The Pharma Innovation Journal 2023; SP-12(9): 2373-2379

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(9): 2373-2379

www.thepharmajournal.com
Received: 24-07-2023
Accepted: 29-08-2023

G Zaman
Professor & Head, Department of Animal Genetics and Breeding, College of Veterinary Science, Khanapara, Assam Agricultural University, Guwahati, Assam, India

Arundhati Phookan
Assistant Professor (Stage 3), Department of Animal Genetics and Breeding, College of Veterinary Science, Khanapara, Assam Agricultural University, Guwahati, Assam, India

Bula Das
Professor, Department of Animal Genetics and Breeding, College of Veterinary Science, Khanapara, Assam Agricultural University, Guwahati, Assam, India

Arpana Das
Professor, Department of Animal Genetics and Breeding, College of Veterinary Science, Khanapara, Assam Agricultural University, Guwahati, Assam, India

S Laskar
Professor, Department of Animal Genetics and Breeding, College of Veterinary Science, Khanapara, Assam Agricultural University, Guwahati, Assam, India

Farzin Akhtar
Scientist, Goat Research Station, Assam Agricultural University, Burnihat, Assam, India

Corresponding Author:
Arundhati Phookan
Assistant Professor (Stage 3), Department of Animal Genetics and Breeding, College of Veterinary Science, Khanapara, Assam Agricultural University, Guwahati, Assam, India

Genetic structure and diversity study of indigenous cattle population of northeast India

G Zaman, Arundhati Phookan, Bula Das, Arpana Das, S Laskar and Farzin Akhtar

Abstract
Genetic diversity study of three indigenous cattle population of northeast India *viz.* Assam local cattle (ALC), Arunachal Pradesh Local cattle (APLC) and Manipur local cattle (MLC) was carried out using 24 microsatellite markers. A total of 120 individuals were genotyped and 300 alleles were identified. The overall mean observed and expected numbers of alleles were found to be 4.917±0.069 and 1.880±0.084; 3.333±1.274 and 2.166±0.077; and 4.857±1.796 and 2.034±0.311 in ALC, APLC and MLC respectively. The overall means for observed and expected heterozygosities were 0.448±0.023 and 0.476±0.024; 0.634±0.233 and 0.690±0.165; and 0.544±0.248 and 0.578±0.187 in ALC, APLC and MLC respectively. The moderate to high mean heterozygosity suggested that the three cattle populations possess high level of genetic diversity. The mean Polymorphism information content values and Shannon's information index were found to be 0.355±0.097 and 0.660±0.037, 0.417±0.086 and 0.775±0.034 and 0.394±0.071 and 0.732±0.139 in ALC, APLC and MLC respectively. The sign rank test for bottleneck analysis indicated that cattle populations under study has not undergone any recent bottle neck. The genetic distance revealed that ALC and APLC population formed a separate cluster indicating their closeness as compared to MLC which can be considered genetically more unique. The Analysis of Molecular Variance showed that 43% of the total variation was due to differences between genetic groups.

Keywords: Indigenous cattle of Assam, indigenous cattle of Arunachal Pradesh, indigenous cattle of Manipur, heterozygosity, microsatellites

Introduction
North east India comprising eight states possesses a large variety of livestock genetic resources. These resources are maintained mostly in the rural areas by the poor and marginal farmers with small land holdings under unrecognized husbandry practices. The native germplasm are well known for their tropical adaptability and disease resistance. The cattle of North east India have never been assessed for which these animals are very often termed as nondescript. The state of Assam alone constitutes 75.12% of the total population of cattle from North east India of which however, a sizeable proportions (94.38%) are indigenous. The proportion of indigenous cattle in Arunachal Pradesh and Manipur state are 93.75 and 75.22% respectively.

These animals relatively poor in milk yield, peak yield and other reproductive traits but produces quality milk that are rich in fat, solids-not fat and total solids. The bullocks were mostly used in agricultural works for ploughing, harrowing and threshing. The importance of indigenous cattle populations lies in their adaptation to local biotic and abiotic stresses and to traditional husbandry systems. The indigenous genetic diversity constitutes a buffer against changes in the environment and is a key in selection and breeding for adaptability and production on a range of environments. Our indigenous breeds may produce less milk or meat than improved breeds, but are capable to fulfill wider range of functions for their owners and are much easier to manage. Country to crossbred cattle, bullocks produced by local cow are well suited for work under harsh conditions.

