*Original Research*

# Genetic Diversity and Relatedness among Different Four Cattle Breeds Reared in Rajasthan

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**Abstract**

*Genetic diversity and relationship between population of one hundred nine individuals from four cattle breeds i.e. Rathi, Tharparkar, Gir and Kankrej were studied using 18 microsatellite markers, proposed by FAO and ISAG. A total of 144, 135, 147 and 155 alleles were identified across 18 microsatellite loci in Rathi, Tharparkar, Gir and Kankrej cattle, respectively. Means of observed and expected heterozygosity were found to be 0.52±0.24 and 0.75±0.20 in Rathi, 0.47±0.32 and 0.7±0.28 in Tharparkar, 0.5±0.29 and 0.68±0.32 in Gir and 0.62±0.29 and 0.72±0.27 in Kankrej cattle, respectively. The average PIC (Polymorphic Information Content) value was found to be highly informative amongst all the cattle breeds. The Mean value of F-statistics significantly deviated from zero indicating that breeds are well separated. The present study indicates a significant deficit of heterozygotes in studied cattle breeds. The Nei's Genetic distance analysis of the four cattle breed population revealed close genetic similarity between Rathi-Tharparkar in comparison to other breeds with maximum genetic distance between Tharparkar and Gir. Phylogenetic analysis based on UPGMA method further supported close genetic relationship between Rathi-Tharparkar as they clustered in one group.*

**Key words:** Cattle Breeds, F-Statistics, Genetic Diversity, Heterozygosity, Microsatellite Marker, Polymorphism, Phylogenetic Analysis

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## Introduction

Animal species are very important for food and agriculture production and the domestication process of animal species has continued for almost last 12,000 years (http://www.fao.org/dad–is/html). There are around more than 40 domestic livestock species available but amongst them only 14 % contribute to the world's food and agriculture production. India is rich in farm animal diversity and cattle is considered to be the most important farm animal with 219.64 million cattle population which is 16.21% of world cattle

population (FAO 2001). But cattle genetic diversity is declining from last century (Sodhi *et al.*, 2008). Decreasing genetic diversity in cattle population prevents farmers from selecting stocks or developing new breeds in response to environmental change, threats of disease, new knowledge of human nutrition requirements, changing market conditions and societal needs, all of which are largely unpredictable. There are thirty seven phenotypically recognized breeds of indigenous cattle in India (NBAGR-Annual Report 2014). Indigenous breeds have distinct merits over exotic breeds, like better disease resistance, low Rajasthan state stands 2nd in the country in terms of milk production and 4th in productivity of milk from indigenous cattle breeds (Basic Animal Husbandry Statistics, 2014).

