academicJournals

Vol. 16(4), pp. 171-178, 25 January 2017 DOI: 10.5897/AJB2016.15785 Article Number: 292290362539 ISSN 1684-5315 Copyright © 2017 Author(s) retain the copyright of this article http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Genetic diversity among Sawakni, Berberi and Najdi sheep breeds in Saudi Arabia using microsatellites markers

Ahmed Hossam Mahmoud¹*, Ayman swelum², Mohammad Abul Farah¹, Khalid. Alanazi¹, Ahmed Rady¹, Mahmoud Salah³, Nabil Amor⁴ and Osam Mohammed⁴

¹Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia.
²Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia.
³Biology Department, College of Science, Jazan University, Jazan, Saudi Arabia.
⁴KSU Mammals Research Chair, Department of Zoology, College of Science, King Saud University, Riyadh Saudi Arabia.

Received 16 November, 2016; Accepted 19 January, 2017

The present study was conducted to assess the genetic diversity and population genetic structure of three sheep populations namely; Sawakni (SW), Berberi (BR) and Najdi (NJ), in Saudi Arabia, utilizing 45, 18 and 31 individual blood DNA extractions, respectively. Seventeen microsatellite markers were used to genotype these 94 sheep individuals. There were 195 alleles generated employing the 17 microsatellites loci with a mean of 11.47 alleles per locus, with a range of observed and expected heterozygosity from 0.651 to 0.989 and 0.590 to 0.816, respectively. The total number of alleles of 169, 127 and 111, and their means of effective number of alleles of 4.983, 4.192 and 3.781 were observed in SW, BR and NJ populations, respectively. Thirteen of the microsatellites loci studied in SW, seven loci in BR and five loci in NJ were found to be deviated from Hardy-Weinberg Equilibrium. The fixation genetic indices (F_{ST}) among the three populations were very low, ranging from 0.029 (between SW and BR) to 0.038 (between NJ and BR), indicating low population differentiation among the three sheep populations. The present study showed that the microsatellite markers are powerful tools in breeding programs, however more microsatellites may be needed for a broad judgment on the genetic status of the sheep populations in Saudi Arabia.

Key words: DNA, genetics, microsatellite, Saudi sheep.

INTRODUCTION

Inbreeding may lead to the loss of genetic variation within the livestock breeds and that the breed itself may become extinct. To prevent the extinction of a breed and conserving its merit, molecular biological approaches,

*Corresponding author. E-mail: ahmdhusam@hotmail.com.

Author(s) agree that this article remains permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> such as the polymerase chain reaction (PCR) technique (Nicholas et al., 1996), utilizing various DNA markers (Erlich, 1991), has been established to study the genetic variation within and between different animal resources. Genetic markers such as DNA fingerprinting (DFP), randomly amplified polymorphic DNA (RAPD) and microsatellites have been used in studying genetic variability, parentage verifications and genome mapping projects in llama, goat, sheep, cattle, chicken and camel (Groenen et al., 2000; Jianlin et al., 2000; Sasse et al., 2000; Krüger et al., 2002; Geng et al., 2003; Li et al., 2004; Yang et al., 2004; Peter et al., 2007; Mahmoudi et al., 2010 and Spencer et al., 2010). In sheep, genetic diversity have been investigated by several studies using primers developed for amplification of microsatellite loci (Diez-Tascon et al., 2000; Hassan et al., 2003; Arora and Bhatia, 2004; Gutiérrez-Gil et al., 2006; El-fawal, 2006; Peter et al., 2007).

In Saudi Arabia, sheep population exceeding 7.2 million head (Ayadi et al., 2014) and plays an important role in sustenance of life of many local communities. Several breeds of sheep have been identified in different regions in Saudi Arabia based on some morphological characteristics. They are well adapted to the prevailing adverse environment of Saudi Arabia; the most popular native breeds are Najdi, Naeimi, and Herri breeds (Abouheif et al., 1989). Sawakni and Berberi are two exotic breeds introduced to the sheep populations of the Kingdom of Saudi Arabia from Sudan, and Somalia, respectively. They became popular choices for many Saudi in the last decade, comparable to Najdi breed for many cases.

The genetic variations within and among these sheep populations are poorly documented. Therefore, the present study was conducted to investigate the genetic diversity within and among three sheep populations (Sawakni, Berberi and Najdi), as a way for further development programs of sheep breeding in the Kingdom of Saudi Arabia.

