STUDY OF GENETIC DIVERSITY USING MICROSATELLITE MARKERS IN IRAQI SHEEP BREEDS

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ABSTRACT

Genotypes and allele frequency values were studied to determine the genetic diversity of three microsatellite markers (BM1329, BM134 and CSSM66) in two local Iraqi sheep breeds (50 Awasi ewes (AW) and 50 Naimi ewes (NA)) and breed in one Agricultural Stations in middle of Al-Furut Iraq (Karbala Governorate). The Genetic analyses for the study were carried out at the Biotechnology Research Center / Al-Nahrain University using PCR technology to obtain alleles for the studied microsatellites. The results of this study indicate that there is a multiplicity of genotypes and alleles resulting from these markers among the members of the single breed and between the studied breeds. It was revealed that the allele frequency values showed the presence of alleles dominant on the rest of alleles produce in this sample studied, the BM1329 microsatellite of the Awassi breed produced the dominant allele frequency in 195(0.25) and 320 (0.25), While in the Naimi breed were the values of the dominant allele frequency of the195(0.22), 220(0.21) and 230(0.24), and the dominant allelic frequency of (BM134) marker in the Awassi breed was 118 and 135 and repeated (0.21 and 0.26, respectively). For CSSM66 microsatellite, the dominant allelic results and their allelic frequency values were (195 (0.20), 205 (0.23) and 220 (0.25)) for Awasi breed, whereas it was 180 (0.24), 195 (0.28) and 220 (0.22) for Nuaimi breed. These results enable us to use these markers as a means of determining the diversity and genetic relationships between individuals of a single breed as well as between breeds.

Keywords: genotype, allele frequency, PCR, Awasi ewes, Naimi ewes.

مجلة العلوم الزراعية العراقية -2020 :51 (5):1367-1374 **هادي وآخرون**

دراسة التنوع الوراثي باستخدام واسمات التتابعات الدقيقة (Microsatellites) لعدد من سلالات الأغنام العراقية

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المستخلص

تم دراسة التراكيب الوراثية والتكرار الاليلي بهدف معرفة مدى التنوع الوراثي لثلاثة من واسمات المايكروستلايت (BM1329,BM134 and CSSM66) في سلالتين من سلالات الأغنام العراقية المحلية (50 نعجة عواسية (Awasi(AW) و50 نعجة نعيمية (Naaimi(NA) والمربأة في احدى المحطات الزراعية في منطقة الفرات الأوسط العراق (محافظة كربلاء). اجريت الفحوصات الوراثية الخاصة بالدراسة في مركز بحوث التقنيات الأحيائية / جامعة النهرين وباستخدام تقانة التفاعل البلمرة المتسلسل (PCR) للحصول على الاليلات الخاصة بالمايكروستلايت المدروسة. دلت نتائج هذه الدراسة الى وجود تعدد في التراكيب الوراثية والاليلات الناتجة من هذه الواسمات فيما بين افراد السلالة الواحدة وما بين السلالات المدروسة، ولوحظ ان قيم التكرار الاليلي قد اظهر وجود اليلات سائدة على بقية الاليلات الناتجة بهذه العينة المدروسة اذ ان المايكروستلايت BM1329 لسلالة العواسي أنتج تكرار اليلي سائد للاليل 195(0.25) والاليل 230(0.25) بينما في سلالة النعيمي فكانت قيم التكرار الاليلي السائد للاليل 195(0.22)، الاليل 220(0.21) والاليل 230(0.24)، وكانت قيم التكرار الاليلي السائد للماركر BM134 في سلالة العواسي 118 و 135 وبتكرار (0.21 و 0.26 على التوالي) و في سلالة النعيمي ظهرت الاليلات السائدة وبتكرار (118(0.22)، 125(0.23) و 130(0.23))، و اما المايكروستلايت CSSM66 فان نتائج الاليلات السائدة وقيم التكرار الاليلي لها فكانت (195(0.20)، 205(0.23) و 220(0.25)) لسلالة العواسي و في حين انها كانت بسلالة النعيمي (180(0.24)، 195(0.28) و 220(0.22)). هذه النتائج المتحصل عليها تمكننا من استخدام هذه الماركرات كوسيلة لتحديد مدى التنوع والعلاقة الوراثية فيما بين الافراد للسلالة الواحدة وكذلك فيما بين السلالات.

