Assessment of Genetic Variation of Native Cattle in Middle Euphrates in Iraq Using SSR markers

Ruqaya Gani Hadi[1] and Hayder Raheem Alnajm[2]

[1,2]Department of Animal Production Techniques, Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Babylon, Iraq.

[2]E-mail: haider.raheem@atu.edu.iq

Abstract

A genetic diversity study in the native cattle populations of three regions in the Middle Euphrates in Iraq was carried out using 8 simple sequence repeats (SSRs) markers. A total of 60 individuals were genotyped and 149 alleles were identified. The overall mean observed (Na) and expected (Ne) numbers of alleles were found to be 6±1.17, 6.12±1.40, 6.5± 2.01, 2.166±0.077, 4.30± 1.10, 4.62±1.27, and 4.91±1.21 in Babylon, Karbala, and Najaf, respectively. The mean Shannon's information index (I) and fixation index (Fis) values were found to be 1.55±0.21, 1.63±0.22, 1.67± 0.25; 0.28±0.03, 0.31±0.05 and 0.26±0.04 in Babylon, Karbala and Najaf respectively. The overall means for observed (Ho) and expected (He) heterozygosity were 0.53±0.11, 0.52±0.12, 0.58±0.14, 0.76±0.05, 0.78±0.04 and 0.80±0.05 in Babylon, Karbala, and Najaf cities, respectively .The mean F-statistics includes Fixation indices (Among populations) (FIS), Fixation indices (Among individuals within populations) (FIT), and Fixation indices (Within individuals) (FST) values were found to be 0.284, 0.309, and 0.029 respectively. The Analysis of Molecular Variance (AMOVA) showed that 4.16% of the total variation was due to differences among native cattle genetic groups.

Keywords: Genetic diversity, Heterozygosity, SSR markers, Shannon's index.

Introduction

The cattle are an essential part of the human diet and daily life, as they are raised worldwide to provide milk and meat, which offer amino acids, minerals, and vitamins important for human nutrition [1]. Globally, 81% of milk production and 21% of meat production come from cows [2]. In Iraq, there are four known native cattle breeds: Rostaki, Sharabi, Janoubi, and Karadi. The latest available statistics on the number of cattle in Iraq date back to 2022, with approximately 1,770,967 heads, accounting for 23% of the total animal population in Iraq [3]. The cattle population has declined in recent decades due to the harsh conditions in Iraq. It is essential to preserve and sustainably use their genetic resources, as they possess tolerance to high temperatures, resistance to diseases and endemic parasites, and genetic diversity [4]. The conservation of cattle is crucial for present and future production systems of various animal products [5]. Additionally, genetic diversity is necessary for animals to conform to diverse environmental conditions such as diseases and climate [6]. Among the primary objectives of cattle breeders are to enhance production traits and maximize economic benefits for the population. To accomplish this task, breeders utilize genetic markers in addition to the traditional selection program. Breeders can use genetic markers to more accurately identify their preferred cattle breeds [7, 8, 9]. The simple sequence repeats (SSRs) markers have been widely used to study the genetic diversity of cattle populations as they are useful in studying relationships between individuals and

determine the genetic diversity within native cattle populations in some cities of the Middle Euphrates using SSR markers for their importance in genetic improvement programs, selection, and preservation of native genetic resources.

Material and Methods

Sample Collection

Blood samples were collected from 60 animals in the Middle Euphrates region in Iraq there are three cities: Babylon (n = 20), Karbala (n = 20), and Najaf (n = 20). They collected 5 mL of blood from the jugular vein of the animals using tubes containing EDTA as an anticoagulant and stored at − 20 °C until DNA isolation.

DNA Extraction and SSR Genotyping

The genomic DNA was isolated using the kit (Geneaid, USA) according to the manufacturer's instructions. In this research, we used eight SSR loci, which were recommended by the International Society for Animal Genetics (ISAG) [12], and included ETH10, ETH3 [13], CSSM66, BM1824 [14], ILSTS006 [15], BM2113, CSRM60, and TGLA227 [16], that all SSR loci were autosomal. The labeled primer sequences and chromosomal localization of each SSR locus are provided in complementary Table 1. The usual standard protocol for PCR amplification was used [17].

breeds successfully]10]. SSR markers are commonly used to estimate genetic diversity within and between breeds, levels of inbreeding, similarity and differences, genetic differentiation, and admixture among breeds [11]. This study aimed to

Table 1. Microsatellite loci, position in chromosome, sequence, and annealing.