MATERIALS AND METHODS

A total of 94 individuals of sheep belonging to three populations: Sawakni (45 SW), Berberi (18 BR) and Najdi (31 NJ) were selected from five different localities in Saudi Arabia. Blood samples (10 mL) were collected from each sheep by jugular venipuncture into vacuum EDTA tubes. DNA was extracted from blood samples according to the manufacturer's instructions of a QIAgen DNeasy Kit (Hilden, Germany). Table 1 shows the seventeen microsatellite primer-pairs, a part of a section of markers recommended by the International Society of Animal Genetics (ISAG)/FAO (FAO, 2011), used to investigate the sheep genotypes. Purity and concentration of each sample was quantified using NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA). These markers were selected by taking into account the level of polymorphism previously detected in other studies and the location on different chromosomes (Peter et al., 2007; Ferrando et al., 2014; Yilmaz et al., 2015). Polymerase chain reaction (PCR) amplifications were carried out in a 25 µl reaction volume containing100 ng of template DNA and 2 µl of each 10 µM primer.

To reduce the possibility of cross contamination and variation in the amplification reactions, master mixes containing all PCR reagents including the Kapa Taq polymerase enzyme (KAPA Biosystems, Boston, MA, USA) except DNA templates and primers were used. The amplification program was performed using the Gene Amp PCR system 9700 thermocycler (Applied Biosystems, Warrington, UK). The amplification protocol was an initial denaturation step for 2 min at 94°C, followed by 35 cycles at 94°C for 30 s, 55 °C annealing temperature (Table 1) for 30 s and 72°C for 30 s. The final step of the amplification protocol was the extension step at 72°C for 5 min. All the reactions were carried out on 96 well PCR plates (Applied Biosystems, Warrington, UK). The microsatellite primers were labeled with dyes FAM, TAMN, HEX and ROXN and microsatellite data were analyzed in the ABI Prism® 3500 Genetic analyzer (Applied Biosystems, Warrington, UK). Each analyzed PCR reaction contained GeneScan® LIZ 500 molecular weight standards (Applied Biosystems, Warrington, UK). The quantity and quality of DNA were checked by spectrophotometer (Jenway Genova Spectrophotometer Krackler Scientific Incorporation, USA).

Statistical analyses

The basic parameters for each locus and populations, allele frequencies, observed number of alleles (Na), effective number of alleles (Ne), observed (Ho), expected (He) heterozygosities and Polymorphism Information Content (PIC), were measured using Cervus version 3.0.3 (Kalinowski et al., 2007). Deviations from Hardy-Weinberg equilibrium (HWE) and Wright's F-statistics (Fis, F_{ST} , and F_{IT}) within and among the sheep populations were calculated by using GenePop version 4.0.10 (Raymond and Rousset, 1995). We used the Bayesian clustering method implemented in Structure v. 2.3.1 (Pritchard et al., 2000) to evaluate the number of genetic units within the 94 individuals of the three studied sheep populations. The likelihood of a specific number of homogenous genetic clusters (K) in the dataset, and the relative contribution of each individual to each cluster was estimated under admixture model with Markov Chain Monte Carlo (MCMC) of 2.1 × 10^6 iterations after a burn-in of 1 x 10^5 , for K = 1 to K = 6. Ten independent simulations for each K (1-6) were performed. The most likely number of genetic units was assessed using the resulting likelihood, as well as by examining the modal distribution of DeltaK (ΔK) (Evanno et al., 2005).

RESULTS

The 94 sheep individuals of the three populations: (SW), (BR) and (NJ) were genotyped using 17 microsatellite loci. The seventeen microsatellite loci were polymorphic. Table 2 shows, for the three populations, the values of the total number of alleles (Na), mean effective number of alleles (Ne) and observed (Ho) and expected (He) heterozygosities. A total of 195 alleles were detected in which 169, 127, and 111 alleles were observed respectively in SW, BR and NJ populations. Out of these 195 alleles, 61 were designated as private alleles in which 39 were found in SW, 15 in BR and 7 in NJ populations. The numbers of effective alleles averaged 4.893, 4.192 and 4.781 in SW, BR and NJ sheep breeds, respectively.