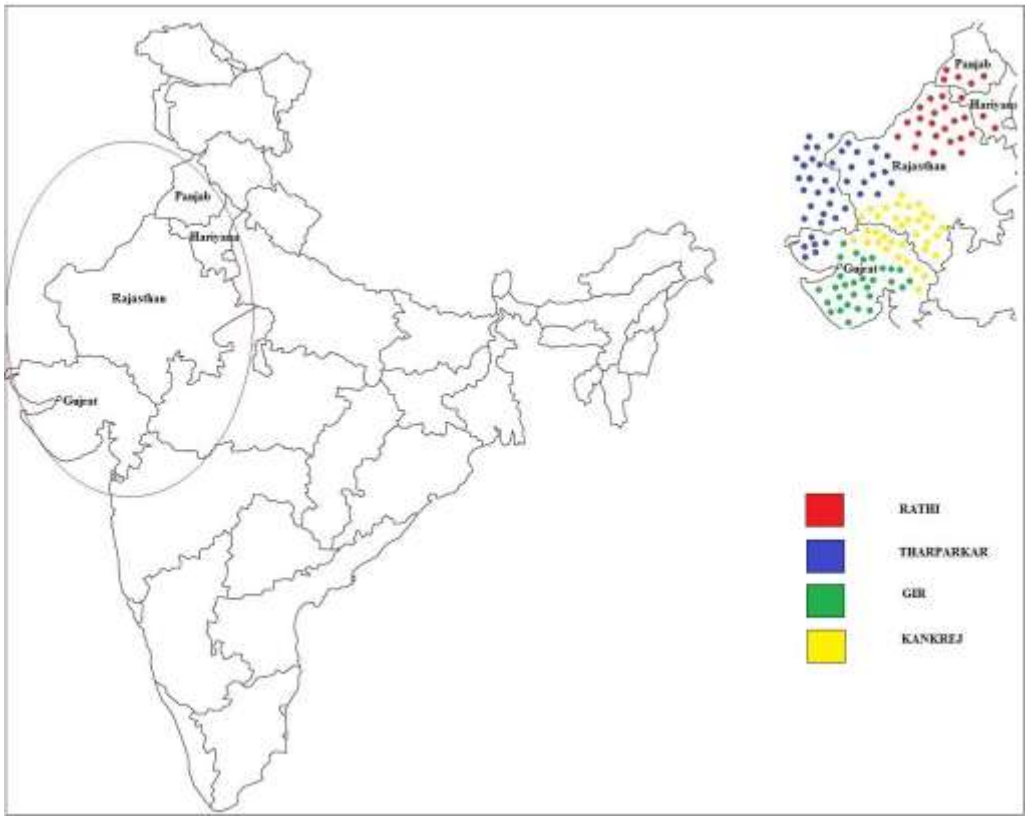


**Fig. 1**: Distribution of cattle population

It is the only state where the population of indigenous cattle is more than 90% of the total cattle population. The population of indigenous cattle has decreased by 8.94 per cent (19th annual census 2012 statistics). Despite of the fact that the state owes important indigenous cattle breeds like Rathi, Tharparkar, Gir, Kankrej, Haryanvi, Malvi, Naguri, Sahiwal etc., more than 50% of total indigenous cattle are still being rated as non-descript. It is therefore important that indigenous breeds of cattle are conserved, developed and proliferated through professional breeding programs and farm management. Microsatellite markers have high degree of polymorphism and co dominance making them extraordinarily informative for





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discrimination of breeds (Vaiman *et al.*, 1994). It is widely used to assess the genetic diversity and population structure amongst the species for ex-situ and in-situ conservation procedures (Kantanen *et al*., 2000).The present study focused on genetic diversity among four native indigenous cattle breeds i.e. Rathi, Tharparkar, Kankrej and Gir using microsatellite markers and also estimated the informativeness of markers with inbreeding estimation (heterozygote deficiency) within and between selected cattle breeds population.

## Material and Methods

### Sample Collection

From the native breeding grounds of i.e. Rathi, Tharparkar, Gir and Kankrej, a total of 109 blood samples were collected. All the animals were randomly selected, taking into consideration that the animals should be genetically unrelated. The information of the animals was collected after consulting pedigree records maintained and interviewing the owners in detail. Blood samples were collected in tubes having EDTA as anticoagulant and further processed for DNA extraction. DNA extraction was carried out using rapid salt extraction method with some slight modifications (Aljanabi *et al*., 1997).

### Microsatellite Analysis

A total of 18 microsatellite marker loci were chosen for the present study to detect polymorphism in breeds. PCR amplification was carried out in 25 ul reaction mixture having 1.5 mm $MgCl_2$, 200 µm dNTPs, 10 pMol of forward and reverse primers and 1u/µl of *Taq*DNA polymerase and 50-100 ng of genomic DNA samples from each individual. All the microsatellites were amplified using eppendroff thermal cycler with the following conditions: initial denaturation at 94˚C for 5 min, 30 repeated cycles of 1 min at 94˚C, 1 min for different annealing temperatures for each microsatellite loci, 72˚C for 30 seconds and a final extension of 10 min. at 72˚C. Amplified PCR products were resolved on 8% non-denaturing polyacrylamide gels. Allele size and genotypes were scored manually and data was further analyzed using different statistical tools.

### Statistical Analysis

Allele frequency, observed number of alleles (Na), effective number of alleles (Ne), private alleles, observed heterozygosity (Ho) and expected heterozygosity (He) were calculated by GenAlEx 6.5 version(Peakall and Smouse, 2006; 2012). Genetic variability among the breeds were calculated by Wright's F-statistics (Fis/Fit/Fst) (Nei's, 1972). Genetic distance and genetic distance based phylogenetic tree were analysed by Neighbor-joining algorithm using population genetic analysis software POPGENE-1.32 version (32 bit) (Yeh *et al*., 1997). Hardy-Weinberg equilibrium (HWE) was estimated using GENEPOP 1.2 version (Raymond and Rousset, 1995). Fixation Index (Fis) was estimated using FSTAT





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version 2.9.3 (Goudet, 2001). Polymorphic Information content (PIC) for each locus was estimated according to (Botstein *et al*., 1980).

