Global Veterinaria 11 (5): 634-639, 2013
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2013.11.5.8128

Assessment of Genetic Diversity among Saudi Sheep Breeds for Characterization and Conservation Purposes

[1]Jamal M. Sabir, [2]Nabil S. Awad,
[2]Ehab E. Alhallous and [1]Mohammed H.Z. Mutawakil

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, KSA
[2]Biotechnology and Genetic Engineering Unit, Scientific Research Deanship, Taif University, KSA
[3]Biological and Geological Department, Faculty of Education, Alarish,
Suez Canal University, Egypt

Abstract: Characterization and evaluation of genetic diversity is the first step in the conservation and utilization of indigenous genetic resources. The present study aims to characterize genetic constitution of Naeimi, Harri and Habsi Saudi sheep breeds and estimate the genetic diversity within and between these breeds. Thus, Random amplified polymorphic DNA (RAPD) technique was adopted with ten animals for each breed. The detected polymorphism were ranged from 80% with primer (OP- B6) to 96.55% with (OP- B4) primer. The highest homogeneity was observed within Harri breed followed by Habsi and Naeimi breeds (40% and 24.2%) respectively. This study will also permit to setup a baseline data that would be of influential importance for inevitable for future animal genetic resource management program. Further larger studies are needed to characterize and evaluate the genetic diversity among all Saudi sheep breeds.

Key words: Saudi Sheep · Genetic Diversity · Characterization · RAPD-PCR

INTRODUCTION

Mutton is the meat of choice among Saudi Arabian citizens followed by chicken, camel, fish and beef. Therefore, sheep and goats occupy a special niche in Saudi Arabian agribusiness [1, 2]. The population of sheep is about 5.2 millions head [3]. The government of KSA encourages the investments in the field of agribusiness since 1980s. It is observed that, estimated number of sheep in KSA was increased during period from 2002-2005 but then decreased from 2005 to 2010 at accelerated pace [1, 3]. Accelerated decline of biodiversity worldwide was reported and 20% of the domestic animal breeds are at risk of extinction [4-8]. This decline in biodiversity is critical because the lose of genetic diversity is in-compensable [4].This loss of biodiversity might be due to economic reasons and/or socio-political [9]. In particular for sheep breeds status, it is estimated that 180 sheep breeds (14%) are extinct [6, 7]. Thus, conservation and maintenance of animal genetic biodiversity of local breeds will facilitate the effective management of Farm Animal Genetic Resources. Different reports worldwide confirmed that, indigenous sheep breeds are a valuable source of genetic material due to adaptation to local, sometimes, harsh environmental conditions, nutritional fluctuations and resistances to diseases and parasites [10-13]. Several studies were conducted and encouraged the conservation of indigenous genetic resources including sheep breeds [14-17]. Characterization and evaluation of genetic diversity is the first step in the conservation and utilization of indigenous sheep breeds [7, 18, 19]. Moreover Estimation of genetic diversity is a prerequisite for improving of any species or genetic material. There are many attempts have been conducted to characterize and estimate the genetic diversity among sheep breeds via different morphological, cytological and/or biochemical markers [8, 21-22]. However these markers were not considered suitable for large scale utilization mainly because of their limited number and/or difficult, expensive

Corresponding Author: Nabil S Awad, Biotechnology and Genetic Engineering Unit, Scientific Research Deanship, Taif University, KSA.

and time consuming assay procedures. Moreover, it does not reveal much polymorphism to differentiate the breeds/species to that extent [23-25]. Molecular markers have been utilized in order to characterize sheep breeds and evaluation genetic diversity within and among sheep populations using different PCR techniques based on nuclear and /or mitochondrial genomes [26-28]. Random amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR) based fingerprinting technique that amplifies random DNA fragments with single short primers of arbitrary nucleotide sequence under low annealing stringency [29, 30]. RAPD technique has an extra advantage that it does not require any sequence information on the target genome. The RAPD markers have been described as a simple and easy method to use for estimation of genetic variability among breeds or species [21, 31]. Although, it is widely accepted that detailed molecular data on within- and between breed diversity are essential for effective management of [32-36]. Naeimi, Harri and Habsi sheep breeds are Saudi local sheep breeds and mainly utilized for mutton production. These breeds reflect good adaptive traits to the local environmental conditions in KSA and meet the Saudi consumer needs.

The aim of the present study is to characterize the Saudi sheep breeds (Naeimi, Harri and Habsi) and estimate the genetic diversity within and between these breeds. This study will also permit to setup a baseline data that may be important for future animal genetic resource management program.

