GENETIC DIVERSITY OF IRAQI LOCAL COWS AND THEIR COMPARISON WITH IMPORTED COWS USING MICROSATELLITE MARKERS

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ABSTRACT

This study was conducted to identify the genetic diversity of microsatellite markers CSSM66, ETH10 and ILSTS34 using 100 cows of several breeds distributed as follows: 30 local Iraqi cows (equally divided into Restaki, Janoubi and Sharabi) as well as 50 Holstein cows (German) and 20 Brahman bulls after amplification in PCR reaction, 16 alleles of ETH10, CSSM66 and ILSTS34 markers were detected in a sample of studied cattle and they were 7, 6 and 5 alleles, respectively. The total Shannon (I) was 1.5400, and it was found that the average value of Obs_Hom is smaller than the average value of Exp_Het, being 0.2600 and 0.7544, respectively. This indicates the presence of high genetic heterogeneity or diversity among cows. The average values of FIS, FIT and FST and genetic migration Nm -0.6257, -0.0162, 0.3749 and 0.4168, respectively. These results showed through the UPGMA tree a common ancestry between domestic cattle and Brahman.

Keywords: microsatellite, ILSTS34 , ETH10,AL- Sharabi cows.
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التنوع الوراثي في الابقار المحلية العراقية ومقارنتها مع الابقار المستوردة باستعمال واسمات المايكروستلات

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المستخلص.

أجريت الدراسة بهدف الكشف وتحديد التنوع الوراثي باستخدام لواسمات التتابعات الجزيئية الدقيقة (Microsatellite) CSSM66 و ETH10 و ILSTS34 باستخدام 100 بقرة من عده سلالاتَ موزعة كما يلي 30 بقره عراقية محليه قسمت بالتساوي(رستاكي , جنوبي , شرابي) و50 بقره الهولشتاين (ألمانية المنشأ) , 20 ذكر من ماشية البراهما بعد تضخيمها في تفاعل PCR تم الكشف عن 16 اليل للواسمات ETH10 وCSSM66 و ILSTS34 في عينة لابقار المدروسه وكانت 7 و6 و5 اليل على التوالي, وجد ان متوسط الغزارة الأليلية الكلي (na) وعدد الأليلات المؤثرة الكلي (ne) و دليل شانون الكلي(I) كانت 6.0000 و 4.1310 و 1.5400 على التوالي ووجد ان متوسط قيمة Obs_Hom اصغر من متوسط قيمة Exp_Het اذ بلغت 0.2600 و 0.7544 على التوالي ، مما يدل على وجود تغاير او تنوع وراثي عالي بين الابقار , سجلت متوسط قيم FIS و FIT و FST والهجرة الجينية Nm −0.6257 و−0.0162 و 0.3749 و 0.4168 على التوالي, اوضحت النتائج ومن خلال شجره UPGMA عن اصل مشترك بين الابقار المحلية والبراهما.

الكلمات المفتاحية: مايكروستلات، ILSTS34 ، ETH10، ابقار الشرابي.
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INTRODUCTION

