Journal of Animal and Veterinary Advances 10 (14): 1789-1800, 2011
ISSN: 1680-5593

Genetic Markers and Their Application in Buffalo Production

[1]M.A. Alim, [1]D.X. Sun, [1]Yi Zhang and [2]M.O. Faruque
[1]State Key Laboratory of Animal Genetics and Breeding, Ministry of Agriculture,
Department of Animal Genetics and Breeding, College of Animal Science and Technology,
China Agricultural University, 100193 Beijing, China
[2]Department of Animal Breeding and Genetics, Bangladesh Agricultural University,
2202 Mymensingh, Bangladesh

Abstract: In genetic analysis, various types of genetic markers such as morphological, chromosomal, biochemical and molecular markers are used in buffalo production. Mainly the molecular markers, (hybridization-based markers and PCR-based markers) have tremendous uses which are capable of pinpointing genes and traits and diversity and evolution at the genetic level. In recent years, the demonstration of genetic polymorphism at the DNA sequence level has provided a large number of marker techniques with variety of applications. Genetic marker is a potential tool to geneticists and breeders to evaluate the existing germplasm and to manipulate it to create animals as desired and needed by the society. Now, genetic marker has brought a new dimension to animal breeding, especially in direct identification of the gene. This review focuses on application of different genetic markers in buffalo production.

Key words: Genetic marker, molecular marker, DNA, buffalo, focuses, applications

INTRODUCTION

In recent years, various types of genetic markers such as morphological, chromosomal and biochemical and molecular markers are used to increase the productivity of animals. Morphological (e.g., pigmentation or other features) and chromosomal (e.g., structural or numerical variations) markers usually show low degree of polymorphism and hence are not very useful for genetic markers. Biochemical markers have been tried out extensively but have not been found encouraging as they are often sex limited, age-dependent and are significantly influenced by the environment. Sometimes, the various genotypic classes are indistinguishable at the phenotypic level owing to dominance effect. Furthermore, these markers reflect variability in their coding sequences that constitute <10% of the total genome. The molecular markers, capable of detecting the genetic variation at the DNA sequence level have not only removed these limitations but also possess unique genetic properties that make them more useful than other genetic markers. Moreover, they are numerous and distributed ubiquitously throughout the genome. These follow a typical Mendelian inheritance which usually expresses in a co-dominant fashion and are often multiallelic giving mean heterozygosity of >70%. They remain unaffected by the environmental factors and generally do not have pleiotropic effects on Quantitative Trait Loci (QTL). Since, gene expression is not a prerequisite, virtually the entire genome including the noncoding regions can be visualized. Genetic markers by definition are stable inherited variations that can be measured or detected by a suitable method and can be used to evaluate the specific genotype or phenotype other than itself. These markers are numerous and distributed ubiquitously throughout the genome. In some cases, genetic markers are breed-specific but more often although, there are breed overlaps in genetic-marker existence, there are marked differences in the frequencies of the controlling genes. Genetic marker based on unique sequence DNA mutation located within protein coding regions have long been used in genetic population studies with most of the markers being biochemical polymorphisms identified on the basis of electrophoretic differences (Baker and Manwell, 1977). Recent developments in DNA technologies have made it possible to uncover a large number of genetic polymorphisms at the DNA sequence level and to use them as markers for evaluation of the genetic basis for the observed phenotypic variability. Since, the 1st demonstration of DNA-level polymorphism

Corresponding Author: D.X. Sun, State Key Laboratory of Animal Genetics and Breeding, Ministry of Agriculture, Department of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, 100193 Beijing, China

known as the Restriction Fragment Length Polymorphism (RFLP) (Grodzicker *et al*., 1974), an almost unlimited number of molecular markers have accumulated. Currently, more powerful and less laborious techniques to uncover new types of DNA markers are steadily being introduced. The introduction of Polymerase Chain Reaction (PCR) (Saiki *et al*., 1988) in conjunction with the constantly increasing DNA sequence data also represents a milestone in this endeavour. The present review focuses on genetic markers and their various uses in buffalo production.

Genetic marker: Genetic marker can be defined as any stable and inherited variation that can be measured or detected by a suitable method and can be used subsequently to detect the presence of a specific genotype or phenotype other than itself which otherwise is non measurable or very difficult to detect. Such variations occurring at different levels, i.e., at the morphological, chromosomal, biochemical or DNA level can serve as the genetic markers (Mitra, 1994).

Molecular markers: The markers revealing variations at DNA level are referred to as the molecular markers. Molecular markers are found at specific locations of the genome. They are used to flag, the position of a particular gene or the inheritance of a particular characteristic.

Classification of molecular marker: The markers revealing variations at the DNA level are referred to as the molecular markers and on the basis of techniques used for their detection, these have been classified into two major categories: hybridization-based markers and PCR-based markers.

The hybridization-based markers: These include the traditional Restriction Fragment Length Polymorphism (RFLP) analysis (Botstein *et al*., 1980) as well where in appropriately labeled probes for the genes of importance (e.g., cDNA or genomic DNA sequences) are hybridized on to filter membranes containing Restriction Enzyme (RE)-digested DNA separated by gel electrophoresis and subsequently transferred onto these filters by Southern blotting.

The polymorphisms are then visualized as hybridization bands. The individuals carrying different allelic variants for a locus will show different banding patterns. Hybridization can also be carried out with the probes (e.g., genomic or synthetic oligonucleotide) for the different families of hypervariable repetitive DNA sequences namely, minisatellite (Jeffreys *et al*., 1985), simple repeats (Ali *et al*., 1986), Variable Number of Tandem Repeats (VNTR) (Nakamura *et al*., 1987) and microsatellite (Litt and Luty, 1989) to reveal highly polymorphic DNA Fingerprinting Patterns (DFP).

The PCR-based markers: These have however, removed the necessity of probe-hybridization step and have led to the discovery of several useful and easy-to-screen methods. Depending on the type of primers (i.e., primers of specific sequences targeted to a particular region of a genome or primers of arbitrary sequences) used for PCR, these markers can be further sub-divided into the following 2 groups:

The sequence-targeted PCR assays: In this assay system, a particular fragment of interest is amplified using a pair of sequence-specific primers. In this category, PCR-RFLP or Cleaved Amplified Polymorphic Sequence (CAPS) analysis is a useful technique for screening of sequence variations that give rise to the polymorphic RE sites. Such analysis involves amplification of a specific region of DNA encompassing the polymorphic RE site and digestion of the amplified DNA fragment with the respective RE.