MATERIALS AND METHODS

Blood Samples Collection: Three breeds (Naeimi, Harri and Habsi) were recruited in the present study. Ten blood samples of each breed were collected on 5 ml EDTA tubes from jugular vein. Blood samples were stored on -20°C until DNA extraction step.

DNA Extraction and RAPD-PCR Analysis: Genomic DNA was extracted from blood samples according to instructions of Blood DNA Preparation Kit (Jena Bioscince; Germany). PCR reactions were carried out using 2X superhot PCR Master Mix (Bioron; Germany) with 10 Pmol of each 5 different arbitrary 10-mer primers (Operon technologies Cologne, Germany). The names and sequences of the used primers are listed in Table 1. PCR Amplifications were performed in a Eppendorf® thermal cycler using the following PCR program: 1 cycle at 94°C, 4 min; 35 additional cycles consisting of 94°C 5 sec, 37°C 20 sec and 72°C 20 sec.

Analysis of the PCR Products: After the amplification, the PCR reaction products were electrophoresed with 100 bp ladder marker (Fermentas, Germany) on 10 X 14 cm 1.5%-agarose gel (Bioshop; Canada) for 30 min using Tris-borate-EDTA Buffer. The gels were stained with 0.5µg/ml of ethidium bromide (Bioshop; Canada), visualized under the UV light and documented using a GeneSnap 4.00- Gene Genius Bio Imaging System (Syngene; Frederick, Maryland, USA).

Data Analysis: All gels were visualized and documented using a GeneSnap 4.00-Gene Genius Bio Imaging System. PCR amplified DNA fragments were scored across the lanes as variables. Each RAPD-PCR fragment was assumed to represent a single allele. The digital image files were analyzed using Gene Tools software from Syngene. Each band was scored as: present (1); or absent (0) to generate the data matrix. The generated data matrix was used to calculate the similarity matrix based on Jaccard's Coefficient [37]. Hierarchical cluster analysis was performed to produce a dendrogram using the unweighted pair-group method with arithmetical (UPGMA).

Table 1: List of primers code, sequences,% and average of polymorphism and number of produced bands among three studied sheep breeds

		Breeds							
Primer code	Primer sequence	Naeimi	Harri	Habsi	Total cored bands	Amplified bands	Polymorphic markers	Monomorphic markers	Polymorphism%
OPB1	GTTTCGCTCC	75	76	52	203	28	24	4	85.7
OPB3	CATCCCCCTG	56	61	40	157	29	27	2	93.1
OPB4	GGACTGGAGT	78	76	80	234	29	28	1	96.55
OPB5	TGCGCCCTTC	45	70	36	151	31	27	4	87
OPB6	TGCTCTGCCC	72	91	61	224	30	24	6	80
Total	326	374	269	969	147	130	17		
Average									88.4

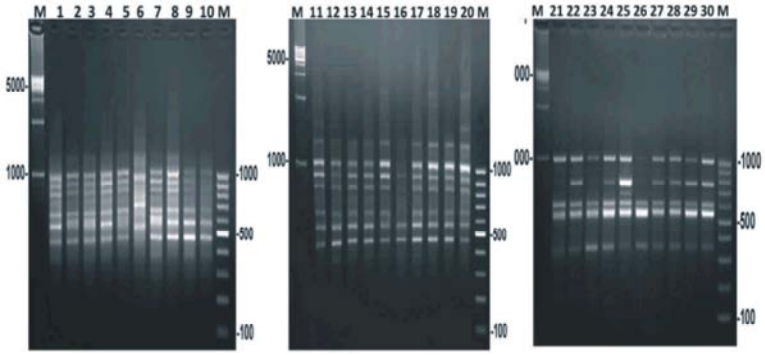

Fig. 1: RAPD fingerprint generated from Habsi (1-10), Harri (11-30) Saudi sheep breeds using OP-B6 primer

