



REVIEW ARTICLE

Molecular markers and their applications in cattle genetic research: A review



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Abstract India is one the world's five largest producers of livestock and poultry meat. The livestock sector contributes 28% of the country's agriculture gross domestic product and about 5% of the country's overall gross domestic product. Genetic improvement of livestock has been mainly dependent on the selective breeding with superior phenotypes. The use of molecular genetics techniques in association with conventional animal breeding tools are important to balance the process of selection and thus to optimize the animal breeding program. In this regard, the use of molecular markers is significantly important and it certainly has added advantages over conventional breeding techniques. On the basis of techniques used for the detection of molecular markers, two major categories have been identified: hybridization-based and polymerase chain reaction-based markers. Identification and use of markers for milk quality and production traits, disease resistance, and thermo-tolerance will ensure better health and productivity. Also, markers for fertility and carcass quality traits ensure faster and preferred growth in cattle. Apart from these, the use of different markers such as microsatellites for assessment of biodiversity will help the conservation of our indigenous germplasm. The present review deals with molecular markers and their application in versatile aspects that will prove beneficial for researchers and scientists to undertake further research to improve cattle health and production.

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Introduction

India's livestock sector plays a noteworthy role from the economic point of view, and contributes 28% and 5% of the country's agricultural and total gross domestic product, respectively. India has the world's largest bovine population with 199 million cattle,¹ which is about 14.7% of the world's total cattle population. India is the largest producer of milk, with 121.8 billion kg in 2010–11, of which cattle contribute 45% of the total production, i.e., 54.9 billion kg. However, the average productivity of Indian cows is far less than that of other developed countries. This may be attributable to several reasons, but the major concern of the country is the low productivity of indigenous animals. It is certain that the development of increasingly advanced statistical methods and use of assisted reproductive technologies have helped to maximize selection for genetic gain.

The focus on the main activities in animal breeding started changing from quantitative to molecular genetics in the 1990s throughout the globe. In order to optimize the animal breeding program, it is important to balance molecular genetic techniques with conventional animal breeding techniques.^{2,3} Recent developments in the fields of molecular biology and molecular technology involve the use of genetic (molecular) markers for the improvement of production traits holistically. This takes into consideration most of the factors that may affect the breeding program.^{4,5} In biotechnological language, a molecular marker is a DNA fragment in association with a certain location in the genome and can also be called a genetic marker; the marker is used in identifying partial DNA sequence in an unknown DNA pool.⁶ Several types of DNA molecular markers have been explored from 1980 onwards, all of them playing significant roles for evaluating farm animal genetic diversity that have become specifically advantageous for the geneticist.⁷ In 1990, scientists in the USA started using the rapid amplification of polymorphic DNA (RAPD) markers and amplified fragment length polymorphisms. A variety of genetic hybridization based markers [restriction fragment length polymorphism (RFLP) and minisatellites] and polymerase chain reaction (PCR) based markers [microsatellites and single nucleotide polymorphisms (SNPs)] have been used widely.^{8,9} The major challenge that faces molecular geneticists is to identify markers for genes that control the phenotypic variation in the target traits. The present review deals with the various molecular markers and the role played by them in: the selection of traits as well as animals for better productivity; disease resistant breeding; conservation and biodiversity; and geographical distribution of cattle breeds.

What is a molecular marker?

Genetic marker is a broad term for any visible or assayable phenotype or the genetic basis for assessing of the observed phenotypic variability. Genetic markers are classified: based on visually evaluated traits (morphological and productive traits), based on gene product (biochemical markers), and founded on DNA analysis (molecular markers).¹⁰ Molecular marker is a term used to refer to a specific DNA variation between individuals that has been

found to be associated with certain characteristics. These variations include insertions, deletions, translocations, duplications, and point mutations. They have characteristic biological properties that can be detected and measured in any parts of the body such as the blood or tissue at any stage^{11,12} and they are not confounded by environment, pleiotropic, or epistatic effects.¹³

