based on blood group and protein polymorphism.

Gwakisa *et al.,* (1997) studied the genetic diversity in some indigenous cattle breeds of East Africa by RAPD. The cattle breeds and their respective locations in parenthesis were Boran (Kongwa, central Tanzania), Maasai Zebu (Kongwa, central Tanzania), Iringa Red (Iringa, southern Highlands of Tanzania), Dwarf Chagga Zebu (Moshi, Kilimanjaro, northern Tanzania), Tarime (also called Sukuma, Tabora and Shinyanga; western Tanzania) and Ankole (Tabora, western Tanzania). Also included were Boran and Sahiwal breeds from Kenya and -West African N'Dama breeds sampled from the International Livestock Research Institute (ILRI) in Kenya. Twenty cattle (10 of each sex) were sampled from each breed. They have adapted to fragile environments and are influenced only by natural selection. In this study, random amplified polymorphic DNA (RAPD) marker was used to analyze Creole cattle genome polymorphism. A comparative analysis using the RAPD technique was performed in pooled DNA of three cattle breeds (Holstein Friesian, Creole and Hereford) in order to evaluate their amplification patterns. A primary screening of RAPD primers allowed to select and use those with higher percentage of GC base composition. A total of 215 loci ranging between 300 and 2500 bp were amplified. Bandsharing frequency (BSF) among breeds showed that less related fingerprints were observed between Creole and Hereford cattle (0.77), while the highest similarity frequency corresponded to Holstein Friesian compared to Hereford (0.81). Specific RAPD bands were identified in the three DNA pools and they were tested in every individual of each breed. It may be possible to isolate and sequence these bands to create breed-specific molecular markers. The identification of multiple alleles of the MSCYP 21 in Creole cattle with a heterozygosity of He = 0.846 supported the variability of this genetic resource. The use of molecular marker RAPD was proposed to establish genetic distance among American Creole cattle and possibly related ancestral Iberian breeds.

Genetic marker types that provide different estimates of genetic diversity information. The choice of the marker to use for genetic diversity is quite often dictated by the power of the method used to generate a reproducible polymorphism that can either be tracked in a Mendelian fashion or can show segregation of a phenotypic trait in a predictable manner (Hannotte and Jianlin, 2005). The choice can also be influenced by the availability of specialized equipment, operating of equipment and assays, and technical competence. The first biomarkers to be widely used in livestock characterization were protein polymorphisms known as allozymes (Queller *et al.,* 1993). Several livestock breeds have been characterized for variations in different proteins

(Di Stasio, 1997). Molecular DNA polymorphisms are now the tools of choice for the assessment of genetic diversity among livestock breeds. They have great potential for discovery of fundamental parameters or characteristics important in conservation, including past effective population size (Garrigan *et al.*, 2002), past bottlenecks (Luikart and Cornuet, 1998), inbreeding status (Ellegren, 1999; Lynch and Ritland, 1999), and sex-specific gene flow. According to Hannotte and Jianlin (2005), important assumptions that are needed for the use of genetic markers include: (i) neutrality of the polymorphisms and (ii) the use of a relatively small number of independently segregating marker loci are a good predictor of the overall genomic diversity of a population. Randomly Amplified Polymorphic DNA (RAPD) technique, like microsatellites, involves the use of PCR for genetic typing and to assess diversity (Williams *et al.*, 1990). In one study involving indigenous African cattle, Gwakisa *et al.* (1994) used RAPD markers to characterize the local Zebu (Bos indicus) cattle breeds of Tanzania. Using RAPD markers, the relatedness among the three local breeds of Tanzania was quantified. One of the primers, ILO 1127, amplified a RAPD fragment in 61% of the Tanganyika Shorthorned Zebu animals but less than 6% in the other breeds. Another primer, ILO 1065, revealed a DNA segment common to 89% of the Boran animals and less than 30% in the other two breeds evaluated. Further, the study revealed that ILO 1065 primer could be a Bos indicus-specific Y-linked polymorphism. They also showed that RAPD analysis could detect introgression. In another similar study, Yu et al. (2004) used RAPD analysis to estimate genetic diversity and relatedness of two native cattle breeds from the Yunnan province of China (DeHong cattle and DiQing cattle) and four introduced beef cattle breeds (Brahman, Simmental, MurryGrey, and ShortHorn). Using 10 primers, it was shown that the Yunnnan DeHong cattle breed was closely related to the Brahman (Bos indicus), and the Yunnan DiQing cattle breed was closely related to the Simmental, ShortHorn, and MurryGrey (Bos taurus) breeds. Under the FAO recommendation, most of the livestock variation studies that have been done used microsatellite markers. The major drawback of RAPDs is that they are dominant markers and heterozygote are typically scored as homozygote, which decreases their information content. However, RAPD analysis could benefit most of African laboratories in the studies of livestock due to their simplicity, affordability and relatively low technical requirements.

