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Review Article

Genetic Diversity Studies Using Microsatellite Markers and Their Contribution in Supporting Sustainable Cattle Breeding Programs: A Review

U. Vinod[1], B. Punya Kumari[2]* and G. Bharathi[3]

[1]Department of Animal Genetics and Breeding, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, INDIA
[2]Livestock Research Station, Palamaner, Chittoor, INDIA

*Corresponding Author: punya67@yahoo.co.in

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Abstract

In genetic characterization studies microsatellite markers have become the most frequently used markers among all other markers. Three different groups described microsatellites at the same time as special type of VNTR's consisting of tandemly repeated sequences with many di-to hexa nucleotides. Microsatellite markers distributed evenly over the genome. Microsatellite DNA markers are the most helpful tools for genetic diversity studies owing to their high variability and abundance throughout the genome. The various parameters developed so far to measure genetic diversity within and among populations are effective number of alleles, observed and expected heterozygosities, F- statistics, polymorphic information content, test for Hardy-Weinberg equilibrium and genetic distance and phylogenetic or tree building approach. The objective of this review is to quantify the genetic diversity studies of cattle populations using microsatellite markers and their contribution in supporting sustainable cattle breeding programs.

Keywords: Heterozygosity, Molecular Markers, Microsatellites and Polymorphic Information Content

Introduction

Traditionally the animal improvement was dependent on quantitative evaluation of breeding values from animals own or individual performance, ancestors, sibs and progeny performance. Although significant genetic progress has been obtained and continues to be achieved by utilising these quantitative genetic strategies, this approach has its own limitation because the environmental factors hide the phenotypic expression of trait there by reducing accuracy of selection. Moreover, it is not clearly understood how many genes are actually involved in the expression of a particular quantitative trait or how each gene contributes to the trait and where it is located in the genome. Such knowledge is important for overall improvement of animals.

Genetic characterization of livestock breeds and species allows the evaluation of genetic variability, a fundamental component in working out breeding strategies and genetic conservation plans. Microsatellite markers have been widely used for population genetic analyses of livestock species, as they are informative and can successfully interpret the relationships between individuals and populations. Microsatellites have been commonly used to estimates within-breed genetic diversity and inbreeding levels, introgression from other species, genetic differentiation and admixture among breeds (Edwards *et al*., 2000; Garcia *et al.*, 2006; Tapio *et al*., 2006; Gingia *et al*., 2009; Li and Kantanen 2009; Qi *et al*., 2009; Tapio *et al.*, 2010 and Ghazy *et al.*, 2013).

Marker

Markers are broadly classified into three main groups, namely, biochemical markers, morphological and nucleic acid (especially DNA based) markers (Fig. 1). Biochemical markers include isozymes and to some extent the use of secondary metabolites. As both these categories are based on the expression of genes, they are environment and developmental stage dependent, whereas DNA based markers are neutral and are independent of any, environmental cues or developmental stage (temporally and spatially independent) (Sarwat *et al.*, 2012). Molecular markers provide information ranging from diversity at the nucleotide level (SNPs) to gene and allele frequencies (genotype information), extent and distribution of genetic diversity, and population structure. Such information gained can be utilized for devising a proper conservation strategy and management of gene bank.