The conservation of domestic animal diversity is essential to meet future needs in India. Since, maintaining genetic diversity is an insurance package against future adverse conditions. Genetic diversity is necessary for the long-term survival of the species and populations because it provides the raw material for adaptation and evolution, especially when environmental conditions have changed (Rajora and Mosseler, 2001) [18]. It has been established that genetic characterization of native genetic groups is the first step towards prioritization of genetic groups for conservation.

Moreover, the genetic diversity has provided the material for every successful breed improvement programme of the developed countries in the 19th and 20th century.

Materials and Methods

Experimental Materials

Blood samples of three cattle population were randomly collected from their native breeding tract at different localities in Assam, Arunachal Pradesh and Manipur (Table 1), northeast India. The samples were collected between July 2013 and December 2013.

Sampling and preservation

A total of 120 blood samples from unrelated animals belonging to each of the three cattle populations from northeast India were collected aseptically (Table 1) in EDTA coated vacutainers and stored at 4 °C until processed for further analysis.

DNA isolation and quantification

Genomic DNA was isolated from whole blood samples by using standard phenol-chloroform method (Sambrook *et al*. 1989) [19] with minor modifications. DNAzol (Invitrogen) reagent was used instead of SDS and proteinase K. The DNA was observed on 0.8% agarose gel containing ethidium bromide. The quantification of DNA was done using ND-1000 spectrophotometer (Thermo scientific).

Selection of microsatellite markers

A total of 24 well-characterized microsatellite markers (Table 2) developed for bovine (FAO, http://dad.fao.org/en/refer/library/guidelin/marker.pdf), were used based on their level of polymorphism, allele size range and reliability of allele to evaluate genetic structure in indigenous cattle populations of northeast India. A minimal distance of 30 cM was maintained when two markers were at the same chromosome. The forward primer of each marker was fluorescently labeled with FAM, ROX, TAMRA or HEX dye. All microsatellite markers were first checked individually to evaluate their performance.

Amplification of microsatellite regions and genotyping

The basic PCR reaction mixture (15 µl) containing 20-50 ng of template DNA; 1.5 mM MgCl2; 5 picomoles each of forward and reverse primers; 1 unit of taq DNA polymerase and 200 mM dNTPs was prepared for amplification each specific loci. Amplification was carried out with initial denaturation at 95 °C for 2 min followed by 30 cycles of denaturation (95 °C for 30 sec), annealing (55 °C for 30 sec) and extension (72 °C for 45 sec) using Applied Biosystems VeritiTM 96- well thermal cycler. After confirming the amplified products on 2.0% agarose, products were further prepared for genotyping in an automated DNA Sequencer.

Each 1µl of PCR product was mixed with 0.3 µl of size standard fluorescent dye Gene Scan Liz 500 (Applied Biosystems, USA) and the volume was made up to 10 µl with Hi-Di formamide. Samples were denatured for 5 minutes at 95ºC and snap chilled on ice for 5 minutes before being run on ABI HITACHI 3500 (Applied Biosystems, USA) genetic analyzer and data were analyzed using Gene Mapper v. 4.0 (Applied Biosystems) to generate genotype calls for each locus by using GS 500 (- 250) LIZ as size standard.

Statistical analysis

GenAlex v6.5 (Peakall and Smouse 2008) [17] was used to estimate genetic diversity and differentiation parameters. Parameters estimated include number of alleles (N_a), allele frequencies, effective number of alleles (N_e), Private alleles (N_p), observed (H_o) and expected (H_e) heterozygosity, F-statistics, Shanon's information index (I), Hardy-Weingberg equilibrium (HWE), Principal component analysis (PCA) and Analysis of Molecular Variance (AMOVA). Polymorphic Information Content (PIC) was calculated using Excel Microsatellite Toolkit 3.1 (Park 2001) [16]. The Bottleneck v1.2.03 (Cornuet and Luikart 1996) [3] was used to know whether these cattle populations exhibited a significant number of loci with the excess of heterozygosity. Phylogenetic and molecular evolutionary analyses were conducted using MEGA v5.05 (Tamura, Peterson *et al*. 2011) [25].

Results and Discussion

In the present study, 24 set of microsatellite markers recommended by FAO-ISAG was used for analyzing the genetic diversity, relationship among and within the three cattle population.