However for the screening of the sequence variations that do not lead to creation or abolition of restriction sites, other approaches namely allele specific PCR (AS-PCR) (Nichols *et al*., 1989), PCR Amplification of Specific Alleles (PASA) (Sarkar *et al*., 1990), Allele Specific Oligonucleotide (ASO) hybridization assay (Saiki *et al*., 1986), Amplification Refractory Mutation System (ARMS) (Newton *et al*., 1989) and Oligonucleotide Ligation Assay (OLA) (Landegren *et al*., 1988) are used. These assays are based on the principle of high specificity of PCR to selectively amplify specific alleles using primers that match the nucleotide sequence of one but mismatch the sequence of other allele. The sequence-targeted PCR approach is also employed to reveal Simple Sequence Length Polymorphism (SSLP), using a pair of primers that flank the Simple Sequence Repeat (SSR) motifs. If cloned and sequenced microsatellite loci can be subjected to PCR amplification and such microsatellite loci can be recovered by PCR such loci are termed as Sequence Tagged Microsatellite Site (STMS) (Beckmann and Soller, 1990) markers.

The arbitrary PCR assays: In this assay system, however unlike the standard PCR protocol, randomly designed single primer is used to amplify a set of anonymous polymorphic DNA fragments. It is based on the principle that when the primer is short (usually 8-10 mm) there is a high probability that priming may take place at several sites in the genome that are located within amplifiable

distance and are in inverted orientation. Polymorphism detected using this method is called Randomly Amplified Polymorphic DNA (RAPD) (Williams *et al*., 1990). Based on this principle, several techniques which do not require any prior sequence knowledge have been developed. However, they differ in number and length of primers used, stringency of PCR conditions and the method of fragment separation and detection. In Arbitrary Primed PCR (AP-PCR) (Welsh and McClelland, 1990), slightly longer primer is used (e.g., universal M13 primer) and amplification products are detected by radiactive or nonradioactive method following polyacrylamide gel electrophoresis.

In DNA Amplification Fingerprinting (DAF) (Caetano-Anolles *et al*., 1991) analysis, shorter primer is used (5-8 mm) which reveals relatively greater number of amplification fragments by polyacrylamide gel electrophoresis and silver staining. All these techniques having similar features can be described by a common term Multiple Arbitrary Amplicon Profiling (MAAP) (Caetano-Anolles *et al*., 1992).

Besides these, a number of modifications of the basic MAAP assays (namely, template endonuclease cleavage MAAP and RAPD-RFLP) have been developed as well.

In addition to arbitrary primers, semi-arbitrary primers designed on the basis of RE sites or sequences that are interspersed in the genome such as repetitive sequence elements (Alu repeats or SINEs), microsatellites and transposable elements are also used. In the Amplified Fragment Length Polymorphism (AFLP) assay (Zabeau, 1993), template DNA is digested with two REs and the resulting restriction fragments are then ligated with adapters and subsequently, PCR amplification is carried out using specially designed primers which comprise; a unique part corresponding to selective bases and a common part corresponding to the adapters and the RE site. Microsatellite-Primed PCR (MAP-PCR) assay is carried out using microsatellite as the primer.

Different markers and their application in buffalo production: There are several genetic markers used in buffalo populations pointing genes and traits and monitoring diversity and evolution at the genetic level. These have been described in subsequent sections.

Blood protein polymorphism: Blood protein polymorphism was used extensively for Parentage test, breed identification and phylogenic study of domestic animals during the 60 and 70's decay of last century. In buffaloes, blood protein was also used for breed identification and phylogenic studies by a number of investigators.

Giri and Pillai (1956) from India were the 1st to report blood protein polymorphisms in domestic water buffaloes. Since then, many scientists from different parts of the world especially Australia, India (Khanna, 1969, 1973; Naik *et al*., 1969; Basavalah, 1970), apan (Amano *et al*., 1980, 1981, 1986, 1992, 1998), Italy (Masina *et al*., 1971) and Bulgaria (Makaveev, 1968, 1970) reported about blood protein polymorphisms of domestic water buffaloes (Table 1).

Though, there is variation in methods and nomenclatures used, nevertheless most of the scientists reported about polymorphic loci for blood proteins and enzymes of domestic water buffaloes.

Table 1: List of blood protein examined electrophoretically

Blood proteins	Locus	Source	Elec. system	References
Alb	Serum albumin	Serum	PAG	Gahne *et al*. (1977)
Tf	Serum transferrin	Serum	PAG	Gahne (1963)
S-α2	Serum slow-α2 monoglobulin	Serum	SG	Gahne *et al*. (1977)
Hb-α and β	Hemoglobin-α and β	RBC	IEF	Chernoff and Pettit (1964)
CA	Cell carbonic anhydrase	RBC	IEF	Shaw and Prasad (1970)
Cp	Serum ceruloplsmin	Serum	SG	Schroffel *et al*. (1968)
Alp	Serum alkaline phosphate	Serum	SG	Gahne (1963)
Amy-I and II	Serum amylase-I and II	Serum	PAG	Gebicke-Harter and Geldermann (1977)
Acp	Cell acid phosphate	RBC	SG	Jr. Karp and Sutton (1967)
LDH-A and B	Cell Lactate Dehydrogenase-A and B	RBC	SG	Shotake (1974)
MDH	Cell malate dehydrogenase	RBC	SG	Shotakeand Nozawa (1974)
Dia-I and II	Cell NADH diaphorase-I and II	RBC	SG	Tucker and Crowley (1978)
6-PGD	Cell gluconate dehydrogenase	RBC	SG	Ishimoto (1972)
Es-D	Cell esterase-D	RBC	SG	Hopkinson *et al*. (1973)
Pep-B	Cell peptidase-B	RBC	SG	Lewis and Harris (1967)
CEs-I and II	Cell esterase-I and II	RBC	SG	Shaw and Prasad (1970)
GPI	Cell glucose phosphate isomerase	RBC	SG	Detter *et al*. (1968)
AK	Cell adenylate kinase	RBC	SG	Fildes and Harris (1966)
To	Cell tetrazolism oxidase	RBC	SG	Fildes and Harris (1966)
AXP	Serum X Protein	Serum	SG	Gahne *et al*. (1977)

*Starch Gel electrophoresis (SG); *Polyacrylamide Gel (PAG); *Isoelectric Focusing (IEF)

Chromosome polymorphism: Prakandze was the 1st to research with buffalo cytogenetics in Asia giving a brief account on the chromosome number. Later, Makino (1944) reported correctly the chromosome number of swamp buffalo of Taiwan and he mentioned it as 48. Ulbrich and Fischer (1966) were the 1st to report about the details of buffalo karyotypes.

Since, then many researchers in Japan (Amano *et al*., 1983, 1987), India (Gupta and Chaudhury, 1978; Chakrabarti and Benjamin, 1980; Yadav and Balakrishnan, 1982), Sri Lanka (Scheurmann *et al*., 1974), Malaysia (Harisah *et al*., 1989), China (Youjun *et al*., 2001) and Thiland (Chavananikul, 1989) have conducted research on cytogenetics of Asian buffaloes. From their studies, it now clear and well established that that river buffaloes and swamp buffaloes differ in their karyotypes.

The diploid chromosome number of river buffaloes is fifty. Their karyotypes consists of five pairs of submetacentric chromosomes, ninteen pairs of acrocentric chromosomes and a pair of acrocentric sex chromosomes.