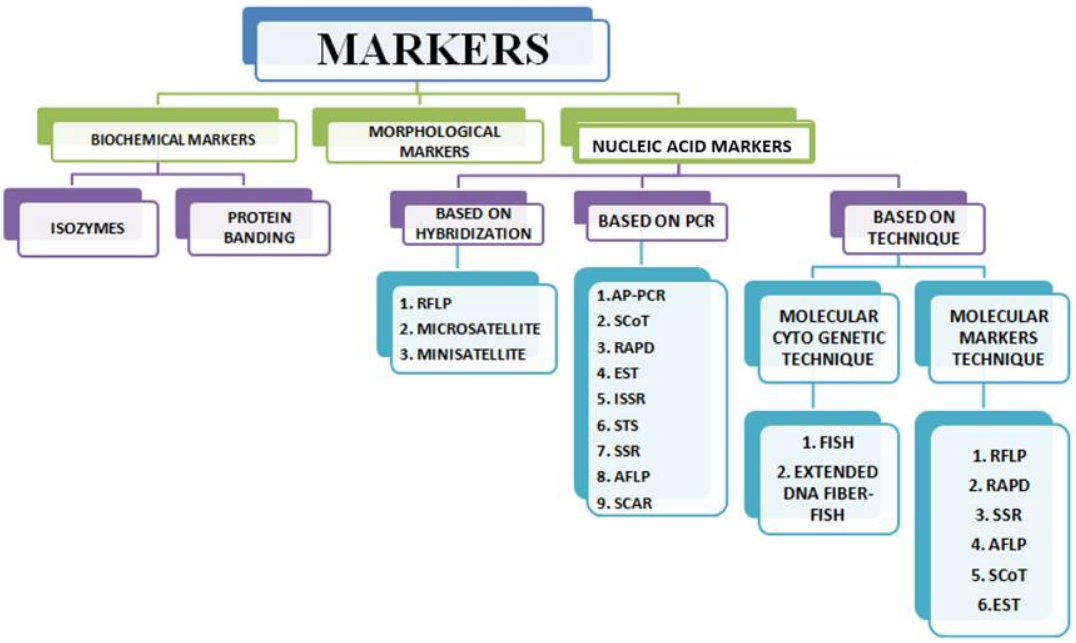

Figure 1: Classification of markers

Genetic Markers

The genetic marker is a known DNA sequence or gene located on the chromosome which can be applied in the identification of individual species or breeds or we can use it in the discovery of other genes or DNA sequences.

Classification of genetic markers:

1. Type I markers
2. Type II markers

Type I markers are associated with genes of known function, while type II markers are related with anonymous genomic segments (O'Brien, 1991).

Type I Genetic Markers

Under this classification, most of the RFLP markers are type I markers because they were recognized during analysis of known genes. Similarly, allozyme markers (the protein they encode has known function) and EST markers (represent transcripts of genes) are also categorized under type 1 markers (O'Brien, 1991) and serve as a bridge for comparison and convey genomic information from a map rich species into a relatively map-poor species.

Type II Genetic Markers

RAPD (Random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) markers are type II markers because they are amplified from anonymous genomic regions via the polymerase chain reaction (PCR). Microsatellites are type II markers and also known as simple sequence repeats (SSR). These markers are abundant, allocate throughout the genome, co-dominant and are highly polymorphic, as well as being species-specific. SNP markers are mostly type II markers are evolved from expressed sequences (eSNP or cSNP). Normally type II markers such as RAPDs, microsatellites, and AFLPs are considered to be non-coding. Such markers are found predominantly and used in population genetic studies (Brown and Epifanio, 2003).

Microsatellite Markers

The term Microsatellite was first coined by Lit and Lutty (1989). Microsatellites or simple sequence repeated (SSR) loci, which have been referred as variable number of tandem repeats (VNTRs), short tandem repeats (STR), and simple sequence length polymorphisms (SSLPs), are found throughout the nuclear genomes of most of eukaryotes and to a lesser extent in prokaryotes. Microsatellites are varied from one to six nucleotides in length. The sequences of di-, tri- and tetra nucleotide repeats are the most common choices for molecular characterization studies. They are tandemly repeated (usually 5-20 times) in the genome with a minimum repeat length of 12 bps.

Applications of Microsatellite Markers

Microsatellite markers are ideal for population – level studies for number of reasons (Fig. 2):

- Microsatellite markers more variable and informative than RFLP, RAPD and AFLPs.
- They are randomly distributed throughout the genome, commonly occurring in non-coding regions and typically selectively neutral.
- Microsatellite loci are often hyper variable within populations and show much higher mutation rates than other nucleolar regions (Weber and Wong, 1993). The high mutation rates, upto 10-2 per generation (Jarne and Lagoda, 1996) allows them to be useful for studies of population structure.
- Microsatellite markers are highly polymorphic, follow Mendelian inheritance and are unaffected by environmental factors.
- Microsatellite alleles show codominant inheritance, making them relatively easy to score directly.
- Using the technique of PCR-based require only low quantities of template DNA.
- These markers are considered to be the best marker system for the detection of intervarietal polymorphisms.