Within population diversity

A total of 24, 24 and 21 microsatellite loci were analyzed for ALC, APLC and MLC respectively. Most of the loci investigated were polymorphic in nature. The number of observed alleles (N_a) in ALC, APLC and MLC ranged from 2 (HEL9, ILSTS033, INRA063, HEL51, ILSTS054 and ILSTO34) to 10 (MM8); 2 (ILSTS033, HEL1 HAUT27, BM1824, ILSTS054, ETH152, ILSTS034 and ILSTS005) to 6 (INRA005 and HAUT24) and 2 (HEL9, ILSTS033 and BM1818) to 8 (CSRM60). The overall mean N_a found to be 4.917±0.069, 3.333±1.274 and 4.857±1.796 in ALC, APLC and MLC respectively (Table 3). Comparable N_a in Hariana (4.2), Sahiwal (4.1) and Tharparker (3.6) cattle reported by (MacHugh *et al*., 1997) [12] and in Ongole (4.5) and Deoni (4.1) by Metta *et al.* 2004 [13]. However, higher N_a as compared to present study reported by Sodhi *et al*. 2006 [23] in Red Kandhari (5.82) and Deoni cattle (5.86); Sharma *et al.* 2015 [22] in Indian cattle population (8.784±0.25); and Karthickeyan *et al.* (2019) [11] in Malaimadu cattle of Tamil Nadu (9.04±3.28). Number of alleles for each locus ranged from 4 (BM1824) to 19 (CSRM60) and the effective number of alleles ranged from 1.8611 (ILSTS011) to 8.3805 (TGLA122) with a mean of 4.11±1.72 (Karthickeyan *et al.*, 2019) [11]. Joshi *et al.* 2018 observed that the number of alleles per locus varied from 2 to 14, with an overall mean of 8.2±1.143. The range of N_a in was found to be 4 to 10 alleles in Kherigarh cattle (Pandey *et al*. 2006) [15], 3 to 15 alleles in Nimari cattle having (Sharma *et al*. 2010) [21] and 4 to 18 alleles in Malvi cattle (Thakur *et al*. 2010) [26].

The effective number of alleles (Ne) in ALC, APLC and MLC ranged from 1.242 (BM1824) to 2.938 (ILSTS011); 1.846 (INRA035) to 2.985 (MM12); and 1.674 (INRA005) to 2.991 (HEL9) respectively. The mean Ne found to be 1.880±0.084, 2.166±0.077 and 2.034±0.311 in ALC, APLC and MLC respectively (Table 3). The Ne reported by Joshi *et al.* 2018 in Nagori cattle (2.655±0.140) corroborated with the present finding. However, Ne value reported by Sharma *et al.* 2015 [22] was higher (4.082±0.139). The values of heterozygosity indicate the diversity level of the molecular markers and thus the existing genetic variation in the population. To describe the genetic diversity within the populations, the observed (H_0) and expected heterozygosities