كلمات مفتاحية: تركيب وراثي، تكرار اليلي، تفاعل البلمرة المتسلسل، نعاج عواسية، نعاج نعيمية.

***Received:11/10/2019, Accepted:21/1/2020**

INTRODUCTION

Diversity is essential in domesticated animals, including genetic differences between breeds as well as within species used in agriculture and food provision (10). The importance of continuing diversity in farm is due to the human need to maintain genetic diversity in order to meet future and unknown challenges associated with animal production and to minimize the impact of changing climate conditions and other environmental factors (14). Microsatellite markers are important tools in conservation and genetic diversity programs in animal herds where they are suitable for studying both genetic variation and ratios among individuals as they are highly polymorphic, thus, we get multiple allele frequencies that enable the identification of individuals studied (11). Microsatellite is termed a number of labels, sometimes called short tandem repeats (STRs) or simple sequence repeats (SSRs), which are repetitive regions on DNA and are characterized by high genetic variation (polymorphism) (9,13,23). Sheep breeding is an important source of livelihood and food security for most rural people, especially in developing countries (27). Sheep are one of the main farm animals raised in Iraq (6633904 head) (15). It is a major source of livestock, which is a major source of meat production and a secondary source of milk and wool in Iraq, meat produced from sheep is the main quality and desirable for consumption by the Iraqi consumer compared to the meat of other animals or even imported live and frozen meat (17). The origins of Iraqi sheep to the Asian sheep with a broad mechanism and spread in Iraq, three breeds of sheep mainly name Awasi, Arabi and Alkradi while the Nuaimi breed is part or a genetic group back to the Awasi breed, but smaller in size, as well as the Hamdani breed is part of the breed Alkradi (25). The Awassi breed is widely spread across the eastern Mediterranean. It is the main breed in Iraq and Syria, represents a contributing part of sheep breeds in Turkey (3.5% of the total sheep population) and the only local breed in Jordan (16). Awassi sheep in Iraq represent more than half of the number of sheep breeds in Iraq (4), The Awassi sheep are characterized by the quality of meat produced in terms of palatability besides their preference by the Iraqi consumer compared to other meat (26). The Al-Naimi breed is part of the Al-Awasi breed, but it is characterized by being smaller in size and also has a higher ability to control food shortage (feed) (6). The Nuaimi breed is widespread in western Iraq and is also found in Saudi Arabia and some neighboring countries and it has a high level of milk production (18). Therefore, this study aimed to study the genetic diversity in the Awasi and Naimi breed based on the types of alleles and the frequency of these alleles among their members and between the breeds.

MATERIALS AND METHODS

This experiment was conducted in the department of medical and molecular biotechnology at Biotechnology Research Center/ Al- Nahrain University. Blood samples were collected from 50 Awasi ewes and 50 Naimi ewes at a station in Karbala Governorate, southwest of Baghdad / Iraq. Blood samples were collected from the jugular vein of sheep using sterile EDTA containers. The samples were kept refrigerated until transferred to the laboratory to perform DNA extraction from blood samples and using a special extraction kit (Geneaid extraction kit, Korea). Three microsatellites (BM134, CSSM66, BM1329) were detected using primers for each of the microsatellites shows in Table 1. The reaction solution was made with a total volume of 25µl consisting of 5 µl of PCR reaction solution plus 5 µl of DNA template and 1 µl of the forward and reverse primers. The solution was supplemented to the final volume by adding 13 µl of distilled water. The PCR program was used according to Table 2 to obtain the final output and by using the annealing temperature of each of the three microsatellites shows in Table 1 in the reaction program. The PCR product samples were deported with 2% agarose gel.The cutting lengths of the studied microsatellites were determined using known ladder volumes (50bp). The results were recorded that the individual with one bundle shows pure genotype (Homozygosity) and the individual with two bundles is a hybrid genotype (Heterozygosity). Allele frequency values were calculated based on (29)