The average number of alleles per locus was 11.470, ranging between 7 (locus OarCP34) and 18 (locus HUJ616) alleles. Twelve out of the 17 loci studied have

Locus name	Sequences 5' \rightarrow 3' forward/ reverse	Label	Allele size (bp)	Chromosomal location	
ILSTS005	GGAAGCAATGAAATCTATAGCC	56FAM	174-218	7	
12313003	TGTTCTGTGAGTTTGTAAGC	JUL AM	174-210		
MCM527	GTCCATTGCCTCAAATCAATTC	56-TAMN	165-187	5	
WOW527	AAACCACTTGACTACTCCCCAA	JO-TAMIN	100-107		
SRCRSP5	GGACTCTACCAACTGAGCTACAAG	5HEX	5HEX 126-158	18	
	GTTTCTTTGAAATGAAGCTAAAGCAATGC	ONEX		10	
OarFCB128	ATTAAAGCATCTTCTCTTTATTTCCTCGC	56FAM	96-130	2	
	CAGCTGAGCAACTAAGACATACATGCG	001710	301 AWI - 30-130	2	
HUJ616	TTCAAACTACACATTGACAGGG 56-ROXN 11	114-160	13		
1100010	GGACCTTTGGCAATGGAAGG		111100		
OarHH47	TTTATTGACAAACTCTCTTCCTAACTCCACC	56-TAMN	130-152	18	
Carint	GTAGTTATTTAAAAAAATATCATACCTCTTAAGG		100 102		
ILSTS11	GCTTGCTACATGGAAAGTGC	56FAM	256-294	9	
	CTAAAATGCAGAGCCCTACC	0017	200 201	C C	
BM8125	CTCTATCTGTGGAAAAGGTGGG	56-TAMN	110-130	1	
2	GGGGGTTAGACTTCAACATACG				
OarFCB226	CTATATGTTGCCTTTCCCTTCCTGC	5-HEX	119-153	2	
	GTGAGTCCCATAGAGCATAAGCTC				
OarAE129	AATCCAGTGTGTGAAAGACTAATCCAG	56FAM	133-159	5	
	GTAGATCAAGATATAGAATATTTTTCAACACC				
OarJMP29	GTATACACGTGGACACCGCTTTGTAC	56-ROXN	96-150	24	
	GAAGTGGCAAGATTCAGAGGGGAAG		HEX 119-153 FAM 133-159 ROXN 96-150		
SRCRSP9	AGAGGATCTGGAAATGGAATC	56FAM	99-135	12	
	GCACTCTTTTCAGCCCTAATG		56FAM 96-130 56-ROXN 114-160 56-TAMN 130-152 56FAM 256-294 56-TAMN 110-130 5-HEX 119-153 56FAM 96-150 56-ROXN 99-135 56FAM 174-282		
MAF214	GGGTGATCTTAGGGAGGTTTTGGAGG	56FAM	174-282	16	
	AATGCAGGAGATCTGAGGCAGGGACG				
OarCP34	GCTGAACAATGTGATATGTTCAGG GGGACAATACTGTCTTAGATGCTGC	56-ROXN	112-130	3	
	CCCTAGGAGCTTTCAATAAGAATCGG				
OarFCB304	CGCTGCTGTCAACTGGGTCAGGG	5-HEX	150-188	19	
	GATCACAAAAAGTTGGATACAACCGTGG				
MAF209	TCATGCACTTAAGTATGTAGGATGCTG	5-HEX	109-135	17	
	AAAGGCCAGAGTATGCAATTAGGAG				
MAF65	CCACTCCTCCTGAGAATATAACATG	56-TAMN	123-163	15	
	UCAUTUUTUUTUAUAATATAAUATU				

Table 1. Primers sequences and labels of the 17 primer pairs used to amplify microsatellite regions in the Ovis aries of the present study.

more than 10 alleles (ILSTS005, MCM527, SRCRSP5, OarFCB128, HUJ616, OarHH47, OarFCB226, OarJMP29, SRCRSP9, MAF214, OarFCB304 and MAF65), and the other five loci possess less than 10 alleles (ILSTS11, BM8125, OarAE129, OarCP34 and MAF209). Observed heterozygosity (Ho) and expected heterozygosity (He) averaged 0.851 and 0.746, respectively (Table 2).