On the other hand, karyotypes of swamp buffaloes consists of a single pair of metacentric chromosomes, four pairs of submetcentric chromosomes and eighteen pairs of acrocentric chromosomes and a pair of acrocentric sex chromosome. The cross between two types produces a number of chromosome polymorphisms in F_1 and F_2. The details of chromosome polymorphisms have been shown in Table 2.

Mitochondrial DNA (mtDNA) polymorphism: A survey of two types of water buffaloes revealed distinct lineages of mtDNA. The sequence divergence between swamp and river buffaloes was similar to the relationship estimated from RFLP analysis of the entire mtDNA (Tanaka *et al*., 1995). Genetic diversity among swamp and river buffaloes was studied by Amano *et al*. (1984) in Bangladesh by DNA sequences analysis of the mitochondrial gene for cytochrome b, the results showed that each of the 2 groups has mitochondrial DNA (mtDNA) with specific cytochrome b haplotype.

Table 2: Chromosome number of the offspring produced from different types of mating between swamp and river buffaloes

Types of mating	Chromosome no.
Purebred cross mating	
Swamp x river	Parent (2n) : 48×50
Meiosis	Gamete (n) : (24) + (25)
F_1 crossbred	Offspring (2n) : 49
Backcross to swamp	
F_1×Swamp	Parent (2n) : 49×48
Meiosis	Gamete (n) : (24), (25) + (24)
BIS	Offspring (2n) : 48 or 49
Backcross to river	
F_1×river	Parent (2n) : 49 48
Meiosis	Gamete (n) : (24), (25) + (25)
BIR	Offspring (2n) : 49 or 50
Inter se mating	
F_1×F_1	Parent (2n) : 49×49
Meiosis	Gamete (n): (24), (25) + (24), (25)
F_2	Offspring (2n) : 48 or 49 or 50

The pairwise nucleotide sequence divergence was calculated to be 2.67% between swamp and river buffaloes, suggesting that they might have diverged from the ancestral populations of the Asiatic domestic water buffaloes, approximately 1 million years ago. Mitochondrial DNA is maternally inherited. Changes in the nucleotide sequence of mtDNA occur faster than nuclear DNA (Faruque, 2003).

Amano *et al*. (1994) reported the genetic variants of ribosomal DNA and mtDNA between river buffaloes and swamp buffaloes and concluded that only mtDNA provides adequate means for classification of water buffaloes. So, analysis of mtDNA provides useful information regarding relationships between and within populations/breeds/species (Faruque, 2003). Bhat *et al*. (1990) described the cleavage pattern of mtDNA of Indian Murrah buffaloes.

Out of thirteen restriction endonuclease enzymes tested only one enzyme viz., Bg L^{-1} had polymorphic sites. Hu *et al*. (1997) described the restriction endonuclease patterns of mtDNA of three local types buffalos of Yunnan province of China. They tested 18 enzymes and identified polymorphisms for BamHI, EcoRI and ScaI sites. From their result, they concluded that Chinese water buffaloes belong to swamp type with genetic variance among different local buffalo populations.

The finding of Bhat *et al*. (1990) and Hu *et al*. (1997) agree to the findings of Tanaka *et al*. (1995). Tanaka *et al*. (1995) used fifteen enzymes for swamp buffaloes and river buffaloes. They identified five restriction cleavage sites two for river buffaloes and three for swamp buffaloes. The additional site they mentioned is PstI. The restriction endonuclease cleavage analysis of mtDNA makes it clear that genetic variability exists in different buffalo populations of Asia.

Microsatellite marker maps/Short Tandem Repeats (STR): Microsatellites, alternatively known as Simple Sequence Repeats (SSRs), Short Tandem Repeats (STRs) or Simple Sequence Length Polymorphisms (SSLPs) are tandem repeats of sequence units generally <5 bp in length, e.g. (TG)n or (AAT)n. Micro satellite are sequences characterized by the tandem repetition of short motifs from 1-4 base pair in length which is why

they are also referred to as Short Tandem Repeats (STR). Bovine autosomal microsatellite markers were tested for Polymerase Chin Reaction (PCR) amplification in African buffalo for population genetic studies. Amplification was observed for 139 markers (83%) and 101 markers were studied further with 91 (90%) being polymorphic.

Considering the overall high level of polymorphism, it was concluded that most bovine microsatellite markers are applicable in African buffalo (Van Hooft *et al*., 1999).

RFLP studies in buffaloes: The wish to improve disease resistance has been primary impetus for Major Histocompatibility Complex (MHC) research in farm animals as numerous associations between MHC polymorphism and disease susceptibility have been found.

Buffalo Lymphocyte antigen (BuLa), the MHC of buffalo has been analyzed by RFEL technique employing Pst, Pvull, HindIII and MspI restriction enzymes and human class II *a-chain* gene (HLA-DRA) c DNA probe. The hybridization of buffalo genome revealed the existence of homologous sequences and corresponding region in buffaloes.

Highly polymorphic pattern was revealed by using Msp I restriction enzyme whereas with other restriction enzyme a non polymorphic pattern was observed (Kumar *et al*., 1993). RFEL studies on Growth Hormone (GH) and Prolactin (PRL) loci in Murrah and Nili-Ravi buffaloes indicated that both the breeds were homozygous for GH and PRL loci suggesting that monomorphism, at these 2 loci may be characteristic feature of buffaloes (Mitra *et al*., 1995).

Chikuni *et al*. (1994) amplified buffalo genomic DNA by PCR using primers based on the nucleotide sequences of *k-casein* gene. Sequencing of the portion of the amplified gene from exon 3-4 revealed polymorphism at nucleotide position 516, resulting in glutamine/aspertic acid in the casein precursor.

Mitra *et al*. (1998) also using PCR/RFEL amplified a 379 bp fragment of buffalo Casein *CSN3* gene and reported one polymorphism at codon 135 (Thr-ACC-Ile ATC) in buffaloes *kappa casein* gene. The frequency of 135 The/Ile alleles was estimated to be 0.88 and 0.12, respectively.

The polymorphism of MHC class II *DRB* gene of riverine buffalo (Bubalus bubalis) was studied. Second exon sequences from the buffalo DRB locus, homologous to the cattle *DRB3* gene were amplified and characterized. The MHC of buffalo has been mapped to chromosome 2 p (Iannuzzi *et al*., 1993a). Compared to other ruminant species, buffalo MHC loci are poorly characterized. Only PCR-RFLP types have been investigated in two Indian buffalo breeds (Aravindakshan *et al*., 2000).

Random Amplified Polymorphic DNA (RAPD): More recently, the Random Amplified Polymorphic DNA (RAPD) technique has emerged as another means for studying genetic variation at the population and species level (Welsh and McCelland, 1990). RAPD markers are based on amplification of genomic DNA by PCR using short primers homologous to random target sites in the genome.

Polymorphisms can be simply identified as the presence or absence of an amplified product on ethidium bromide stained agarose gels. RAPD may be used to detect DNA variability at different levels, ranging from single base changes to deletions and insertions (Williams *et al*., 1990).

Rao *et al*. (1996) used this technique to reveal polymorphism in buffalo and cattle genomic DNA. They used 14 random primers to amplify DNA fragments. Whereas high level of polymorphism was observed between species within species variation was less.