Locus/ Chrs	Primers (5´-3´)	Annealing (˚C)
ETH10 (5)	**F:** GTTCAGGACTGGCCCTGCTAACA **R:** CCTCCAGCCCACTTTCTCTTCTC	58
CSSM66 (14)	**F:** ACACAAATCCTTTCTGCCAGCTGA **R:** AATTTAATGCACTGAGGAGCTTGG	60
ILSTS006 (7)	**F**: TGTCTGTATTTCTGCTGTGG **R:** ACACGGAAGCGATCTAAACG	55
BM1824 (1)	**F:** GAGCAAGGTGTTTTTCCAATC **R:** CATTCTCCAACTGCTTCCTTG	54
BM2113 (2)	**F:** GCTGCCTTCTACCAAATACCC **R:** CTTCCTGAGAGAAGCAACACC	67
CSRM60 (10)	**F:** AAGATGTGATCCAAGAGAGAGGCA **R:** AGGACCAGATCGTGAAAGGCATAG	60
TGLA227 (18)	**F:** GGAATTCCAAATCTGTTAATTTGCT **R:** ACAGACAGAAACTCAATGAAAGCA	55
ETH3 (19)	**F:** GAACCTGCCTCTCCTGCATTGG **R:** ACTCTGCCTGTGGCCAAGTAGG	65

The total reaction volume of 10 mL comprised: 0.5 mL 10 PCR buffer, 0.5 mL dNTP (1 mmol/L), 0.5 mL MgCl2, 0.5 mL of primer master mix, 0.1 mL of Smart Taq DNA polymerase (Cyntol, Russia),1 pmol of each primer (Forward and Reverse) and 6 µL

ddH2O, and 2 µL of genomic DNA. The PCR amplification (Biometra, Germany) included initial denaturation (95 ˚C for 7 min), 35 cycles (94 ˚C for 1 min, annealing based on reducing the temperature from 75 ˚C to 50 ˚C for 60 sec, and 72 ˚C for 1 min), and a final extension (72 ˚C for 5 min). PCR results were determined on 2% agarose gels stained in 1X TAE buffer. Allele sizes were estimated using an 11-line ladder (50 - 755 bp) (Cyntol, Rusia).

Data Analysis

Raw allele bands were determined using the UVdoc 99.02 analysis software (UVI Tech, Cambridge, UK), and allele frequencies and genetic variation parameters were calculated using POPGENE version 1.31 [18], to calculate the Analysis of Molecular Variance (AMOVA) using the GenAlEx version 6.5 [19], the F-statistics (FIS, FIT and FST) using ARLEQUIN version 3.5.2.2 [20].

Rustles and Discussion

After amplification, PCR products were first studied with 1% agarose gel, and the amplification was good, 2-5 µL of the samples were loaded on 2% agarose gel for further analysis (Figure. 1).