- They are also useful for parentage analysis and for estimating the degree of relatedness of individuals or groups.
- Microsatellite markers have certain disadvantages viz., expensive, laborious and time-consuming and homoplasy.

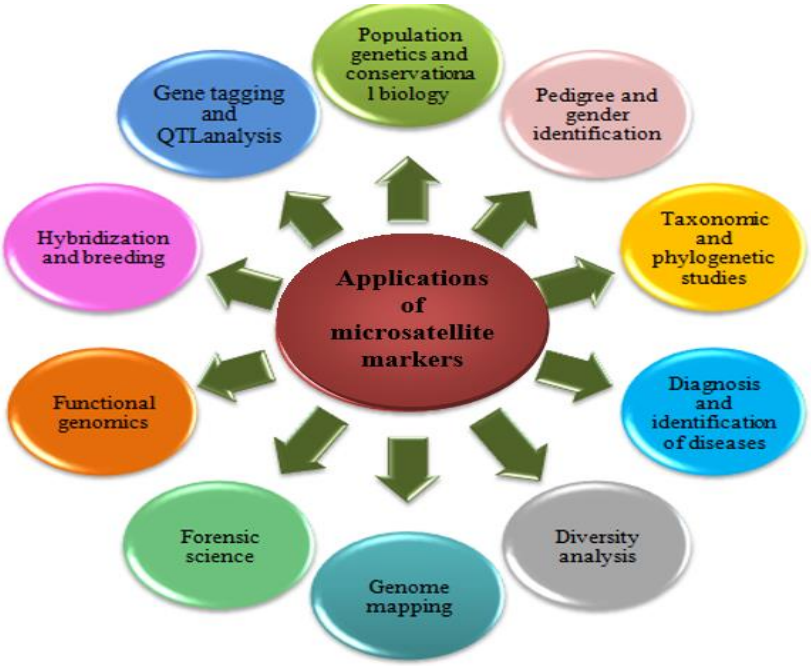

Figure 2: Applications of microsatellite markers

Breed Characterization Studies Using Microsatellites

Molecular characterization has a role in understanding of gene flow, the movement of alleles within and between populations of the same or related species, and its consequences (Toro *et al*., 2009). Molecular characterization helps in genetic management of small populations, to avoid excessive inbreeding and improving conservation strategies (Hanotte and Jianlin, 2005).

Some of the parameters which can help to study genetic diversity within a population are the mean number of alleles per locus, the average expected and observed heterozygosity values (Halima *et al.*, 2012) and testing for deviations from Hardy– Weinberg equilibrium (HWE) per population. The deviation from HWE provides information about those primary forces viz., natural selection, mutation, migration; genetic drift and non-random mating that derive evolutionary change. Earlier research work published on allele diversity, observed (H_o) and expected heterorozygosity (H_e) within population, inbreeding estimate (F_{IS}), Mean Polymorphism Information Content (PIC) and Hardy-Weinberg equilibrium estimates (HWE) reported in various cattle breeds are presented Table 2.

Cattle (*Bostaurus*) have been economically and culturally important farm animal species since their domestication. Many studies have evaluated the diversity of cattle in Asia (Sharma *et al*., 2016; Nishimaki *et al*., 2013; Shah *et al*., 2013), Europe (Mateus *et al*., 2004; Maudet *et al*., 2002), Africa (Dadi *et al*., 2008; Edea *et al.*, 2015), Korea (Suh *et al*., 2014), China (Wang *et al*., 2015; Li *et al*., 2007) and South America (Acosta *et al*., 2013; Brasil *et al*., 2013; Piccoli *et al*., 2014)