Gene mapping: Molecular markers have three-fold applications in gene mapping: a marker allows the direct identification of the gene of interest instead of the gene product and consequently, it serve as a useful tool for screening somatic cell hybrids use of several DNA probes and easy-to-screen techniques, a marker also helps in physical mapping of the genes using *in situ* hybridization and the molecular markers provide sufficient markers for construction of genetic maps using linkage analysis.

Genetic maps are constructed on the basis of two classes of molecular markers: type I markers that represent the evolutionary conserved coding sequences (e.g., classical RFLPs and SSLPs) are useful in comparative mapping strategies where polymorphism is not an essential prerequisite. However, these are mostly Single Locus and Di-Allelic (SLDA) and thus are not useful for linkage analysis. On the other hand, the type II markers (like microsatellites markers) have higher polymorphism information content (PIC, a measure of the usefulness of a marker for linkage studies) than conventional RFLPs and can be generated very easily and rapidly. A beginning in gene mapping in buffaloes has already been made through the pioneering work of Iannuzi and his associates in Italy. A few genes have been mapped recently on specific chromosomes of buffaloes using the

Table 3: Status of physical gene map in buffaloes

Chromosomes	Gene identified	Locations	References
1	*β defensin* gene cluster	1p12	Iannuzzi *et al.* (1996a, b)
	DEFB (β-defensin)	1p	Iannuzzi *et al.* (1998b)
	HAS 8 painting probe	-	Hassanane *et al.* (1994)
	IFNG (Interferon Gamma)	1p 24-26	El-Nahas *et al.* (1997)
	Adenine Nucleotide Translocator 1(ANT1)	1p	-
	Antigen CD 18	1q	De Hondt *et al.* (1997)
	Lymphocyte function-associated antigen	-	-
	Antigen CD71	1q	El-Nahas *et al.* (1996b)
	Transferrin receptor	-	Ramadan *et al.* (2000)
	Uridine monophosphate syntase	1q31	Iannuzzi *et al.* (1994)
	DNA segment (MAF 46, ovine)	1q	De Hondt *et al.* (1997)
2	BF	2p22	Di Meo *et al.* (2000)
	EDN1	2p24	Di Meo *et al.* (2000)
	GSTAI	2p22	Di Meo *et al.* (2000)
	OLADRB (MHC)	2p22	Di Meo *et al.* (2000)
	Crystallin α-polypeptide	2q	El-Nahas *et al.* (1996a)
	Fibronectin (FN1)	2q	Othman and El Nahas (1999)
	Fucosidase α-L-1 tissue	2q	El-Nahas *et al.* (1996a)
	Prolactin (PRL)	2p	Othman and El Nahas (1999)
	Villin	2q33	Iannuzzi *et al.* (1997e)
3	Omega (IFNW) and trophoblast (IFNT) interferon genes	3q15	Iannuzzi *et al.* (1993b)
	NF1, CRYB1, CHRNB1, TP53, P4HB and GHI	3p	Iannuzzi *et al.* (1999)
	Growth hormone	3p24	Iannuzzi *et al.* (1999)
	Trophoblast interferon	3q15	Iannuzzi *et al.* (1993b)
	Tumor protein p53	3p21	Iannuzzi *et al.* (1999)
4	KRT	4q21	Di Meo *et al.* (2000)
	IFNG	4q23	Di Meo *et al.* (2000)
	IGFI	4q31	Di Meo *et al.* (2000)
	IFNG (Interferon Gamma)	River buffalo	Hassanane *et al.* (1994)
	HSA 10 painting probe	4q 23-26	Iannuzzi *et al.*(1998b)
	α skeletal actin (ACTA1)	4p	El-Nahas *et al.* (1996a)
	Conglutinin (CGN1)	4p16	El-Nahas *et al.* (1996a)
	Gamma interferon (IFNG)	4q23-26	De Hondt *et al.* (1991)
	Lactate dehydrogenase B	4q	De Hondt *et al.* (1991)
	Triose-phosphate isomerase 1	4q	El-Nahas *et al.* (1993)
5	HSA 11 painting probe	5p	Iannuzzi *et al.* (1998b)
	Lysozyme gene cluster (LYZ)	5p23	Iannuzzi *et al.* (1993a-c)
	Bovine workshop cluster 11	-	Abou-Mossallem (1999)
	Lactate dehydrogenase A	5p	El-Nahas *et al.* (1999)
	Opoid binding and Cell Adhesion Molecule (OCAM)	5p	El-Nahas *et al.* (1999)
6	Bovine Microsat. IDVGA53, BAT3/U6	6q15	Iannuzzi *et al.* (1997a-e)
7	*α-S2 casein* gene (CSN1S2)	7q32	Iannuzzi *et al.* (1996a, b)
	GNRHR	7q32	Di Meo *et al.* (2000)
	MTP	7q21	Di Meo *et al.* (2000)
	PDE6B	7q36	Di Meo *et al.* (2000)
8	BCP	8q32	Di Meo *et al.* (2000)
	CLCNI	8q34	Di Meo *et al.* (2000)
	IGFBP3	8q24	Di Meo *et al.* (2000)
	Bovine microsat. IDVGA41,U13	8q34	Iannuzzi *et al.* (1997a-c)
	Inhibin β-A	-	Othman and El Nahas (1999)
9	Elongation factor 2 (EEF2)	9q15	Iannuzzi *et al.* (1997a)
	Low-density lipoprotein receptor (LDLR)	-	De Hondt *et al.* (1997)
10	Glycoprotein hormone α-polypeptide (CGA)	-	De Hondt *et al.* (1997)
	Connexin 43 (CJA1)	10q17	De Hondt *et al.* (1991)
	Malic enzyme		El-Nahas *et al.* (1998)
11	β-N acetyl glucosaminidase (HEXA)	-	El-Nahas (1996)
	Nucleoside phosphorylase	-	El-Nahas *et al.* (1998)
	Pyruvate kinase muscle 2	-	El-Nahas *et al.* (1998)
12	Abelson murine viral oncogene homolog (ABL1)	-	El-Nahas *et al.* (1996a)
	Argininosuccinate Synthetase(ASS)	-	El-Nahas *et al.* (1996a)
	Antigen CD 14, LPS-binding protein (CD 14)	-	Iannuzzi *et al.* (1997a)
	α-galactosyltansferase 1	12q36	Othman and El Nahas (1999)
13	Bovine Microsat.IDVGA41, BAT12/U27	13q15	Iannuzzi *et al.* (1997a-d)
	Coagulation factor X (F10)	-	Oraby *et al.* (1998)
14	Prion protein gene (PRNP)	14q15	Iannuzzi *et al.* (1998b)
	Prepro-oxytocin (OXT)	-	De Hondt *et al.* (1997)