Conventional tools versus molecular marker based genetic study

Conventionally selective breeding is a main focus to improve the genetics of animals. This includes progeny testing and various selection programs. Assisted reproductive technologies such as artificial insemination, multiple ovulation, and embryo transfer have been applied and there has been a dramatic improvement in the productivity of animals from the selective breeding of animals.^{14–21} However, traditional breeding techniques in dairy cattle take many years and do not efficiently take into account all sources of genetic variability. Similarly, in sex-limited, low-heritable, or late-expressed traits, the impact of traditional breeding is limited. The use of molecular markers will help to address the problems associated with traditional selection and thus help to select genetically superior animals. For better productivity and disease resistance, molecular markers have contributed much.²² The use of microsatellite markers and identification of the particular biomarkers associated with various diseases and economically significant clinical conditions (such as mastitis) has helped to increase the specificity and accuracy of disease resistant breeding and to enhance productivity.²³

Advantages of using molecular markers

Recent developments in biotechnology have made it possible to unravel a large number of genetic polymorphisms at the DNA level. As a result, researchers and scientists have been encouraged to use them as markers in order to evaluate genetic basis for the observed phenotypic variability.²⁴ 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.²⁵ They have a wide range of immediate applications: parentage determination; estimation of genetic distance; twin zygosity determination and freemartinism; sexing of pre-implantation embryos and disease carrier identification; and gene mapping as well as marker-assisted selection.²⁶ Molecular markers can easily be used as reference points in transgenic breeding and to identify the animals having the specific transgenes. Thus, the overall improvement in livestock species is greatly aided by the use of molecular markers.^{27,28}

Various types of molecular marker used in cattle genetic research

On the basis of techniques used for the detection of molecular markers two major categories have been identified:

hybridization-based and PCR-based markers. The traditional RFLP, wherein probes appropriately labelled are used for identification of the important genes for genetic improvement. Various families of hypervariable repetitive DNA sequences, such as microsatellites and minisatellites, can be subjected to hybridization in order to reveal DNA fingerprinting patterns. The PCR-based markers are further subdivided into sequence-targeted PCR assays and arbitrary PCR assays.¹² 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, RAPD-RFLP, and microsatellite-primed PCR.²⁹ Microsatellites are the most popular markers currently in studies associated with livestock genetic characterization due to their easy application using PCR and electrophoresis.³⁰ The estimates of within and between-breed genetic diversity and genetic admixture among breeds are possible due to the high mutation rate of these markers. Hypervariable microsatellite in the bovine steroid 21-hydroxylase gene is a classic example wherein it is indicative that dinucleotide blocks may be an abundant source of DNA polymorphism in cattle.^{31,32} SNPs have gained high popularity in the past decade but they are a biallelic type of marker. They are the most abundant of all marker systems known so far, both in animal and plant genomes. Besides their abundance, SNPs have received attention due to their genetically stability and amenability to high-throughput automated analysis.³³

Application of molecular markers in cattle genetic research

Markers in milk quality and production

Conventionally, phenotypic as well as biochemical markers have been used to identify an animal with high genetic merit for economic traits in cattle. These face the main problem that the polymorphic information carried by these markers is meager and restricted to the coding region of the chromosome. Also, they are sex and age dependent. Nowadays, molecular markers are being identified on a vast variety of genes of economic importance and are widely accepted. Analysis on animal databases shows that around 344 quantitative trait loci (QTL) are associated with milk traits and 71 with mastitis related traits.³⁴ Association between DNA polymorphism and milk production traits have been studied for a number of genes, including: prolactin³⁵; leptin³⁶; diacylglycerol acyltransferase (DGAT1)^{37–39}; stearoyl-CoA desaturase⁴⁰; bovine leukocytic antigen (BoLA)-DRB3,^{41,42} growth hormone receptor gene,⁴³ casein α s1 (CSN1S1)⁴⁴; ATP-binding cassette, subfamily G, member 2 (ABCG2) gene^{45–47}; protease inhibitor gene⁴⁸; osteopontin gene⁴⁹; proliferator-activated receptor gamma, coactivator (CoA) 1 α gene⁵⁰; growth hormone (GH) gene⁵¹; signal transducer and activator of transcription (STAT) 1^{52,53}; oxidized low density lipoprotein receptor 1⁵⁴; cytochrome P450, subfamily XI B, polypeptide 1⁵⁵; fatty acid synthase⁵⁶; caspase recruitment domain-containing protein 15⁵⁷; bovine K-casein gene CSN3,⁵⁸ thyroglobulin gene⁵⁹; β -lactoglobulin gene⁶⁰; POU class 1 homeobox 1⁶¹; STAT5A⁶²; and stearoyl CoA desaturase⁶³ (Table 1).^{35–63} There are 14 major