In one of the few studies reported, Mwacharo *et al.,* (2006) phenotypically characterized two breeds of Zebu in Kenya. They used a total of 12 morphometric measurements to show that the Masaai Zebu was different from the Kamba Zebu in Kenya. The apparent wide within-breed and between-breed variations in linear body dimensions that were observed in the study were indicative of the large genetic diversity inherent in the small East African Zebu cattle, with clearly well-differentiated breeds. The advent of molecular techniques has led to an increase in the studies that focus on the genetic characterization of domestic breeds using genetic markers (Giovambattista *et al.,* 2001). As a tool used in evaluating genetic variation, markers can provide useful information at different levels and purposes such as structure of animal populations, levels of gene flow, phylogenetic relationships, patterns of historical biogeography, and parentage (Feral, 2006). In addition, genetic assessment is also of interest for the design of genetic improvement programs including appropriate choice of breeds for crossbreeding (Mwacharo *et al.*, 2006). Though understanding genetic diversity is of importance to Africa, only a limited number of studies in livestock have been done in some African countries. For example, Gwakisa *et al.* (1994) described a study that involved characterization of Zebu cattle in Tanzania using randomly amplified polymorphic DNA (RAPD) markers. The cattle breeds included in their study were Tanganyinka Shorthorned Zebu, Boran, and the Mpwapwa. They reported that divergence between the Mpwapwa and Tanganyinka Shorthorned Zebu was on average lower than all other between comparisons. In a study of Ethiopian cattle breeds including Horro, Sheko, Arsi, Abigar and Guraghe, Hassen *et al.* (2007) reported that genetic diversity between Guraghe and Abigar were smallest followed by Guraghe and Arsi. The data developed here will provide a resource for scientists interested in germplasm conservation and will add to previous reports of genetic variation in Tanzanian and Kenyan cattle.

Jeon *et al.,* (2010) Reported that the current animal industry is both technology-intensive and globalised. Efficient molecular tools, such as DNA markers, are in demand to strengthen competitive power by maximizing the improvement of livestock and obtaining the trust of customers by the verification of product origins. This review describes the present techniques applying DNA markers in the animal industry, with a focus on beef cattle and pigs. Preliminary data from an individual traceability assay for Hanwoo (Korean cattle) using 20 microsatellite markers is described. The industrial application of DNA techniques is limited at present,

however, it is expected that DNA markers originating from trait genes, especially those of low heritability and difficult-to-measure traits, may contribute to maximizing the improvement of the major economic traits of animals in the future. The aim is to better understand opportunities for genetic improvement of small ruminants by the resource-poor farmers in traditional smallholder and pastoral farming systems. Dismal performance of programmes involving breed substitution of exotics for indigenous breeds and crossbreeding with temperate breeds have stimulated a recent re-orientation of breeding programmes in tropical countries to utilize indigenous breeds, and most programmes are incipient. The success rate of some breeding programmes involving native breeds is encouraging.

Van and Nei, (2003) reported that the development of molecular biological techniques has created new possibilities for the selection and genetic improvement of livestock. The discovery of the PCR had a major impact on the research of eukaryotic genomes and contributed to the development and application of various DNA markers. DNA markers have already found wide application in parentage verification, individual identification and control of genetic disorders. The ultimate use of DNA markers would be to identify quantitative trait loci (QTL) in order to practice genotypic selection. This paper reviews DNA markers (RAPD, DFP, RFLP AFLP, minisatellites, microsatellites, SNP) and provides a brief overview of the current application of these markers in animal breeding.

Crawford, (2003) reported that genetic modification technologies, developed initially in laboratory strains of selected bacteria and viruses, are essential tools for understanding the genomes of livestock. These tools allow researchers to: isolate sequence and characterise any livestock gene; locate genes on chromosomes; follow the inheritance of any gene and/or chromosomal region in any pedigree; detect phenotypic variation due to, or associated with, variation in the DNA sequence of a gene and identify the genetic alteration causing this. Most of the many thousands of genes identified in livestock vary between individuals.

Brufort *et al.*, (2003) reported that a series of recent genetic studies has revealed the remarkably complex picture of domestication in both New World and Old World livestock. By comparing mitochondrial and nuclear DNA sequences of modern breeds with their potential wild and

domestic ancestors, we have gained new insights into the timing and location of domestication events that produced the farm animals of today. The real surprise has been the high number of domestication events and the diverse locations in which they took place - factors which could radically change our approach to conserving livestock biodiversity resources in the future.

MATERIALS AND METHODS

The experiment was carried out at the Animal Culture and Molecular Biotechnology laboratory, Khulna University, Khulna. Bangladesh. The details of materials and methods used are described as follows.

3.1. Animal selection and blood sample collection

A total of 20 local cattle were selected from different regions for this study. Five ml of blood was collected from each animal in a 06 ml EDTA vaccutainer. The blood was gently mixed with EDTA (present in the vacationer as an anticoagulant agent) and kept on ice box. Subsequently the blood samples were transported to the laboratory and stored at -20°C until further use.

3.2. Isolation of DNA

In the present investigation, DNA was extracted from blood samples according to Roe *et al.* 1990.

Table. 1. Accession number, cattle identity and sex of the experimental cattle.