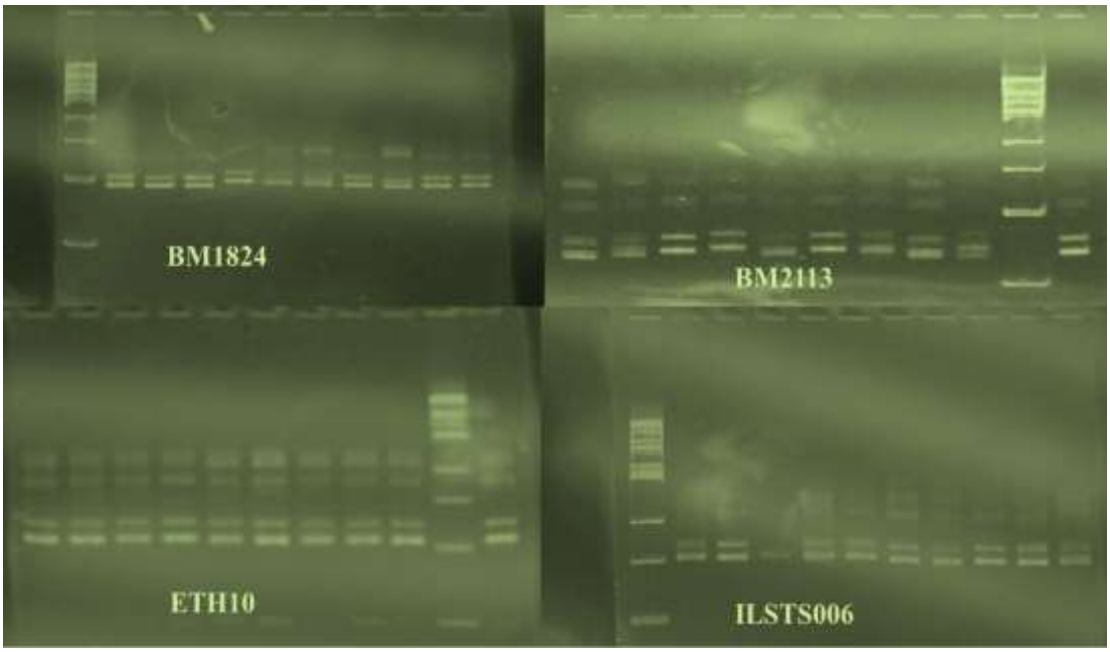

Figure. 1. Agarose gel image shows SSR markers in Iraqi native cattle.

All SSR loci were highly polymorphic for the Iraqi cattle population in the three regions. The allelic frequencies of the 8 SSR loci in the native cattle population are shown in Table 1. The results showed that the highest allele frequencies were in the TGLA227 and ETH3 SSR markers, but the lowest allele frequency was in the ILSTS006 marker (Table 2). The result showed that all the studied SSR markers contain high polymorphism. These results are similar to [21;22].

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Table 2. The allelic frequencies of the 8 SSR loci in the three cattle populations.

Locus	Overall Allele Frequency								
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
ETH10	0.108	0.116	0.216	0.091	**0.341**	0.125			
CSSM66	**0.308**	0.116	0.158	0.025	0.091	0.108	0.191		
ILSTS006	0.025	**0.400**	0.191	0.200	0.183				
BM1824	**0.333**	0.016	0.091	0.008	0.008	0.158	0.066	0.316	
BM2113	0.258	0.058	0.033	0.058	0.191	0.050	**0.350**		
CSRM60	0.108	0.075	0.166	0.025	0.050	**0.250**	0.083	0.241	
TGLA227	0.141	0.050	0.058	0.166	0.066	**0.216**	0.066	0.116	0.116
ETH3	0.158	0.075	0.100	0.125	0.166	0.025	0.083	0.025	**0.241**

The Allelic diversity parameters evaluated for the three regions are shown in Table 3. A total of 149 alleles were observed in the all-animal population from the 8 SSR loci studded. The means numbers of alleles per locus (Na) in Babylon, Karbala, and Najaf were 6±1.17, 6.12±1.40, and 6.5± 2.01, respectively, while the effective number of alleles (Ne) average were 4.30± 1.10, 4.62±1.27, and 4.91±1.21, respectively. The allelic diversity detected in the three cattle populations is relatively higher than reported in]23; 24], but lower than]25; 26]. The mean Shannon's Information index (I) in the Babylon, Karbala, and Najaf were 1.55±0.21, 1.63±0.22, and 1.67± 0.25, respectively (Table 3). The result indicates that the SSR loci selected for the study are polymorphic and useful for studying the genetic diversity of native Iraqi cows. This result lower than]27; 28], while higher than]29; 30]

Table 3. Allelic diversity in the Iraqi native cattle population

Population regions	Babylon			Karbala			Najaf		
Markers	**Na**	**Ne**	**I**	**Na**	**Ne**	**I**	**Na**	**Ne**	**I**
ETH10	5	2.98	1.25	5	4.14	1.51	6	4.81	1.59
CSSM66	6	4.25	1.59	5	3.80	1.46	7	5.16	1.78
ILSTS006	4	3.17	1.25	5	3.33	1.39	5	3.72	1.34
BM1824	6	3.77	1.49	6	4.51	1.60	5	3.37	1.36
BM2113	6	3.46	1.47	6	3.61	1.50	5	3.86	1.45
CSRM60	6	5.19	1.74	8	5.36	1.84	8	5.44	1.86
TGLA227	7	5.67	1.80	7	6.45	1.90	7	6.25	1.95
ETH3	8	5.92	1.85	7	5.83	1.84	9	6.72	2.05
Mean	**6**	**4.30**	**1.55**	**6.12**	**4.62**	**1.63**	**6.5**	**4.91**	**1.67**
SD	**1.17**	**1.10**	**0.21**	**1.40**	**1.27**	**0.22**	**2.01**	**1.21**	**0.25**

Na: Observed number of alleles, Ne: Effective number of alleles, I = Shannon's Information index, SD: Standard deviation.