## Results and Discussion

### Genetic Diversity in Terms of Mean Number of Alleles

Overall 18 microsatellite markers were successfully amplified in four cattle breeds except INRA005 in Rathi, ILSTS002 and INRA005 in Tharparkar, HAUT24, ILSTS006 and INRA005 in Gir and BM1818 and ILSTS006 in Kankrej and are in accordance with the results reported by (Upreti *et al.,* 2012) but on the contrary HAUT24 was successfully amplified in Kankrej population in the present study. A total of 236 alleles ranging 4 – 23 (Mean= 13.1) were found across 18 microsatellite loci. The most polymorphic marker was INRA035 with a total of 23 alleles.

**Table 1**: Diversity measures in four Indian cattle across 18 microsatellite marker

| Locus | Ho(Observed) | He(Expected) | Na (Observed) | Ne (Effective) | PIC Overall |
|---|---|---|---|---|---|
| HEL9 | 0.632 | 0.775 | 6.5 | 4.769 | 0.866 |
| CSRM60 | 0.688 | 0.78 | 7 | 4.568 | 0.862 |
| ETH225 | 0.682 | 0.794 | 8 | 5.028 | 0.884 |
| ETH10 | 0.663 | 0.738 | 7.25 | 4.287 | 0.876 |
| HAUT24 | 0.433 | 0.557 | 4 | 2.932 | 0.915 |
| BM1818 | 0.242 | 0.549 | 4 | 2.851 | 0.898 |
| ILSTS002 | 0.539 | 0.556 | 4.5 | 2.993 | 0.894 |
| ILSTS006 | 0.083 | 0.265 | 1.5 | 1.097 | 0.93 |
| INRA005 | 0.25 | 0.215 | 2.25 | 1.761 | 0.991 |
| HEL5 | 0.675 | 0.885 | 13 | 9.325 | 0.93 |
| BM2113 | 0.28 | 0.863 | 10.5 | 7.414 | 0.925 |
| ETH3 | 0.642 | 0.837 | 10 | 6.628 | 0.901 |
| ETH152 | 0.568 | 0.843 | 10.25 | 7.034 | 0.924 |
| HEL1 | 0.459 | 0.843 | 10.75 | 6.481 | 0.918 |
| ILSTS022 | 0.606 | 0.858 | 12.25 | 7.167 | 0.936 |
| INRA035 | 0.669 | 0.897 | 15 | 10.349 | 0.939 |
| INRA063 | 0.523 | 0.838 | 9.5 | 6.212 | 0.911 |
| TEXAN15 | 0.911 | 0.8 | 9 | 6.286 | 0.925 |
| Mean | 0.53 | 0.716 | 8.069 | 5.399 | 0.913 |

*Ho= Observed heterozygosity, He= Expected heterozygosity, Na= Observed number of allele, PIC= Polymorphic information content*

Breed-wise, the highest total number of alleles was detected in Kankrej (155) with a mean of 8.6 ± 4.8. Tharparkar had lowest total number of alleles (135) with mean 7.5 ± 4.3. Deepika *et al.* (2014) reported mean number of alleles 10.3 in Kankrej and 9 in Tharparkar. The subsequent allelic numbers and mean observed number of allele in Rathi and Gir were 144 with a mean of 8.0 ± 3.4 and 147 with a mean 8.1 ± 4.7, respectively (Table 2). Of all the considered cattle breeds, Kankrej displayed substantially high genetic diversity in terms of mean number of alleles (MNA = 8.6). However the three breeds, Rathi and Tharparkar and Gir displayed relatively lower diversity (MNA = 8.0, 8.0 and 8.1) which is higher than the other reported





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indigenous cattle breeds (Mukesh *et al.*, 2004; Panday *et al.*, 2006) as well as European cattle breed (Moazami *et al.*, 1997; MacHugh *et al.*, 1997; Kantanen *et al.*, 2000). Similarly, lower allelic diversity has also been reported in exotic cattle-Burlina-6.7 (Dalvit *et al.*, 2008), Brown Swiss-5.4 (Schmid *et al.*, 1999) and Creole cattle-7.2 (Egito *et al.*, 2007). However, allelic diversity of similar magnitude has been reported from cattle populations of state of Orissa in India (Sodhi *et al.*, 2008; Sharma *et al.*, 2012). Allelic diversity in cattle breeds studied lower than that reported by Sharma *et al.* (2015). Furthermore, 53 private alleles were also detected in four cattle breeds *i.e.* 11 in Rathi, 8 in Tharparkar, 15 in Gir and 19 in Kankrej suggesting genetic distinctiveness in the population sampled. The effective number of allele (Ne) at each locus was less than the observed number of allele. Ne ranged from 1.097 (ILSTS006) to 10.349(INRA035) with a mean value of 5.39. Breed wise mean effective number of alleles in four breeds *i.e.* Rathi, Tharparkar, Gir, and Kankrej was 5.40±2.50, 5.5±3.5, 5.2±3.1, 5.5±3.0, respectively (Table 2).