Table 1. The microsatellite used and its sequence

Microsatellite	Primer sequence	Annealing Tm	References
BM1329	F- TTGTTTAGGCAAGTCCAAAGTC	62*	(8)
	R- AACACCGCAGCTTCATCC		
BM143	F- ACCTGGGAAGCCTCCATATC	63*	(28)
	R- CTGCAGGCAGATTCTTTATCG		
CSSM 66	F- ACACAAATCCTTTCTGCCAGCTGA	60*	(24)
	R- ATTTAATGCACTGAGGAGCTTGG		

Table 2. Thermal cycle steps of PCR program

Step	Temperature	Time	cycle
Start denaturation	95	5 min	1
Denaturation	94	30 sec	
Annealing	*	30 sec	35
Extension	72	45 sec.	
end Extension	72	7 min.	1
Finish	4	Infinity	

RESULTS AND DISCUSSION

The results of Figure1, 2, 3 and Table 3 show the presence of genetic diversity for the microsatellite studied within the same breed as well as between the two breeds, as it was noted that the number of alleles found in the Awasi breed reached 19 alleles and the Naimi breed 16 alleles. The distribution of alleles for their microsatellite was 7 alleles for BM1329, 6 alleles for BM143 and CSSM66 and Awassi breed, and 6 alleles for BM1329, BM143 and 4 alleles for CSSM66 microsatellite for Al-Naimi breed. The Awassi breed contained alleles 180, 195, 200, 210, 230, 245 and 250 for the microsatellite BM1329 and alleles 108, 118, 120, 125, 130 and 135 for the microsatellite BM143 and for the microsatellite CSSM66 the alleles 175, 180, 190, 195, 205 and 220, The Naimiya breed contained alleles 187, 195, 200, 210, 220 and 230 for the microsatellite BM1329 and alleles 108, 110, 118, 120, 125 and 130 for BM143, while the CSSM66 showed alleles 180, 185, 195, 205 and 220. There was a variation in the alleles obtained between the two breeds as it was found that the microsatellite BM1329 in the Awasi breed contained allele 180, 245 and 250 did not appear in the Naimi breed that containing the allele 187 and 230 and did not appear in Awasi breed, while microsatellite BM143 was found the allele 135 in the Awasi breed and did not appear in the Al-Naimi breed and 110 appeared in Al-Naimi breed and did not appear Awasi breed, The Awassi breed contained alleles 175 and 190 for the microsatellite CSSM66, which was not found in the Naimi breed where the allele 185 was found and the later was not found in the Awassi breed. The allelic frequency of the Awassi breed showed that the dominant alleles were (195 and 230) for the microsatellite BM1329 and the alleles 118 and 135 for the BM143 while the microsatellite CSSM66 was the dominant alleles 195, 205 and 220, whereas in the Naimi breed the dominant alleles had the highest allelic frequency of the microsatellite BM1329 was 220,230 and 195 and BM143 was 118, 125 and 130 and for CSSM66 it was 180, 195 and 220. The genetic markers used in this study was showed the ability to indicate a genetic diversity between the studied breeds and the individuals within the same breed. These results were similar to previous studies conducted to study genetic diversity among Iraqi breeds based on microsatellite markers, which indicated that they contain a genetic diversity at a certain level when they are detected using genetic markers (microsatellite). The study carried out by Mohammed (22) was revealed four breeds of Iraqi sheep (Awassi, Hamdani, Naimi and Karadi) using 24 microsatellites, these microsatellites were distributed in their genetic sites on 19 chromosomes, these breeds belonged to different geographical areas of northern Iraq, where it was found that there was a genetic diversity of these sites in these four breeds. The results of Owaid (25) were conducted on four breeds of Iraqi sheep (Arabi breed from southern Iraq), Awasi (from central Iraq), Hamdani and Karadi (from northern Iraq), these included 15 microsatellites, all of which showed a high genetic diversity among the breeds and the individuals studied were selected within these breeds had not to be relative, while the marker CSSM47 had not