SL. No.	Cattle No.	Location from where sample was collected	Cattle identity	Sex
1	Mad -12	Shibchar, Madaripur	Local	female
2	Mad -34	Shibchar, Madaripur	Local	female
3	Mad -45	Shibchar, Madaripur	Local	male
4	Mad -56	Shibchar, Madaripur	Local	female
5	Mad -04	Shibchar, Madaripur	Local	female
6	Mad -78	Shibchar, Madaripur	Local	female
7	Mad -21	Madaripur sadar	Local	female
8	Mad -55	Madaripur sadar	Local	female
9	Mad -89	Madaripur sadar	Local	male
10	Far-79	Bhanga, Faridpur	Local	female
11	Far-01	Bhanga, Faridpur	Local	female
12	Far-03	Bhanga, Faridpur	Local	female
13	Far-04	Sadarpur, Faridpur	Local	female
14	Far-31	Sadarpur, Faridpur	Local	male
15	Far-77	Sadarpur, Faridpur	Local	male
16	Sar-87	Plang, sariatpur .	Local	female
17	Sar-05	Plang, sariatpur	Local	male
18	Sar-10	Plang, sariatpur .	Local	male
19	Sar-35	Plang, sariatpur .	Local	female
20	Sar-11	Plang, sariatpur .	Local	male

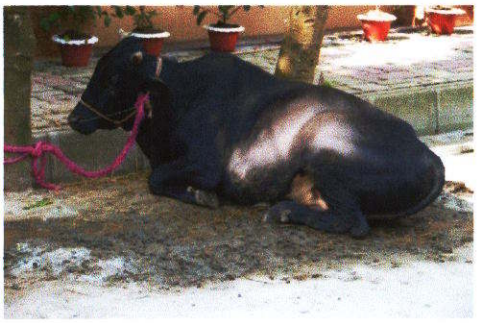

Fig 1.1: Acc No: 89-Bull (Madaripur Sadar) Fig 1.2: Acc No: 12-Bull (Shibchar, Madaripur)

Fig 1.3: Acc No: 56-Cow (Shibchar, Madaripur) Fig 1.4: Acc No: 56-Cow (Bhanga, Faridpur)

Fig 1.5: Acc No: 87-Cow (Plang, Sariatpur)

3.3 Method for Isolation of Total Genomic DNA from Whole Blood of Cattle

This method was adopted from Bruce A. Roe, 1996 with a little modification.

1. The frozen samples were thawed and 0.8ml of 1X SSC buffer with 1 ml of blood was added in a 1.5 ml centrifuge tube and mix well by overtaxing.Tubes were then centrifuged at 12000 rpm for 1 min.
2. One ml of the supernatant was removed and discarded into disinfectant.
3. One ml of 1X SSC buffer was added, vortexed and centrifuged as above for 1 min and all the supernatant was rmoved.
4. Three hundred and seventy five microlitre of 0.2M sodium acetate was added with each pellet and vortexed briefly. Then 25µl of 10% SDS and 5µl of proteinase K was added, vortexed briefly and incubated for 1 h at 55°C.
5. One hundred and twenty microlitre of phenol: chloroform: isoamyal alcohol was added and vortexed for 30 sec. the Sample for 2 min at 12000 rpm was centrifuzed.
6. Carefully the aqueous layer to a new 1.5 ml microcentrifuge tube was added and 1 ml of ice cold 100% ethanolwas added and mixed and incubated for 15 min at -20°C.
7. It was centrifuge for 2 min at 12000rpm and decant the supernatant and drained.
9. 0ne hundred and eighty microlitre of TE (10:1) buffer was added, vortexed and incubated at 55°C for 10 min.
10. Twenty microlitre of 2M sodium acetate was added and mixed, added 500 of cold 100% ethanol, mixed and centrifuged for 1 min. at 12000 g in a microcentrifuge.
11. The supernatant was decant and rinsed the pellet with 1ml of 80% ethanol and centrifuged for 1 min. at 12000 g in a microcentrifuge.
12. The supernatant was decant, and dried the pellet in a Speedy-Vac for 10 minutes (or until dry).
13. The pellet was resuspended by adding 200 of TE (10:1) buffer. It was incubated overnight at 55°C, overtaxing periodically to dissolve the genomic DNA. The samples were stored at -20^0 C.

3.4. Measuring of DNA Concentration and Quality

Spectrophotometer is commonly used in laboratories for the measurement of DNA concentration and purity. The concentration and purity of extracted DNA was measured by spectrophotometeric method. The DNA purity was measured by dividing the absorbance was taked at 260 nm and calculated the amount of DNA using the following formula:

DNA concentration = A260 × Dilution factor × Conversion factor

$$= A260 \times \frac{\text{Volume of distilled water (µl)}}{\text{Amount of the DNA sample (µl)}} \times 50$$

= (µg/ml)

= (ng/µl) [∴ µg/ml = ng/µl]

The quality of DNA was also measured by spectrophotometric method.The absorbance was taken at both 260 and 280 nm. The ratio 260/280 showed the range between of 1.8 – 2.0. Therefore, the extracted DNA was enough pure to carry out the RAPD analysis.

3.5. PCR reaction

3.5.1 Primer selection

In this study 2 RAPD primers was screened out of 10 primers. The primers sequences are shown in Table 2:

Table 2. List of primers used in the experiment.