milk proteins reported in cattle.⁶⁴ These proteins show variability at DNA level perpetuating a protein change chemically, but the allele-specific effects are dependent on genetic background and experimental model (single locus vs. multi locus effects). Some of them such as β -casein⁶⁵ and κ -casein⁶⁶ have proven valuable in assessing the milk quality and lactation of dairy cattle and are economically significant.^{67,68} Recently, our laboratory reported that polymorphisms of β -casein (CSN2) A1A2 alleles are exist among Ongole (Indian zebu) and Frieswal (Holstein Friesian \times Sahiwal crossbred) cattle.⁶⁹

Out of the different marker studies, molecular markers for prolactin,³⁵ leptin,^{71,72} DGAT1,³⁷ and ABCG2⁴⁵ genes are reported by many authors for their association with milk traits. Leptin is considered as a powerful biomolecule for enhancing productivity in farm animals. Its role in lactogenesis, colostrum secretion, galactopoiesis, and immunity to mastitis has made it an important candidate gene for genetic studies.⁷¹ Many SNPs are detected on the promoter region as well as exon regions of the leptin gene, and are found to be highly associated with different milk traits.⁷² Singh et al⁷³ reported that C/BspEI/T and C/HphI/T polymorphisms of leptin gene is associated with milk protein percentage, whereas the C/HphI/T locus of leptin is significantly associated with Solid non fat (SNF) percentage.

Prolactin, which plays an important role in mammary gland development, milk secretion initiation, and maintenance of lactation, is another potential quantitative trait locus affecting milk production traits in dairy cattle. Based on the SNP analysis it has been suggested that CHBP2 and diplotype H2H8 of prolactin would be useful genetic markers in a selection program on milk production traits in Holstein dairy cattle³⁵; the DGAT1 gene—another important candidate gene—is involved in triglyceride synthesis. Research has been conducted to elucidate the DGAT1 polymorphism and its association with milk production traits.^{37–39,74,75} It is now recognized as an important candidate marker particularly for fat content³⁷ in dairy cattle.^{22,76} Again, the ABCG2 gene has been found to have a significant effect on milk fat and protein.^{45,77}

Apart from QTL analysis, application of microsatellite markers has been found to be fruitful in determining the effect of specific molecular markers on milk quality.^{29,77} For example, QTL such as BTA6 have an effect on milk fat and milk protein. Several microsatellite markers have been developed for the identification of the specific region of BTA6.⁷⁸

Even though much research has been carried out on different genes in search for the polymorphism and its association with different milk or other economic traits, there are very few studies that attempted to explain the probable reason for the underplaying mechanism behind the association between polymorphism and the observed phenotype. One reason could be that searches for the molecular markers are carried out at intronic regions or at unknown locations and are looking for a statistical association with the economic trait under study. Also, data from different sources are often fragmented and controversial. Advantageously, cattle QTL databases are now available. It is high time to integrate information from different sources and search for direct markers and find the causative sites for the QTL along with findings for all probable variation, which may help to

elucidate the reason behind the difference in the economic trait variation among the different variants.

