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Applications of molecular markers in livestock improvement: A Review

Ankur Singh[1*], Lalit Mohan Jeena[2], Sandeep Rahangdale[3]
[1-3] Animal Biotechnology Centre, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

Abstract
Molecular genetics deals with the genetic makeup of individuals which includes the identification and mapping of molecular genetic markers and genetic polymorphisms. Molecular genetic markers (DNA markers) are the powerful means for the genomic analysis and allow the connection of hereditary traits with genomic variation. Molecular techniques have led to the identification of multiple genes or markers associated with candidate genes that affect traits of interest in livestock, including genes for single-gene traits and QTL or genomic regions that affect quantitative traits. The discovery of genetic polymorphism at the DNA sequence level has been exploited as markers to explain the observed phenotypic variability in animals. Developments in molecular technologies like genomic selection may help overcome several of the limitations of traditional breeding programs and will be beneficial in breeding for lowly heritable disease traits that only manifest themselves following exposure to pathogens or environmental stressors. Application of molecular markers includes genetic diversity conservation, identification of disease carriers, parentage determination, marker-assisted selection, transgenesis, sex-determination and many more. The present review deals with the applications of molecular markers in versatile aspects that will prove beneficial to undertake further research to improve livestock health and production.

Keywords: polymorphism, marker-assisted selection, transgenesis and candidate gene

1. Introduction

In India, livestock sector contributes about 28% of the country's agriculture gross domestic product and about 5% of the country's overall gross domestic product. Livestock genetic improvement mainly depends on the selective breeding with superior phenotype. The use of molecular genetics techniques in association with traditional animal breeding tools are important to balance selection process and thus to optimize the animal breeding program. Recent developments in the fields of molecular biology and molecular technology involve the use of genetic (molecular) markers for the enhancement of production traits holistically. Some common molecular markers include Restriction Fragment Length Polymorphism (RFLP), Minisatellites, Microsatellites, Randomly Amplified Polymorphic DNA (RAPD), and Amplified Fragment Length Polymorphisms (AFLP). Development of molecular markers suggests their potential use for genetic improvement in livestock species.

2. Molecular 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 further used to detect the presence of a specific genotype or phenotype other than itself, which otherwise is very difficult to detect (Mitra, 1994) [12]. Such variations occurring at different levels, i.e. at the morphological, chromosomal, biochemical or DNA level can serve as the genetic markers. The markers revealing variations at the DNA level are referred to as the molecular markers.

3. Types of molecular markers used in livestock research

Based on the techniques used for the detection of molecular markers two major categories have been identified- Hybridization-based and PCR-based markers. The RFLP, wherein probes appropriately labelled are used for identification of the important candidate genes for genetic improvement. Various families of hyper variable repetitive DNA sequences, such as Microsatellites and Minisatellites, can be subjected to hybridization in order to study DNA fingerprinting patterns. The PCR-based markers are further subdivided into sequence-targeted PCR assays and arbitrary PCR assays (Deb *et al.*, 2012) [4-5]. Cleaved amplified polymorphic sequence, allele specific PCR, PCR amplification of specific alleles, simple sequence length polymorphism, and sequence targeted microsatellite site are in the former category. Arbitrary PCR assays include RAPD-RFLP, RAPD and microsatellite-primed PCR. Microsatellites serves as the most popular markers nowadays in studies associated with livestock genetic characterization due to their easy application using PCR and electrophoresis.

4. Benefits of using molecular markers

Developments in molecular biology unravel large number of genetic polymorphisms as a result of which scientists and researchers have been encouraged to use them as markers to study and evaluate genetic basis for the observed phenotypic variability (Yadav and Mitra, 1999) [18]. The unique genetic properties as well as methodological advantages of molecular markers make them useful and amenable, to a greater extent, for genetic research when compared to other genetic markers. They can also be used easily as reference points in transgenic breeding program and to identify the animals having the specific transgenes.

5. Applications of molecular markers in livestock improvement

I. Marker-Assisted Selection

The main potential for use of markers is to improve genetic improvement in livestock through within-breed selection which requires markers that trace within-breed variability. Marker-Assisted Selection (MAS) is applied for the indirect selection of superior breeding animals and depends on identifying association between genetic marker and linked Quantitative traits loci (QTL). The marker and QTL association mainly depends on distance between marker and target traits. As early as markers linked to QTL have been identified, they can be used in selection program and is beneficial when the traits are difficult and expensive to measure, has low heritability and are recessive in nature.

II. Markers in milk production

Phenotypic and biochemical markers used to identify an animal with high genetic merit for economic traits are restricted to the coding regions of chromosome and they are sex and age limited. Analysis studies on animal databases reveals that around 344 quantitative trait loci (QTL) are associated with milk traits and 71 with mastitis related traits (Ogorevc *et al.,* 2009) [13]. Association between DNA polymorphism and milk production traits have been studied for a number of genes, including: prolactin (He *et al.,* 2006*)* [10], diacylglycerol acyltransferase (DGAT1) (Grisart *et al.,* 2002) [8], bovine leukocytic antigen (BoLA)-DRB3 (Sharif *et al.,* 1999) [14], growth hormone receptor gene (Blott *et al.,* 2003) [3]. Based on the SNP analysis it has been concluded that CHBP2 and diplotype H2H8 of prolactin would act as useful genetic markers in a selection program on milk production traits in Holstein dairy cattle (He *et al.,* 2006) [10].