Primer No.	Sequence (5'-3')
01	AATSSGCTGG
02	TCAGCCACGT
03	**TCTGCCATCC**
04	GTTGCCACCC
05	**CCTGCCATTC**
06	TTCCCCCCAG
07	TCTGCCATCC
08	GGCCCTACAT
09	CTTATCAGGG
10	AACGGTGACC
11	GGCCCTACAT
12	TTGCGGCGAT

3.5.2 PCR amplification

The amplification conditions were based on Williams *et al.* (1990) with some modifications. PCR reactions were performed on each DNA sample in a 20 µl reaction mix containing 4ng genomic DNA, 2 µl of 10x reaction buffer, 2 U units of Taq DNA polymerase 1 µl (100picomol) of primer, 11 µl of sterile water. The PCR samples were overlaid with one drop of mineral oil. DNA amplification was performed by thermal cycler. The amplification program included an initial denaturation step of 94°C for 3 minutes followed by 40 cycles of 94°C (denaturation), 1 minute; 37°C (annealing), 1 minute and 72°C (extention) for 2 minutes. The last cycle was followed by 10 minutes incubation at 72°C and then held at 8°C.

3.5.3 Gel electrophoresis of PCR amplified Product

PCR products from each sample were confirmed by running 2 % agarose gel in IX TAE buffer at 40V for 80 minutes. Loading dye (3 µl) was added to the PCR products and loaded in the wells. Two molecular weight markers DNA (ladder) was also loaded on one side of the gel. After electrophoresis, the gel was taken out carefully from the electrophoresis tank and stained the gel with ethidium bromide. After staining the gel, DNA fragments were observed under UV transilluminator and photographed using gel documentation unit.

3.3.4 Data analysis

RAPDs were scored according to the presence (1) or absence (0) of polymorphic DNA fragments. The scores obtained using all primers in the RAPD analysis were then combined to create a single data matrix. This was used for estimating polymorphic loci, Nei's (1973) gene diversity, genetic distance (D) and constructing a UPGMA (Unweight Pair Group Method of Arithmetic Means) dendogram among the populations using computer program COMPONENT (Yeh *et al.*, 1999).

Result and Discussion

4.1. DNA extraction and quantity determination

Extraction of DNA was performed using Roe.*et.al.* 1996. The quality of DNA was confirmed by 2% agarose gel electrophoresis

PCR is the technique of choice for nucleic acid quantification. The DNA quantity was determined by spectrophotometer absorbance at 260 nm and subjected to PCR amplification. However, successful quantification depends on the quality of DNA. The spectrophotometric absorbance readings at 260 nm of twenty experimental cattle are shown in Table. 3.

Table 3. Spectrophotometric absorbance readings at 260 nm and quantity of DNA of experimental cattle sample.

Serial No.	Accession or tag no.	Absorbance reading at 260 nm	Quantity of DNA(ng/µl)
1	Mad-12	0.036	178.20
2	Mad-34	0.045	222.75
3	**Mad-45**	**0.096**	**475.20**
4	Mad-56	0.033	163.35
5	Mad-04	0.067	331.65
6	Mad-78	0.048	237.60
7	Mad-21	0.078	386.10
8	Mad-55	0.090	445.50
9	Mad-89	0.046	227.70
10	Far-79	0.087	430.65
11	Far-01	0.056	277.20
12	Far-03	0.074	366.30
13	Far-08	0.044	217.80
14	**Far-31**	**0.031**	**153.45**
15	Far-77	0.065	321.75
16	Sar-87	0.075	371.25
17	Sar-05	0.062	306.90
18	Sar-10	0.049	242.55
19	Sar-35	0.067	331.65
20	Sar-11	0.080	396.00

The higher DNA concentration was recorded in Mad-45 cattle and the lowest DNA concentration was obtained from Far-31 cattle. The quantified DNA sample subsequently diluted to 2ng/µl as per requirements for ideal PCR. The PCR did run using twelve primers and among them two primers showed reproducibility. The PCR product reproducibility and banding pattern was confirmed by 2% agarose gel electrophoresis with 100 bp ladder.

4. 2. Molecular characterization

In the present study, a polymerase chain reaction (PCR) based randomly amplified polymorphic DNA (RAPD) marker was used to amplify cattle DNA using 10 arbitrary oligonucleotide primers. The method was able to detect the heterogeneity of amplified DNA from experimental cattle.The present study also indicates the effectiveness of RAPD analysis and the data recorded from DNA amplification were very sound for molecular characterization of twenty experimental cattle.