Genetic variation findings according to native cattle populations are given in Table 4. The mean observed (Ho) as well as expected heterozygosity (He) was very good and high

(Table 4). The mean value of Ho across loci in Babylon City was 0.53±0.11, with estimates per locus ranging from 0.35 (ETH10, and BM1824) to 0.71 (ETH3), as well as, the mean Ho in Karbala City was 0.52±0.12, with ranged from 0.34 (TGLA227) to 0.81 (ETH3), in addition to mean in the Najaf city 0.58±0.14, and ranged from 0.30 (BM1824) to 0.85 (CSSM66). The mean value of Expected heterozygosity (He) across loci in Babylon City was 0.76±0.05, with estimates per SSR loci ranging from 0.68 (ETH10) to 0.85 (ETH3), as well as, the mean He in Karbala City was 0.78±0.04, with ranged from 0.71 (ILSTS006) to 0.86 (TGLA227), in addition to mean in the Najaf city 0.80±0.05, and ranged from 0.72 (BM1824) to 0.87 (ETH3). Most of the SSR markers studied had moderate to high values in terms of heterozygous values (>0.50). Our results were greater than]31] and less than]32; 33]. The mean fixation index (Fis) values were found to be 0.28±0.03, 0.31±0.05, and 0.26±0.04 in Babylon, Karbala, and Najaf respectively (Table 4). The values of Fis estimates were positive at 6 SSR markers indicating the within-all population heterozygotes deficiency, whereas two loci were characterized by negative Fis values in Najaf city. Thus, the studied native cattle population in the three cities (Babylon, Karbala, and Najaf) was characterized by a substantial heterozygote deficiency (28, 31, 26%) respectively. Only two SSR loci (CSSM66 and ILSTS006) significantly contributed to observed heterozygote deficiency in the Najaf cattle population. This result is highest from]34; 35] while lowest from [36].

Table 4. Observed heterozygosity (Ho), expected heterozygosity (He), and estimated heterozygosity deficit (Fis) at different loci in the three cattle populations

Population regions	**Babylon**			**Karbala**			**Najaf**		
Markers	**Ho**	**He**	**Fis**	**Ho**	**He**	**Fis**	**Ho**	**He**	**Fis**
ETH10	0.35	0.68	0.47	0.50	0.77	0.34	0.55	0.81	0.30
CSSM66	0.69	0.78	0.08	0.45	0.75	0.38	0.85	0.82	-0.05
ILSTS006	0.60	0.70	0.12	0.50	0.71	0.28	0.75	0.75	-0.02
BM1824	0.35	0.75	0.52	0.50	0.79	0.35	0.30	0.72	0.57
BM2113	0.50	0.72	0.29	0.50	0.74	0.30	0.50	0.76	0.32
CSRM60	0.65	0.82	0.19	0.60	0.83	0.26	0.50	0.83	0.38
TGLA227	0.45	0.84	0.45	0.34	0.86	0.58	0.65	0.86	0.22
ETH3	0.71	0.85	0.15	0.81	0.85	0.03	0.60	0.87	0.29
Mean	**0.53**	**0.76**	**0.28**	**0.52**	**0.78**	**0.31**	**0.58**	**0.80**	**0.26**
SD	**0.11**	**0.05**	**0.03**	**0.12**	**0.04**	**0.05**	**0.14**	**0.05**	**0.04**

Ho: Observed heterozygosity, He: Expected heterozygosity, Fis: fixation index, SD: Standard deviation

The F-statistics (FIS, FIT, and FST) in the three regions are shown in Table 5. The total population FIT and subpopulation FST values displayed positive values with mean values of 0.309 and 0.029. The average inbreeding coefficient of an individual related to the whole population (FIT) varied between 0.154 for ILSTS006 and 0.489 for BM1824, and the measurement of population differentiation (FST) ranged from 0.014 (BM1824 and

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ETH3) to 0.061, obtained for ETH10. Although the positive FIS value indicates that individuals in a population are high related than expected. The positive F value obtained in our study for three regions (Babylon, Karbala, and Najaf) that are closed and small cattle populations is not that relevant and shows that null alleles probably obscure the real picture. The mean FST value (0.029) explained that 2.9% of the genetic diversity was genetic variation among the population and also indicated the existence of subpopulations. These results are similar to those obtained for [37; 38; 39].

