African Journal of Biotechnology Vol. 11(10), pp. 2581-2586, 2 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3196
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Characterization of four indigenous sheep breeds ofBalochistan, Pakistan by random amplified polymorphic DNAs

Mohammad Masood Tariq[1]*, Masroor Ahmad Bajwa[1], Khaleel Jawasreh[2], Mohammad Arif Awan[1], Ferhat Abbas[1], Abdul Waheed[3], Majed Rafeeq[1], Abdul Wadood[1], Khalid ullah Khan[4], Nadeem Rashid[1], Mohammad Adnan Atique[1] and Farhat Abbas Bukhari[1]

[1]Center for Advanced studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan.
[2]Department of Animal Production, Faculty of Agriculture, Jordan University of Science and Technology (JUST), Jordan.
[3]Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan.
[4]Ministry of Education, Diversity and Equity Branch, Victoria BC V8W 9H3, Canada.

Accepted 16 December, 2011

Genetic diversity among four indigenous breeds of sheep namely Mengali, Balochi, Beverigh and Harnai of Balochistan was detected by random amplified polymorphic DNA (RAPD) technique. Nineteen RAPD primers were initially applied and seventeen were chosen for further analysis, based on band pattern quality, reproducibility and presence of bands. An average of 92 RAPD fragments were obtained by using 17 primers and out of the 36 fragments (39.13%) illustrated monomorphism while, 56 bands (60.87%) were polymorphic in all the four sheep breeds. The number of bands amplified in all the sheep breeds ranged from 2 to 10. The highest number of polymorphic loci 40 was observed in the Mengali breed, while the lowest 28 was in Balochi. Further, 33 and 31 polymorphic loci were seen in Beverigh and Harnai breeds, respectively. The overall gene diversity was highest in the Mengali (0.1474) while the lowest in Balochi breed (0.0998). Results of genetic similarities showed closer proximity between Balochi and Beverigh (0.992), Balochi and Harnai (0.992), and between Beverigh and Harnai (0.996). The resemblance was observed between Mengali and Balochi (0.918), between Mengali and Beverigh breeds (0.931) and between Mengali and Harnai breeds (0.925). The high level of genetic similarity between Balochi, Beverigh and Harnai sheep indicated the close relationship that might be due to common habitat. Further the present study highlighted the presence of diversity among and within breeds that can be used in the selection or crossbreeding programs of sheep. The present study suggests that RAPD-PCR can effectively be used to determine the genetic distances among the sheep breeds.

Key words: Genetic distance, polymorphism, random amplified Polymorphic DNA.

INTRODUCTION

Pakistan has vast animal genetic resources as it is reflected by the availability of 31 breeds of (thin and fat tail) sheep. Indigenous and locally developed sheep breeds are important assets for many reasons because, over the years, they have developed unique combinations of adaptive traits to best respond to the pressure of the local environments (Isani and Baloch, 1996). Reports suggested that local breeds of a region exist with different names with same phenotypic characteristics. There is a need to genetically re-evaluate these breeds to assess the existing population structure and differences among the important sheep breeds which would serve as an initial guide to facilitate the conservation programs in an effective and meaningful way (Williams et al., 1993). Markers that are an important tool in the evaluation of genetic variation can provide valuable information at different levels such as structure of animal population,

*Corresponding author. E-mail: tariqkianiraja@yahoo.com. Tel. 00922812855679. Fax: 009229213134.

levels of gene flow, phylogenetic relationships, patterns of historical biogeography, and parentage (Feral, 2002).

Application of the random amplified polymorphic DNA (RAPD) technique has greatly increased the ability to understand the genetic relationships within species at molecular level. Knowledge on genetic relationships in livestock within species is very essential for genetic improvement and breeding programs (Appa Rao et al., 1996). Molecular genetic techniques are also used to detect the DNA variation among different breeds and species of the animals. The RAPD-PCR technique was effectively used to evaluate genetic distances between sheep breeds (Cushwa et al., 1996).