(H_e) were calculated. The overall means for observed (Ho) and expected (He) heterozygosities were 0.448±0.023 and 0.476±0.024 in ALC, 0.634±0.233 and 0.690±0.165 respectively in APLC and 0.544±0.248 and 0.578±0.187 in MLC (Table 3). The high Ho and He indicated that the three cattle populations possess high level of genetic diversity. Ho ranged from 0.033 (INRA063) to 0.900 (HAUT24); 0.400 (ILSTSO54) to 0.943 (MM8) and 0.067 (HEL9) to 1(BM1824) respectively in ALC, APLC and MLC. He ranged from 0.033 (INRA063) to 0.794 (MM8); 0.200 (ILS005) to 1 (MM8) and 0.180 (HEL9) to 0.816 (HAUT27) in ALC, APLC and MLC respectively. A similar He to ALC and MLC reported in Ongole (0.46±0.1) and Deoni (0.50±0.1) by Metta *et al.* 2004 [13]. Comparable Ho and He were found in Kenkatha cattle (0.47±0.24 and 0.62±0.21) and Gaolao cattle (0.53±0.17 and 0.68±0.14) by Chaudhari *et al.* 2009 [2]. Hepsibah *et al*. 2014 [7] reported a similar Ho and He in Hallikar cattle (0.6068±0.05 and 0.6863±0.03) as compared to APLC. A similar Ho with APLC was reported by Deepika and Salar (2012) [4] in Indian grey cattle breeds (0.693±0.56) and Karthickeyan *et al*. 2019 [11] in Malaimadu cattle of Tamil Nadu (0.65±0.19). Joshi *et al.* 2018 reported H_0 of 0.610 in Nagori cattle which was found to be similar to APLC. Comparable H_e was reported in Deoni (0.59), Sahiwal (0.61) and Hariana (0.66) by Mukesh *et al*., 2004; and Umblachery cattle (0.61) by Karthikeyan *et al*., 2007. However, Sharma *et al*. 2015 [22] reported a higher estimates of Ho (0.653±0.014) and He (0.685±0.012) and Karthickeyan *et al.* 2019 [11] observed a higher He in Malaimadu cattle of Tamil Nadu (0.73±0.11). The Polymophism Information Content (PIC) acts as a good index for genetic diversity evaluation. The PIC value in ALC, APLC and MLC ranged from 0.175 to 0.585 (ILSTSo11); 0.353 (INRA035) to 0.590 (MM12 and ILSTS054) and 0.321(INRA005) to 0.591 (HEL9). The mean PIC values were found to be 0.355±0.097, 0.417±0.086 and 0.394±0.071 in ALC, APLC and MLC respectively (Table 3). Moderate to high PIC value indicated that heterozygosity lies in the selected population. Metta *et al.* 2004 [13] reported the PIC values of the polymorphic loci ranged from 0.15 to 0.79 in Ongole and 0.13 to 0.80 in Deoni breeds. A higher PIC was reported by Karthickeyan *et al*. 2009 [9] in Kangayam (0.5628±0.03); Sharma *et al*. 2010 [21] in Nimari cattle (0.64) and Gaolao cattle (0.65±0.15); Karthickeyan *et al.* 2019 [11] in Malaimadu cattle of Tamil Nadu (0.68±0.14). Shannon's information index (I) was found to be as 0.660±0.037, 0.775±0.034 and 0.732±0.139 respectively in ALC, APLC and MLC (Table 3).The high I indicated high level of diversity. The *chi* square test for HWE revealed that 18 out of 24 loci deviated from equilibrium in ALC, 22 out of 24 loci deviated from equilibrium in APLC and 16 out of 21 loci deviated from equilibrium in MLC. The within population inbreeding estimates (FIS) observed were 0.042, 0.046 and 0.062 in ALC, APLC and MLC respectively and it indicated insignificant heterozygote shortfall in the population under study. A comparable value for FIS (0.048±0.017) with ALC and APLC was given by Sharma *et al*. 2015 [22] in Indian cattle. A relatively higher value of F_{IS} was observed in Ongole and Deoni breed of cattle (0.117) for Gaolao (0.2121) and Kenkatha cattle (0.2248) by Chaudhury *et al.* 2009; Hallikar (0.131±0.05) by Hepsibha *et al.* 2014 [7] and Malaimadu cattle of Tamil Nadu (0.1130±0.19) by Karthickeyan *et al.* 2019 [11]. The sign rank test was carried out for bottleneck analysis and it indicated that under IAM, TPM and SMM models, the number of loci with heterozygosity excess were 12, 9 and 7 in ALC; 13, 12 and 10 respectively in APLC and 12, 8 and 7 respectively in MLC (Table 4). This suggests that cattle populations under study have not undergone any recent bottle neck. A similar report was given for Malaimadu cattle of Tamil Nadu by Karthickeyan *et al.* 2019 [11] where no bottleneck observed.

Genetic distance between cattle population

The genetic distance tends to be least (1.763) between ALC and APLC and widest (2.470) between APLC and MLC (Table 5). The dendrogram revealed (Fig.1) that ALC and APLC population formed a separate cluster indicating their closeness. Compared to ALC and APLC, MLC can be considered genetically more unique as it separated out of cluster. The genetic differentiation between different pairs of populations was significantly different from zero. Principal component analysis also showed the clustering of the cattle populations according to their geographical origin (Fig.1 and Fig.2). Therefore, the geographical origin of the populations should be taken into consideration while deciding conservation and improvement options for these populations. Sharma *et al.* 2015 [22] carried out genetic distance study in Indian cattle and found that Ongole was distinct from the rest of populations, Hariana and Mewati were closer and fall in a different quadrant along with Gaolao whereas, Kenkatha, Ponwar, Kherigarh, Gangatiri, Bachaur, Shahabadi and Purnea clustered together in one quadrant. The AMOVA revealed that percentage of variation among populations was 43% among population, 30% among individuals and 27% within population. Variance components among population were highly significant for all the studied loci (Table 6).