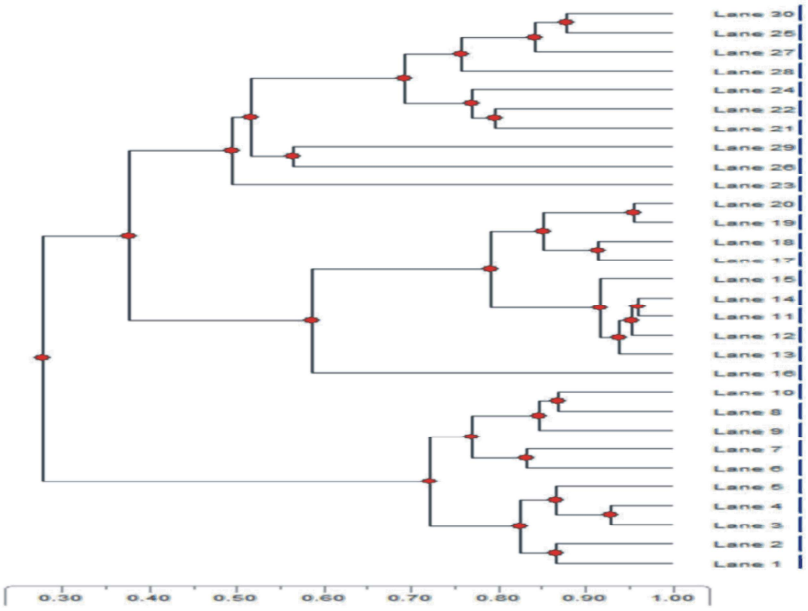

Fig. 2: Phylogenetic relationship and genetic distance within and between the studied Habsi (1-10), Harri(11-20) and Naeimi (21-30) sheep breeds with UPGMA dendrogram based on Jaccard's coefficient

RESULTS AND DISCUSSION

All used RAPD primers showed successful PCR amplification. Five primers produce distinctive reproducible bands in all breeds. Some of the used primers produced high polymorphic patterns where others produced less polymorphic fragments. Total number of produced bands and scored percentages of polymorphism for each primer among studied sheep breeds illustrated in Table 1. Each used primer produced informative electrophoretic profile. An example of the analyzed gels is shown in Figure 1. Where, it shows the range of bands as well as differences and similarity. The used primers produced 969 PCR bands among 147 amplified bands (Table 1). Out of which 130 and 17 bands were polymorphic (88.4%) and monomorphic (11.6) respectively. The five used primers produced multiple band profiles with a number of amplified DNA fragments ranging from 28 to 31. The size and number of amplified fragments also varied from 200bp to 4000bp with different primers. The maximum number (234fragments) was amplified with primer (OP- B4) and the minimum number (151 fragments) was amplified with primer (OP- B5). The detected polymorphism were ranged from 80% with primer (OP- B6) to 96.55% with (OP- B4) primer. Variations in band numbers and profile not only found between breeds but also found within breeds.

However most of the bands were not variable between individuals of the same breed. The RAPD data were used for construction of dendrogram among the studied breeds. The resulted dendrogram in the Figure 2 showed that, there are three main separate clades. Each 10

individuals belongs to the same breed were clustered together. The first clade includes (1-10 Habsi individuals). While the second comprised from (11-20) Harri individuals and the third clade includes (21-30) Naeimi individuals. The genetic similarity between first clade individuals was the highest; it was up to 72%. While it was the lowest among third clade individuals (38%). However the second clade individuals revealed 58% similarity. Habsi and Harri breeds are genetically close, whereas Naiemi is more distant. Although RAPD-PCR system uses random primers under low specificity conditions, it yields different information, since it analyze different sequences and detect different types of variations along the entire genome not only in particular sequences. Thus, this system is helpful in characterization of different type of genetic resources [30, 38].

Detected variation of the number and size of amplified fragments between and within breeds could be a result of nucleotide changes at the primer annealing site or due to addition or deletion between two priming sites [39]. On the other hand these variations indicate different pattern of amplification of used primers. Moreover, it explore the genetic heterogeneity between and within the studied breeds. In addition to revealed ability of used RAPD primers to investigate the genetic polymorphism among studied breeds. Furthermore, the RAPD markers used in the present investigation proved to be quite powerful in distinguishing different individuals belong to different breeds [8, 20, 21, 40]. Each breeds (10 samples clustered together in one separate clade. Which revealed the fact that each sample belongs to the same breed and high similarity between individuals [41, 42]. In other words this results is due to high percentage of homogeneity within each breed and high level of genetic variability among studied breeds. The observed interbreed variability may be due to individual variations, a difference in the population architecture and/or might be due to genetic stratification [8, 43-46].

CONCLUSION

The generated data using RAPD markers revealed genetic variability and relationship among three sheep breeds found in Saudi Arabia. The obtained results introduce line of evidence that RAPD-PCR is useful in the characterization, estimation of genetic diversity and phylogenetic relationship of animal genetic resources conservation programs.