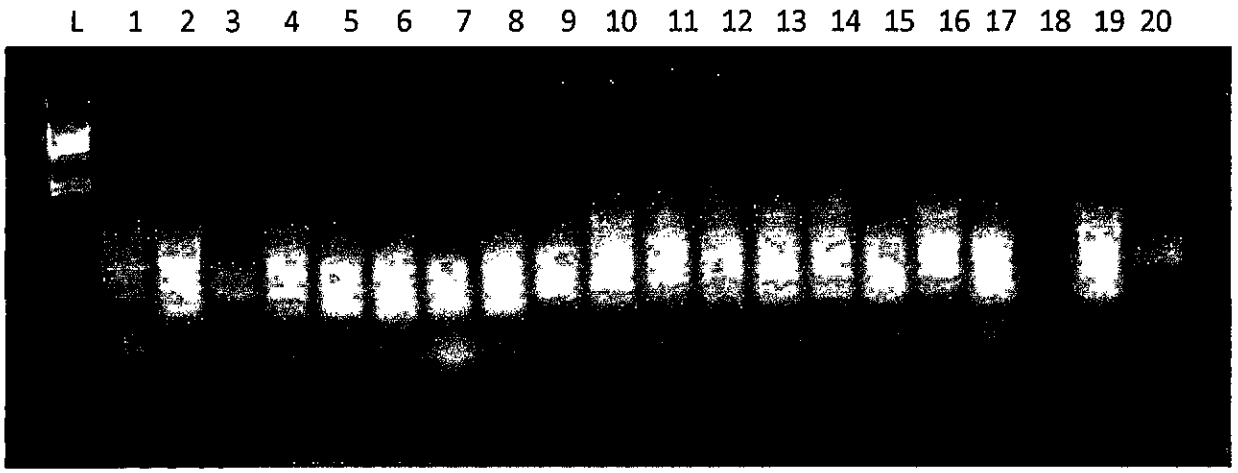

Fig 2: RAPD profile of twenty experimental cattle using primer 5. The number corresponds to the serial number of the genotypes.

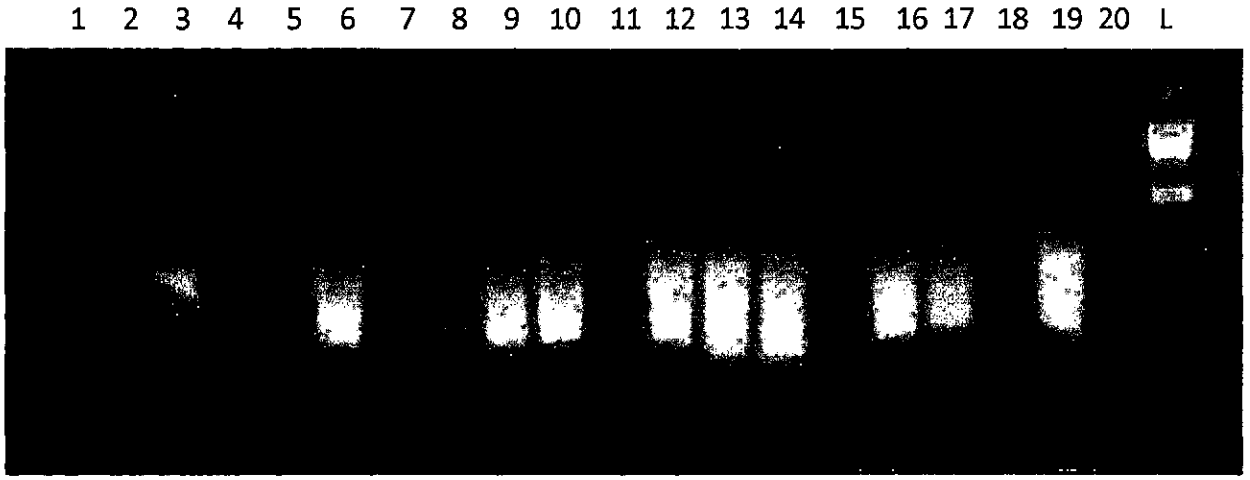

Fig 3: RAPD profile of experimental cattle using primer 3. The number corresponds to the serial number of the genotypes.

4.2.1. DNA fingerprinting

Primer selection: Randomly 10 primers were tested for the selected samples and among which two primers were matched (Table 4) and showed polymorphisms. Primers that used in RAPD analysis of twenty experimental cattle amplified 95 different reproducible bands. Different primers generated various banding patterns ranging from 2 to 10 and the average bands per primer was above 2.0 and of the 95 bands scored, 72 (75.79%) were found to be polymorphic and 23 bands (24.21%) were found to be common in nature. The frequencies of polymorphic bands varied from primer to primer. Though no sample-specific marker was identified, the high level of polymorphism revealed by the proportion of polymorphic loci (83.33%) indicated that RAPD markers could be considered as effective tools for estimating genetic diversity of experimental cattle population. The cattle number could be distinguished by a combination fragments and differences between clusters reflected in frequencies rather than presence or absence of specific fragments.

Table4. List of primers and their different bands.

Sample no.	Primer Code	No. of total band	Number of polymorphic band	Proportion of polymorphic loci (%)
Mad-12	3,5	4	3	75
Mad-34	3,5	2	2	100
Mad-45	3,5	5	4	80
Mad-56	3,5	5	4	80
Mad-04	3,5	2	2	100
Mad-78	3,5	6	5	83.33
Mad-21	3,5	2	2	100
Mad-55	3,5	5	4	80
Mad-89	3,5	5	4	80
Far-79	3,5	7	5	71.43
Far-01	3,5	3	3	100
Far-03	3,5	8	6	75
Far-08	3,5	10	7	70
Far-31	3,5	10	6	60
Far-77	3,5	2	2	100
Sar-87	3,5	7	4	57.14
Sar-05	3,5	7	5	71.43
Sar-10	3,5	4	4	100
Sar-35	3,5	6	5	83.33
Sar-11	3,5	2	2	100
Total	2*20=40	95	72	1666.66
Average		2.38	1.8	83.33

Mufti *et al* (2009) reported polymorphisim and genetic relationship in different cattle populations using primers and showed a total of 92% olymorphisims which is higher than 83.33%. It suggests that within this period of time, the gene segregation rate has increased in these animals. In another study involving indigenous African cattle, Gawkisa *et.al.* (1994) used RAPD markers to characterize the local cattle (*Bos indicus*) cattle breeds of Tanzanian (using ILO 1127 primer) and showed 72% polymorphisims which is almost similar to the present study. The degree of polymorphisms 61% compared to taurine cattle 29% in population (Talle *et al.*2005). Estimation of higher level of genetic variation in the breeds might be consistent with the fact that they are highly polymorphic animals.