Table 1: Summary of sampling locations and number of cattle sampled

Population	Acronyms	Sampling sites	Latitude and longitude	Number of samples
Assam local cattle	ALC	Entire state of Assam	24º3/-27º58/ N and 89º5/ - 96º1/ E	40
Arunachal Pradesh local cattle	APLC	Entire South regions of Arunachal Pradesh	26º28/ - 29º30/ N and 91º30/ - 97º30/ E	40
Manipur local cattle	MLC	Entire state of Manipur	23º83/ - 25º68/N and 93º03/ - 94º78/E	40
Total				120

Table 2: Outline of markers used in the present study

Locus	Repeat motif	Primer Sequences (5' → 3')	Dye	T_a (°C)	Size range (bp)
INRA005	(TG)n	5'-CAATCTGCATGAAGTATAAATAT-3' 5'-CTTCAGGCATACCCTACACC-3'	ROX	55	138-150
HEL9	(CA)n	5'-CCCATTCAGTCTTCAGAGGT-3' 5'CACATCCATGTTCTCACCAC-3'	TAMARA	55	138-172
CSSM663	(CA)n	5'-ACACAAATCCTTTCTGCCAGCTGA-3' 5'-AATTTAATGCACTGAGGAGCTTGG-3'	FAM	55	176-210
INRA035	(TG)n	5'-ATCCTTTGCAGCCTCCACATTG-3' 5'-TTGTGCTTTATGACACTATCCG-3'	FAM	55	099-123
ETH3	(TG)n	5'-GAACCTGCCTCTCCTGCATTGG-3' 5'-ACTCTGCCTGTGGCCAAGTAGG-3'	FAM	55	97-141
MM12	(CA)n	5'-CAAGACAGGTGTTTCAATCT-3' 5'-ATCGACTCTGGGGATGATGT-3'	TAMARA	55	91-129
HAUT24	(CA)n	5'-CTCTCTGCCTTTGTCCCTGT-3' 5'-AATACACTTTAGGAGAAAAATA-3'	HEX	55	103-127
ILSTS033	(TG)n	5'-TATTAGAGTGGCTCAGTGCC-3' 5'-ATGCAGACAGTTTTAGAGGG-3'	ROX	55	136-162
INRA063	(CA)n	5'-ATTTGCACAAGCTAAATCTAACC-3' 5'-AAACCACAGAAATGCTTGGAAG-3'	ROX	55	171-189
HEL1	Ann	5'-CAACAGCTATTTAACAAGGA-3' 5'-AGGCTACAGTCCATGGGATT-3'	HEX	55	84-120
ILSTS011	(TG)n	5'-GCTTGCTACATGGAAAGTGC-3' 5'-CTAAAATGCAGAGCCCTACC-3'	FAM	55	258-272
HAUT27	(CA)n	5'-TTTTATGTTCATTTTTTGACTGG-3' 5'-AACTGCTGAAATCTCCATCTTA-3'	HEX	55	140-150
ETH10	(CA)n	5'-GTTCAGGACTGGCCCTGCTAACA-3' 5'-CCTCCAGCCCACTTTCTCTTCTC-3'	FAM	55	205-219
BM1824	(TG)n	5'-GAGCAAGGTGTTTTTCCAATC-3' 5'-CATTCTCCAACTGCTTCCTTG-3'	TAMARA	55	180-196
HEL51	Ann	5'-GCAGGATCACTTGTTAGGGA-3' 5'-CTGCAGTTCTGCATATGTGG-3'	FAM	55	118-154
ILSTA030	(CA)n	5'-CTGCAGTTCTGCATATGTGG-3' 5'-CTTAGACAACAGGGGTTTGG-3'	TAMARA	55	150-158
ILSTS006	(TG)n	5'-TGTCTGTATTTCTGCTGTGG-3' 5'-ACACGGAAGCGATCTAAACG-3'	FAM	55	276-302
ILSTS054	(TG)n	5'-GAGGATCTTGATTTTGATGTCC-3' 5'-AGGGCCACTATGGTACTTCC-3'	FAM	55	125-145
CSRM60	(CA)n	5'-AAGATGTGATCCAAGAGAGAGGCA-3' 5'-AGGACCAGATCGTGAAAGGCATAG-3'	FAM	55	69-115
ETH152	(TG)n	5'-AGGGAGGGTCACCTCTGC-3' 5'-CTTGTACTCGTAGGGCAGGC-3'	TAMARA	55	192-206
ILSTS034	(TG)n	5'-AAGGGTCTAAGTCCACTGGC-3' 5'-GACCTGGTTTAGCAGAGAGC-3'	HEX	55	138-196
ILSTS005	(TATATG)n	5'-GGAAGCAATGAAATCTATAGCC-3' 5'-TGTTCTGTGAGTTTGTAAGC-3'	FAM	55	177-193
MM8	Ann	5'-CCCAAGGACAGAAAAGACT-3' 5'-CTCAAGATAAGACCACACC-3'	ROX	55	128-142
BM1818	(TG)n	5'-AGCTGGGAATATAACCAAAGG-3' 5'-AGTGCTTTCAAGGTCCATGC-3'	HEX	55	235-285