REFERENCES

1. Abouheif, A., G. Abdo, M. Basmaeil and A. Al-Sobayel, 1989. Identification of the preference patterns of different breeds of sheep for consumption in Saudi Arabia. Asian Australian Journal of Animal Science, 2: 129-132.
2. Abouheif, A., N. Al-Owaimer, M. Shafey, A. AlShaikh and S. Aljumaah, 2010. Polymorphism of Booroola FecB gene in prolific individuals from Najdi and Naeimi sheep breeds of Saudi Arabia. Journal of Animal and Veterinary Advances, 10: 1262-1264.
3. Saudi Ministry of Agriculture, 2010. http:// www.moa.gov.sa/ files/stat24/4/2.htm.
4. FAO, 2000a. Domestic Animal Diversity Information System (DAD-IS 2.0): http://dad.fao.org/dad-is/home.htm.
5. FAO, 2000b. In: Scherf, B. (ed), World Watch List for Domestic Animal Diversity, 3rd ed., Rome.
6. Cardellino, A., 2004. Conservation of farm animal genetic resources a global view. Farm Animals Genetic Resources, 30: 1-14.
7. FAO, 2007. The State of the Worlds Animal Genetic Resources for Food and agriculture. FAO, Rome, Italy.
8. Kunene, W., C. Bezuidenhoutb and V. Nsahlaic, 2009. Genetic and phenotypic diversity in Zulu sheep populations: Implications for exploitation and conservation. Small Ruminant Research, 84: 100-107.
9. Taberlet, P., A. Valentini, R. Rezaei, S. Naderi, F. Pompanon, R. Negrini and A. Marsani, 2008. Are cattle, sheep and goats endangered species. Mol. Ecol., 17: 275-284.
10. Nsoso, J., B. Podisi, E. Otsogile, S. Mokhutshwane and B.Ahmadu, 2004. Phenotypic characterization of indigenous Tswana goats and sheep breeds in Botswana: continuous traits. Tropical Animal Health and Production, 36: 789- 800.
11. Kunene, W. and A. Fossey, 2006. A survey on livestock production in some traditional areas of Northern KwaZulu-Natal in South Africa. Livestock Research for Rural Development, 18: 108- 113.
12. Kosgey, S. and O. Mwai, 2007. Genetic improvement of small ruminants in low input, smallholder production systems: technical and infrastructural issues. Small Ruminant Research, 70: 76-88.
13. Galal, S., O. Gürsoy and I. Shaat, 2008. Awassi sheep as a genetic resource and efforts for their genetic improvement review. Small Ruminant Research, 79: 99-108.