Iraq possesses a wide variety of livestock for different types of farm animals such as buffalo, cows, camels, sheep, and goats, as these types spread in all regions of Iraq and constitute significant sources to provide the population with the necessary nutrition of meat and milk, the proportion of the cows out of the total animals in Iraq reached (21,10%),the numbers of farm animals have declined over the past few decades due to the abnormal conditions in Iraq and the cross-breeding process with other breeds, it was found that it is necessary to preserve and use them sustainably. (5, 23, 25, 31) because of their ability to withstand high temperatures, resistance to endemic diseases and parasites, and genetic distinction (4,5,6). Breeding cows is one of the most important elements of animal production in Iraq, and it is an important part of the overall economic wealth(1, 19, 20). Molecular markers have revolutionized the ability to characterize genetic variation and shorten genetic selection (7,13). It evaluates breeds and increases the electoral yield in genetic improvement programmes (2). Markers have been widely used to access genetic diversity because they contributed information about each region of the genome regardless of the level of gene expression, the use of microsatellite markers is one of the most powerful methods for studying genetic diversity, calculating genetic distances, and discovering the percentage of the hybrid animal, the reason is the high degree of polymorphism and random distribution across the genome (29). A group of essential markers have been recorded and recommended for their study and extensive use in livestock to address a specific problem not yet addressed in native species, a set of 30 microsatellite markers, including ETH10, CSSM66, and ILSTS 34, may be recommended for use in such work so that joint analysis of future data is possible (21). The structure of ETH10 consists of diploid repeats (ACn) and consists of five alleles with different sequences located in the one exon of the signal transducer and transcription activator gene (STAT6) located on bovine chromosome 5 (15). D14S31-CSSM66 structure consists of diploid repeats (ACn) and four alleles of different sequences, the CSSM66 locus on chromosome 14 is proximal to the Bos taurus autosome 14 (BTA14) quantitative traits and genes related to economically important traits (8,11,36). The ILSTS34 structure consists of diploid repeats (GTn) and is located on chromosome 5 in bovine and is one of the microsatellites markers for assessing genetic diversity and is extremely useful in genetics and conservation breeds due to its high range of polymorphism (32). Given the importance of preserving the genetic diversity of local cows for the purpose of preserving and using them sustainably to ensure the continuation of the process of improvement and selection, this study aims to study the genetic diversity in a sample of local cows and compare this diversity with two widely spread breeds of cows, namely the Holstein and Brahman breed, using three microsatellites markers.

MATERIALS AND METHODS

the Genetic Resources Conservation Directorate, Directorate of Animal Resource, and the Ministry of Agriculture was consulted to deteet the locations of the animals. They were of different ages and different breeds. From these samples, 30 cows were selected according to the scientific and formal specifications, and 50 cows of the Holstein breed, white and black spotted, and 20 Brahman bulls Figure (1).

Figure 1. Model of the Iraqi local cows used in the study

Blood sampling

Blood samples (3 ml) were collected his jugular vein puncture using K2EDTA tubes from all animals and stored under - 18^{o} c until assay.

DNA Isolation

DNA was isolated from the blood according to the instructions of the diagnostic kit (kit) supplied by the Korean company Geneaid in the laboratory of the Faculty of Agricultural Engineering Sciences.

PCR and Gel Electrophoresis

Primers were selected for three of the genetic markers for micro-sequences Table (1) which includes primer sequences a replicon ,size , degree of annealing and remittance Table (2) the materials used in molecular detection using an enzyme reaction sequential PCR of the studied genes using the PCR GreenMasterMix KIT diagnostic kit with a volume of 25 microliters, and placed in the PCR device according to the reaction conditions for each duplicated gene segment. After completion of the PCR, the electrophoresis of the PCR product was carried out using the agarose gel preparation method to migrate the DNA genetic material. The electrophoresis process began after the PCR reaction and DNA amplification, as 7 lµ of the PCR product is taken and placed in the pits with the use of a ladder with a size of 25 and 100 nitrogen bases (DNA Marker 100 -25bp). The PCR product was passed on an agarose gel at a concentration of 2.5 mm in TAE (1X) solution and the voltage was fixed at 90 volts and 60 amperes for an hour and a half, then the gel was immersed in a solution containing ethidium bromide dye for 20 minutes.

Statistical analysis

The results were analyzed using POPGENE (software Version 3.2).