The traditional characterization by means of phenotypic characteristics can now be complemented by molecular markers and the development of sophisticated statistical techniques for data analysis. Microsatellite based studies on genetic characterization of animal breeds have been conducted in developed countries more frequently (Diez-Tascon et al., 2000). Unfortunately, only few of such studies have been conducted in Pakistan because of limited resource available (Rehman, 2002). The RAPD primers have been successfully used for genotyping, construction of genetic maps, exploring genetic relationships within and between plant species (Heun et al., 1991).

The four breeds of sheep namely Mengali, Balochi, Beverigh and Harnai included in the present investigation have significant importance to the farmer community in Balochistan, Pakistan. These breeds are fat tailed, with peculiar phenotypic and genotypic characteristics. Mengali and Balochi sheep breeds have dwell in common habitat, that is, Queeta, Kalat and Sibi divisions, whereas Beverigh and Harnai breeds are well populated in Zhob and some parts of Sibi division. In the present study, genetic diversity among four breeds of sheep was determined by RAPD-PCR analysis. It was an attempt to differentiate rarely recognizable Mengali breed of sheep through RAPD-PCR and to highlight the current genetic diversity including other three important breeds of sheep in Balochistan.

MATERIALS AND METHODS

The research work was accomplished at Hi-Tech Laboratory in the Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan. A total of 48 (n = 12 from each breed) blood samples were aseptically collected from the jugular vein with the help of disposable syringes (5 ml from each animal) from four sheep breeds viz. Mengali, Balochi, Beverigh and Harnai from Quetta, Mastung, Nushki and Loralai districts. Blood Samples were collected according to their geographical distributions keeping in view the breed characterization and where possible, pedigrees were consulted. Samples were presserved in properly labeled falcon tubes containing anticoagulant (5% EDTA) and were then stored at -20°C. The genomic DNA was extracted from the blood samples using DNA purification kit (PUREGENE-Gentra System, USA). The purified genomic DNA samples were stored in cryogenic vials (1.5 ml) at -20°C. The optical density (OD) of the total genomic DNA was measured at 260 nm wavelength using spectrophotometer (Pharmaspec Schimedzu, Japan). The quality of DNA was further checked by running 10 ng DNA on 1.5% agarose gel in 1x TAE (Tris-Acetate-EDTA) buffer. All DNA samples of good quality were used for the RAPD analysis. Due to economic constraints, only genomic DNA of the animals (n=12) was selected from each breed and used for the further process.

The RAPD-PCR amplification was performed by following the method as described by Williams et al. (1990) using thermal cycler (Model 2720, Applied Bio-system) by using 25 µl PCR master mixture containing PCR graded water (H2O) (Sigma) 16.25 µl; PCR Buffer (10X) (Vivantus USA) 2.5 µl; $MgCl_2$ (50mM) (Vivantus USA) 1 µl; dNTPs (10 mM) (Vivantus USA) 1 µl; each primer (Gene link USA) 10 picomoles 2 µl; Taq Polymerase (Vivantus USA) 0.25 µl and DNA (Template) 2 µl. A total of 19 primers were used (Table 1) for this analysis following the technique described by Ali (2003). The thermal cycler was programmed for 2 min of initial denaturation at 94°C, followed by 45 cycles at 94°C for 30 s each, for DNA denaturation. Annealing was done with each primer (Table 1) following extension at 72°C for 30 s and final extension at 72°C for 10 min was carried out. The samples were cooled at 4°C. The amplified DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. The amplified pattern was finally viewed using UV trans-illuminator (Dolphin view-Wealtec, USA). PCR products were scored across the lane as variables. The presence of an amplified DNA band was scored as '1' and the absence as '0'. The data of primers were used to estimate the standard genetic diversity (similarity and distance) on the basis of number of shared amplified products and calculation of matrix (Nei and Li, 1979). The calculation was made using the formulae 1: F = Nxy/Nx +Ny; (Where F is the similarity coefficient; Nxy is the number of bands shared between individuals x and y and Nx and Ny are the number of bands in individuals x and y respectively) and 2: D = 1–F; where D is the genetic distance.