Microsatellite Allele Diversity

The mean number of alleles is a good indicator of the genetic polymorphism within the population (Halima *et al*., 2012) and it depends on sample size of the population because of the potential presence of unique alleles in a population that may occur at low frequencies (Qwabe, 2011). The mean number of observed and expected alleles of different cattle populations, as reported by various authors are summarized in Table 2, which varied from 2.04 (El-Sayed *et al*., 2016) to 22.66 (Hussain *et al*., 2016) and 2.5 (Ramesha *et al*., 2016) to 7.6 (Teneva *et al.*, 2005) respectively, whereas allele size varied from 64 (Selvi *et al*., 2004) to 382 (Thiagarajan *et al.*, 2012). The number

of detected alleles may increase with an increase in population size. Mean number of alleles that indicate the genetic polymorphism within the studied microsatellites were reported for several cattle populations (Table 2). A high number of alleles imply more genetic variation (Nei, 1987) and it is the key relevance in conservation programs. However, though those reports explain the existence of high polymorphism, the average number of alleles depends on sample size; number of observed alleles tend to increase with increasing population size (Aljumaah *et al.*, 2012). Therefore, it is important that sample population sizes should be more or less equal for comparison (Qwabe, 2011). Microsatellite markers are currently the markers of choice for molecular genetic studies. The microsatellites recommended by International Society of Animal genetics (ISAG / FAO) were used widely for these studies (Table 1).

Table 1: The primer sequences and chromosomal localization of the microsatellites used (FAO, 2011)

S. No.	Microsatellite Locus	Primer sequence (5' ⟶3')	Chromosome number	Accession number	Annealing temperature (Oc) (reported)
1	BM1818	F: AGCTGGGAATATAACCAAAGG	23	G18391	57.5
		R: AGTGCTTTCAAGGTCCATGC			
2	BM1824	F: GAGCAAGGTGTTTTTCCAATC	1	G18394	57
		R: CATTCTCCAACTGCTTCCTTG			
3	BM2113	F: GCTGCCTTCTACCAAATACCC	2	M97162	57
		R: CTTCCTGAGAGAAGCAACACC			
4	CSSM66	F: AAACTGTATTCTCTAATAGCTAC	14	--	55-65
		R: GCAAGACATATCTCCATTCCTTT			
5	CSRM60	F: AAGATGTGATCCAAGAGAGAGAGGCA	10	Z14042	63.5
		R: AGGACCAGATCGTGAAAGGGCATAG			
6	ETH 3	F: GAACCTGCCTCTCCTGCATTGG	19	222744	55-65
		R: ACTCTGCCTGTGGCCAAGTAGG			
7	ETH10	F: GTTCAGGACTGGCCCTGCTAACA	5	Z22739	66
		R: CCTCCAGCCCACTTTCTCTTCTC			
8	ETH152	F: TACTCGTAGGGCAGGCTGCCTG	5	Z14040	64
		R: GAGACCTCAGGGTTGGTGATCAG		G18414	
9	ETH185	F: TGCATGGACAGAGCAGCCTGGC	17	Z14042	66
		R: GCACCCCAACGAAAGCTCCCAG			
10	ETH225	F: GATCACCTTGCCACTATTTCCT	9	Z14043	58
		R: ACATGACAGCCAGCTGCTACT			
11	HAUT24	F: CTCTCTGCCTTTGTCCCTGT	22	X89250	52-55
		R: AATACACTTTAGGAGAAAAATA			
12	HAUT27	F: AACTGCTGAAATCTCCATCTTA	26	X89252	52
		R : TTTTATGTTCATTTTTTGGACTGG			
13	HEL 1	F: CAACAGCTATTTAACAAGGA	15	X65202	52
		R: AGGCTACAGTCCATGGGATT			
14	HEL5	F: GCAGGATCACTTGTTAGGGA	21	X65204	52-57
		R: AGACGTTAGTGTACATTAAC			
15	HEL9	F: CCCATTCAGTCTTCAGAGGT	8	X65214	52-57
		R: CACATCCATGTTCTCACCAC			
16	HEL13	F: TAAGGACTTGAGATAAGGAG	11	X65207	52-57
		R: CCATCTACCTCCATCTTAAC			
17	ILSTS005	F: GGAAGCAATGAAATCTATAGCC	7	L23481	54.5
		R: TGTTCTGTGAGTTTGGTAAGC			

