Donnish Journal of Agricultural Research. Vol 1(1) pp. 001-004 October, 2014.
http://www.donnishjournals.org/djar

Full Length Research Paper

Measuring the Biodiversity in Iraqi Sheep Breeds Using Microsatellites: Detection of Silent and Private Alleles

***Talib Ahmed Jaayid and Jaafer Mohammed Owaid**

Genetic Engineering Laboratory, College of Agriculture, University of Basrah (UOB), Basrah 320, Iraq.

Accepted 4th October, 2014.

This study was conducted in the genetic engineering lab., college of agriculture, university of Basrah (UoB). Four Iraqi breeds were included in this study, Arabi, Awasi, Hamdani, and Karadi, twenty Blood samples were collected from each breed. DNA was extracted using a kit from Invitrogen company, U.S.A, using Four microsatellite markers (ILSTS005, OarAE129, OARCP34 and CSSM31) to detect the silent and private alleles. A total of 166 and 25 silent and private alleles was obtained for the four molecular markers, respectively. The larger number of silent alleles (51) was observed with CSSM31 molecular marker, while the lower number of silent alleles (28) was recorded with the marker OARCP34 . The number of private alleles with different sizes were 23 alleles for all molecular markers. The larger number of private alleles (8) was with ILSTS005 molecular marker, while the lower number of private alleles (5) was detected with OARCP34 molecular marker, so it's possible to say that we can use OARCP34 molecular marker in biodiversity studies of Iraqi sheep breeds.

Keywords: Microsatellites technique, Iraqi sheep breeds, silent allele, private allele, genetic polymorphism.

INTRODUCTION

Food and agricultural organization have reported that somewhere in the world, at least one breed of traditional livestock is lost every week, as farmers began to focus on new breeds of cattle, pigs, sheep and chickens. 16 % of them have become extinct, as well as 15% have become rare. The estimated number of existing animal breeds 617. From them 474 breeds have been extinct since 1892 and more than 80 cattle breeds found in Africa were replaced by some exotic breeds because farmers believe that their original animals do not meet the needs of the market and lack good genotypes, therefore there is a large potential loss resulting from the breeding programs used to improve the cattle performance (Collins and Qualset, 1999).

Phenotypic or morphological markers are the oldest genetic markers used in animal breeding programs followed by chromosomal markers, biochemical markers and finally molecular markers (molecular genetics) based on genetic materials (Jordana et al., 2003). Molecular genetics techniques benefit in selecting and measuring the degree of genetic similarity within the breed, as well as the genetic linkage between genes (Steffen et al., 1993). The process of comparison among animal species using DNA techniques are very useful to know the genetic diversity within breeds and amongst them (Mehmeti, 2000). Molecular techniques, particularly microsatellites used in the study of genetic diversity among breeds, populations and individuals, are considered to be stable and polymorphic, with easy analysis when applied in studying genetic linkage (Crawford and Littlejohn, 1998; Jouquand et al., 2000; Moioli et al., 2001; Kumar et al., 2006). Alleles produced from microsatellites techniques are often a high variation accompanied by high rates of mutagenesis, thus its highly genetically polymorphic in

Corresponding Author: taleb1968@yahoo.com

most mammalian species (Weber, 1990; Jeffreys et al., 1994).

Molecular genetics applications techniques may open great prospects for investigators from the world to find out genetic material, whatever genetic material and technique (Jaayid and Saker, 2012, Jaayid, 2013, Jaayid et al., 2013). One of them is a microsatellites (Jaayid and Dragh, 2013). It has greatly increased the ability to understand the genetic relationships between species and also between the same species at the molecular level. Microsatellites as DNA markers are advantageous over many other markers as its highly polymorphic, highly abundant, co-dominant inheritance, simpler to analyze and easy to score, but nevertheless this type of marker has disadvantages such as null alleles, or size homoplasy (Schlotterer, 2004).