T_a, Annealing temperature

Table 3: Microsatellite analysis in pooled cattle populations

Name of the Population	Total No. of Allele	Parameters						
		Na	Ne	Ho	He	PIC	I	F_{IS}
Assam Local cattle	118	4.917±0.069	1.880±0.084	0.448±0.023	0.476±0.024	0.36±0.097	0.660±0.037	0.042
Arunachal Local cattle	80	3.333±1.274	2.166±0.077	0.634±0.233	0.690±0.165	0.42±0.086	0.775±0.034	0.046
Manipur Local cattle	102	4.857±1.796	2.034±0.311	0.544±0.248	0.578±0.187	0.39±0.071	0.732±0.139	0.062
Pooled Mean	300	4.369±0.898	2.027±0.143	0.542±0.093	0.581±0.107	0.39±0.031	0.722±0.058	0.050

N_a: Number of alleles; N_e: Effective number of alleles; H_o: Observed Heterozygosity;
H_e: Expected Heterozygosity; I: Shannon's Information Index.

Table 4: Bottleneck analysis in three populations

Population	Model	Sign rank test - Number of loci with heterozygosity excess		
		Expected	Observed	Probability
ALC	IAM	11.17	12	0.01874
	TPM	13.24	9	0.01083
	SMM	12.46	7	0.00012
APLC	IAM	11.20	13	0.39017
	TPM	11.62	12	0.40131
	SMM	12.25	10	0.21122
MLC	IAM	11.21	12	0.42681
	TPM	11.31	8	0.18266
	SMM	11.22	7	0.01135

ALC: Assam local cattle; APLC: Arunachal Pradesh local cattle; MLC: Manipur local cattle

Table 5: Genetic Distance (below diagonal) and paired FST values (above diagonal) between the populations

Cattle population	Assam Local Cattle	Arunachal Local Cattle	Manipur local cattle
Assam Local Cattle	- - -	0.293**	0.305**
Arunachal Local Cattle	1.763	- - -	0.326**
Manipur local cattle	2.380	2.470	- - -

** Highly significant (p<0.01)

Table 6: AMOVA analysis in three populations

Source of variation	Degree of freedom	Sum of squares	Variance component	Percentage of variation
Among Pops	2	775.624	4.878	43
Among Individual	114	1128.286	3.425	30
Within Pops	117	356.5	3.047	27
Total	233	2260.410	11.350	100