**Table 2**: Observed number of alleles, effective number of alleles and private allele

| Locus | Na | Ne | PA | Na | Ne | PA | Na | Ne | PA | Na | Ne | PA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HEL9 | 8 | 4.523 | 0 | 5 | 3.545 | 0 | 8 | 7.127 | 0 | 5 | 3.879 | 0 |
| CSRM60 | 7 | 4.545 | 0 | 6 | 3.986 | 0 | 9 | 4.811 | 2 | 6 | 4.927 | 0 |
| ETH225 | 8 | 3.84 | 0 | 7 | 5.762 | 0 | 9 | 6.113 | 0 | 8 | 4.396 | 0 |
| ETH10 | 4 | 2.667 | 0 | 9 | 5.544 | 2 | 6 | 3.121 | 0 | 10 | 5.818 | 1 |
| HAUT24 | 6 | 4.05 | 0 | 6 | 4.167 | 0 | 0 | 0 | 0 | 4 | 3.512 | 0 |
| BM1818 | 7 | 4.5 | 2 | 4 | 3.556 | 0 | 5 | 3.347 | 1 | 0 | 0 | 0 |
| ILSTS002 | 5 | 3.408 | 0 | 0 | 0 | 0 | 9 | 5.007 | 4 | 4 | 3.556 | 0 |
| ILSTS006 | 3 | 2.571 | 1 | 3 | 1.815 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| INRA005 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 7.043 | 9 |
| HEL5 | 14 | 10.316 | 0 | 15 | 12.519 | 1 | 12 | 8.464 | 1 | 11 | 6 | 0 |
| BM2113 | 9 | 6.759 | 1 | 9 | 6.877 | 0 | 10 | 6.877 | 1 | 14 | 9.143 | 0 |
| ETH3 | 11 | 7.707 | 2 | 8 | 4.091 | 0 | 9 | 6.426 | 1 | 12 | 8.288 | 2 |
| ETH152 | 9 | 6.259 | 0 | 6 | 4.122 | 0 | 13 | 8.224 | 0 | 13 | 9.529 | 1 |
| HEL1 | 11 | 7.686 | 0 | 8 | 6.533 | 0 | 12 | 5.17 | 1 | 12 | 6.533 | 3 |
| ILSTS022 | 12 | 7.2 | 2 | 12 | 7.84 | 0 | 11 | 8.041 | 0 | 14 | 5.586 | 0 |
| INRA035 | 10 | 6.426 | 0 | 15 | 12.25 | 0 | 18 | 10.965 | 3 | 17 | 11.756 | 1 |
| INRA063 | 9 | 5.939 | 1 | 9 | 6.323 | 0 | 10 | 7.024 | 0 | 10 | 5.563 | 1 |
| TEXAN15 | 11 | 8.471 | 2 | 13 | 9.561 | 4 | 6 | 2.947 | 1 | 6 | 4.167 | 1 |
| Total | 144 | | 11 | 135 | | 8 | 147 | | 15 | 155 | | 19 |
| Avg. | 8 | 5.4 | | 8 | 5.5 | | 8.1 | 5.2 | | 8.6 | 5.5 | |
| SD | 3.4 | 2.5 | | 4.4 | 3.5 | | 4.7 | 3.1 | | 4.8 | 3 | |

*Na= Observed number of allele, Ne= Effective number of allele*

**Polymorphic Information Content**

Overall loci were highly polymorphic with a 100% value of polymorphism in all the four populations. Highest percentage of polymorphic loci were found in Rathi (94.44%) population and polymorphic information content (PIC) value ranged between 0.536 (ILSTS006) and 0.895 (HEL5). Likewise in population of Tharparkar, Gir and Kankrej, polymorphic loci detected were 88.89%, 83.33% and 88.89% respectively. The overall PIC value indicating that all the markers considered for the study are highly





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informative (>0.5) to characterize the given four cattle breed populations. Whereas, marker INRA005 was only amplified in Kankrej breed signifying breed specific marker. The mean percentage of polymorphic loci was detected as 88.89% among the four cattle population.