The animal genetic resources available in the world are in a tragic situation, 16 % of them have been lost over the past hundred years, and currently constitute endangered breeds about one-third of the remaining (FAO, 2000). The threats of animal genetic resources in the developing world have increased in recent years, creating an urgent need to take action to reduce the loss of biodiversity. These rates are higher in developing countries compared to other countries. The sheep are still part of Iraqi heritage. But this heritage has changed, so there was a great desire to preserve this heritage by using molecular genetics. Iraqi sheep back to Asian sheep with fat tailed. Sheep comes in first place in Iraq in terms of numbers reaching to 12093063, its constitute about 63.86% of the total livestock (Al-Fahad and Abbas, 2009). Iraqi Sheep are divided into four main breeds, Awasi, Arabi, Karadi and Hamdani. However, a substantial part of the Iraqi sheep is completely hybreed because of mixing between them, which makes existence many number of hybrid populations having little genetic differences (Hussein et al., 2011).

Our study was to investigate the existence of biodiversity in sheep local breeds especially the presence of private and silent alleles.

MATERIALS AND METHODS

Blood Samples Collection

This study was conducted in the genetic engineering Lab., college of agriculture, university of Basrah (UoB). Blood samples were collected from four Iraqi sheep breeds: Arabi (college of agriculture, UoB), Awassi (college of agriculture, university of Qadisiyah), Hamdani (Nineveh province) and Alkradi (college of agriculture, university of Sulaymaniyah) and by 20 samples of each strain and by 5 ml of each animal from the jugular vein using a medical syringe. DNA was extracted from blood samples using a kit from Invitrogen, USA.

PCR, Electrophoresis and Genotyping Techniques

The primers chosen for these experiments were obtained from Eurofins MWG Operon, AL, USA. PCR analysis was performed by using ILSTS005, OarAE129, OARCP34 and CSSM31 molecular markers. The genomic DNA was amplified using a thermal cycler (Veriti™ Thermal Cycler, Applied Biosystems, USA). Agarose gel electrophoresis technique used to determine the amplifying DNA. Formamide method used to determine alleles loci (Salem, 2005).

Statistical Analysis

Data for each molecular marker and size of alleles recorded in the tables. Statistical analysis was conducted using the Popgene program, (Yeh et al., 1991). Silent alleles calculated through the samples were not able to be amplified, while the private alleles calculated had frequency less than 5% (source).

Results and Discussion

Four molecular markers used in this study dedicated to Iraqi sheep breeds (figures 1). The results revealed the presence of silent and private alleles as well as genetic variation for these molecular markers depending on the microsatellites technique. This does not mean failure in producing alleles, it's related to the animals. Presence of point mutation in amplifying places causing it. With respect to the presence of private alleles, these alleles may be an important resource of biodiversity in the Iraq sheep breed because these alleles didn't spread widely among sheep.

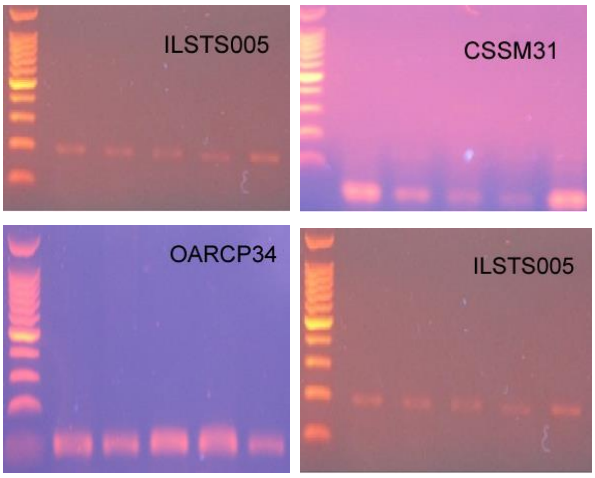

Figure 1: PCR profiles of Iraqi sheep breeds amplified by molecular marker ILSTS005, OarAE129,, OARCP34 and CSSM31. Amplification products were electrophoresed on a 2 % agarose gel with TBE (0.09 M Tris, pH 8.5, 0.09 M boric acid, 2.5 mM EDTA) and detected by staining with ethidium bromide. The gels were illuminated with UV light and taken photographs by Photonyx S140 direct copy system (Nyx Technik company, USA).