Table 3:Continued

Chromosomes	Gene identified	Locations	References
	Normal host prion protein	-	Iannuzzi *et al.* (1998b)
15	Brain Specific Protein η amino chain (BSPN)		De Hondt *et al.* (1997)
16	Bovine Microsat. IDVGA32, BAT15/U19	16Q25	Iannuzzi *et al.* (1997a-c)
	Beta-follicle stimulating hormone	-	Oraby *et al.* (1998)
	Beta hemoglobin	-	Oraby *et al.* (1998)
17	Bovine zinc finger protein 164 (U23)	-	Iannuzzi *et al.* (1997b)
18	Bovine zinc finger protein X81804 (U9)	-	Iannuzzi *et al.* (1997b)
19	Microtubule Associated Protein (MAP1B)	19q13	Iannuzzi *et al.* (1998a)
20	Immmunoglobulin Gamma Heavy Chain (IGHG)	20q23-25	Hassanane *et al.* (1993)
21	Cathelicidin (CATHL)	21q24	Iannuzzi *et al.* (1998a)
	DNA segment (CSSM6)	-	Hondt *et al.* (2000)
	DNA segment (CSSM41)	-	Hondt *et al.* (2000)
22	Major histocompatability complex class II	2p22	Hondt *et al.* (2000)
	DR β 1	2p22	Iannuzzi *et al.* (1993c)
	Antigen CD81 (TAPA-1)	-	Abou-Mossallem (1999)
	Myelin basic protein	-	El-Nahas *et al.* (1996a)
	Yamagushi sarcoma viral oncogene homolog	-	El-Nahas *et al.* (1996a)
	DNA segment (COSAE7)	22q24	Iannuzzi *et al.* (1998a)
23	Bovine Microsat. IDVGA59, BAT26/U26	23q22	Iannuzzi *et al.* (1997a-e)
24	HSA 16+7 painting probe	-	Iannuzzi *et al.* (1998b)
	BovineMicrosat. IDVGA71,U8	24q13	Iannuzzi *et al.* (1997a, b)
X	cosmids (cIOBT 314,945,1489)	Both arms	Prakash *et al.* (1997)
	DNA segment (IDVGA82)	Xq44	Iannuzzi *et al.* (1998a)
Y	DNA segment (IDVGA50)	-	Iannuzzi *et al.* (1998a)

techniques like somatic cell hybridization and *In situ* Hybridization (ISH). Present status of gene mapping in buffaloes has been shown in Table 3.

CONCLUSION

The genetic improvement of animals is a continuous and complex process. Ever since, the domestication of animals by man, he has always remained busy in improving his animals. In this pursuit, many methods have been developed and tested. In recent years, the demonstration of genetic polymorphism at the DNA sequence level has provided a large number of marker techniques with variety of applications. This has in turn prompted further consideration for the potential utility of these markers in animal breeding. However, utilization of marker-based information for genetic improvement depends on the choice of an appropriate marker system for a given application. Selection of markers for different applications are influenced by several factors, viz., the degree of polymorphism skill or expertise available, possibility of automation, radioisotopes used reproducibility of the technique and finally the cost involved. Presently, the pace of development of molecular markers is tremendous and the trend suggests that explosion in marker development will continue in the near future in buffalo population.

NOMENCLATURE

RBC = Red Blood Cell
BF = Complement Factor B
EDN1 = Endothelin 1
GSTAI = Glutathione S-Transferase Class-Alpha
OLADRB (MHC) = Similar to MHC Class II Antigen
NF1 = Neurofibromatosis
CRYB1 = Crystalline Beta Polypeptide 1
CHRNB1 = Cholinergic Receptor, nicotinic, Beta Polypeptide 1
TP53 = Tumor Protein P^{53}
P4HB = 2-oxoglutarate, 4-dloxygenase, Beta Polypeptide
GNRHR = Gonadotropin-Releasing Hormone Receptor
MTP = Microsomal Triglyceride Transfer Protein
PDE6B = Phosphodiesterase 6B
IGFBP3 = Insulin-Like Growth Factor Binding Protein 3

REFERENCES

Abou-Mossallem, A.A., 1999. Assignment of genes coding for leukocyte surface markers to Egyptian buffalo chromosomes. Ph.D. Thesis, Cairo University, Cairo, Egypt.

Ali, S., C.R. Muller and J.T. Epplen, 1986. DNA finger printing by oligonucleotide probes specific for simple repeats. Hum. Genet., 74: 239-243.

Amano T., M. Katsumata, S. Suzuki, K. Nozawa and Y. Kawamoto *et al.*, 1981. Morphological and genetical survey of water buffaloes in Indonesia. The Research Group of Overseas Scientific Survey, The Origin and Phylogeny of Indonesian Native Livestock, Part II. Tokyo. pp. 31-54.

Amano, T., K. Kurogi, K. Tanaka, T. Namikawa, Y. Yamamoto and C.B. Loc *et al*., 1998. Constitution of genes controlling blood protein types of Vietnam native water buffaloes and their phylogenic study. Rep. Soc. Res. Native Livestock, 16: 33-48.

Amano, T., K. Nozawa, T. Namikawa, M.A. Hasnath, K.G. Mostafa and M.O. Faruque, 1984. Blood Protein Polymorphism of Water Buffaloes in Bangladesh. In: Genetic Studies on the Breed Differentiation of Native Domestic Animals in Bangladesh, Amano, T. (Ed.). Tokyo University of Agriculture, Tokyo, pp: 2542.

Amano, T., T. Namikawa and H. Martojo, 1983. Body measurements, blood groups and blood protein polymorphisms of water buffaloes and anoas in Indonesia. Rep. Soc. Res. Native livestock, 10: 82-97.

Amano, T., T. Namikawa and S. Suzuki, 1980. Genetic differences between swamp and river buffaloes in the electrophoretic variations of albumin and transferrin. Proc. Japan Acad., 56: 463-468.

Amano, T., T. Namikawa, I. Okada, M.A. Hasnath, M.O. Faruque and M.A. Majid, 1987. Karyotyptes and Blood Protein Polymorphisms of Native Water Buffaloes in Bangladesh. In: Genetic Studies on the Breed Differentiation of Native Domestic Animals in Bangladesh. Part II, Okada, I. (Ed.). Hiroshima University, Hiroshima, pp: 117-129.

Amano, T., T. Namikawa, T. Shotake and H.W. Cyril, 1986. Blood protein polymorphisms of water buffaloes in Sri Lanka. Rep. Soc. Res. Native Livestock, 11: 117-128.

Amano, T., T. Namikawa, Y. Maeda, K. Tsunoda and Y. Yamamoto *et al*., 1992. Constitution of genes controlling blood protein types of Nepalese water buffaloes and their phylogenic classification. Rep. Soc. Res. Native Livestock, 14: 89-100.

Amano, T., Y. Miyakoshi, T. Takada, Y. Kikkawa and H. Suzuki, 1994. Genetic variants of ribosomal DNA and mitochondrial DNA between swamp and river buffaloes. Anim. Genet., 25: 29-36.

Aravindakshan, T.V., A.M. Nainar and S.N. Sivaselvam, 2000. Polymorphism in exon 2 of the BuLA-DRB3 gene in Indian buffalo (*Bubalus bubalis* var. indicus) detected by PCR-RFLP. Anim. Sci., 70: 221-226.