**Observed Heterozygosity and Expected Heterozygosity**

The heterozygosity estimates at individual loci are present in Table 1. The average expected gene diversity ($H_{exp}$) ranged from 0.215 to 0.897 with an overall mean of 0.716. Observed heterozygosity ($H_{obs}$) across 18 loci ranged between 0.083 and 0.911 with a mean of 0.530. Breed-wise, the mean $H_{obs}$ ($H_{exp}$) were 0.52 (0.75) in Rathi, 0.47 (0.70) in Tharparkar, 0.50 (0.68) in Gir and 0.62 (0.72) in Kankrej, respectively (Table 3).

**Table 3**: Observed heterozygosity, expected heterozygosity and PIC

| Population | Rathi | | | Tharparkar | | | Gir | | | Kankrej | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | Ho | He | PIC | Ho | He | PIC | Ho | He | PIC | Ho | He | PIC |
| HEL9 | 0.364 | 0.779 | 0.749 | 0.542 | 0.718 | 0.668 | 0.857 | 0.86 | 0.844 | 0.765 | 0.742 | 0.699 |
| CSRM60 | 0.56 | 0.78 | 0.749 | 0.583 | 0.749 | 0.708 | 0.846 | 0.792 | 0.768 | 0.762 | 0.797 | 0.766 |
| ETH225 | 0.667 | 0.74 | 0.706 | 0.591 | 0.826 | 0.804 | 0.722 | 0.836 | 0.816 | 0.75 | 0.773 | 0.747 |
| ETH10 | 0.429 | 0.625 | 0.572 | 0.63 | 0.82 | 0.781 | 0.783 | 0.68 | 0.642 | 0.813 | 0.828 | 0.864 |
| HAUT24 | 0.667 | 0.753 | 0.715 | 0.4 | 0.76 | 0.725 | 0 | 0 | 0 | 0.667 | 0.715 | 0.664 |
| BM1818 | 0.333 | 0.778 | 0.753 | 0.25 | 0.719 | 0.667 | 0.385 | 0.701 | 0.647 | 0 | 0 | 0 |
| ILSTS002 | 0.273 | 0.707 | 0.589 | 0 | 0 | 0 | 0.885 | 0.8 | 0.775 | 1 | 0.719 | 0.667 |
| ILSTS006 | 0.333 | 0.611 | 0.536 | 0 | 0.449 | 0.407 | 0 | 0 | 0 | 0 | 0 | 0 |
| INRA005 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.858 | 0.843 |
| HEL5 | 0.857 | 0.903 | 0.895 | 0.846 | 0.92 | 0.916 | 0.478 | 0.882 | 0.874 | 0.519 | 0.833 | 0.815 |
| BM2113 | 0.357 | 0.852 | 0.835 | 0.071 | 0.855 | 0.842 | 0.357 | 0.855 | 0.839 | 0.333 | 0.891 | 0.882 |
| ETH3 | 0.824 | 0.87 | 0.858 | 0.6 | 0.756 | 0.733 | 0.643 | 0.844 | 0.826 | 0.5 | 0.879 | 0.868 |
| ETH152 | 0.385 | 0.84 | 0.823 | 0.692 | 0.757 | 0.72 | 0.36 | 0.878 | 0.868 | 0.833 | 0.895 | 0.887 |
| HEL1 | 0.571 | 0.87 | 0.858 | 0.143 | 0.847 | 0.829 | 0.407 | 0.807 | 0.793 | 0.714 | 0.847 | 0.835 |
| ILSTS022 | 0.583 | 0.861 | 0.85 | 0.786 | 0.872 | 0.861 | 0.464 | 0.876 | 0.864 | 0.593 | 0.821 | 0.808 |
| INRA035 | 0.571 | 0.844 | 0.862 | 0.929 | 0.918 | 0.913 | 0.786 | 0.909 | 0.865 | 0.391 | 0.915 | 0.91 |
| INRA063 | 0.714 | 0.832 | 0.811 | 0.5 | 0.842 | 0.824 | 0.333 | 0.858 | 0.846 | 0.545 | 0.82 | 0.799 |
| TEXAN15 | 1 | 0.882 | 0.871 | 0.929 | 0.895 | 0.887 | 0.714 | 0.661 | 0.63 | 1 | 0.76 | 0.723 |
| Total | 9.49 | 13.53 | 13.03 | 8.49 | 12.7 | 12.28 | 9.02 | 12.24 | 11.9 | 11.18 | 13.09 | 12.78 |
| Avg. | 0.52 | 0.75 | 0.724 | 0.47 | 0.7 | 0.683 | 0.5 | 0.68 | 0.661 | 0.62 | 0.72 | 0.71 |
| SD | 0.24 | 0.2 | 0.21 | 0.32 | 0.28 | 0.275 | 0.29 | 0.32 | 0.313 | 0.29 | 0.27 | 0.268 |