Grigaliunaite et al. (2003) reported that when unique allele has a frequency below 0.1, it means that this allele can be present in some of the population at low frequency and can also exist in other breed, in the case of these populations, it constitutes a large number. Hoda and Marsan (2012) reported that there are a large number of private alleles observed in Albanian sheep, their frequencies alleles didn't exceed 10%, while Peter (2005) found two private alleles in Albanian sheep (Bardhoka breed) and one allele in Ruda breed, while

didn't find any private alleles in Shkodrane breed. Therefore, the investigators noted that these alleles don't provide sufficient genetic information, but provide important information about the genetic originate phenomenon.

Table 1: Silent alleles from studied molecular markers and their distributions in Iraqi sheep breed

Molecular marker	Different alleles No. in size	Silent alleles				Total No. of alleles
		Arabi	Awasi	Hamdani	karadi	
ILSTS005	17	6	17	8	8	39
OarAE129	19	10	10	19	9	48
OARCP34	16	8	5	7	8	28
CSSM31	21	13	16	8	14	51
Total No. of alleles	73	37	48	42	39	166

Table (1) shows that the total number of silent alleles was 166 distributed among four molecular markers. Large number of silent alleles appeared in molecular marker CSSM31 (51 alleles), while molecular marker OARCP34 gave the lowest number of silent alleles (28 alleles). Regarding the number of alleles of different sizes, OARCP34 marker produced 16 silent alleles. This further evidence that OARCP34 have little variation compared to the rest of the molecular markers.

Table 2: Private alleles from studied molecular markers and their distributions in Iraqi sheep breed (Alleles frequency was 0.0250)

Molecular marker	Size range (bp)	Different alleles No. in size	Private alleles				Total of alleles No.
			Arabi	Awasi	Hamdani	karadi	
ILSTS005	213-175	7	7	4	2	2	8
OarAE129	175-145	5	5	3	0	2	6
OARCP34	122-100	5	5	0	1	1	5
CSSM31	187-42	6	6	1	5	0	6
Total No. of alleles		23	23	8	8	5	25

Table (2) shows that the number of private alleles of different sizes was 23 distributed among four molecular markers. ILST005 marker gave 8 private alleles, while OARCP34 marker gave the lowest number (5 alleles), therefore this marker had a small number of private alleles. This fact was supported by production, lower silent alleles for the same marker. So this marker can promise high value, quality if used widely in Iraqi sheep breeds, because it proved to be low in production of silent and private alleles compared to the rest of investigated molecular markers.

Thus marked with the fewest number OARCP34 marker. This fact is supported for this marker even with respect to silent alleles, then produced the lowest number of them. This can promise markers of high value, quality if used widely in sheep populations, meaning the use of large concentrations of Iraqi sheep breeds because it proved to be low in the production of silent and private alleles in sheep breeds compared to the rest of molecular markers studied. Our results agreed with Al-Barzinji et al. (2011). He was found several private alleles in Hamdani breed with frequencies ranged between 0.0160 to 0.0080. El Nahas et al. (2008) found private alleles in Egyptian Barki breed with frequencies ranged from 0.026, while they did not find any private alleles in Al osimi sheep breed. Sharma et al. (2006) found private alleles in Indian Hassan sheep, as alleles frequencies varied from 0.0143 to 0.0429.