4.3.2. Genetic Distance Determination

Estimates of (Nei's & Li, 1979) genetic distance calculated all possible pairs-wise genetic distance values by the following formula:

$$d_{xy} = 1 - \{2n_{xy}/(n_x + n_y)\}$$

Where, n_x and n_y are the numbers of bands amplified in individuals x and y, respectively, and $2n_{xy}$ is the number of bands shared by those individuals.

Table.4. Pair wise estimation of genetic distance among experimental cattle using Nei's Equation.

Sample No.	Mad-12	Mad-34	Mad-45	Mad-56	Mad-04	Mad-78	Mad-21	Mad-55	Mad-89	Far-79	Far-01	Far-03	Far-08	Far-31	Far-77	Sar-87	Sar-05	Sar-10	Sar-35	Sar-11
Mad-12	-																			
Mad-34	0.6667	-																		
Mad-45	0.5556	1	-																	
Mad-56	0.3333	0.4286	0.5556	-																
Mad-04	0.6667	0.50	0.7143	0.4286	-															
Mad-78	0.80	0.75	0.6364	0.4545	0.75	-														
Mad-21	0.6667	0.50	0.7143	0.7143	0.50	0.75	-													
Mad-55	0.5556	0.4286	0.60	0.60	0.7143	0.4545	0.4286	-												
Mad-89	0.5556	0.7143	0.40	0.60	1	0.6364	0.7143	0.40	-											
Far-79	0.4545	0.5556	0.50	0.1667	0.5556	0.3846	0.7778	0.50	0.3333	-										
Far-01	0.4286	0.60	0.75	0.25	0.20	05556	0.60	0.75	1	0.40	-									
Far-03	0.6667	0.60	0.3846	0.3846	0.60	0.2857	0.80	0.3846	0.3846	0.20	0.6364	-								
Far-08	0.5714	0.6667	0.4667	0.3333	0.6667	0.25	0.8333	0.4667	0.6667	0.1765	0.5385	0.1111	-							
Far-31	0.4286	0.6667	0.3333	0.3333	0.6667	0.25	0.6667	0.3333	0.3333	0.1765	0.5385	0.1111	0.10	-						
Far-77	0.6667	0.50	0.7143	0.4286	0	0.75	0.50	0.7143	1	0.5556	0.20	o.60	0.6667	0.6667	-					
Sar-87	0.2727	0.7778	0.3333	0.3333	0.7778	0.5385	0.7778	0.50	0.1667	0.1429	0.60	0.3333	0.2941	0.1765	0.7778	-				
Sar-05	0.6364	0.5556	0.50	0.3333	0.7778	0.2308	0.7778	0.3333	0.3333	0.1429	0.60	0.20	0.1765	0.1765	0.7778	0.2858	-			
Sar- 0	0.75	0.6667	0.3333	0.5556	1	0.60	1	0.5556	0.3333	0.4545	1	0.3333	0.4286	0.4286	1	0.4545	0.2727	-		
Sar- 5	0.80	0.50	0.4545	0.4545	0.75	0.3333	0.75	0.2727	0.2727	0.2308	0.7778	0.1429	0.25	0.25	0.75	.3846	0.0769	0.20	-	
Sar- 11	0.3333	1	0.7143	0.7143	1	0.75	0.50	0.7143	0.7143	0.7778	0.60	1	0.8333	0.6667	1	0.5556	0.7778	1	1	-

The values of pair-wise comparisons of Nei's (1979) genetic distance among experimentantal cattle genotypes were computed from combined data sets for the 2 primers ranging from 0.00 to 1.00 (Table 5). Comparatively higher genetic distance (1.00) was found among (Madaripur-45,89), (Faridpur- 01, 77), (Sariatpur-10, 11) versus Madaripur-34, 04, 21, 89, Faridpur- 04, 03, 77 and Sariatpur-05, 10 due to discriminating breeding among the selected breeds and it may be happened due to the environmental effects of the regions. The lowest genetic distance (0.00) was found between Faridpur-77 and Madaripur-04 that is why there is a possible reason to migrate the people from one region to another region and it can be happened due to sell or buy the cattle from different regions.

In this study there was a high level of genetic variation among the studied samples of cattle as indicated by the proportion of polymorphic loci. Estimation of higher level of genetic variation in the cattle might be consistent with the fact that it is a highly polymorphic animal. Kemp and Teale, 1994 identified population specific DNA polymorphisms to detect *Bos indicus* in West Africa. They estimated the allele frequency characteristics of cattle breeds using DNA markers (Buitkamp.*et al.* 1991). More or less similar values of heterozyosity varying from 0.010 in the pantagonia Creole cattle to 0.843 in the Argetinean Creole cattle breed were reported by Liron.*et al.* (2002) where they used five loci related to milk production and found similar genetic distances of average animals like the present experiment.