In order to obtain the genetic variation between and within breeds, two genetic analyses were conducted; the first analysis of the data was conducted using (n = 12) sheep from all the investigated breeds and was aimed at estimating variation between individuals. A similarity matrix was estimated using Jaccards' coefficient and the dendrogram obtained by clustering according to the Un-weighted Pair-Group Method with Arithmetic Averages (UPGMA) using SPSS software, version 11.0. In order to detect the diversity within and between the four lines or breeds, an alternative analysis was conducted using PopGene Version 1.31 (Yeh et al., 2000). The UPGMA dendrogram for breeds was constructed according to the method described by Nei (1972).

RESULTS AND DISCUSSION

In the present study, RAPD-PCR method was used, the genetic variation among four sheep breeds was analyzed using nineteen primers. Two primers could not be amplified hence not used. An average of 92 RAPD fragments was obtained by using 17 primers. The number of fragments amplified in four breeds ranged from 2 to 10 fragments per primer. The number of DNA fragments amplified with each primer found in the present study is in agreement with several investigators (Chen et al., 2001; Rehman, 2002; Appa Rao et al., 1996; Ali, 2003; Kumar et al., 2003; Saifi et al., 2004; Mahfouz, 2008). Kumar et al. (2003) used six oligonucleotide primers for RAPD fingerprinting in order to estimate genetic relationship among four different breeds of sheep. All the six primers

Table 1. List of the primers (Gene link USA) used their nucleotide sequences and annealing temperatures.

Primers S/N	Sequence (5′-3′)	Annealing temperature ºC/time (s)
1	ATG ACG TTGA	
2	GGG CTA GGG T	
3	ACC GGG AAC G	45/30
4	AGC AGG TGG A	
5	AGG CCC CTG T	
6	ATG CCC CTG T	
7	AAA GCT GCG G	28/30
8	ACC GCC GAA G	
9	GGC ACT GAG G	
10	CGC TGT CGC C	
11	AGT CCT CGC C	
12	TGG TGG ACC A	45/30
13	GAA TGC GAC G	
14	CTG AGG AGT G	
15	CGA GCC CTT CCA GCA CCC AC	
16	GAA ACG GGT GGT GAT CGC AG	54/30
17	GGT GAC GCA GGG GTA ACG CC	
18	GGA CTG GAG TGT GAT CGC AG	58/30
19	GGA CTG GAG TGG TGA CGC AG	

Table 2. Quantitative characteristics and genetic diversity using (n=19) RAPD markers.

Sheep breed	The number of polymorphic loci	The percentage of polymorphic loci (%)	H = gene diversity	I = Shannon's index
Pooled(four breed)	56	60.87	0.1467	0.2331
Mengali	40	43.48	0.1474	0.2217
Balochi	28	30.43	0.0998	0.1498
Beverigh	33	35.87	0.1227	0.1832
Harnai	31	33.7	0.1104	0.1662

showed polymorphism in producing bands. Mahfouz et al. (2008) also reported fifty-seven amplified bands using five random primers with a mean of 11.4 bands per primer.

Contrary to the present study, low number of fragments was amplified as reported by Elmaci et al. (2007) and Khalidi et al. (2010). William et al. (1990) and Meunier (1993) reported that polymorphic loci are useful for study the genetic variation between breeds. Generally, the size and number of the fragments produced is dependent upon the nucleotide sequence of the primer used and also the source of the template DNA. Polymorphism revealed by RAPD could be a result of nucleotide changes at the primer annealing site or due to addition or deletion between two priming sites.