This is clear evidence that the procedure or technique used in this study has no effect on presence or absence of silent and private alleles. Peter et al. (2005) found 6 silent alleles in several German breeds, they attributed reasons to the presence of empty alleles in DNA, while Mukesh et al. (2004) attributed this phenomenon to increase homozygous genotypes and decrease the heterozygous. But Nei (1978) believed to the location where animals live under selection pressure to produce desirable genotypes. Sharma et al. (2006) stated exist several private alleles with frequencies ranged between 0.00109 to 0.00576 on the Hassan sheep breed. They attributed reasons to use the automatic genetic analysis , while Rosa et al. (2013) have found 14 private lleles with frequencies ranged between 0.0009-0.0456. used 24 molecular markers.

On the contrary, others didn't find any private alleles in sheep breeds such as Arora and Bhatia, 2006). Muigai et al. (2009) did not find any private alleles in his study on Kenyan fat tailed sheep breeds. Many investigators reported that presence of silent allele doesn't mean there is a problem in the method or its accuracy, but it's about genetic side. It means there is a mutation occurred in the genetic material (Hoda and Marsan 2012). El Nahas et al. (2008) found silent

alleles in Egyptian Barki sheep breed while they didn't find any silent alleles in Al-Osimi sheep breed.

REFERENCES

1. Al-Barzinji Y M S, Lababidi S, Rischkowsky B, Al-Rawi A A, Tibbo M, Hassen H and Baum M (2011)**.** Assessing genetic diversity of Hamdani sheep breed in Kurdistan region of Iraq using microsatellite markers. African Journal of Biotechnology.10: 15109-15116.

2. Al-Fahad Y and Abbas T (2011). Statistical atlas agricultural roadmap for agricultural development of the green economy. Ministry of planning, Iraq.

3. Arora R and Bhatia S (2006). Genetic diversity of Magra sheep from India using microsatellite analysis. Asian-Aust. J. Anim. Sci. 19 (7): 938 – 942.

4. Collins W W and Qualset C O (1999)**.** Biodiversity in agro ecosystems. CRC Press. (CRC Press LLC, 2000 Corporate Blvd., N.W. Boca Raton, Florida 33431, USA).

5. Crawford A M, Dodds K G, Ede A J, Pierson C A, Montgomery G W, Garmonsway H G, Beattie A E, Davies K, Maddox J F, Kappes S W, Stone R T, Nguyen T C, Penty J M, Lord E A, Broom J E, Buitkamp J, Schwaiger W, Epplen J T, Matthew P, Matthews M E, Hulme D J, Beh K J, McGraw R A and Beattie C W (1995). An autosomal genetic linkage map of the sheep genome. Genetics. 140: 703-724.

6. El Nahas S M, Hassan A A, AbouMossallam A A, Mahfouz E R, Bibars M A, Oraby H A S and de Hondt H A (2008). Analysis of genetic variation in different sheep breeds using microsatellites. African Journal of Biotechnology. 7 (8):1060-1068.

7. FAO (2000). World watch list for domestic animal diversity. Food and Agriculture Organization, Rome.

8. Grigaliunaite I, Haidja M T, Grislis V Z, Kantanen J and Miceikien I (2003). Microsatellite variation in the Baltic Sheep breeds. Veterinarija Jr Zootechnika. T.21: 1-8.

9. Hoda A and Marsan P A (2012). Genetic characterization of Albanian sheep breeds by microsatellite markers, analysis of genetic variation in animals, Prof. Mahmut Caliskan (Ed.), ISBN: 978-953-51-0093-5.

10. Hoda A, G Hykaj L, Sena and Delia E (2011). Population structure in three Albanian sheep breeds using 36 single nucleotide polymorphisms. Acta Agriculture Scand Section A 61: 12-20.

11. Hussein M J, Zowaid C and Mehdi R (2011). Sheep breeding and improvement of Iraq. Ministry of Agriculture, Iraq.

12. Jaayid T A and Dragh M A (2013). Genetic Biodiversity in Buffalo Population of Iraq Using Microsatellites Marker. Journal of Agricultural Science and Technology. A 4: 297-301. USA.