Table 1. Sequences of primers supplied by Macrogen

Marker	Chr.	Prirmer sequence (5 – 3) Forward Reverse	Degree of annealing (C^{o})	size (bp)	Reference
ETH10	5	F:GTTCAGGACTGGCCCTGCTAACA R:CCTCCAGCCCACTTTCTCTTCTC	58.2	185 - 221	(37)
CSSM66	14	F:ACACAAATCCTTTCTGCCAGCTGA R:AATTTAATGCACTGAGGAGCTTGG	59	168 - 207	(9)
ILSTS34	5	F:AAGGGTCTAAGTCCACTGGC R:GACCTGGTTTAGCAGAGAGC	55	138 - 212	(9)

Table 2. Materials used and their volumes in the PCR Technique

reaction materials		Volume
Master mix (Promega , USA)		12.5 µl
Primers	Forward	1 µl
	Reverse	1 µl
Nuclease Free Water		7.5 µl
DNA		3 µl
Total volume		25 µl

RESULTS AND DISCUSSION

PCR product amplification

The results of the PCR migration revealed the presence, diversity, size, and distribution of alleles of the three studied markers for the local and imported cattle breeds in Iraq. Six alleles of different sizes were found for CSSM66, seven alleles for the ETH10 and five alleles for ILSTS34 noticed markers were in Table (3) Figure (2).

Table 3. The distribution, allele code and size resulting from PCR and electrophoresis of the markers CSSM66, ETH10 and ILSTS34 in the studied cows

Marker	Allele Code	bp allele size	Presence of the allele in cows				
			SH	R	G	P	H
CSSM66	E	170	1	1	0	0	0
	B	200	1	1	0	0	1
	F	205	0	1	0	0	0
	C	180	0	0	1	1	1
	D	210	0	0	1	1	0
	A	140	0	0	0	0	1
EHT10	L	240	1	1	1	1	0
	Q	215	1	1	1	1	0
	Y	210	1	1	1	1	0
	H	205	0	0	0	0	1
	K	235	0	0	0	0	1
	G	225	0	0	0	0	1
	P	200	0	0	0	0	1
ILSTS34	T	200	1	0	0	0	1
	M	210	0	1	0	1	1
	S	240	0	0	1	0	0
	Z	170	1	1	1	0	0
	W	190	0	0	0	1	1

SH = Sharabi cows, R = Restaki cows, G = Southern cows, P = Brahman cows, H = Holstein cows, 1 = presence of the allele, 0 = absence of the allele