Results of the F-statistics for each of the 17 analyzed loci in the three sheep populations are shown in Table 3. Mean values for F_{IS} , F_{IT} and F_{ST} were -0.145, -0.097 and 0.042, respectively. The low F_{IS} and F_{IT} mean values, which are very close to zero, indicated low level of inbreeding within and among the populations. It also points towards low genetic differentiation among the

populations as indicated by the very low values of the pairwise fixation genetic indices (Fst) among the three populations. Fst values ranged from 0.029 (between SW and BR, and between SW and NJ) to 0.038 (between NJ and BR) as indicated in Table 4. HWE results (Table 5) showed that 4, 8 and 12 loci in SW, BR and NJ sheep breeds respectively followed HWE, and the rest are deviated from the HWE at p<0.05. The mean of Polymorphic Information Content (PIC) for the 17 microsatellite marker was 0.754, ranged from 0.627 (marker OarAE129) to 0.863 (marker OarFCB226) (Table 4).

Bayesian clustering assignment, on SW, BR and NJ sheep individuals, revealed that Ln L(K) increased with the number of clusters tested (K) and reached the highest

1	SW			BR			NJ					
Locus	Na	Ne	Но	Не	Na	Ne	Но	He	Na	Ne	Но	Не
ILSTS005	9.000	5.031	0.933	0.801	9.000	5.786	0.889	0.827	6.000	3.922	0.548	0.745
MCM527	11.000	4.994	0.911	0.800	8.000	4.909	1.000	0.796	9.000	5.045	0.774	0.802
SRCRSP5	9.000	4.099	1.000	0.756	5.000	3.880	1.000	0.742	5.000	3.360	0.452	0.702
OarFCB128	13.000	5.140	0.400	0.805	8.000	5.143	0.778	0.806	8.000	4.711	0.774	0.788
HUJ616	15.000	7.219	1.000	0.861	12.000	6.231	1.000	0.840	5.000	2.758	0.645	0.637
OarHH47	12.000	4.816	1.000	0.792	4.000	2.339	0.833	0.573	9.000	3.105	0.935	0.678
ILSTS11	7.000	4.436	0.933	0.775	7.000	4.596	1.000	0.782	5.000	3.867	0.871	0.741
BM8125	6.000	3.415	1.000	0.707	6.000	3.028	1.000	0.670	8.000	3.891	0.677	0.743
OarFCB226	16.000	7.910	0.733	0.874	11.000	3.682	0.778	0.728	8.000	5.125	0.935	0.805
OarAE129	6.000	2.560	0.978	0.609	6.000	3.115	1.000	0.679	3.000	2.750	0.516	0.636
OarJMP29	10.000	4.436	0.756	0.775	8.000	3.904	1.000	0.744	9.000	4.281	0.839	0.766
SRCRSP9	9.000	5.728	0.933	0.825	8.000	4.291	1.000	0.767	5.000	3.041	0.742	0.671
MAF214	10.000	3.750	0.756	0.733	4.000	2.167	0.722	0.539	4.000	1.990	0.516	0.497
OarCP34	6.000	4.219	0.978	0.763	7.000	4.985	0.944	0.799	6.000	4.418	0.742	0.774
OarFCB304	12.000	5.769	1.000	0.827	10.000	4.985	1.000	0.799	10.000	5.653	0.645	0.823
MAF209	8.000	4.383	0.911	0.772	7.000	3.904	1.000	0.744	6.000	3.041	0.645	0.671
MAF65	10.000	5.273	1.000	0.810	7.000	4.320	1.000	0.769	5.000	3.325	0.968	0.699
Mean	9.941	4.893	0.895	0.782	7.471	4.192	0.938	0.741	6.529	3.781	0.719	0.716
SE	0.725	0.315	0.038	0.015	0.536	0.272	0.024	0.020	0.501	0.240	0.038	0.020

Table 2. Number of alleles (Na), Mean effective number of alleles (Ne), observed (H_0) and expected (H_e) heterozygosities for each locus of the three different sheep populations, SW, BR and NJ.

Table 3. F-statistics analysis for each of 17 microsatellite markers amor	١g
SW, BR and NJ sheep.	