Baker, C.M. and C. Manwell, 1977. Heterozygosity of the sheep: Polymorphism of malic enzyme, isocitrate dehydrogenase (NADP+), catalase and esterase. Aust. J. Biol. Sci., 30: 127-140.

Basavalah, P., 1970. Genetic studies on blood groups, haemoglobin and transferrin polymorphism in Indian water buffaloes. M.Sc. Thesis, Punjab University, Chandigarh, Punjab.

Beckmann, J.S. and M. Soller, 1990. Toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. Nat. Biotechnol., 8: 930-932.

Bhat, P.P., B.P. Mishra and P.N. Bhat, 1990. Polymorphism of mitochondrial DNA (mtDNA) in cattle and buffaloes. Biochem. Genet., 28: 311-318.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

Caetano-Anolles, G., B.J. Bassam and P.M. Gressoff, 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio/Technology, 9: 553-557.

Caetano-Anolles, G., B.J. Bassam1 and P.M. Gresshoff, 1992. DNA fingerprinting: MAAPing out a RAPD redefinition? Bio/Technology, 10: 937-937.

Chakrabarti, S. and B.R. Benjamin, 1980. Chromosomal studies on the Indian water buffaloes. Cheiron, 9: 138-140.

Chavananikul, V., 1989. Cytogenetic aspect of crossbreeding in Buffalo. Paper Presented in the International Symposium on Buffalo Genotypes for Small Farms in Asia. UPM, Kuala Lumpur, Malaysia.

Chernoff, A.I. and N.M. Pettit Jr., 1964. The amino acid composition of hemoglobin. 111. A qualitative method for identifying abnormalities of the polypeptide chains of hemoglobin. Blood, 24: 750-756.

Chikuni, K., T. Tabata, M. Saito and M. Monma, 1994. Direct sequencing of the water buffalo (*Bubalus bubalis*) k-casein gene. Anim. Sci. Technol., 65: 652-655.

De Hondt, H.A., A.A. Bosma, M. den Bieman, N.A. de Haan and L.F.M. van Zutphen, 1991. Gene mapping in the river buffalo (*Bubalus bubalis* L.). Genet. Sel. Evol., 23: S104-S108.

De Hondt, H.A., D. de Gallagher, H. Oraby, O.E. Othman, A.A. Bosma, J.E. Womack and S.M. El Nahas, 1997. Gene mapping in the river buffalo (*Bubalus bubalis* L.): Five syntenic groups. J. Anim. Breed. Genet., 114: 79-85.

Detter, J.C., P.O. Ways, E.R. Giblett, M.A. Baughan, D.A. Hopkinson, S. Povey and H. Harris, 1968. Inherited variations in human phosphohexose isomerase. Ann. Hum. Genet., 31: 329-338.

Di Meo, G.P., A. Perucatti, L. Schibler, D. Incarnato, L. Ferrara, E.P. Cribiu and L. Iannuzzi, 2000. Thirteen type I loci from HSA4q, HSA6p, HSA7q and HSA12q were comparatively FISH-mapped in four river buffalo and sheep chromosomes. Cytogenet Cell Genet., 90: 102-105.

El-Nahas, S.M., F.M. Abdel-Tawab, M.M. Zahran, F.S. Soussa, M.A. Rashed and S.M. Ali, 1998. Gene mapping of river buffalo by somatic cell hybridization. Egypt. J. Genet. Cytol., 27: 171-179.

El-Nahas, S.M., H.A. Hondt, O.S. de Othman, A.A. Bosma and N.A. de Haan, 1993. Assignment of genes to chromosome 4 of the rive buffalo with a panel of buffalo-hamster hybrid cells. J. Anim. Breed. Genet., 110: 182-185.

El-Nahas, S.M., H.A. Hondt, S.F. de Soussa, S.F. Hassan and A.A. El Ghor, 1999. Assignment of new loci to river buffalo chromosomes confirms the nature of chromosome 4 and 5. J. Anim. Breed. Genet., 116: 21-28.

El-Nahas, S.M., H.A. Oraby, H.A. Hondt, A.M. de Medhat, M.M. Zahran, E.R. Mahfouz and A.M. Karim, 1996a. Synteny mapping in river buffalo. Mammalian Genome, 7: 831-834.

El-Nahas, S.M., H.A. Ramadan, A.A. Abou-Mossallem, E. Kurucz, P. Vilmos and I. Ando, 1996b. Assignment of genes coding for leukocyte surface molecules to river buffalo chromosomes. Vet. Immunol. Immunopathol., 52: 435-443.

El-Nahas, S.M., H.O. Oraby, O.E. Othman, H.A. Hondt, A.A. de Bosma and J.E. Womack, 1997. Use of molecular markers for the identification of river buffalo chromosomes: Chromosome one. J. Anim. Breed. Genet., 114: 451-455.

Faruque, M.O., 2003. Phylogenetic studies of Bangladeshi water buffaloes based on morphological variation, blood protein and mtDNA polymorphisms. Ph.D. Thesis, Tokyo University of Agriculture, Japan.

Fildes, R.A. and H. Harris, 1966. Genetically determined variation of adenylate kinase in man. Nature, 209: 261-263.

Gahne, B., 1963. Genetic variation of phosphatase in cattle serum. Nature, 199: 305-306.

Gahne, B., R.K. Juneja and J. Grolmus, 1977. Horizontal polyacrylamide gradient gel electrophoresis for the simultaneous phenotyping of transferrin, albumin and post-albumin in blood plasma of cattle. Anim. Blood Groups Biochem. Genet., 8: 127-137.

Gebicke-Harter, P.J. and H. Geldermann, 1977. Inheritance of amylases in blood serum of cattle. Biochem. Genet., 15: 59-73.

Giri, K.V. and N.C. Pillai, 1956. Multiple haemoglobins in the blood of animals. Nature, 178: 1057-1057.

Grodzicker, T., J. Williams, P. Sharp and J. Sambrook, 1974. Physical mapping of temperature-sensitive mutations of adenoviruses. Cold Spring Harb. Symp. Quant. Biol., 39: 439-446.

Gupta, P. and R. Chaudhury, 1978. Robertsonian changes in chromosome of Indian Murrah buffalo. Nucleus, 21: 80-97.

Harisah, M., T.I. Azmi, T.A. Bangso, M. Hilmi and M.K. Vidyadaran *et al*., 1989. Chromosome make up of water buffalo breed-types of South East Asia. Paper Presented in the International Symposium on Buffalo Genotypes for small farms in Asia, UPM, Kuala Lumpur, Malaysia.

Hassanane, M.S., B.P. Chowdhary, F. Gu, L. Andersson and I. Gustavsson, 1994. Mapping of the interferon gamma (IFNG) gene in river and swamp buffaloes by *in situ* hybridization. Hereditas, 120: 29-33.