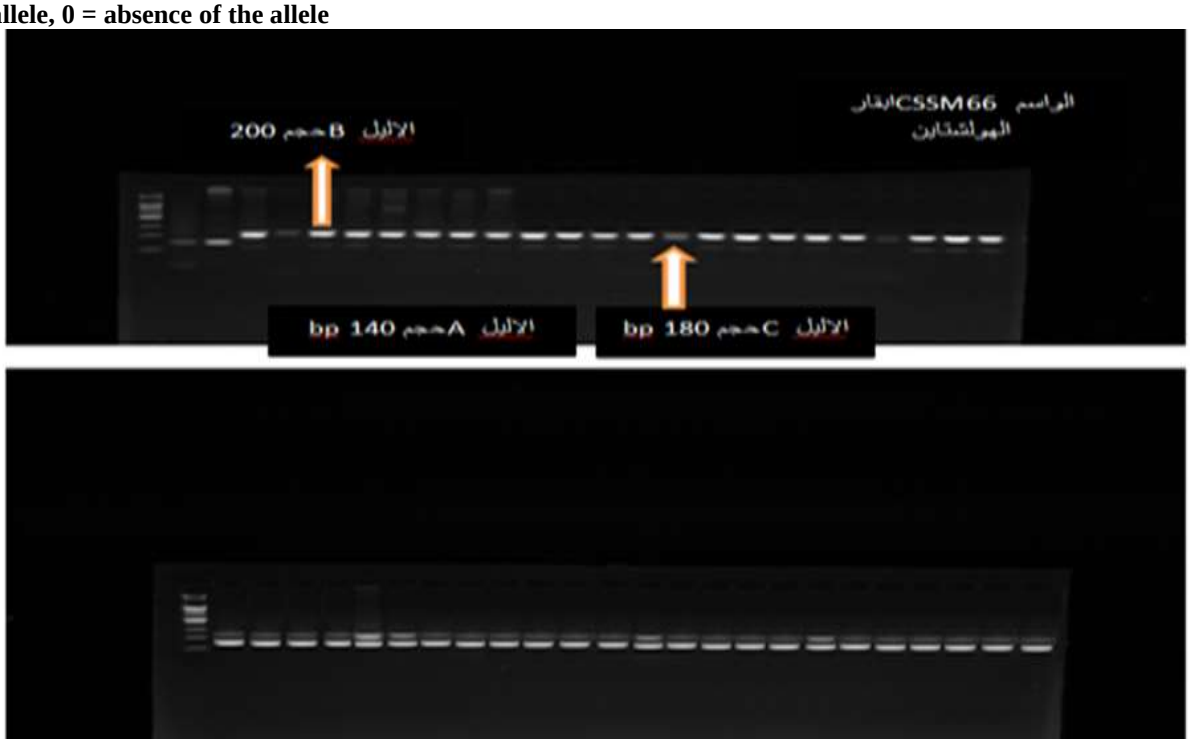

Figure 2. Model of PCR migration and allele size for CSSM66 markers Holstein cows

Mean allelic abundance (na), number of influential alleles (ne) and Shannon index (I) for ETH10, CSSM66 and ILSTS34 markers

The mean total allelic abundance (na) for all three microsatellites markers (ETH10, CSSMS66 and ILSTS34) for the studied sample was 6.0000, and the total number of influential alleles (ne) was 4.1310. The total Shannon information index I means the probability of homology to distinguish Variation of individuals (27) 1.5400 (Table 4), the allelic abundance values were distributed as 7.0000, 6.0000 and 5.0000 for the markers ETH10, ILSTS34 and CSSM66, respectively, that the high diversity in alleles indicated a high genetic diversity in animals found in Iraq, and this is consistent with the study of (14), which reflects allelic abundance as a measure of allelic diversity or the number of distinct or rare alleles expected in a random subsample taken from the community. It is close to

4.8924, 3.2315 and 4.2689, respectively. As for Shannon's index, the values of homology were high and close to all markers, as it reached 1.6824, 1.3637, and 1.5739, which reflects the high diversity in alleles and differentiation. For Iraq breeds, this diversity is considered important and the need to preserve it for the purpose of developing livestock and helping to develop improvement plans for Iraqi animals. This is consistent with the study of Radhika (2021) (30), as it indicated that the number of alleles present in a group is a measure of genetic diversity. Management and sustainable development of agricultural animal genetic resources.

Table 4. Mean allelic abundance (na), number of influential alleles (ne), and Shannon's index (I) for ETH10, CSSM66 and ILSTS34 markers for different Iraqi local and imported cows

animal type	groups of cows Sharabi Restaki Southern Holstein Brahman			
Marker	number	*Na	ne*	*I
ETH10	200	7.0000	4.8924	1.6824
ILSTS34	200	5. 0000	3.2315	1.3637
CSSM66	200	6. 0000	4.2689	1.5739
ST ± mean	200	1.0000 ± 6.0000	0.8390 ± 4.1310	0.1621 ± 1.5400