The selected primer can be used for analysis in other cattle germplasm. The wide range of dissimilarity value 0.00 to 1.00 suggests that the germplasm collection represents a wide genetically diverse population. RAPD markers are randomly distributed throughout the genome, but most of the genome 90% are not expressed at the phenotypic level (Williams *et al.* 1990). As a result, markers like RAPDs may accurately assy the degree of genetic change between two genomes, but they may be not necessarily reflect the divergence in term of changes in traits of agronomic importance.

For a more efficient determination of genetic diversity in cattle varieties, a higher number of decamer primers showing maximum number of polymorphic band or other available marker systems could be utilized. Nevertheless, the information about the

genetic diversity obtained from the present experimental animals could be used successfully in future breeding and germplasms conservation programme for cattle production systems of Bangladesh like high yielding milk and meat production animals of Bangladesh.

4.2.3. Cluster Analysis of the Genotypes Based On RAPD Analysis

Component dendrogram was constructed according to the morphological data. The dendrogram (Fig: 5) shows that all the breeds were grouped into two major clusters (I) and (II). Cluster I sub divided into I A and I B. Again, cluster I A subdivided into I A (1) and I A (2).

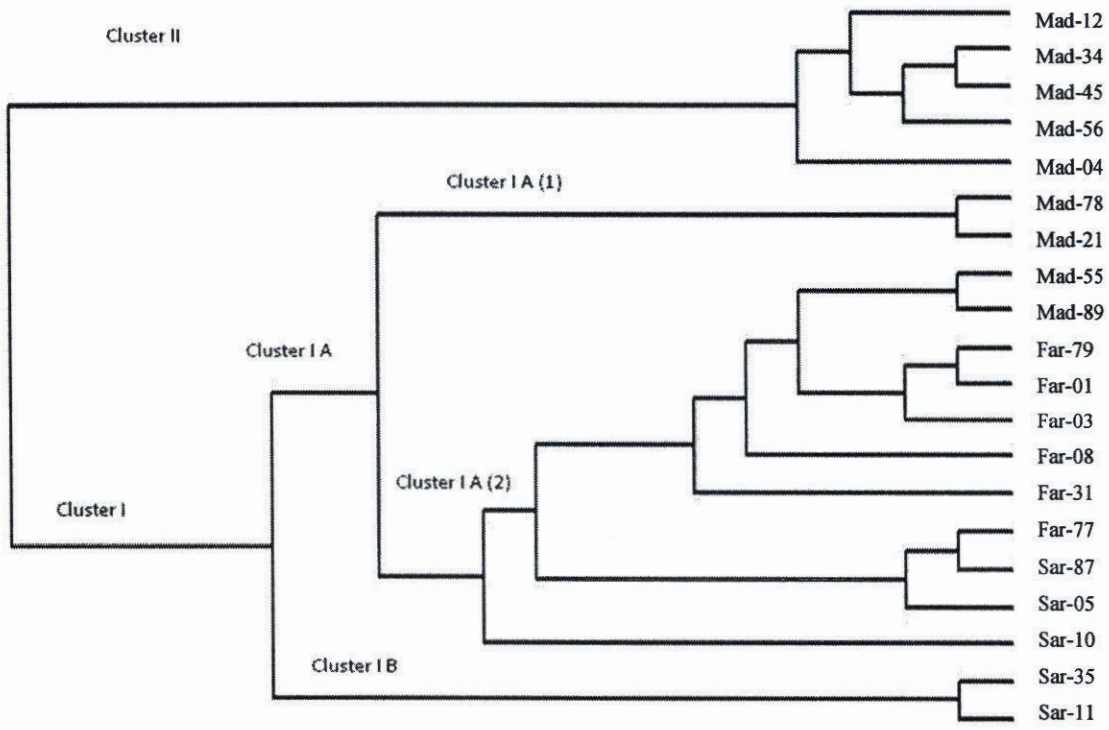

Fig 4: The unweighted pair group method of analysis (UPGMA) dendrogram based on summarized data regarding differentiation among experimental cattle.

Table 06. Distribution of twenty experimental local cattle using different clusters

Cluster No.			Total No. of Samples in cluster	Samples included in different clusters
I	I A	I A(1)	Mad-34	Mad-34, Mad- 55
		I A(2)	Far-01	Mad-56, Mad-04, Mad-89, Far-79, Far-01, Far-03, Far-08, Far-31 Far-31, Far-77, Sar-87, Sar-05
	I B	I B	Mad-34	Mad-45, Mad-89
II	II		Mad-04	Mad-03, Mad-78, Mad-21, Sar-10, Sar-11