showed a genetic diversity in all samples of different breeds as it was a single allele for all samples. Also the results reported by AL-Brkat (5) who studied the Awassi sheep in southern Iraq using microsatellite markers and found it was have a genetic diversity. The study conducted by Al-Atiyat et al (3) in Saudi Arabia on five breeds of sheep, including the breed of Naimi and foreign breed (Awassi Jordanian sheep) to determine the structure and genetic diversity of these breeds using microsatellite that basis of our work, in their work, they obtained that the BM1329 had a genetic diversity, as the Al-Naimi breed of Saudi Arabian showed the presence of 8 YLs and the Awasi breed of Jordanian contained 4 YLs of this microsatellite and that these two breeds were closer together in terms of structure hereditary breeds from the other breeds despite the different geographical location. The results of the BM1329 study indicate that there are variations in the number and size of alleles, which can be used in the study of genotypes and the relationship between individuals per breed and between breeds. These results are accordance with that obtained by Yilmaz et al (30) when studying four breeds of Turkish sheep using microsatellite markers to determine the genetic relationship between them and it was found that the microsatellite BM1329 22 Allele for the four breeds and showed a genetic diversity. The results were reported by Sun et al (28) when studying the Chinese Hu sheep using a number of microsatellite markers and how they relate to the Litter Size attribute, the BM1329 marker showed the presence of 5 alleles within this breed. Also, Elfawal et al (12) found that when studying three breeds of Egyptian sheep to find out their genetic diversity using microsatellite markers, BM1329 showed a genetic diversity of 15 alleles at a rate of 5 alleles per breed. The results of the study, which are attributed to the microsatellite BM134, showed that there were six genotypes and six alleles for each of the studied breeds and the allele 135 was characterized by its presence in the Awasi breed while the allele 110 was apparent only in the Naimi breed, these findings suggest that it can be used to identify animals with good genetic makeup, quantitative traits in animals, and to take advantage of this diversity. The results obtained by Al-Atiyat et al (1) when studied the breed of Awassi sheep in Jordan and ten different geographic regions that these sheep have shown a genetic diversity in relation to the microsatellite used and it has been divided into four major genetic groups instead of back to ten different geographical areas due to genetic convergence between them and that the microsatellite BM134 has shown the presence of 12 Allele in this breed and all the studied areas. Al-Atiyat et al (2) was found in their study of the Australian Marino sheep breed and for three different groups with important characteristics (control population (CR), low parasite resistance (LR) and high parasite resistance (HR), using 28 microsatellite markers and to determine the genetic variation between these three groups, BM134 produced 8 alleles in both CR and LR and 6 alleles in the HR group.The results also showed by Sun et al (28) when the use of four microsatellites to study the Chinese sheep and how the relationship of these microsatellite with the characteristic Litter Size to determine the genotypes that have high susceptibility to performance and the microsatellite BM134 produced 9 alleles within the breed. A study conducted by Kevorkian et al (19) on four breeds of Roman sheep to determine genetic diversity and extent of genetic relationship between them by the use of 11 microsatellites, it was found that microsatellite BM134 produced different numbers of alleles 6,9,11 and 12 in different breeds. The results found by Arranz et al (7) when studying six Spanish sheep breeds using 18 microsatellites to determine the genetic variation between these breeds, showed that microsatellite BM134 observed different numbers of alleles of these breeds 6,9,10,10,11 and 11. CSSM66 microsatellite results showed that there were eight genotypes and it belongs to six alleles in the studied animals of the Awasi breed, while the animal sample of the Naimi breed contained eight genotypes and it belongs to five alleles, this indicates the diversity of the number of genotypes produced. A study indicated by Arranz et al (7) that used 18 microsatellites to determine the genetic diversity and the extent of genetic variation between the six breeds of Spanish sheep,