13. Jaayid T A and Saker D K (2012). Detection of species adulteration in imported meat from farm animals and pork using polymerase chain reaction with species specific repeat (PCR-SSR). Basrah J. Agric. Sci., 25(2): 151-183.

14. Jaayid T A, Yousief M Y, Muhssen E M and Saker D K (2013). Analysis of DNA Polymorphisms in Arabic camel (Camelusdromedarius) using Random Amplified Polymorphic DNA polymerase chain reaction technique. Basrah J. Agric. Sci., 26 (1): 14-22.

15. Jeffreys A J, Tamaki K, Macleod A, Manechton D G, Neil D L and Armour J A (1994). Complex gene conversion events in germline mutation at human minisatellites. Nature Genetics. 6: 136-145.

16. Jordana J, Alexandrino P, Beja-Pereira A, Bessa I J, Canon J, Carretero Y, Dunner S, Laloe D, Moazami-Goudarzi K, Sanchez A and Ferrand N (2003). Genetic structure of eighteen local south European beef cattle breeds by comparative F-statistics analysis J. Anim. Breed. Genet. 120: 73-87.

17. Jouquand S, Priat H C, Lachaume P, Andr C and Galibert F (2000). Identification and characterization of a set of 100 tri- and dinucleotide microsatellites in the canine genome. Anim. Genet. 31: 266-272.

18. Kumar S, Gupta T, Kumar N, Dikshit K, Navani N, Jain P and Nagarajan M (2006). Genetic variation and relationships among eight Indian riverine buffalo breeds. Mol. Ecol. 15: 593-600.

19. Levinson G and Gutman G A (1987). Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Molecular biology and evolution 4: 203.

20. Moioli B, Georgoudis A, Napolitano F, Catillo G, Giubilei E, Ligda C H and Hassnane M (2001). Genetic diversity between Italian, Greck and Egyptian buffalo populations. Livestock Prod. Sci. 70: 203-211.

21. Muigai A W T, Okeyo A M, Kwallah D, Mburu and Hanotte O (2009). Characterization of sheep populations of Kenya using microsatellite markers: Implications for conservation and management of indigenous sheep populations. South African Journal of Animal Science. Vol. 39, No. 1: 93-96.

22. Mukesh M, Sodhi M and Bhatia S (2006). Microsatellite-based diversity analysis and genetic relationships of three Indian sheep breeds. J. Anim. Breed. Genet. 123: 258-264.

23. Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetic. 89: 583-590.

24. Peter C (2005). Molekular genetische Charakterisierung von Schafrassen Europas und des Nahen Ostens.

25. Peter C, Bruford M, Perez T, Dalamitra S, Hewitt G and Erhardt G (2007). Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. Int'l Soc. Anim. Genet. 38: 37-44.

26. Rosa A J M, Sardina M T, Mastrangelo S, Tolone M and Portolano B (2013). Parentage verification of Valle del Belicedairy sheep using multiplex microsatellite panel. Small Ruminant Research. 113: 62- 65.

27. Salem Z G (2005). Genetic variations in native cattle. MSC thesis. Al-Azhar University. Egypt.

28. Schlotterer C (2004). The evolution of molecular markers-just a matter of fashion? Nature Reviews Genetics 5: 63-69.

29. Sharma R, Pandey A K, Kumar D, Jain A, Malik G, Gour D S and Ahlawat S P S (2006). Genetic variation analysis of Hassan sheep population using microsatellite marker. Korean J. Genetics. 28(1):43-51.

30. Steffen P, Eggen A, Dietz A B, Womack J E, Stranzinger G and Fries R (1993). Isolation and mapping of polymorphic microsatellites in cattle. Anim. Genet. 24 (2): 121-124.

31. Weber J L (1990). Human DNA polymorphisms based on length variations in simple sequence tandem repeats. Genome Analysis. 1: 159-181.