18	ILSTS006	F: TGTCTGTATTTCTGCTGTGG	7	L23482	57
		R: ACACGGAAGCGATCTAAACG			
19	INRA005	F: CAATCTGCATGAAGTATAAATAT	10	X63793	52.5
		R: CTTCAGGCATACCCTACACC			
20	INRA23	F: GAGTAGAGCTACAAGATAAACTTC	3	X67830	55
		R: TAACTACAGGGTGTTAGATGAACTC			
21	INRA 032	F: AAACTGTATTCTCTAATAGCTAC	11	X67823	55-58
		R: GCAAGACATATCTCCATTCCTT			
22	INRA035	F: TTGTGCTTTATGACATATCCG	16	X68049	57.5
		R: ATCTTTGCAGCCTCCACATTG			
23	INRA037	F: GATCCTGCTTATATTTAACCAC	10	X71551	53
		R: AAAATTCCATGGAGAGAGAAAC			
24	INRA063	F: ATTTGCACAAGCTAAATCTAACC	18	X71507	58
		R: AAACCACAGAAATGCTTGGAAG			
25	MM12	F: CAAGACAGGTGTTTCAATCT	9	Z30343	53
		R: ATCGACTCTGGGGATGATGT			
26	SPS115	F: AAAGTGACACAACAGCTTCTCCAG	15	FJ828564	61.5
		R: AACGAGTGTCCTAGTTTGGCTGTG			
27	TGLA53	F: GCTTTCAGAAATAGTTTGCATTCA	16	-	58
		R: ATCTTCACATGATATTACAGCAGA			
28	TGLA122	F: CCCTCCTCCAGGTAAATCAGC	21	-	55-58
		R: AATCACATGGCAAATAAGTACATAC			
29	TGLA126	F: CTAATTTAGAATGAGAGAGGCTTCT	20	-	55-58
		R: TTGGTCTCTATTCTCTGAATATTCC			
30	TGLA227	F: CGAATTCCAAATCTGTTAATTTGCT	18	-	59
		R: ACAGACAGAAACTCAATGAAAGCA			

Within Population Inbreeding Estimates

High positive F_{IS} indicates a high degree of homozygosity, while negative values indicate low level of inbreeding (Dorji *et al.*, 2012). The means for within population inbreeding estimates of various cattle breeds reported in earlier literature are detailed in Table 2, and the inbreeding estimates varied from -0.83 (Hussein *et al.*, 2015) to 0.97 (Vinod *et al.,* 2019).

Heterozygosity

The observed heterozygosity, the proportion of heterozygotes observed in a population and the expected heterozygosity, proportion of heterozygotes expected in a population following the Hardy Weinberg proportions (Ojango *et al.,* 2011) are the most widely used parameters to measure genetic diversity in a population (Toro, *et al.*, 2009). The mean observed and expected heterozygosity in various cattle populations, as reported by earlier authors are presented in Table.2, which varied from 0.02 (Vinod *et al.,* 2019) to 0.78 (Teneva *et al.*, 2005) and 0.47 (Chaudhari *et al.*, 2009) to 0.92 (Thiagarajan *et al.,* 2012), respectively. Literature suggests that heterozygosity estimates having greater than 0.5 heterozygosity values are believed to be appropriate for genetic diversity studies (Dávila *et al.*, 2009; Dorji, *et al*., 2012). Small population size, inbreeding, high selection pressure in closed population and minimal or null immigration of new genetic material into the population might be the reasons for low heterozygosity in some population. (Canon *et al.,* 2006). Observed heterozygosity values are almost closer to, or lower than, the expected heterozygosity in most of the breeds under study indicating no overall loss of heterozygosity (allele fixation) (Araujo *et al.,* 2006) and the populations are in HWE.