Out of 92 fragments, 36 fragments showed monomorphism among four sheep breeds (39.13%). While, the rest of 56 bands (60.87%) were polymorphic. The highest polymorphic loci were observed in Mengali was 40, followed by Beverigh 33 and Harnai 31, respectively. Further, the lowest bands 28 were observed in Balochi breed (Table 2).

The findings of the present study are not in agreement with other researchers such as Elmaci et al. (2007) who revealed that percentage of polymorphic loci were 80.49, 78.05, and 73.17%, for Kivircik, Gökçeada and Sakiz sheep breeds, respectively. Mahfouz et al. (2010) analyzed Egyptian sheep breeds, and reported that fifty six out of 57 total amplified fragments (98.25%) were polymorphic loci and the rest were monomorphic. Pandey et al. (2010) also showed the genetic diversity of Shahabadi sheep population in Bihar, India through RAPD-PCR technique using eighteen microsatellite markers. The results of the

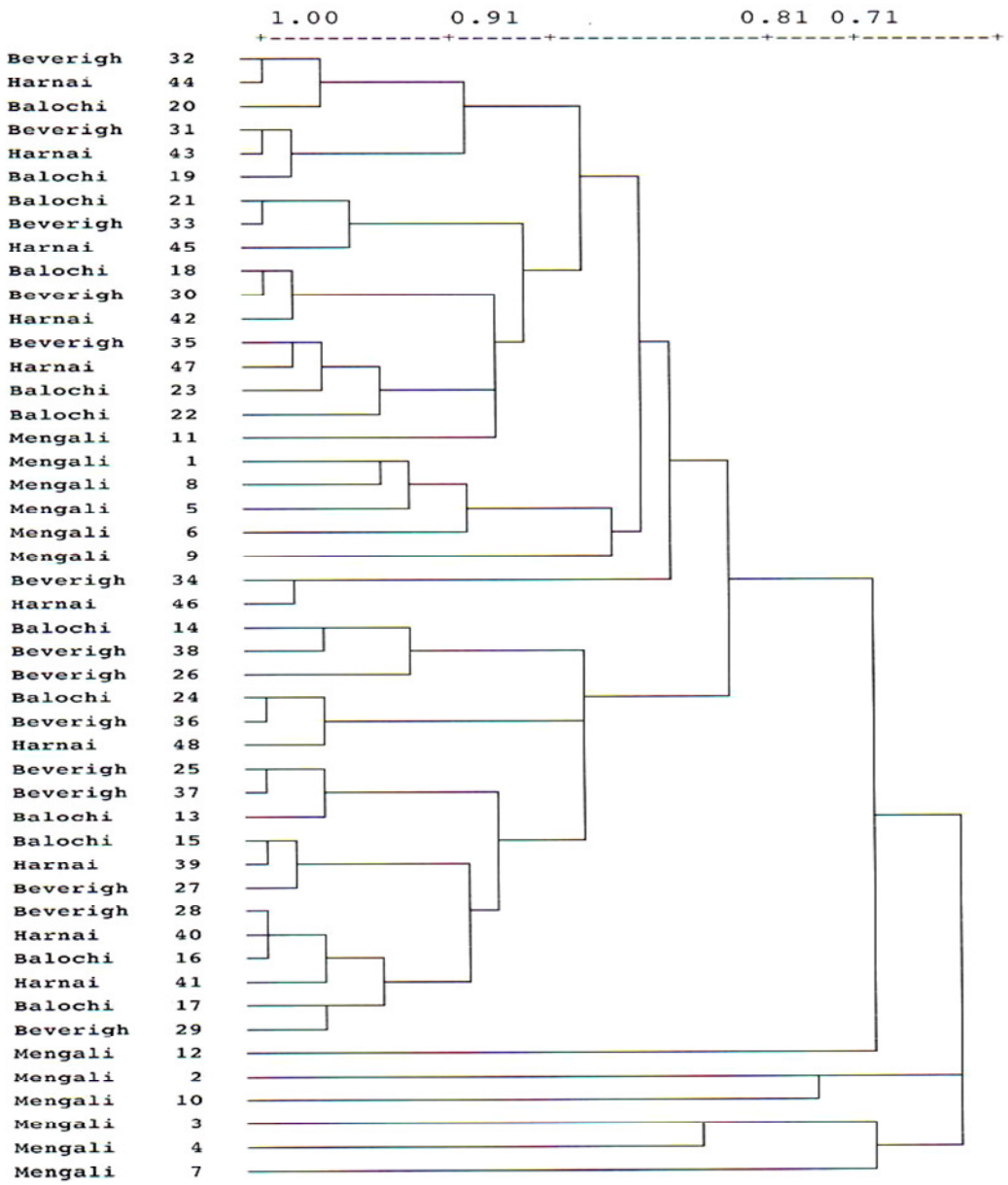

Figure 1. Dendrogram for individuals from four sheep breeds using seventeen polymorphic RAPD primers and based on Jaccard coefficient of similarity.

study illustrated that microsatellites were highly polymorphic with a mean allelic number of 5.56 ± 1.79. Ali (2003) carried out a study on genetic analysis using RAPD markers in order to investigate variation in four breeds of sheep (Baladi, Barki, Rahmani and Suffolk). Nineteen random primers were used to amplify DNA fragments in these breeds. The RAPD-PCR patterns with a significant level of polymorphism were detected between breeds. Khalidi et al. (2010) analyzed three ovine breeds in Tunisia, in total, 59 loci were amplified, out of which 47 (79.66%) were found polymorphic.