Disease resistant cattle by marker-aided selection/breeding

Inadequate animal production significantly contributes to food shortage as well as poverty in the developing nations of the world. It is predicted that demand for animal products will increase by 50% by 2020. Unsustainable disease control measures is a contributory factor for deterioration of the situation that can be checked by breeding for disease resistance. In this regard molecular markers find their importance and the impacts may include a reduction in pathogen or parasite abundance contributing to improved productivity as well as welfare and robustness.^{79,80}

Molecular markers associated with mastitis

Out of many QTL studied for mastitis, a good association between DNA sequence variation and mastitis resistance or

susceptibility has been found for around 15 candidate genes: BoLA-DRB3^{42,81,82}; CXCR2⁸³; bovine lactoferrin gene⁸⁴; CCL2, IL8, CCR2, and IL8RA⁸⁵; caspase recruitment domain-containing protein 15; TLR4 gene^{86,87}; CXCR1⁸⁸; osteopontin⁸⁹; haptoglobin-matrix metalloproteinase 9 complex (Hp-MMP 9)⁹⁰; BRCA 1^{91,92}; and CACNA2D1.^{93,94} Also, amplified fragment length polymorphism marker analysis showed that 24 markers are associated with clinical mastitis of which CGIL4 is recognized as the most promising marker.⁸⁶ Studies have shown that BoLA-DRB3, IL8RA, TLR4, and LTF are highly associated with mastitis and can be considered as important candidate genes for cattle mastitis studies. Also, BoLA-DRB3 and LTF genes show an association with both mastitis and production traits. The BoLA gene complex shows varying results when investigated for its association with mastitis. This gene is also reported to be associated with parasitic infestations,⁹⁵ foot-and-mouth disease resistance,⁹⁶ and lameness and is related with many defensive factors.⁹⁷ Our team is presently working on identification of genetic polymorphism in the host receptor gene of foot-and-mouth disease in cattle. Toll-like receptors (TLRs) 2 and 4 can recognize pathogen associated

Table 1 Certain important bovine markers associated with lactogenic parameters in cattle.

Serial (SL) No.	Marker/polymorphic genes	Chromosomal location	Linked traits	Refs
1.	PRL (prolactin)	23	Milk production performance overall	35
2.	LEP (leptin)	4	Better milk yield with good energy balance and fertility	36
3.	DGAT1 (diacylglycerol O-acyltransferase 1)	14	Milk yield and composition	37–39
4.	SCD1 (stearoyl-CoA desaturase)	26	Milk fatty acid composition	40
5.	BoLA-DRB3 (<i>Bos taurus</i> major histocompatibility complex, class II, DRB3)	23	Somatic cell count and altered milk production traits	41,42
6.	GHR (growth hormone receptor)	20	Milk yield and composition	43
7.	CSN1S1 (casein α s1)	6	Milk protein expression	44
8.	ABCG2 (ATP-binding cassette, subfamily G, member 2)	6	Milk yield and composition	45–47
9.	PI (protease inhibitor)	21	Milk production and reproduction traits	48
10.	Osteopontin/SPP1 (secreted phosphoprotein 1)	6	Milk production traits	49
11.	PPARGC1A (proliferator-activated receptor gamma, coactivator 1 α)	6	Milk fat synthesis	50
12.	GH (growth hormone)	19	Milk production traits	51
13.	STAT1 (signal transducer and activator of transcription 1)	2	Milk protein synthesis and fat metabolism	52,53
14.	OLR1 (oxidized low density lipoprotein receptor 1)	5	Milk fat yield and fat percentage	54
15.	CYP11B1 (cytochrome P450, subfamily XI B, polypeptide 1)	14	Milk production traits	55
16.	FASN (fatty acid synthase)	19	Milk fat	56
17.	CARD15/NOD2 (nucleotide-binding oligomerization domain containing 2)	18	Milk production traits	57
18.	CSN3 (casein κ)	6	Milk production related traits	58
19.	TG (thyroglobulin)	14	Intra muscular fat and milk production traits	59
20.	LGB (β -lactoglobulin)	11	Milk protein concentration	60
21.	PIT 1/POU1F1 (POU class 1 homeobox 1)	1	Milk yield and productive life	61
22.	STAT5A (signal transducer and activator of transcription 5A)	19	Milk composition	62
23.	SCD (stearoyl CoA desaturase)	6	Milk production traits	63