Table 2: Genetic variability measures in various cattle breeds

S. No.	Country of origin	Allele size	N_O	N_e	H_e	H_O	F_{IS}	PIC	No of micro-satellite markers	Author
Mafriwal dairy cattle	Malaysia	64-300	6.56	5.16	0.8	0.52			50	Selvi *et al.* (2004)
Hallikar cattle	India	102-294	6.36		0.78	0.75		0.75	19	Naveen *et al.* (2005)
Bulgarian grey cattle	Bulgaria	78-260	7.6	7.6	0.86	0.78		0.72	11	Teneva *et al.* (2005)
Krishna valley cattle	India	94-300	4.72	4.21	0.65	0.66		0.62	25	Karthickeyan *et al.* (2006)
Kenkatha cattle	India		5.95	3.4	0.68	0.54	0.21	0.63	21	Pandey *et al.* (2006)
Tharparkar	India	102-296	6.2	3.23	0.67	0.57	0.14	0.6	25	Sodhi *et al.* (2006)
Umblachery cattle	South India	94-300	4	2.91	0.61			0.56	25	Karthickeyan *et al.* (2007)
Burlina cattle	Italy		6.7		0.67	0.67	-0.003		12	Dalvit *et al.* (2008)
Gaolao cattle	India	86-301	9.52	3.99	0.688	0.530.21	0.21	0.65	25	Chaudhari *et al.* (2009)
Kenkatha cattle	India	86-297	7.92	3.53	0.47	0.62	0.22	0.59	25	Chaudhari *et al.* (2009)
Punganur cattle	India	77-255	6		0.66	0.68	.	0.62	11	Chenna Kesvulu *et al.* (2009)
Kangayam cattle	India	94-300	4.04	2.9	0.61		-0.08	0.56	25	Karthickeyan *et al.* (2009)
Hariana cattle	Pakistan	75-309	4.59	2.87	0.67	0.51	0.25		27	Rehman and Khan (2009)
Hissar cattle	Pakistan	75-309	4.37	2.89	0.63	0.47	0.25		27	Rehman and Khan (2009)
Colombian cattle breeds	Colombia	112-264	14.2		0.84	0.63	0.25		10	Montoya *et al.* (2010)
Hallikar cattle	India	122-302	7.8	3.75	0.68	0.59	0.13	0.65	5	Chandra shekar *et al.* (2011)
Latin American creole cattle	Spain		6.92	4.06	0.73	0.71	0.02		19	Delgado *et al.* (2011)
Istrian cattle	Croatia	102-301	9.1		0.72	0.64	0.11	0.53	9	Ivankovic *et al.* (2011)
Kuman hill cattle	India		9.65	4.31	0.73	0.66	0.08		23	Pandey *et al.* (2011)
Motu cattle	India		10	4.73	0.74	0.66	0.11		23	Pandey *et al.* (2011)
Umblachery cattle	india	95-382	10.25	8.77	0.92		0.14		7	Thiagarajan *et al.* (2012)
Ghumusari	India		12.19	5.54	0.75	0.68	0.1	0.72	21	Deepika and Raj kumar (2013)
Binjharpuri	India		11.43	5.3	0.76	0.76	0.01	0.73	21	Deepika and Raj kumar (2013)
Hariana	India		11.81	5	0.76	0.76	0.01	0.74	21	Deepika and Raj kumar (2013)