Hassanane, M.S., F. Gu, B.P. Chowdhary, L. Andersson and I. Gustavsson, 1993. *In Situ* hybridization mapping of the immunoglobulin gamma heavy chain (IGHG) gene to chromosome 20q23-q25 in river buffaloes. Hereditas, 118: 285-288.

Hondt, H.A., O.E. de, M.F. Othman, A.A. Abdel Samad, H.A. Hassan, S.M. Oraby and S.M. El Nahas, 2000. Mapping of eight molecular markers to river buffalo chromosomes and the confirmation of the nature of chromosomes 2 and 3. J. Egypt German Soc. Zool., 31: 39-48.

Hopkinson, D.A., M.A. Mestriner, J. Cortner and H. Harris, 1973. Esterase D: A new human polymorphism. Ann. Hum. Genet., 37: 119-137.

Hu, W., B. Xu and L. Lian, 1997. Polymorphism of mitochondrial DNAs of yunnan domestic water buffaloes, *Bubalus bubalis*, in China, based on restriction endonuclease cleavage patterns. Biochem. Genet., 35: 225-231.

Iannuzzi, L., D.S. Gallagher, G.P. Meo, A.M. Ryan and A. Perucatti *et al*., 1993a. Chromosomal localization of the lysozyme gene cluster in river buffalo (*Bubalus bubalis* L.). Chromosome Res., 1: 253-255.

Iannuzzi, L., D.S. Gallagher, A.M. Ryan, G.P. di Meo and J.E. Womack, 1993b. Chromosomal localization of omega and trophoblast interferon genes in cattle and river buffalo by sequential R-banding and fluorescent in situ hybridization. Cytogenet Cell Genet., 62: 224-227.

Iannuzzi, L., D.S. Gallagher, J.E. Womack, G.P. di Meo, L.C. Skow and L. Ferrara, 1993c. Chromosomal localization of the major histocompatibility complex in cattle and river buffalo by fluorescent in situ hybridization. Hereditas, 118: 187-190.

Iannuzzi, L., D.S. Gallagher, G.P. di Meo, G. Diamond, C.L. Bevins and J.E. Womack, 1996a. High-resolution FISH mapping of beta-defensin genes to river buffalo and sheep chromosomes suggests a chromosome discrepancy in ffttle standard karyotypes. Cytogenet Cell Genet., 75: 10-13.

Iannuzzi, L., D.S. Gallagher, J.E. Womack, G.P. di Meo, C.P. Schelling and M.A.M. Groenen, 1996b. FISH mapping of the á-S2 casein gene on river buffalo and cattle chromosomes identifies a nomenclature discrepancy in the bovine karyotype. Chromosome Res., 4: 159-162.

Iannuzzi, L., D.S. Gallagher, L.S. Liou, G.P. di Meo, A.M. Ryan, K.N. Sastry and J.E. Womack, 1994. Chromosomal localization of conglutinin (CGN1) gene to river buffalo by sequential RBH-banding and FISH. Hereditas, 120: 283-286.

Iannuzzi, L., D.S. Jr. Gallagher, D.P. di Meo, Y. Yang, J.E. Womack, S.K. Davis and J.F. Taylor, 1999. Comparative FISH-mapping of six expressed gene loci to river buffalo and sheep chromosomes. Cytogenet Cell Genet., 84: 161-163.

Iannuzzi, L., G.P. di Meo and L. Ferretti, 1997a. Six bovine cosmid-derived microsatellites mapping different syntenic groups are fluorescence *in situ* hybridization mapped to six river buffalo chromosomes. Chromosome Res., 5: 541-543.

Iannuzzi, L., G.P. di Meo, C. le Chalony and G. Goubin, 1997b. FISH mapping of bovine zinc finger protein 164 (U23) and X81804 (U9) genes to river buffalo chromosomes 17 and 18 respectively. Chromosome Res., 5: 569-570.

Iannuzzi, L., H. Hayes, G.P. di Meo, D. Mercier and K. Kohno, 1997c. Chromosomal localization of alpha-galactosyltransferase 1 (GGTA1) and elongation factor 2 (EEF2) genes in river buffalo by FISH. Chromosome Res., 5: 274-276.

Iannuzzi, L., L. Ferretti, G.P. di Meo and A. Perucatti, 1997d. FISH Mapping of Bovine U21, U1 and U7 Molecular Markers to River Buffalo Chromosomes 3p, 5q and 5p. Chromosome Res., 5: 337-340.

Iannuzzi, L., L. Skow, G.P. di Meo, D.S. Gallagher and J.E. Womack, 1997e. Comparative FISH-mapping of villin (VIL) gene in river buffalo, sheep and goat chromosomes. Chromosome Res., 5: 199-202.

Iannuzzi, L., G.P. di Meo, A. Perucatti, B. Castiglioni and L. Ferretti, 1998a. Eight molecular markers from bovine syntenic groups U2, U5, U24, U14, U12, U28, X and Y were fluorescence *in situ* mapped to eight river buffalo chromosomes. Chromosome Res., 6: 656-659.

Iannuzzi, L., R. Palomba, G.P. di Meo, A. Perucatti and L. Ferrara, 1998b. Comparative FISH-mapping of the prion protein gene (PRNP) on cattle, river buffalo, sheep and goat chromosomes. Cytogenet Cell Genet., 81: 202-204.

Ishimoto, G., 1972. Blood protein variants in Asian macaques. II. Red cell enzymes. J. Anthropol. Soc. Nippon, 80: 337-350.

Jeffreys, A.J., V. Wilson and S.L. Thein, 1985. Hypervariable minisatellite regions in human DNA. Nature, 314: 67-73.

Jr. Karp, G.W. and H.E Sutton, 1967. Some new phenotypes of human red cell acid phosphatase. Am. J. Hum. Genet., 19: 54-62.

Khanna, N.D., 1969. Studies on transferrin polymorphism in Indian water buffaloes. Indian J. Anim. Sci., 39: 21-26.

Khanna, N.D., 1973. Biochemical polymorphism and blood groups in Indian buffaloes (*Bubalus bubalis*). Ph.D. Thesis, Haryana, India.

Kumar, S., P.N. Bhat, P.P. Bhat, T.J. Rasool, A.K. Rawat and Renu, 1993. Nucleotide diversity in the DQ b subregion of buffalo Lymphocyte Antigen (BuLA) system. GMF Int'l Seminar on Gene/Genome mapping. Calicut, India, July 22-23.

Landegren, U., R. Kaiser, C.T. Caskey and L. Hood, 1988. DNA diagnostics-molecular techniques and automation. Science, 242: 229-237.

Lewis, W.H.P. and H. Harris, 1967. Human red cell P Peptidases. Nature, 215: 315-355.

Litt, M. and J.A. Luty, 1989. A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am. J. Hum. Genet., 44: 397-401.

Makaveev, T., 1968. Study of biochemical polymorphism and blood groups in buffalo (*Bubalus bubalis*). Zniuot. Nauk, 5: 3-20.

Makaveev, T., 1970. Albumin, transferrin, serum, amylase and blood groups in Bulgarian water buffalo. Proceedings of the 11th Europ Conference Animal Blood Groups Biochemistry Polymorphism, (ABGBP'70), Warsaw, pp: 235-238.