14. Dovie, K., C. Shackleton and E. Witkowski, 2006. Valuation of communal area livestock benefits, rural livelihoods and related policy issues. Land Use Policy, 23: 260-271.
15. Du Toit, J., 2008. The indigenous livestock of Southern Africa. http:// www.damarasheep.co.za/ files/ ParisRoundtable.pdf.
16. Gizaw, S., H. Komen and M. Van Arendonk, 2008. Selection on linear size traits to improve live weight in Menz sheep under nucleus and village breeding programmes. Livest. Sci., 118: 92-98.
17. Mariante, A., S. Alburquerque, A. Egito, C. McManus, A. Lopes and S. Paiva, 2009. Present status of conservation of livestock genetic resources in Brazil. Livest. Sci., 120: 204- 212.
18. Bjornstad, G. and H. Roed, 2001. Breed demarcation and potential for breed allocation of horses assessed by microsatellite markers. Animal Genetics, 32: 59-65.
19. Notter, R., 1999. The importance of genetic diversity in livestock populations of the future. Anim. Sci., 77: 61-69.
20. Ali, B.A., 2003. Genetics similarity among four breeds of sheep in Egypt detected by random amplified polymorphic DNA Markers. Afr. J. Biotechnol., 2: 194-197.
21. Kumar, S., A. Kolte, R. Yadav, S. Kumar, L. Arora and K. Sigh, 2008. Genetic variability among sheep breeds by random amplified polymorphic DNA-PCR. Indian Journal of Biotechnology, 7: 482-486.
22. Abouheif, A., N. Al-Owaimer, M. Shafey, A. AlShaikh and S. Aljumaah, 2011. Polymorphism of Booroola FecB gene in prolific individuals from Najdi and Naeimi Sheep Breeds of Saudi Arabia. Journal of Animal and Veterinary Advances, 10: 1262-1264.
23. Arranz, J., Y. Bayon and F. San Primitivo, 1996. Comparison of protein markers and microsatellites in differentiation of cattle populations. Anim. Genet., 27: 415-419.
24. Barker, S., S. Moore, D.J. Hetzel, D. Evans, G. Tan and K. Byrne, 1997. Genetic diversity of Asian water buffalo (Bubalus bubalis): microsatellite variation and a comparison with protein-coding loci. Animal Genetics, 28: 103-115.
25. Kantanen, J., I. Olsaker and L. Holm, 2000. Genetic diversity and population structure of 20 North European cattle breeds. Journal of Heredity, 91: 446-457.
26. Hiendleder, S., K. Mainz, Y. Plante and H. Lewaiski, 1998. Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources: No evidence for contribution from Urial and Argali. J. Hered., 89: 113-120.
27. Peter, C., M. Bruford, T. Perez, S. Dalamitra, G. Hewitt and G. Erhardt, 2007. Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. Anim Genet., 38: 37-44.
28. Ong, T., B. Song, H. Qian, L. Wu and W. Whong, 1998. Detection of genomic instability in lung cancer tissues by random amplified polymorphic DNA analysis. Carcino, 19: 233-235.
29. Williams, G., R. Kubelik, J. Livak, A. Rafalski and V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.
30. Awad, N., M. Sally, A. Margueriet, H. Morad and Z. Ayman, 2010. Fingerprinting and assessment of genetic variability of Varroa destructor in Egypt. Journal of Apicultural. Research, 49: 251-256.
31. Ruane, J., 2000. A framework for prioritizing domestic animal breeds for conservation purposes at the national level: a Norwegian case study. Conservation Biology, 14: 1385-1393.
32. Bruford, W., G. Bradley and G. Luikart, 2003. DNA markers reveal the complexity of livestock domestication. Nature Reviews Genetics, 4: 900-910.
33. Simianer, H., 2005. Decision making in livestock conservation. Ecological Economics, 53: 559-572.
34. Toro, M. and A. Caballero, 2005. Characterization and conservation of genetic diversity in subdivided populations. Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences, 360: 1367-1378.
35. Toro, M., J. Fernández and A. Caballero, 2009. Molecular characterization of breeds and its use in conservation. Livestock Science, 120: 174-195.
36. Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Res., 18: 7213-7218.
37. Jaccard, P., 1908. Bull. Soc. Vaudoise Sci. Nat., 44: 223-270.
38. Tariq, M., B. Masroor, K. Jawasreh, A. Awan, F. Abbas, A. Waheed, M. Rafeeq, A. Wadood, K. ullah Khan, N. Rashid, A. Atique and A. Bukhari, 2012. Characterization of four indigenous sheep breeds of Balochistan, Pakistan by random amplified polymorphic DNAs. Afr. J. Biotechnol., 11: 2581-2586.

39. Sharma, D., K. Appa Roa, R. Singh and S. Totey, 2001. Genetic diversity among chicken breeds estimated through random amplified polymorphic DNA. Animal Biotechnol., 12: 111-120.
40. Elmaci, C., Y. Oner, S. Ozis and E. Tuncel, 2007. RAPD analysis of DNA polymorphism in Turkish sheep breeds. Biochem. Genet., 45: 691-696.
41. Khaldi, B., B. Rekik, L. Haddad and S. Zourgui, 2010. Genetic characterization of three ovine breeds in Tunisia using randomly amplified polymorphic DNA markers. Livest. Res. Rural Dev., 22: 3.
42. Halder, I and M. Shriver, 2003. Measuring and using admixture to study the genetics complex diseases. Hum Genet, 1: 52-62.
43. Ziv, E. and G. Burchard, 2003. Human population structure and genetic association studies. Pharmacogenomics, 4: 431-441.
44. Hoggart, J., D. Shriver, A. Kittles, G. Clayton, M. McKeigue, 2004. Design and analysis of admixture mapping studies. Am. J. Hum. Genet., 74: 965-978.
45. Marchini, J., R. Cardon, S. Phillips and P. Donnelly, 2004. The effects of human population structure on large genetic association studies. Nat Genet., 36: 512-517.
46. Jawasreh, Z., M. Al-Rawashdeh, A. Al-Majali, H. Talafha, A. Eljarah and F. wawdeh, 2011. Genetic relatedness among Jordanian local Awassi lines Baladi, Sagri and Blackface and the black Najdi breed using RAPD analysis. Genomics and Quant. Genet., 2: 31-36.