Indigenous grey cattle-10	India		9.00-12.19	4.2-5.6	0.7-0.78	0.68-0.76	0.033		21	Deepika and Raj kumar (2014)
Non descriptive cattle	India		10.4	5.12	0.81	0.76	-0.13	0.93	5	Kumar *et al.* (2014)
Iberian cattle	Spain		4.78	3.76	0.68	0.65	-0.05		21	Martin *et al.* (2014)
Korean native cattle	Korea	77-276	9.2		0.73	0.66		0.69	30	Suh *et al.* (2014)
Turkish breeds	Turkey		13.45		0.78	0.74	0.06		20	Yusuf *et al.* (2014)
Pulikulum	India		7.89	3.73	0.66	0.57	0.15	0.82	18	Barani *et al.* (2015)
Fuga	sudan		7	3.96	0.72	0.77	-0.83	0.66	9	Hussein *et al.* (2015)
Butana	Sudan		5	3.3	0.69	0.73	-0.31	0.63	9	Hussein *et al.* (2015)
Kenana	Sudan		5	3.12	0.65	0.69	-0.19	0.59	9	Hussein *et al.* (2015)
Bali cattle	Indonesia	70-95	8	4.16	0.6	0.41	0.29	0.57	4	Septian *et al.* (2015)
Simmentral cattle	Indonesia	77-190	6.28	3.81	0.68	0.66	NA	NA	12	Augung *et al.* (2016)
siwa cattle	Egypt	127-329	2.75	2.75	0.46	0.09	0.83	0.45	8	El-Sayed *et al.* (2016)
Farafra	Egypt	110-306	2.04	2.85	0.66	0.2	0.69	0.64	8	El-Sayed *et al.* (2016)
Pakistani cattle breeds	Pakistan	-	22.66	6.73	0.81	0.49	0.28	0.81	21	Hussain *et al.* (2016)
Malnad Gidda	India		7.27	3.31	0.68	0.63	NA	0.63	11	Ramesha *et al.* (2016)
Vechur	India		3.54	2.79	0.7	0.59	NA	0.56	11	Ramesha *et al.* (2016)
Punganur	India		2.5	2.5	0.59	0.54	NA	0.5	11	Ramesha *et al.* (2016)
Belahi cattle	India		9.31	4.39	0.72	0.69	0.03	0.71	16	Vohra *et al.* (2017)
Punganur cattle	India	79-309	7.9	6.17	0.83	0.02	0.97	0.81	20	Vinod *et al.* (2019)

N_o= observed number of alleles, N_e= expected number of alleles, He = observed heterozyogosity, Ho= expected heterozyogosity, F_{IS} = within population inbreeding estimates and PIC = polymorphic information content

Polymorphism Information Content (PIC)

PIC value is the statistical evaluation of informativeness of marker. Informativeness of polymorphic DNA markers is determined by the computation of heterozygosity and polymorphism information content (PIC) value. The PIC value may vary from 0 to 1. The PIC values > 0.5 are considered as highly informative, 0.5 to 0.25 as moderately informative and < 0.25 least informative. The mean PIC values of different cattle populations reviewed in Table.2. Loci with many alleles and PIC value of one are most desirable (Botstien *et al.,* 1980). A higher value of PIC indicates more alleles and greater polymorphism at the particular locus. The polymorphism at any locus is created by increasing dinucleotide repeats and mutation (Karthickeyan *et al.*, 2007).

Hardy-Weinberg Equilibrium

A population with the constant gene and genotypic frequencies is said to be in Hardy-Weinberg equilibrium (HWE). The relationship between gene frequencies and genotype frequencies is of the greatest importance because many of the deductions in population and quantitative e genetics rest on it. The natural processes of mutation, migration, non-random mating, genetic drift and both artificial and natural selection are the factors that are known to cause deviations from HWE. Ideal HWE population do not actually occur in nature.

Conclusion

Microsatellite markers are most informative molecular markers as they are highly abundant, very simple to analyse, easy to score and low-cost locus detectection by PCR. Ubiquity, Mendelian co-dominant inheritance, extreme polymorphism and high heterozygosity made microsatellites to play a predominant role as markers in the genome. The various parameters viz., mean number of alleles per locus, observed and expected heterozygosity, PIC, genetic distance and phylogenetic approach are being used to evaluate genetic diversity within and among populations. Microsatellite markers are very powerful genetic markers for identification of genetic structure of cattle population and to study the genetic variation of closely related individuals. Microsatellite marker analysis provides essential requisite information for formulating appropriate breeding strategies and conservation plans for cattle.

Conflict of Interests

There is no conflict of interest.

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