molecular patterns. They stimulate both innate as well as adaptive immune systems. TLR4 recognizes the presence of lipoprotein A from Gram-negative bacteria, together with CD 14 receptor whereas TLR 2 is activated inside the phagosome by peptidoglycans from Gram-positive bacteria and is a crucial molecular marker in bovine mastitis.⁹⁸ Recent studies in our laboratory identified that genetic polymorphisms also exist in the Toll/interleukin-1 receptor of the bovine TLR4 gene among Frieswal crossbred cattle.²³ Lactoferrin, with its strong iron binding properties, has multifunctional roles, such as host defense against microbial infection and anti-inflammatory activity, and is a strong functional candidate for mastitis resistance or susceptibility. Another gene, osteopontin—expressed in a range of immune cells—plays a role in cell attachment and wound healing by mediating cell activation and cytokine production. Bulls with extreme estimated breeding values can be selected for osteopontin (SPP1) polymorphism, which will be of practical relevance to develop control strategies against coliform mastitis.⁸⁹ B-defensin 5 is an early bactericidal effector molecule of the innate system that shows pathogen specificity. It plays a crucial role in chronic sub-clinical mastitis caused by contagious pathogens such as *Staphylococcus aureus* or *Streptococcus agalactiae*. Hp-MMP 9 is also an essential biomarker in bovine mastitis as this protein complex is present in neutrophil granules and, in the serum of dairy cattle, plays a crucial role in acute bacterial sepsis that results in mastitis.⁹⁰ Association analysis conducted in our laboratory identified lactoferrin, BRCA1, and CACNA2D1 as mastitis-resistance molecular markers by performing with Somatic cell count (SCC).⁹⁹

Molecular markers in other diseases

About 200 diseases of cattle, goat, pig, and sheep are thought to be caused by sequence variations in single genes, of which the causal mutations in less than half of them have been elucidated. One of the potential candidate genes that confer innate resistance against various intracellular pathogens is Solute Linked Carrier 11A1 (SLC11A1). It is a transmembrane protein also known as natural resistance associated macrophage protein 1. There exists a significant association of polymorphisms at the 3' untranslated region (UTR) of the SLC11A1 gene with resistance/susceptibility to brucellosis in cattle^{95,100,101} and buffalo.^{102,103} The mannan-binding lectin gene participates as an opsonin in the innate immune system of mammals,¹⁰⁴ which is significantly correlated to somatic cell score. Engracia Filho et al¹⁰⁵ reported the association between dermal mast cells count and host resistance to ticks. By the use of bovine total leukocyte complementary cDNA microarray differences in the clusters of protein kinase C subunits and major histocompatibility complex class I or II related molecules can be identified significantly. Moreover, glyceraldehyde-3-phosphate dehydrogenase is also an important marker to detect susceptibility to trypanosomiasis in cattle. QTL mapping of chromosome 17 and BTA16 are significant to detect tolerance to trypanosomiasis in N'Dama breeds of cattle.^{106,107} The use of SNP chip technology has been found to be useful in order to identify animals having increased bovine tuberculosis resistance. A

genome wide selection strategy can be used for the prediction of the overall genetic merit for tuberculosis.^{108,109} Mapping of the QTL to BTA20 is a major approach to detect the infection status against paratuberculosis in cattle.^{110,111}