Genetic diversity

Table (5) shows the calculated average and the expected values of the homozygotes Obs_Hom and the heterozygous Obs_Het alleles in a sample of groups for the studied cows. It was found that the average value of Obs_Hom that was 0.2600 is lower than the value of Obs_Het that was 0.7400, that were calculated for all three microsatellite markers: ETH10, CSSMS66 and ILSTS34. In addition, the average expected value of Exp_Hom is less than the value of Exp_Het, which was 0.2456 and 0.7544. Moreover, the Nei values, representing the total expected heterozygotes value, that was calculated according to what (25) came up with, were to be close to the value of the estimated Exp_Het, which amounted to be 0.7506. This indicates the presence of heterogeneity or diversity hereditary in the genotypes among animals, which agrees with (18), as the expected average heterozygotes calculated on all sites in a particular population can be considered as a general measure of genetic diversity, and a large number of sites can be examined as it is compared with Ho (computed zygote heterogeneity) , As for each and every name, it was found that the value of the allelic mixture calculated for Obs_Het for ETH10 was superior to Obs_Hom as it reached 1.0000 and 0.0000, respectively, and the expected value of Exp_Hom was also lower than that of ETH10. The value of Exp_Het was 0.204 and 0.7966, respectively, and the Nei values were 0.7956 approximated to the value of Exp_Het allelic mixture, which indicates the presence of a very high diversity within this marker. As for the marker ILSTS34, it was found that the value of Obs_Hom is close to the calculated Obs_Het and was 0.5500 and 0.4400, respectively. The expected value of Exp_Het was higher than the value of Exp_Hom, which was 0.6940 and 0.3060, respectively, and the values of Nei were 0.6905, close to the value of Exp_Het allelic mixing within this marker, and the diversity was high within the marker. As for the name, CSSM66 found a value of allelic for Obs_Het greater than Obs_Hom. It reached 0.7700 and 0.2300, respectively, and the expected value of Exp_Hom was also lower than the expected value of Exp_Het, which amounted to 0.2304 and 0.7696, respectively. Also, the values of Nei were 0.7657, close to the value of the Exp_Het allelic mixture, which indicates the presence of genetic diversity within this marker. It is evident from the results of allelic mixing that there is high genetic variance within the sample of the studied cows in Iraq, It can be used to develop plans for improving and drawing selection breeding methods for local cows with the need to preserve the genetic diversity of local cows, and this is consistent with the study of(1) and is usually considered genetic markers with allelic mixing estimate values greater than 0.5 are helpful in the community, and (26) explained that Genetic diversity is a measure of genetic differences between animals in a population (ie genetic variance), to ensure that a breeding program is still viable in the future it is necessary to monitor and maintain genetic diversity, that

allows selection of superior animals for breeding where it is not feasible in the case of the absence of any genetic diversity, because all animals are genetically similar, and no process of selection and improvement of the next generation can be carried out. In such a case, there is no feasibility in developing programs for breeding and improvement

Table 5. ratios of allelic mixing in groups of Iraqi local and imported cows

animal type	groups of cows Sharabi Restaki Southern Holstein Brahman						
Marker	Number	Obs_Hom	Obs_Het	Exp_Hom	Exp_Het*	Nei**	Ave_ Het
ETH10	40	0.0000	1.0000	0.2004	0.7966	0.7956	0.6017
ILSTS34	40	0.5500	0.4400	0.3060	0.6940	0.6905	0.3589
CSSM66	40	0.2300	0.7700	0.2304	0.7696	0.7657	0.4364
ST ± mean	40	±0.260	±0.740	0.0544 ±0.2456	±0.7544	±0.7506	±0.465
		0.2762	0.2762		0.0544	0.0541	0.1240

Evidence of stability index in a sample of cattle breed within the ETH10, CSSM66 and ILSTS34 markers