In the present study, the breeds have shown variation (Table 2). The distinction in bands profile was observed within and among breeds; however, some of the bands were not variable between individuals of same breeds. It was noted that the majority of RAPD-PCR primers gave distinctly reproducible bands in four breeds. Some of the primers produced highly polymorphic pattern but other produced less polymorphic product. These variations in bands profile of the present study is supported by the research work carried out by Appa Rao et al. (1996), Ali (2003) and Kumar et al. (2008).

The results of dendrogram between the individuals are presented in Figure 1. The dendrogram can be divided into three main clusters. It is observed interestingly that the Mengali individuals clustered together and indicated

Table 3: Nei original measures of genetic similarities and genetic distance between the studied sheep population

Breed	Mengali	Balochi	Beverigh	Harnai
Mengali		0.918	0.931	0.925
Balochi	0.085		0.992	0.992
Beverigh	0.071	0.007		0.996
Harnai	0.077	0.007	0.003	

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

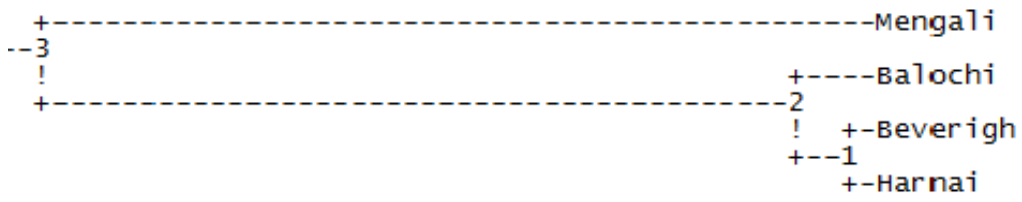

Figure 2. Dendrogram among four sheep population based on Nei' method using seventeen polymorphic RAPD primers.

the high similarity between the individuals. However, the other three breeds scattered between the three clusters that also refer to high similarity between them and the moderate distance between them and the Mengali breed.

The genetic similarity matrix of RAPD data for the four breeds (Mengali, Balochi, Beverigh and Harnai) was constructed according to Nei and Li (1979) (Table 3). The overall genetic similarity in sheep breeds was ranged from 0.918 to 0.996. The highest genetic similarity was found between Harnai and Beverigh breeds (0.996) while the lowest was resolute between Balochi and Mengali breeds (0.918).