where microsatellite CSSM66 appeared in different numbers of alleles between these breeds (Churra 11; Latxa 8; Manchega 12; Rasa-Aragonesa 11; Castellana 10 and Merino 13). The results of Olufunmilayo et al (24) when studying five Nigerian sheep breeds and included imported Merino breed using 26 microsatellites to know the nature of the diversity and genetic relationship between this breed and the results of the microsatellites CSSM66 indicate that it contains 22 alleles produced by members of this studied breed, which belongs to different parts of Nigeria and the lengths of cut alleles ranging from 173-213 base pairs. Lasagna et al (21) was found when they used 30 microsatellites in their study of the Italian Appenninica sheep breed and their genotype using a number of microsatellites and compared with the genotype of three Italian sheep breeds (Sopravissana, Gentile di Puglia and Spanish Merinos) to find out the relationship and genetic convergence between these breeds and found that the microsatellites CSSM66 produced 18 alleles between these breeds and the size of the alleles ranged between 180-202 base pairs. In a study of Kunene et al (20) of Nguni (Zulu) sheep in South Africa to determine the extent of genetic diversity using a number of microsatellites, the microsatellites (CSSM66) found that the number of alleles reached 14 alleles in four clans of this breed and its belong to ten herds to different regions of South Africa.A study was conducted to determine the extent of genetic diversity among the Awasi and Naimi breed by identifying the resulting alleles and the frequency of these alleles for a number of microsatellite markers. This may be attributed in the case of the breed to the breeding conditions and the method used in the production plant from which the samples were collected. As for the inter-breeds, it is due to the fact that the Naimi breed is originally a lineage derived from the original Awassi breed and therefore carries part of the genotype of the original Awassi breed and the common environment in which animals were raised did not lead to the emergence of genotypes to improve animal adaptation to heterogeneous environments.

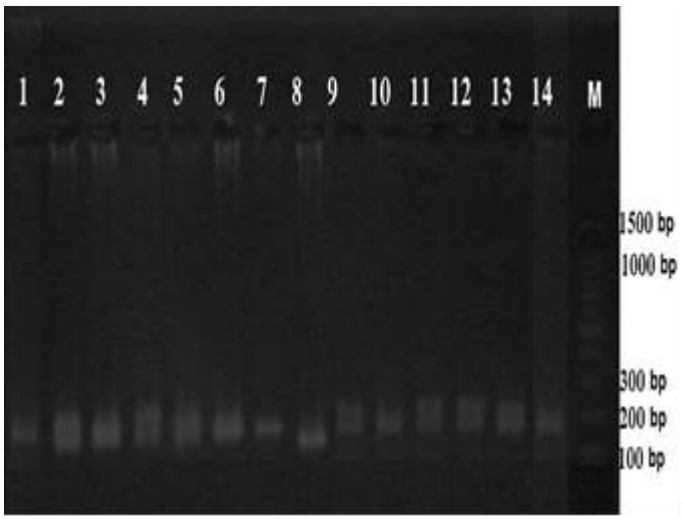

Figure 1. PCR product for BM1329 (M: 100 pb ladder, 1-7 Awassi samples,8-14 Naimi samples).