*Ho= Observed heterozygosity, He= Expected heterozygosity, PIC= Polymorphic Information Content*

Thus, Rathi and Tharparkar breed showed lowest level of heterozygosity value. Where Deepika *et al.* (2014) reported mean $H_{obs}$ ($H_{exp}$) in Tharparkar and Kankrej higher than the present study. Amongst four cattle breeds observed heterozygosity between 0.273 to 1 in Rathi, 0.071 to 0.929 in Tharparkar, 0.333 to 0.885 in Gir and 0.333 to 1.00 in Kankrej respectively. The expected Heterozygosity (He) ranged between 0.611-0.903 in Rathi, 0.449 - 0.920 in Tharparkar, 0.661 to 0.909 in Gir and 0.715 to 0.915 in Kankrej. The present study revealed that expected heterozygosity (He) was higher than observed heterozygosity (Ho). Various





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factors such as inbreeding, genetic hitchhiking, null alleles and occurrence of population substructure (Wahlund effect) have been reported as the reasons for heterozygote deficiency in population (Nei, 1987).

**Genetic Differentiation**

The mean Fis, Fit, and Fst values of all cattle populations were 0.257±0.24, 0.3956±0.24 and 0.1859±0.23 respectively (Table 4).

**Table 4**: F-stats

| Locus | Fis | Fit | Fst |
|---|---|---|---|
| HEL9 | 0.1845 | 0.2366 | 0.0639 |
| CSRM60 | 0.1177 | 0.1647 | 0.0533 |
| ETH225 | 0.1402 | 0.2077 | 0.0785 |
| ETH10 | 0.1013 | 0.2169 | 0.1287 |
| HAUT24 | 0.2221 | 0.5203 | 0.3833 |
| BM1818 | 0.5596 | 0.7245 | 0.3745 |
| ILSTS002 | 0.0307 | 0.3823 | 0.3627 |
| ILSTS006 | 0.6856 | 0.9095 | 0.7121 |
| INRA005 | -0.1655 | 0.7478 | 0.7836 |
| HEL5 | 0.2369 | 0.2695 | 0.0427 |
| BM2113 | 0.6758 | 0.6951 | 0.0596 |
| ETH3 | 0.2338 | 0.2762 | 0.0554 |
| ETH152 | 0.3266 | 0.3805 | 0.0801 |
| HEL1 | 0.4553 | 0.4943 | 0.0717 |
| ILSTS022 | 0.2928 | 0.3422 | 0.0699 |
| INRA035 | 0.2536 | 0.2838 | 0.0405 |
| INRA063 | 0.3755 | 0.4185 | 0.0689 |
| EXAN15 | -0.1391 | 0.0068 | 0.128 |
| Mean | 0.2576±0.24 | 0.3956±0.24 | 0.1859±0.23 |

The overall estimates were significantly (P<0.05) different from zero. Fis statistics, ranged from -0.1655(INRA005) to 0.6856(ILSTS006). Except for two loci (INRA005 and TEXAN15), all other loci contributed significantly to the within population heterozygote deficit. Further, within population inbreeding estimates (Fis) indicated a deficit of heterozygotes ranging from 17% (Kankrej) to 37% (Tharparkar). The highest numbers of loci exhibiting deficiency in heterozygosity were observed in the Rathi and Tharparkar breeds, while the number of such loci was lowest in Kankrej breed and are in accordance with the reports of (Sodhi *et al.*, 2011). The knowledge of the population structure of the considered cattle breeds discovered overall positive value of Fis (0.257), Fit (0.3956), and Fit>Fst (0.1859) recommending departures from arbitrary mating and proper selection of animals for a particular breed. Majority of the loci (16=18) contributed for heterozygote deficiency (Fis>0) with maximum contribution of ILSTS006 (0.6856), BM2113 (0.6758), and BM1818 (0.5596) locus. Less heterozygote deficiency in Kankrej breed detected in present study could be credited to less genetic segregation between the sub-population of this breed from the region of study (nonappearance of Wahlund impact) in accordance (Sodi





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*et al*., 2011). The presence of comparable circumstance couldn't be precluded for rest of the three Indian dairy cattle breeds showing heterozygotes deficiency. Other factors like null alleles (non-amplifying alleles), sample relatedness and linkage with loci under selection (genetic hitchhiking) could be the reason for this heterozygote deficiency. The genetic differentiation value (Fst) per locus varied from 0.0405 to 0.7836 with an average of 0.1859±0.23 across all the loci. Across different breed pairs, maximum differentiation was observed in Tharparkar- Gir pair (0.204) whereas Rathi-Tharparkar (0.137) was the least differentiated pair. The level of genetic differentiation among Indian cattle breeds measured in terms of Fst (18.5%) was moderate and significant.