The average genetic diversity or heterozygosity (0.1467) indicated that the broader genetic diversity between and within populations was highest within Mengali and Beverigh (0.1474 and 0.1227, respectively) and the lowest value 0.0098 was recorded within Balochi individuals. The Shannon's index was also computed to provide relative estimation of variability; its values ranged between 0.1449 and 0.2217. The values of Shannon's index were consistent with the values of heterozygosity (Table 2).

The coefficient of genetic differentiation (GST) varied among the 92 loci typed was indicated that 82% of total genetic variation was within populations and 18% was among populations. Elmaci et al. (2007) found a total diversity of 0.226 between Turkish breeds and recently Jawasreh et al. (2011) reported the GST to be 0.096 between Jordanian Awassi breed lines and Najdi breed.

The phylogeny tree constructed between the four breeds (Figure 1) indicated the high genetic distance between the Mengali and the three other breeds (Balochi, Beverigh and Harnai). It can be observed from the Beverigh and Harnai clustered in separate branch that take aim the high genetic closeness between them as compared between Balochi and Mengali breeds. The results of the phylogeny trees and dendrogram (Figures 1 and 2) confirmed the results obtained through the similarity matrix (Table 3).

The results of genetic diversity of the present study are also in agreement with the findings of many researchers who have studied different breeds of sheep (Ali, 2003; Stephen et al., 2001; Hoda et al., 2009; Padday et al., 2010). Hoda et al. (2009) studied the genetic structure and variability of four local sheep breeds namely Bardhoka, Ruda, Recka and Shkodrane using six microsatellites loci. They reported that the gene distance for each breed ranged from 0.75 in Bardhoka to 0.78 in Recka sheep breeds. Pandey et al. (2010) have reported hetrozygosity in the population ranged (0.279-0.739) with the mean (0.501±0.151), reflecting substantial genetic variation in the population. These results are partially in agreement with the findings of the present study

The high level of genetic similarity between Beverigh, Harnai and Balochi sheep indicated the close relationship due to common habitat as compared to other breeds (Mengali) which reside further parts of Balochistan province. It is further recommended that due to variation in fragments, profile in these sheep breeds therefore are diverse to each other.

Authenticity of pedigree, appropriate selection of molecular technique, availability of prior knowledge about the genome of respective species, stipulation of good working conditions are deficient in Balochistan province of Pakistan. The data presented here will provide a resource for scientists interested in germ plasm conservation and will add reports of genetic variation among sheep breeds of Pakistan.

Conclusion

The present study reflected that the molecular genetic

techniques such as RAPD-PCR can be efficiently used not only to establish genetic diversity among and within breeds but also to find out breed specific genetic markers. Knowledge of genetic distance among animals and breeds, and genetic diversity/structure within breeds could be useful for conservation of genetic resources. The genetic diversity that is herein reported among four sheep breeds should be expanded by exploring more advanced analysis of genetic markers such as simple sequence repeats (SSR) and single nucleotide polymorphism (SNP). The present work provides useful information for future sheep breeding studies in Balochistan.

REFERENCES

Ali BA (2003). Genetics similarity among four breeds of sheep in Egypt detected by random amplified polymorphic DNA Markers. Afr. J. Biotechnol. 2(7): 194-197.

Appa Rao KBC, Bhat KV, Totey SM (1996). Detection of species specific genetic markers in farm animals through random amplifiedpolymorphic DNA (RAPD). Genetic analysis: Bimolecular Engr. 13: 135-138.

Chen S, Li, M, Li Y, Zhao S, Yu C, Fan B, Li K (2001). RAPD variation and genetic distance among Tibetan, Inner Mangolia and Liaoning Cashmere goat. Asian-Aust. J. Anim. Sci. 14: 1520-1522.

Cushwa WT, Dodds KG, Crawford AM, Medrano JF (1996). Identification and genetic mapping of random amplified polymorphic DNA (RAPD) markers to the sheep genome. Mammalian Genome, 7: 580-585.