Table (6) shows the average values of the stability index of the three markers in the sample of the studied cows, as the average values of FIS, FIT, FST and genetic migration were recorded to be Nm= -0.6257, -0.0162, 0.3749 and 0.4168, respectively. It was found from these results that there is no domestic breeding genetic drift and high genetic diversity through negative FIS values, and this is agrees with (35) study. It was shown that the degree of internal breeding was high when the estimate of FIS was a positive number, and in the case of negative FIS value, is an indication of the existence of the external breeding between breeds. The results showed a significant differentiation between breeds of FST values, which agreed with the study of (16) that stated the FST value is considered high and the differentiation is high between breeds when the value is more than 0.15, in addition, these results agree with the study of (28) on Indian cows. The study (28) noted that the proportion of gene flow decreased Nm between breeds, due to the possibility of clear genetic difference between breeds or the possibility of geographical isolation for local animals and the high genetic variance that exists. This also agrees with the study (17) that stated that the high gene flow between breeds is due to the lack of genetic variation between them, which happens in local animals, this lack is due to human intervention. This lack of genetic variation was also observed in the previous tables of the studied Holstein cows, which show a clear extent of human intervention. It was found that the values of FIS markers : ETH10 , CSSMS66 and ILSTS34 were -0.6619, -0.4208, and -0.8166, respectively, indicating the absence of cross-fertilization between relatives due to what was attributed above. inundation, it was found that the FST values were 0.1793, 0.4468, and 0.5206, respectively. Moreover, among the three markers ILSIS34, CSSM66 and ETH10, it was found that the LSIS34 and CSSM66 markers had the highest genetic differentiation unlike ETH10 that had the lowest values, although it was considered a high value. As for the gene flow Nm, it was found that the marker ETH10 recorded the highest flux value of 1.1446 and this shows the possibilities that there was a relationship, common ancestry, or the flow of exogenous genes between animals. However, the two markers CSSM66 and ILSTS34 showed a low gene flow that reached 0.3095 and 0.2302, respectively, and this decrease is probably due to the proximity of the marker location of the quantitative traits in the animal itself or the presence of the special allele for each animal. As (34) indicated the presence of special alleles in domestic cows were considered among the unique gene pools. It is possible to consider that special alleles are an effective tool for genetic identification between the breeds of cows. in addition, the study (34) The presence of more special alleles in a population is an indication of the distinction of that genetic group from the reason for the increase in the rate of migration and gene flow (Nm) led to a decrease in the percentage of specific alleles in many groups of animals and thus a very significant decrease in the frequency of specific alleles.

Table 6. Evidence of stability index in groups of Iraqi local and imported cows

animal type	groups of cows Sharabi Restaki Southern Holstein and Abrahman				
Marker	number	Fis	Fit	Fst	Nm*
ETH10	40	0.6619-	0.3640-	0.1793	1.1446
ILSTS34	40	0.8166-	0.1291	0.5206	0.2302
CSSM66	40	0.4208-	0.2140	0.4468	0.3095
mean	200	0.6257-	0.0162-	0.3749	0.4168

UPGMA trees

Figure (3) shows a rooted tree of four varieties as described by (33) and for varieties 1, 2, 3 and 4, class 4 was considered the basis for classifying the tree according to the light of which the groups of cows branched out. It was POP1 Sharabi, POP2, Restaki, and POP3 Southern. POP4 Holstein and POP5 Brahman and each strain was rooted to a mei branch, the first branch contained class 3 within class 2 clans POP1 Sharabi POP2 Restaki and class 1 clans POP3 Southern and POP5 Brahman the second independent branch contained POP4 Holstein. It was found that the Al-Sharabi and Al-Restaki cows meet in the 2nd class, the Southern and the Brahman in the 1st class, and both types are branched from the 3rd class, which in turn is a branched from the 4th class. It was found that the Sharabi, Restaki, Southern and Brahman cows meet with one origin, which is the type No. 3 belonging to the first branch and its collection goes back to the same appearance it is to the Indian cows, and this applies to the external appearance of the local animals closely related to the Indian cows, and it is possible that it belongs to the Indian breed (*Bos indicus*). The Holstein was unique to one independent breed and did not receive any of the Iraqi and Brahman species, and this indicates a return to the European cattle (*Bos Taurus*), which resolves the controversy about the origin of Iraqi cows and confirms the viewer who says that it belongs to the Indian origin(3), which goes back to the breed 4 Bovine family and independence of Holstein cows found in the second rooted branch of class Four directly.

Figure 3. UPGMA tree showing the original affinity between the studied cows (POP GENE)

C0NCLUSION

In conclusion, the three microsatellite markers : ETH10, CSSMS66 and ILSTS34 can be considered as one of the most important and essential candidate markers to study the genetic diversity of Iraqi local cows,conserve the high genetic diversity of local breeds, to protect from extinction, to make breeding and improvement programs for the local cow.

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