Overall, the mean value of Fst differed significantly from zero, subdivisions within the subpopulation. Fst values ranged from 0.137(Rathi-Tharparkar) to 0.204 (Tharparkar-Gir). The analysis implied that 13.7% to 20.4%, with an average of 18.5% of the total genetic variation, corresponded to breed differences due to unique allelic differences between the breeds while the remaining 81.5% was the result of differentiation among individuals within the breed across 18 markers. The genetic differentiation value in this study has shown higher value than previously reported 0.113 (Mukesh *et al.,* 2004) cattle breeds in India. However, the lower value of (Fst) has been reported in North European breeds Fst = 0.107 (Kantanen *et al.,* 2000), seven European cattle breeds Fst= 0.112 (McHugh *et al.,* 1998), and Swiss cattle Fst= 0.090 (Schmid *et al.,* 1999). While Orissa and hill cattle (Sharma *et al.,* 2012) likewise zebu cattle (Uzzaman *et al.,* 2014) has been reported much lower Fst value. The genetic differentiation value in this study was relatively higher than 0.13 (Sharma *et al.,* 2015). This might be either due to distinct barriers separating the four cattle breeds or proper breeding strategies being employed by the breeders.

**Hardy-Weinberg Equilibrium**

HWE (Hardy-weinberg equilibrium) test depicted that eight microsatellite loci in Rathi cattle, nine loci in Tharparkar, ten loci in Gir and nine loci in Kankrej were in equilibrium state where as the remaining loci significantly deviated ($P<0.01$, $P<0.05$, $P<0.001$ ) from HWE (Table 5).

**Genetic Distance and Dendogram**

The genetic distance analysis amongst the four cattle populations showed the smallest distance between Rathi and Tharparkar (0.597) and maximum distance between Tharparkar and Gir (0.907). This observation was also supported by Phylogenetic tree analysis by using UPGMA method which shows two clusters where Rathi-Tharparkar were clustered in one group of cattle population and Gir-Kankrej were clustered in another group with 100% bootstrapping (Fig. 2).





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**Table 5**: Fixation index (Fis) and Hardy-Weinberg equilibrium

| Locus | Rathi | | | Tharparkar | | | Gir | | | Kankrej | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fis | HWE (P) | HWS (SIG) | Fis | HWE (P) | HWS (SIG) | Fis | HWE (P) | HWS (SIG) | Fis | HWE (P) | HWS (SIG) |
| HEL9 | 0.55 | 0 | *** | 0.265 | 0.035 | * | 0.021 | 0.228 | Ns | 0 | 0.203 | Ns |
| CSRM60 | 0.301 | 0.001 | ** | 0.241 | 0.01 | ** | -0.049 | 0.572 | Ns | 0.068 | 0.929 | Ns |
| ETH225 | 0.12 | 0.185 | ns | 0.306 | 0 | *** | 0.164 | 0.308 | Ns | 0.055 | 0.014 | * |
| ETH10 | 0.347 | 0.076 | ns | 0.25 | 0 | *** | -0.13 | 0.006 | ** | 0.051 | 0.26 | Ns |
| HAUT24 | 0.143 | 0.464 | ns | 0.5 | 0.055 | Ns | NA | NA | mono | 0.111 | 0.001 | ** |
| BM1818 | 0.61 | 0.075 | ns | 0.727 | 0.207 | Ns | 0.483 | 0.176 | Ns | NA | NA | Mono |
| ILSTS002 | 0.628 | 0.001 | ** | NA | NA | mono | -0.086 | 0.577 | Ns | -0.333 | 0.544 | Ns |
| ILSTS006 | 0.524 | 0.083 | ns | 1 | 0.003 | ** | NA | NA | mono | NA | NA | Mono |
| INRA005 | NA | NA | mono | NA | NA | mono | NA | NA | mono | -0.108 | 0.052 | Ns |
| HEL5 | 0.088 | 0.139 | ns | 0.12 | 0.263 | Ns | 0.475 | 0 | *** | 0.394 | 0 | *** |
| BM2113 | 0.605 | 0 | *** | 0.922 | 0 | *** | 0.606 | 0 | *** | 0.639 | 0 | *** |
| ETH3 | 0.084 | 0.026 | * | 0.239 | 0.334 | Ns | 0.256 | 0.016 | * | 0.449 | 0 | *** |
| ETH152 | 0.57 | 0.003 | ** | 0.126 | 0.451 | Ns | 0.603 | 0 | *** | 0.097 | 0.679 | Ns |
| HEL1 | 0.375 | 0.024 | * | 0.842 | 0 | *** | 0.509 | 0 | *** | 0.193 | 0.006 | ** |
| ILSTS022 | 0.361 | 0.144 | ns | 0.136 | 0.015 | * | 0.484 | 0 | *** | 0.296 | 0 | *** |
| INRA035 | 0.356 | 0.061 | ns | 0.026 | 0.503 | ns | 0.153 | 0.01 | ** | 0.587 | 0 | *** |
| INRA063 | 0.177 | 0.015 | * | 0.437 | 0.032 | * | 0.624 | 0 | *** | 0.355 | 0.018 | * |
| TEXAN15 | -0.091 | 0.312 | ns | 0 | 0.094 | ns | -0.044 | 0.006 | ** | -0.268 | 0.22 | Ns |
| Overall | 0.333 | | | 0.37 | | | 0.285 | | | 0.175 | | |