Molecular markers in conservation and biodiversity of cattle breeds

Thirty well recognized breeds of cattle exist in India along with several other breeds in different states that have not yet been characterized and defined, which reflects the enormous as well as diverse cattle genetic resources of the country. This has become the reason to give much importance to the use of molecular markers. Various fluorescent-labeled microsatellite markers have been used to characterize Kenkatha and Gaolao breeds, indicating a substantial shortfall which is about 21% heterozygous in Gaolao and 22% heterozygous in Kenkatha. There is also little genetic differentiation between the two breeds (approximately 2%).¹¹² Several microsatellite markers have also been used for conservation studies concerning certain other important cattle breeds.^{70,113} Markers such as *Miniopiterus schreibersii* clone MM 8, 12; ecdysis triggering hormone — 10, 225; and bovine dinucleotide repeat INRA 005, 063 are remarkable in this regard. A list of microsatellite markers that have been approved by Food and Agriculture Organization (FAO) for the characterization of cattle breed is available.¹¹³

Molecular markers for selection of thermo-tolerant breeds of cattle

The selection of thermo-resistant animals is an intelligent way to improve cattle productivity. It is known that zebu breeds are more heat tolerant in comparison to breeds of European origin.^{114–116} Identification and exploitation of *Bos indicus* genotypes to increase thermo-tolerance in cattle will be of great impact in the changing climatic scenario. Olson et al¹¹⁷ have identified a major gene—designated as the slick hair gene—that is dominant in inheritance and is responsible for producing a very short, sleek hair coat. Cattle inheriting this particular gene are better able to regulate body temperature and have higher milk yields.¹¹⁷

The cell is protected from several stressors by heat shock proteins (Hsp). A series of studies has been conducted to identify genetic polymorphism in Hsp70 genes of cattle. Investigations have been carried out to find out the association between the heat shock response of mononuclear cells in blood and SNPs at the 5' UTR of Hsp 70.1. Such mutation sites as molecular genetic markers are very useful in the selection of heat tolerant cattle.^{118,119} Recently, our team found that the presence of a cytosine deletion at the AP2 box region of Hsp 70 promoter may negatively affect the expression of Hsp70.1 mRNA in peripheral bovine mononuclear cells subjected to *in vitro* heat stress among Frieswal cattle breeds. Our studies show that cows with homozygous wild types had significantly better summer tolerance and higher total milk yield, peak

yield, yield at 300 days, and protein and fat percentages than the deletion type. The results thus suggest that the promoter region of bovine hsp70.1 gene is polymorphic among Frieswal cows and may be useful in the selection of dairy cows for relatively better thermo-tolerance and higher milk production.¹²⁰

We recently evaluated the effect of thermal stress on the relative expression profile of heat shock protein 90 (Hsp90) among Sahiwal (*B. indicus*) and Frieswal (*B. indicus* × *Bos taurus*) breeds of cattle. Our findings revealed that Sahiwal may express higher levels of Hsp90 than Frieswal to regulate their body temperature and increase cell survival under heat stress.^{121,122} Further, we have identified a polymorphism (SNP g.4338T > C) in the intronic region of HSP90 at AB1 gene among Sahiwal and Frieswal cows and the results indicate that TT genotypes are better in terms of heat-tolerance coefficient and milk productivity traits than CC and CT genotypes.¹²³

Heat stress leads to oxidative stress, which in turn causes alteration of plasma sodium and potassium ions. Because ATP1A1 encodes an integral membrane protein involved in electrochemical gradients of sodium and potassium ions across the plasma membrane,¹²⁴ various polymorphism studies have been done on this gene. It was reported that various SNPs at different positions of this gene have a significant association with heat-tolerance traits in dairy cows.^{125,126} Because we are in the initial phase of identification of specific genes controlling heat resistance, further exploitation is needed to draw a conclusion on the molecular basis of thermo-tolerance of cattle.

Molecular markers in cattle fertility

Sex-limitedness and low heritability of reproductive traits make phenotype selection more difficult. Also, selection at the molecular level helps in decision-making early in an animal's life, which will substantially reduce the cost of selection programs. Accordingly, there has been considerable interest in mapping and identifying genes involved in the regulation of reproductive traits and in elucidating their expression and polymorphic patterns. Genes that have been found to play an important role in reproduction include: bovine follicle stimulating hormone receptor¹²⁷; luteinizing hormone/choriogonadotropin receptor; bovine inhibin α ¹²⁸; bovine progesterone receptor¹²⁹; and growth differentiation factor 9.¹³⁰ These genes are associated with the total number of ova produced and number of transferable embryos in super ovulation. Hence, they can be used as potential markers for super ovulation response. Singh et al²² reported that polymorphism C/Nrul/T of bovine leptin gene has a significant association with age at first service and age at first calving.