III. Mastitis associated Molecular markers

Out of many QTL studied for mastitis, association between DNA sequence variation and mastitis susceptibility has been found for around 15 candidate genes like CXCR2 (Youngerman *et al.,* 2004) [21], bovine lactoferrin gene (Wojdak *et al.,* 2006) [17].

IV. Molecular markers in cattle fertility

Sex-limitedness and low heritability of reproductive traits make phenotype selection difficult. Molecular level selection helps in decision-making early in an animal's life, which will substantially reduce the selection programs cost. Genes that have been found to play an important role in reproduction include: bovine follicle stimulating hormone receptor (Yang *et al.,* 2010) [19], bovine inhibin a[42]; bovine progesterone receptor (Yang *et al.,* 2012) [20] and growth differentiation factor 9. These candidate genes are linked with the total number of ova produced and number of transferable embryos in super ovulation. (Ganguly *et al.,* 2012*)* [6] showed that protamine 1 and 2 genes expressed differentially among normal and motility impaired semen of Frieswal bulls.

V. Molecular markers in Disease resistance of livestock

Resistance to infectious diseases is one of the critical determinant of the sustainability of higher production. Studies showed several different QTL with various effects on different disease phenotypes. 14 QTL associated with various indicators of resistance to Marek's disease, including the proliferation of tumours, survival and viraemia has been studied (Vallejo *et al.,* 1998) [16]. Similarly, Hanotte *et al*, (2003) [9] detected 16 QTL for various indicators of tolerance of trypanosomosis in a cross of N'Dama and Boran cattle.

VI. Molecular markers for selection of Thermo-tolerant cattle breed

Selection of thermo-resistant animals is an efficient way to increase cattle productivity. A series of studies has been conducted to identify genetic polymorphism in Hsp70 genes of cattle. Studies have been carried out to investigate the association between the heat shock response of mononuclear cells in blood and SNPs at the 50 UTR of Hsp 70.1. Such mutation sites as molecular genetic markers are very useful in the selection of heat tolerant cattle (Basirico *et al.,* 2011) [2].

VII. Molecular markers in other cattle genetic traits

Polymorphism detection in mitochondrial DNA markers (specifically D loop) helps in identifying the wild progenitors of domestic cattle and facilitates the process of establishing geographic pattern of genetic diversity (Deb *et al.,* 2012) [4-5]. Molecular investigation of the glutathione peroxidase-1 gene in Malvi and Nimari cattle (*B. indicus*) for draft capacity have been carried out and are found to had significant effect on draft ability of animals (Alex *et al.,* 2013) [1].

VIII. Molecular markers in Parentage Testing

Parentage testing using molecular markers yields much higher exclusion probability (> 90%) than the testing with blood groups (70–90%) or other biochemical markers (40–60%). DNA fingerprinting with oligoprobes (OAT18 and ONS1) has been successfully used for determining the parentage of IVF buffalo calf (Mattapallil and Ali, 1988) [18]. (Glowatzki *et al.,* 1995) [7] demonstrated that using two triplex microsatellite co-amplification systems, wrong parentage can be excluded with almost 99% accuracy. Molecular markers also acts as a tool for animal identification, especially for verification of the semen used for artificial insemination.

IX. Sex determination

Sexing of pre-implantation embryos is an important tool for improving herd for a desired purpose. Molecular techniques have potential application in determination of sex of pre-implantation embryos, as the embryos can be sexed using male-specific or Y-chromosome-specific DNA sequence as probes (Vaiman *et al.,* 1988) [15]. Sexing of embryo using PCR-based approach has various advantages like: (i) It can be carried out in less time with almost 100 per cent accuracy (ii) less invasive and requires very small quantities of DNA for PCR assay, which can be isolated from two to eight cells biopsied from the embryo (iii) It can be done at an early stage of embryo e.g. blastocyst stage (6 to 8 days) or even earlier at the 16–32 cell stage.

X. Genetic Conservation

Molecular markers serve as tool to examine the existing germplasm and to manipulate it to develop character-specific strains which provide the basis for effective genetic conservation. Molecular markers are increasingly employed to estimate population genetic parameters of relevance to conservation biology, such as within population heterozygosis, between population gene flow and genetic distinctiveness of taxonomic units.

XI. Mapping Livestock Genetic Diversity

The use of markers for product tracing has been implemented in some industries. Genetic map of an animal or poultry species can show which animals carry specific traits. The capacity to generate dense genetic maps in each species can in principle allow the complete genome to be evaluated for quantitative trait loci (QTL) with a major effect on the phenotype. Molecular markers help researchers to create a genetic linkage map which shows the position of markers and genes on a chromosome and the distance between genes. These genetic maps have been used to select markers that are distributed across whole genome.

6. Conclusion

Genetic improvement of livestock is a complex and continuous process. Ever since the domestication of animals, their productivity is of prime importance. In this pursuit many strategies 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. Utilization of marker-based information for genetic improvement depends on the choice of an appropriate marker. Selection of markers for different applications are influenced by several factors like 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 trend suggests that explosion in marker development will continue in the near future. It is expected that molecular markers will serve as 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.

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