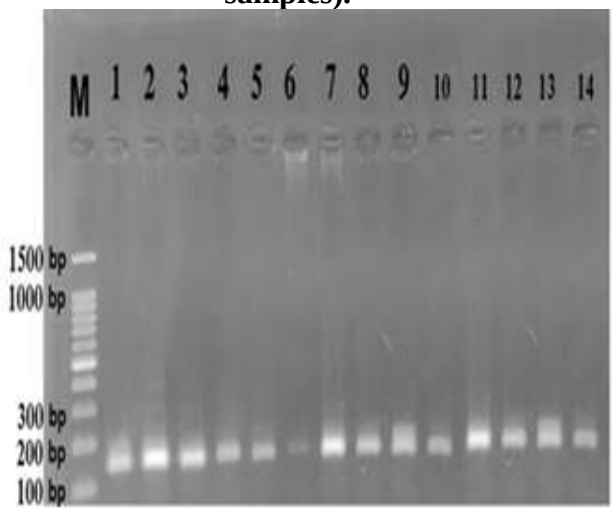

Figure 2. PCR product for BM143 (M: 100 pb ladder, 1-7 Awassi samples,8-14 Naimi samples).

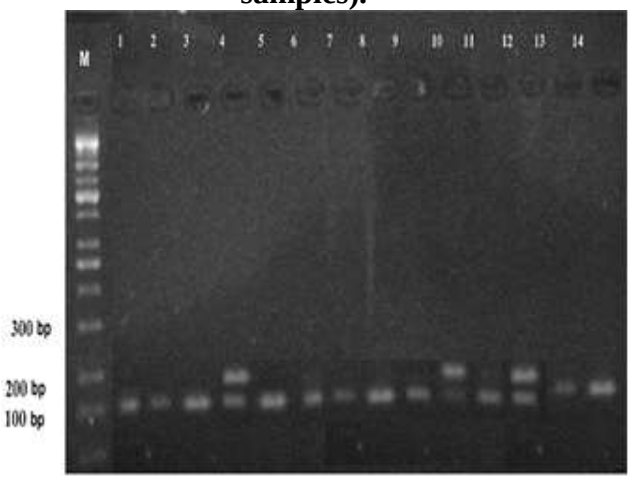

Figure 3. PCR product for CSSM66 (M: 100 pb ladder, 1-7 Awassi samples,8-14 Naimi samples).

Table 3. Microsatellite markers, Chromosome location (Chr.), Size range S.R.(bp), Genotype (number of individuals), Number of alleles (N. A.), Alleles (A.) and Alleles frequency (A. F.).

Locus	Chr.	S. R. (bp)	Genotype	N. A.	A.	A. F.
			Awassi Sheep			
BM1329	6	180-250	180/195(9)	7	180	0.09
			195/195(8)		195	0.25
			200/210(10)		200	0.1
			230/230(7)		210	0.1
			230/245 (11)		230	0.25
			250/250(5)		245	0.11
					250	0.1
BM143	6	108-135	108/108(5)	6	108	0.9
			108/118(9)		118	0.21
			118/118(6)		120	0.11
			120/125(11)		125	0.11
			130/135(12)		130	0.12
			135/135(7)		135	0.26
CSSM66	9	175-220	175/175(5)	6	175	0.10
			180/195(9)		180	0.15
			180/180(3)		190	0.07
			190/195(7)		195	0.20
			195/195(2)		205	0.23
			205/205(6)		220	0.25
			205/220(11)			
			220/220(7)			
			Nuimi Sheep			
BM1329	6	187-230	187/195(10)	6	187	0.1
			195/195(6)		195	0.22
			200/200(7)		200	0.14
			210/220(9)		210	0.09
			220/230(12)		220	0.21
			230/230(6)		230	0.24
BM143	6	108-130	108/118(8)	6	108	0.08
			110/110(6)		110	0.12
			118/118(7)		118	0.22
			120/125(12)		120	0.12
			125/130(11)		125	0.23
			130/130(6)		130	0.23
CSSM66	9	180-220	180/180(3)	5	180	0.24
			180/185(8)		185	0.16
			185/185(4)		195	0.28
			180/195(10)		205	0.10
			195/195(4)		220	0.22
			195/220(10)			
			205/205(5)			
			220/220(6)			

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