In order to obtain a high conception rate, male fertility is equally or more important. It has been reported that early pregnancy wastage due to fertilization failure or embryogenesis failures are potentially of seminal origin.¹³¹ Molecular markers in candidate genes (cation channel of sperm 1, sperm-specific NHE, A kinase, anchor protein 4, pyruvate kinase, cytochrome oxidase, reproductive homeobox 5, cysteine rich secretory protein 2,

phosphatidylethanolamine binding protein 1, Doppel, tissue inhibitor of metalloproteinase, etc.) are important for assessing semen quality and fertility in bulls.^{132,133} Recently, Ganguly et al⁷⁶ showed that protamine 1 and 2 genes expressed differentially among normal and motility impaired semen of Frieswal bulls. Recently we identified that some of the Y specific microsatellite biomarkers are also associated with some of the semen quality traits among crossbred bulls.¹³⁴

Molecular markers in other cattle genetic traits

Detection of polymorphisms in mitochondrial DNA markers (specifically D loop) helps in identifying the wild progenitors of domestic cattle thereby facilitates the process of establishing geographic pattern of genetic diversity.¹⁰³ In beef cattle production, the myostatin gene is an important candidate gene associated with growth and carcass composition of cattle. It significantly affects economic traits such as hot carcass weight, fat depth, and marbling. Other than myostatin, bovine calpastatin,^{135,136} μ -calpain,^{137,138} and thyroglobuline¹³⁹ gene polymorphisms are also reported to be associated with meat tenderness traits and are good makers for beef cattle production. Also, growth differentiation factor 10¹⁴⁰ and prolactin receptor¹⁴¹ genes have potential effects on body measurement traits and affect growth traits in cattle. Molecular marker studies on the draft ability of various breeds are very limited in India and abroad. 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.¹¹²

Identifying disease carriers in bovine is very important especially in the era of artificial insemination where the chance of perpetuating the disease from the carrier bull to the progenies is high. RFLP is considered the method of choice for identification of bovine disease carriers. It has been used for identification of carriers for bovine leukocyte adhesion deficiency, deficiency of uridine monophosphate synthase, complex vertebral malformation, bovine citrullinemia, and factor XI deficiency,¹⁴² which have a significant economic impact on dairy cattle breeding worldwide. Another aspect of cattle research is semen identification and verification, which is done using microsatellite markers and thereby avoids incorrect parentage.¹⁴³

Conclusion and future perspectives

Various molecular markers for improving milk production and their association with disease, and productive and reproductive traits in cattle have proved to be beneficial to the dairy producers and breeders. They can also be used efficiently in breeding and management decisions. The use of various molecular markers in determining the susceptibility to economically important diseases (such as mastitis) along with other diseases caused by a plethora of microbes and parasites proves to be beneficial to check loss of animals and their productivity. Development of technologies to measure polymorphisms at loci along with microsatellite markers and microarray technology provides an idea about

the advancement in the field of biotechnology and genetics to improve cattle health and production. The role played by microsatellite markers in conservation and biodiversity of different breeds and assessing such diversity within and between breeds is incomparable. There has been strong focus on single genes and mapping QTL to make them available in the near future. Integration of information from all sources along with a search for direct markers and finding their causative sites for the QTL is required. The new era of *omics* technology provides us with genomic charts as well as genetic variations among individuals and groups that may prove beneficial processing as well as analysis and integration of a large amount of data. Thereby *omics* technology will provide valuable information regarding the precision of selection of molecular markers in the near future.

Conflicts of interest

All authors declare no conflicts of interest.

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