Table 5. F-statistics (FIS, FIT, and FST) for overall populations.

Locus	FIS	FIT	FST
ETH10	0.368	0.407	0.061
CSSM66	-0.133	0.175	0.048
ILSTS006	-0.125	0.154	0.032
BM1824	0.481	0.489	0.014
BM2113	0.310	0.345	0.050
CSRM60	0.282	0.292	0.013
TGLA227	0.422	0.440	0.031
ETH3	-0.163	0.175	0.014
Mean	**0.284**	**0.309**	**0.029**

FIS: Fixation indices (Among populations), FST: Fixation indices (Among individuals within populations), FIT: Fixation indices (Within individuals)

The AMOVA revealed that the percentage of variation among populations was 4.16% among the population, 29.24% among individuals within populations, and 66.60% within individuals (Table 6, and Figure 3). The low variation among populations is due to the geographical proximity of the animals, the possibility of breeders using the same males, and the ease of migration of animals between the study cities. Our results are lower than [40; 41; 32].

Table 6. AMOVA analysis in three regions.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	2	14.108	0.04675 Va	4.16
Among individuals within populations	57	295.500	1.18377 Vb	29.24
Within individuals	60	169.000	2.81667 Vc	66.60
Total	119	478.608	4.04719	100

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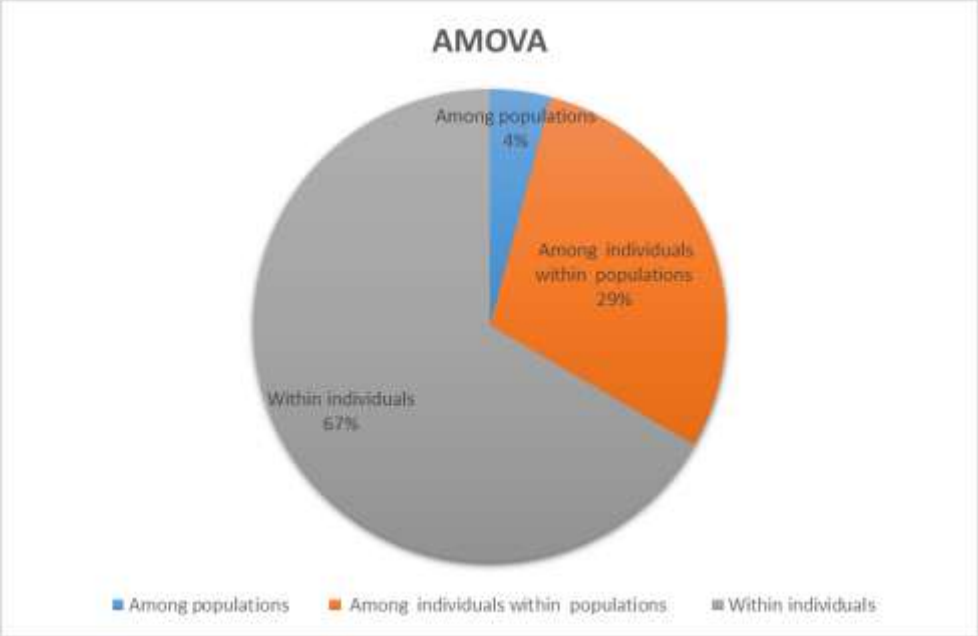

Figure 3. The variation of native cattle population in three regions.

Conclusion

This research includes a detailed analysis of the genetic diversity of the native cattle population of the Middle Al-Furat region in Iraq, especially Babylon, Karbala, and Najaf cities. The present study concluded that the native cattle population under study retained low to moderate levels of genetic variation which might be due to the conservation of an effective population. Genetic variation can be searched scientifically with proper design for improvement in this germplasm.

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