Diez-Tascon C, Littlejohn RP, Almeida PAR, Crawford AM (2000). Genetic variation within the Merino sheep breed: Analysis of closely related populations using microsatellites. Anim. Genet. 31: 243-251.

Elmaci C, Oner Y, Ozis S, Tuncel E (2007). RAPD analysis of DNA polymorphism in Turkish sheep breeds. Biochem. Genet. 45: 691-696.

Feral JP (2002). How useful are the genetic markers in attempts to understand and manage marine biodiversity. J. Exp. Mar. Biol. Ecol. 268: 121-145.

Heun ME, Kennedy JA, Anderson NLV, Lapitan ME, Sorrels M, Kennedy AE (1991). Construction of a restriction fragment length polymorphism map for barely (*Hordeum vulgare*). Genome, 34: 437-447.

Hoda A, Dobi P, Hyka G (2009). Genetic diversity and distance of Albanian local sheep breeds using microsatellite markers. Livest. Res. Rural Dev. 21(6): 93.

Isani GB, Baloch MN (1996). Sheep and goat breed in Pakistan. Press Corporation, Karachi, Pakistan.

Jawasreh KIZ, Al-Rawashdeh IM, Al-Majali A, Talafha H, Eljarah A, Awawdeh F (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.

Khaldi ZB, Rekik B, Haddad L, Zourgui SS (2010).Genetic characterization of three ovine breeds in Tunisia using randomly amplified polymorphic DNA markers. Livest. Res. Rural Dev. 22: 3.

Kumar KG, Kumar P, Bhattacharya TK, Bhushan B, Patel K, Choudhary V, Sharma A (2003). Genetic relationship among four breed of sheep using RAPD-PCR. J. App. Anim. Res. 24: 177-183.

Kumar S, Kolte AP, Yadav BR, Kumar S, Arora AL, Singh VK (2008). Genetic variability among sheep breeds by random amplified polymorphic DNA-PCR. Indian J. Biotechnol. 7: 482-486.

Mahfouz ER, Othman EO, Soheir MEN, Mohamed AAEB (2008). Genetic variation between some Egyptian sheep breeds using RAPD-PCR. Research J. Cell Mol. Biol. 2: 46-52.

Meunier JR, Grimont PA (1993). Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. Res. Microbiol. 144: 373-379.

Nei M (1972). Genetic distance between populations. Am. Nat. 106: 283-292.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms ofrestriction endonucleases. Proceedings of the National Academy of Sciences (United States of America) 76: 5269-5273.

Pandey AK, Sharma R, Singh Y, Mishra BP, Mondal KG, Singh PK, Singh G, Joshi BK (2010). Variation of 18 STR loci in Shahabdi sheep of India. Russian J. Genet. 46: 86-92.

Rehman M (2002) DNA marker studies in cotton. PhD. Thesis. University of Agriculture, Faisalabad Pakistan.

Saifi HW, Bhushan B, Kumar S, Kumar P, Patra BN, Sharma A (2004). Genetic identity between Bhadawari and Murrah breeds of Indian buffaloes (Bubalus bubalis) using RAPD-PCR. Asian-Aus. J. Anim. Sci. 17: 603-607.

Stephen J, Kifaro GC, Gwakisa PS, Wollny CBA (2001). Comparison of RAPD and microsatellite DNA markers in genetic differentiation of Tanzanian local sheep herds. In: Kifaro, G.C. (edition), Farm animal genetic resources in Tanzania. Proceedings of SUA-MU Enreca project workshop, Morogoro, Tanzania, 6 August, pp. 68-80.

Williams JG, Reiter RS, Young RM, Scolnik PA (1993). Genetic mapping of mutations using phenotypic pools and mapped RAPD markers. Nucleic Acids Res. 21: 2697-2702.

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.

Yeh FC, Boyle T, Yang R (1999). POPGENE version 1.31. A Microsoft window based freeware for population genetic analysis. University of Alberta, Edmonton, Canada.