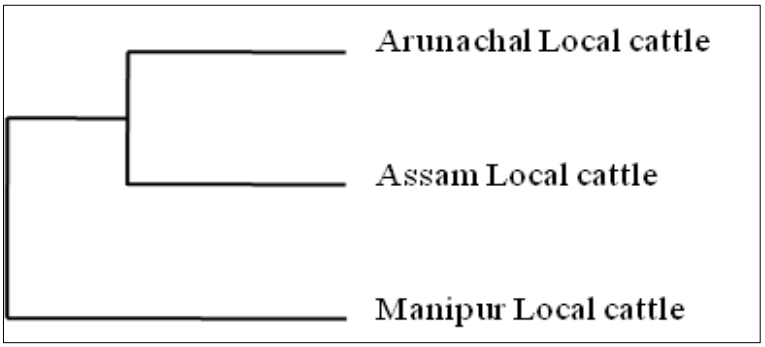

Fig 1: Neighbour- joining consensus tree among three cattle population

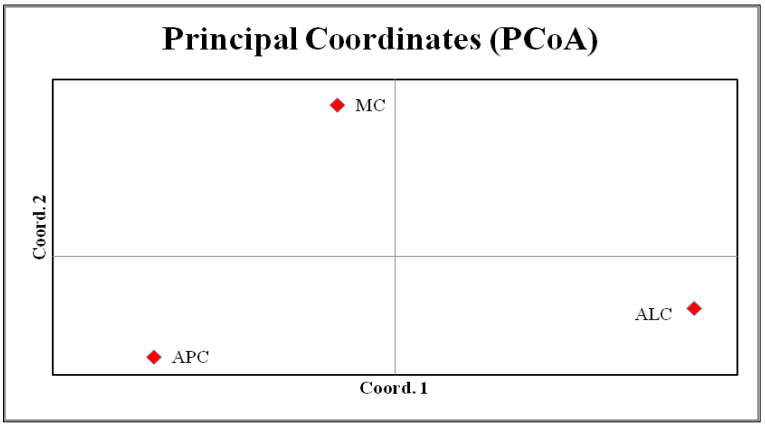

Fig 2: Multidimensional scaling plot constructed on the basis of pair-wise F_{ST} values of cattle population

Conclusion

This study involves detailed analysis of the genetic diversity of Indigenous cattle population of North-eastern India especially Assam Local Cattle, Arunachal Pradesh Local Cattle and Manipur Local Cattle. Conclusion can be drawn from the present study that population under study retained moderate to high levels of genetic diversity which might be due to maintenance of effective population. Genetic diversity can be explored scientifically with proper planning for improvement in these germplasm. Very less inbreeding as well as no recent bottleneck condition detected in these populations. Estimates of differentiation and genetic structure

confirm history of individual population and suggest considerable uniqueness in these groups. Studied population showed clustering of the cattle populations according to their geographical origin. Therefore, the geographical origin of the populations should be taken into consideration while deciding conservation and improvement options for these populations.

Acknowledgement
Authors acknowledge all the herd owners who had cooperated for sample collection from their cattle and College of Veterinary Science, Assam Agricultural University, Guwahati, Assam, India for permitting to carry out the research work.

Funding
The financial support was provided by the Indian Council of Agricultural Research (ICAR) through the ICAR- NBAGR Core Lab Project.

Conflict of interests
The authors declare that they have no conflict of interest.