Due to the higher genetic distance, Mad-56, 04, 89, Far-79, 01, 03, 08, 31, 77, and Sar-87, 05 breeds clustered separately with cluster I's sub cluster IA (1) and IB. Under cluster –II breeds Madaripur-12, 78, 21 and Sariatpur-79, 01 are showed genetically similar and they grouped into same cluster. Under cluster -1 breed Madaripur-34, 45, 56, 04, 55, 89, Faridpur-79, 01, 03, 08, 31, 77, and Sariatpur-87, 05, 35 are showed same characteristics but different from cluster-11. Similarly sub-cluster-1A Madaripur- 34, 56, 04, 55, 89 Faridpur-79, 01, 03, 08, 31, 77, Sariatpur-87, 05 were similar but dissimilar from sub-cluster -1B-Madaripur-45, 89. The higher genetic distance due to indiscriminate crossing/ breeding, immigration of animals, mutation in ancestors or due to different origin. Moreover, due to long genetic variation between Mad-12 and Sar-11, they can be crossed for parent development. The highest gene diversity indicates that the population is alarming to deteriorate with native or exotic blood than other population. On the other hand, the low genetic distance could be due to their same origin as well as lower genetic variability. In another similar study, *Yu et al.* (2004) used RAPD analysis to estimate genetic diversity and relationship of two native cattle breeds from the Yunnan province of China (De Hong cattle and Di Qing cattle) and four introduced beef cattle breeds (Brahman, Simmental, Murry Grey and Short Horn). Using 10 primers, it was observed that the Yunnan De Hong cattle breed was closely related to the Braham (*Bos indicus*), and the Yunnan Di Qing cattle breed was closely related to the Simmental, short horn and Murry grey (*Bos taurus*) breeds.

Summary and Conclusion

The investigation was conducted in order to isolate and quantity of genomic DNA and to determine the polymorphisim. Roe *et. al.*1990. methods were used for DNA isolation using blood samples collected from the indigenous cattle from selected regions. The quality and quantity of DNA were determined by spectrophotomer.

Genetic diversity is of great significance for breeding programmes. For successive genetic relationship morphological & molecular characterization are very effective. RAPD markers are the most effective method to determine the molecular characterization. A maximum of 7 polymorphic bands were obtained in PCR amplification from genomic DNA of the samples used in present study. The wide range of dissimilarity values (0.00 to 1.00) suggests that the samples collection represents a genetically diverse population.

Constructing the COMPONENT dendrogram to estimate the genetic diversity of cattle phenotypically, some effective population size could influence the structure of the variation. Determining the degree of variability within samples is necessary as a preliminary step for studying the genetic diversity among cattle breeds

It is concluded that in this experiment only 75.79% polymorphism were identified among the selected cattle, which is too high when compared with the result of those experiment conducted with other cattle. This high level of polymorphism may be due to discriminating breeding among the selected cattle that is why there is scope of cross breeding for future improvement of local cattle to yield of high milk and meat products.

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APPENDICES

Appendix-1

COMPOSITION OF DNA EXTRACTION SOLUTION

1X Standard Saline Citrate (SSC); pH 7.03 (For 100ml)

NaCl: 0.875gm
Na-citrate: 0.441gm
Dissolved in 60ml distilled water, bring the final volume 100ml.

0.2M Na-acetate (For 100ml)
Na-acetate (anhydrous):1.6406gm
Dissolved in 80ml distilled water, bring the final volume 100ml.

2M Na-acetate (For 100ml)
Na-acetate (anhydrous):16.406gm
Dissolved in 80ml distilled water, bring the final volume 100ml.

10% SDS (For 100ml)
Sodium dodeocyle sulphate (SDS): 10gm
Dissolved in 80ml distilled water, adjust 100ml volume.

Proteinase K
Proteinase K 20mg in 1ml of distilled water.

Detergent
Detergent 20mg in 1ml of distilled water.

Phenol: Choroform: Isoamyl Alcohol (25:25:1)
TE Saturated Phenol: 25ml
Choroform: 25ml
Isoamyl Alcohol: 1ml
Total volume 51ml.

Choroform: Isoamyl Alcohol (25:1)
Choroform: 25ml
Isoamyl Alcohol: 1ml
Total volume 26ml.

TE (10:1) buffer (For 500ml)
1M Tris-HCl (pH7.6):5ml
0.5M EDTA (pH8.0):10ml
Adjust final volume 500ml with distilled water.

100% Cold Ethanol

70% Ethanol
Ethanol: 70ml
Distilled water: 30ml.

50X TAE Buffer, pH 8.03 (For 100ml)
EDTA: 1.8609gm
Glacial acetic acid: 5.4ml
Tris-base: 24.2gm
Adjust 100 ml with distilled water.

1X TAE Buffer (For 400ml):
50× TAE buffer: 8ml
Distilled water: 392ml.

14N HCl:
Concentrated HCl: 127.51 ml in 100 ml of distilled water.
Make the final volume 250 ml with distilled water.

1M NaOH (For 100ml):
NaOH pellets: 4 gm
Distilled water: 80ml
Make the final volume 100 ml with distilled water.

0.5 M EDTA; pH 8.0 (For 250ml)
EDTA: 46.525gm
Dissolved in 100ml distilled water

Adjust pH to 8.0 with 10M NaOH
Add distilled water to 250ml.

1M Tris-HCl; pH 7.6 (For 250ml):
Tris-base: 30.275gm
Dissolved in 100ml distilled water
Adjust pH to 7.6 with concentrated HCl
Make the final volume 250 ml with distilled water.

Appendix -2

Reagents used

Name of Reagent	Name of the Company
NaCl	AnalaR^{R}
Na_2SO_4	Schnchardt,Germany
Tris-base	Hinweis
SDS	Hinweis
EDTA	Hinweis
Na-citrate^{TM} Dihydrate	-----
Agar	Invitrogen^{TM} life technolgies
Gel loading dye	Invitrogen^{TM} life technolgies