Makino, S., 1944. Karyotypes of domestic cattle, Zebu and domestic water buffalo. Cytologia, 13: 247-264.

Masina, P., D. Iannelli and T.M. Bettini, 1971. Serum albumin and transferrin variants in italian water buffalo (*Bos bubalis* L.). Cell. Mol. Life Sci., 27: 587-589.

Mitra, A., 1994. Molecular markers and their applications in livestock improvement. Ph.D. Thesis, National Dairy Research Institute (Deemed University), Karnal, Haryana, India.

Mitra, A., P. Schlee, C.R. Balakrishnan and F. Pirchner, 1995. Polymorphisms at growth hormone and prolactin loci in Indian cattle and buffalo. J. Anim. Breed. Genet., 112: 71-74.

Mitra, A., P. Schlee, I. Krause, J. Blusch, T. Werner, C.R. Balakrishnan and F. Pirchner, 1998. Kappa-casein polymorphisms in Indian dairy cattle and buffalo: A new genetic variant in buffalo. Anim. Biotechnol., 9: 81-87.

Naik, S.N., K.N. Tandon and P.V. Naik, 1969. Transferrin types in Indian water buffalo (*Bubalus bubalis*). Experientia, 25: 1105-1106.

Nakamura, Y., M. Leppert, P. O'Connell, R. Wolff and T. Holm *et al*., 1987. Variable number of tandem repeat (VNTR) markers for human gene mapping. Science, 235: 1616-1622.

Newton, C.R., A. Graham, L.E. Heptinstall, S.J. Powell and C. Summers *et al*., 1989. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Res., 17: 2503-2516.

Nichols, W.C., J.J. Liepnieks, V.A. McKusick and M.D. Benson, 1989. Direct sequencing of the gene for Maryland/German familial amyloidotic polyneuropathy type II and genotyping by allele-specific enzymatic amplification. Genomics, 5: 535-540.

Oraby, H.A., S.M. El Nahas, H.A. de Hondt and M.F. Abdel Samad, 1998. Assignment of PCR markers to river buffalo chromosomes. Genet. Selec. Evol., 30: 71-78.

Othman, O.E. and S.M. El Nahas, 1999. Synteny Assignment of four genes and two microsatellite markers in river buffalo (*Bubalus bubalis* L.). J. Anim. Breed. Genet., 116: 161-168.

Prakash, B., I. Olsaker, I. Gustavsson and B.P. Chowdhary, 1997. FISH mapping of three bovine cosmids to cattle, goat, sheep and buffalo X chromosomes. Hereditas, 126: 115-119.

Ramadan, H.A.I., O.E. Othman, M.F.A. Samad, A.A. Abou-Mossallem and El Nahas, S.M., 2000. Somatic cell hybrids characterization by monoclonal antibodies and assignment of CD71 to the q arm of buffalo and cattle chromosome one. J. Egypt German. Soc. Zool., 31C: 133-143.

Rao, K.B.C.A., K.V. Bhat and S.M. Totey, 1996. Detection of species-specific genetic markers in farm animals through random amplified polymorphic DNA (RAPD). Genet. Anal.: Biomol. Eng., 13: 135-138.

Saiki, R., D.H. Gelfand, S. Stoffel, S.J. Scharf and R. Higuchi *et al*., 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science, 239: 487-491.

Saiki, R.K., T.L. Bugawan, G.T. Horn, K.B. Mullis and H.A. Erlich, 1986. Analysis of enzymatically amplified â-globin and HLA-DQá DNA with allele-specific oligonucleotide probes. Nature, 324: 163-166.

Sarkar, G., J. Cassady, C.D.K. Bottema and S.S. Sommer, 1990. Characterization of polymerase chain reaction amplification of specific alleles. Anal. Biochem., 186: 64-68.

Scheurmann, E.R., R. Wiesner, H. Fischer and M.R. Jainudeen, 1974. Karyotype, C-bands and identification of sex chromosome in Ceylonese water buffalo (*Bubalus bubalis*). Giess Erbpath. Zuchtgs. Hyg, 6: 1-1.

Schroffel., J., A. Kubek and V. Glasnak, 1968. Serum ceruloplasmin polymorphism in cattle. Proceedings of the 11th Europ Conference Animal Blood Groups Biochemistry Polymorphism, (ABGBP'68), Warsaw, pp: 207-210.

Shaw, C.R. and R. Prasad, 1970. Starch gel electrophoresis of enzymes-a compilation of recipes. Biochem. Genet., 4: 297-320.

Shotake, T. and K. Nozawa, 1974. Gnetic polymorphisms in blood proteins in troops of Japanese macaques, *Macaca fuscate*. Cytoplasmic malate dehydrogenase polymorphisms in *Macaca fuscata*. Primates, 15: 219-226.

Shotake, T., 1974. Genetic polymorphisms in blood proteins in troops of Japanese macaques, *Macaca fuscata*, II. Erythrocyte lactate dehydrogenase polymorphism in *Macaca fuscata*. Primates, 15: 297-303.

Tanaka, K., T. Yamagata, J.S. Masangkay, M.O. Faruque and D. Vu-Binh *et al*., 1995. Nucleotide diversity of mitochondrial DNAs between the swamp and the river types of domestic water buffaloes,*Bubalus bubalis*, based on restriction endonuclease cleavage patterns. Biochem. Genet., 33: 137-148.

Tucker, E.M. and C. Crowley, 1978. NADH diaphorase as a genetic marker for sheep red cells. Anim. Blood Groups Biomed. Genet., 9: 161-167.

Ulbrich, F. and H. Fischer, 1966. The chromosomes of the asiatic buffalo (*Bubalus bubalis*) and the African buffalo (*Cyncerus caffer*). Zeitschrift Tierzuchtung Zuchtungsbiologie, 83: 219-223.

Van Hooft, W.F., O. Hanotte, P.W. Wenink, A.F. Groen, Y. Sugimoto, H.H. T. Prins and A. Teale, 1999. Applicability of bovine microsatellite markers for population genetic studies on African buffalo (*Syncerus caffer*). Anim. Genet., 30: 214-220.

Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 18: 7213-7218.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., 18: 6531-6535.

Yadav, B.R. and C.R. Balakrishnan, 1982. On the Y chromosome of the Murrah buffalo (*Bubalus bubalis*). Can. J. Genet. Cytol., 24: 189-192.

Youjun, H., Z. Xianfang, S. Jainghua, L. Mengnui and H. Fenxiang, 2001. Chromosome polymorphism and its mechanism of crossbred progenies between river buffalo and swamp buffalo. Paper Presented in the International Symposium on Sustainable Management of Animal Genetic Resources for Increasing Human Livelihood in Asia: a Planning Workshop. IRLI, Los Baron, Philippines.

Zabeau, M., 1993. Selective restriction fragment amplification: A general method for DNA fingerprinting. European Patent Application, No. 0-534-858-A1, http://www.freepatentsonline.com/EP0534858.html.