References
1. Bora SK, Tessema TS, Girmay G. Genetic Diversity and Population Structure of Selected Ethiopian Indigenous Cattle Breeds Using Microsatellite Markers (2023) Genetics Research Article ID 1106755; c2023. https://doi.org/10.1155/2023/1106755.
2. Chaudhari MV, Parmar SNS, Joshi CG, Bhong CD, Fatima S, Thakur MS, *et al*. Molecular characterization of Kenkatha and Gaolao (*Bos indicus*) cattle breeds using microsatellite markers. Animal Biodiversity and Conservation. 2009;32(2):71-76.
3. Cornuet JM, Luikart G. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics. 1996;144:2001-2014.
4. Kumar D, Salar R. Genetic diversity and bottleneck analysis of indigenous grey cattle breeds of India based on microsatellite data. DHR International Journal of Biomedical and Life Sciences. 2012;3:184-194.
5. Del Bo L, Polli M, Longeri M, Ceriotti G, Looft C, Barre-Dirie A. Genetic diversity among some cattle breeds in the alpine area. J Anim. Breed. Genet. 2001;118:317-325.
6. Ganapathi P, Rajendran R, Kathiravan P. Detection of occurrence of a recent genetic bottleneck event in Indian hill cattle breed Bargur using microsatellite markers. Tropical Animal Health and Production. 2012;44:2007-2013.
7. Hepsibha P, Gogoi A, Karthickyan SMK. Molecular genetic architecture of Hallikar cattle (*Bos indicus*) revealed through microsatellite markers. Indian Journal of Biotechnology. 2014;13:411-413.
8. Joshi Pallavi, Vyas P, Kashyap SK. Molecular characterization of Nagori cattle using microsatellite markers. Journal of Pharmacognosy and Phytochemistry. 2018;7(2):3250-3252.
9. Karthickeyan SMK, Sivaselvam SN, Selvam R, Thangaraju P. Microsatellite analysis of Kangayam cattle (*Bos indicus*) of Tamil Nadu. Indian Journal of Science and Technology. 2009;2:38-40.
10. Karthickeyan SMK, Sivaselvam SN, Selvam R, Raja TV, Rajendran R, Thangaraju P. Umblachery breed of cattle in south India: Genetic assessment through microsatellite markers. Asian Journal of Animal and Veterinary Advances. 2007;2:218-222.
11. Karthickeyan SMK, Kumarasamy P, Chandra ARM, Hepsibha P, Sivaselvam SN. Molecular characterization of Malaimadu cattle. Indian Journal of Animal Sciences. 2019;89(7):795-797.
12. MacHugh DE, Shriver MD, Loftus RT, Cunningham P, Bradley DG. Microsatellite DNA Variation and the Evolution, Domestication and Phylogeography of Taurine and Zebu Cattle (*Bos taurus* and *Bos indicus*). Genetics. 1997;146:1071-1086.
13. Metta M, Kanginakudru S, Gudiseva N, Nagaraju J. Genetic characterization of the Indian cattle breeds, Ongole and Deoni (*Bos indicus*), using microsatellite markers: A preliminary study. BMC Genetics. 2004;5:16. doi:10.1186/1471-2156-5-16.
14. Mukesh M, Sodhi M, Bhatia S, Mishra BP. Genetic diversity of Indian native cattle breeds as analyzed with 20 microsatellite loci. J Anim Breed Genet. 2004;121:416-24.
15. Pandey AK, Sharma R, Singh Y, Prakash B, Ahlawat SPS. Genetic diversity studies of Kherigarh cattle based on microsatellite markers. Journal of Genetics. 2006;85:117-22.
16. Park SDE. The Excel microsatellite toolkit (version 3.1) Animal Genomics Laboratory, University College; Dublin, Ireland; c2001.
17. Peakall R, Smouse PE. A heterogeneity test for fine-scale genetic structure. Mol Ecol Notes. 2008;17:3389-400.
18. Rajora OP, Mosseler A. Molecular markers in sustainable management, conservation and restoration of for rest genetic resources. In Genetic response of forest systems to changing environmental conditions. Springer, Dordrecht; c2001. p. 187-202.
19. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Harbour Laboratory Press; c1989.
20. Schmid M, Saitbekova N, Gaillard C, Dolf G. Genetic diversity in Swiss cattle breeds. J Anim. Breed. Genet. 1999;116:1-8.
21. Sharma R, Parmar SNS, Joshi CG, Thakur MS, Bhong CD, Chaudari MV. Molecular characterization of Nimari cattle using fluorescently labelled microsatellite markers. Indian Journal of Animal Sciences. 2010;80:661-665.
22. Sharma Rekha, Kishore A, Manishi M, Sonika A, Maitra A, Pandey AK, *et al*. Genetic diversity and relationship of Indian cattle inferred from microsatellite and mitochondrial DNA markers. BMC Genetics. 2015;16:73. DOI 10.1186/s12863-015-0221-0.
23. Sodhi M, Mukesh M, Prakash B, Ahlawat SPS, Sobti RC. Microsatellite DNA typing for assessment of genetic variability in Tharparkar breed of Indian zebu (*Bos indicus*) cattle, a major breed of Rajasthan. J Genet. 2006;85:165-170.
24. Srinivas S, Narsimha Rao G, Ahmed N, Ramesh Gupta B. Molecular genetics characterization of Ongole and Deoni cattle using microsatellite markers. Indian Journal of Animal Sciences. 2006;76:649-652.
25. Tamura Koichiro, Peterson Daniel, Peterson Nicholas, Stecher Glen, Nei Masatoshi, *et al*. MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods Mol Biol Evol. 2011;28(10):2731-

2739. DOI: 10.1093/molbev/msr121
26. Thakur SS, Parmar SNS, Joshi CG, Thakur MS, Sharma R, Chaudari MV. Genetic characterization of Malvi cattle using microsatellite markers. Indian Journal of Animal Sciences. 2010;80:138-141.