*Fis= Fixation Index, HWE= Hardy-Weinberg equilibrium, SIG= Significance level*

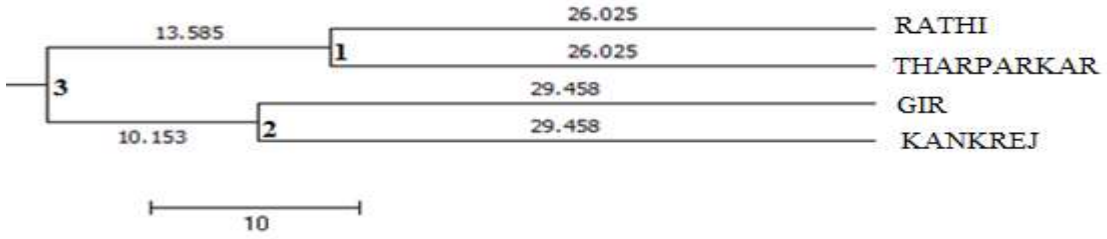


**Fig. 2:** Phylogenetic analysis of four cattle breeds Rathi, Tharparkar, Gir and Kankrej population were based on Nei's (1978) Genetic distance software POPGENE- 1.32 version (32 bit).

Similarly, pair-wise Nei's genetic distance values showed highest extent of divergence between Tharparkar and Gir (0.907) and lowest between Rathi and Tharparkar (0.597) (Table 6).

**Table 6**: Pairwise population matrix of Nei genetic distance

| Rathi | Tharparkar | Gir | Kankrej | |
|---|---|---|---|---|
| 0 | | | | Rathi |
| 0.597 | 0 | | | Tharparkar |
| 0.875 | 0.907 | 0 | | Gir |
| 0.853 | 0.815 | 0.653 | 0 | Kankrej |

Furthermore, Phylogenetic analysis based on Nei's genetic distance revealed that Rathi and Tharparkar are more closely related to each other than Gir and Kankrej in context of genetic relationship.







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## Conclusion

The present study genetic diversification among four cattle breed populations *i.e.* Rathi, Tharparkar, Gir and Kankrej was evaluated using 18 microsatellite markers. The mean polymorphic loci of 88.89% across the four breeds indicated highly informative capability of microsatellite markers used in the study. Further, significant deviation of microsatellite loci studied by HWE (except 8 loci in Rathi, 9 loci in Tharparkar, 10 loci in Gir, 9 loci in kankrej) and the differences between mean observed and expected heterozygosity within the four cattle breeds suggested tendency of markers toward heterozygote deficiency and same was reflected by inbreeding estimation (Fis) within four cattle breeds population. This study inferred that highest genetic diversity was found in Kankrej breed in comparison to other indigenous breeds; however, it is also insinuate that former breeding strategies were being continued for the management of all studied breeds. Hence, there is demand to change breeding strategies and management programs for further improvement of indigenous breeds population of Rajasthan.

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