Locus	Fis	Fit	Fst
ILSTS005	0.001	0.031	0.030
MCM527	-0.120	-0.073	0.042
SRCRSP5	-0.114	-0.093	0.019
OarFCB128	0.186	0.242	0.068
HUJ616	-0.131	-0.057	0.066
OarHH47	-0.355	-0.302	0.039
ILSTS11	-0.220	-0.195	0.020
BM8125	-0.263	-0.224	0.031
OarFCB226	-0.017	0.048	0.064
OarAE129	-0.296	-0.176	0.092
OarJMP29	-0.135	-0.109	0.023
SRCRSP9	-0.182	-0.133	0.041
MAF214	-0.127	-0.037	0.080
OarCP34	-0.140	-0.134	0.006
OarFCB304	-0.080	-0.045	0.033
MAF209	-0.169	-0.130	0.033
MAF65	-0.303	-0.270	0.025
Mean	-0.145	-0.097	0.042
SE	0.031	0.031	0.006

value at K	= 5 groups	(Figure	1a). Base	d on	the ∆K
values, the	result of K	= 2 see	ms to be	the	optimal

number of clusters (Figure 1a). The estimated individual genotype membership coefficient (Q) in each ancestral

Table 4. Pairwise population F_{ST} values among SW, BR and NJ sheep.

Population	SW	BR
BR	0.029	
NJ	0.029	0.038

SW = Sawakni; BR = Berberi; NJ = Najdi.

Table 5. Number of loci significantly deviating from Hardy-Weinberg equilibrium (HWE) and number of alleles at each locus (K) for SW, BR and NJ sheep populations of Saudi Arabia.

Locus	SWa	BR	NJ	К	PIC
ILSTS005	***	*	ns	12	0.787
MCM527	ns	ns	ns	11	0.814
SRCRSP5	***	**	ns	11	0.704
OarFCB128	***	ns	ns	14	0.846
HUJ616	*	ns	ns	18	0.817
OarHH47	ns	ns	ns	12	0.721
ILSTS11	***	ns	*	8	0.750
BM8125	***	***	ns	9	0.691
OarFCB226	***	**	**	16	0.863
OarAE129	***	***	ns	8	0.627
OarJMP29	***	**	ns	13	0.753
SRCRSP9	***	ns	ns	11	0.777
MAF214	***	***	ns	11	0.630
OarCP34	***	**	*	7	0.743
OarFCB304	ns	**	*	14	0.823
MAF209	ns	ns	ns	9	0.722
MAF65	***	ns	*	11	0.756

 $^{\rm a}{\rm The}$ breed abbreviations SW, BR and NJ are as follows: Sawakni, Berberi and Najdi, respectively. *SSR loci deviated from HWE at P < 0.05.

cluster for the optimal K number is represented in Figure 1b. For the three populations, averages of Q coefficient were higher than 90%. In particular, SW and BR breeds were clearly assigned to a single cluster, and the second one includes exclusively NJ individuals (Figure 1b). The net nucleotide distances, based on allele frequencies divergence among populations, recorded between the two clusters reached 2%.

DISCUSSION

Several researchers have investigated the genetic variations among closely related breeds in farm animals using the microsatellite markers (Peter et al., 2007; Blackburn et al., 2011). The number of alleles per locus for the three breeds studied ranged from 7 to18, indicating of genetic polymorphism within the tested

sheep populations. This range was comparable with that observed (6-18) in four Romanian sheep populations (Kevorkian et al., 2010; Jakaria et al., 2012). It was higher than that observed by Pramod et al. (2009) in Vembur sheep population of South India (2-9) and by Radha et al. (2011) in Kilakarsal sheep population (3-12). Yilmaz et al. (2015) found a range of 15 to 31 alleles per locus in Turkish sheep populations (Gökceada, Kıvırcık, Karacabey Merino, and Sakız) whereas Ricardo et al. (2016) has reported a range of 10 to 23 alleles per locus in the Colombian sheep. In the study of Turkish sheep populations by Yelmaz et al. (2015) they observed a total of 352 alleles with a mean number of 20.71 alleles per locus, whereas in the Colombian sheep, Ricardo et al. (2016) showed 157 alleles with a mean number of alleles per a locus of 14.27. do Amaral Crispima et al. (2014) showed 100 alleles with mean 12.5 alleles per locus in Pantaneiro sheep in Brazil, Sassi-Zaidy et al. (2014) found 270 alleles with a mean of 15.88 alleles per locus in Tunisian sheep, and in a Vembur sheep in South India, Pramod et al. (2009) found 147 alleles with mean 5.88 alleles per locus. However, our finding indicated almost mid-point between these values, with actual total number of alleles of 195 and a mean number of alleles per locus of 11.470. Private alleles defined in this study as alleles unique to a single population were observed to be 39, 15 and 7 alleles for SW, BR and NJ sheep populations, respectively. Despite the low frequencies of these alleles, however, they can be distinguished among the three sheep populations and can be good indicators as breed markers. Blackburn et al. (2011) observed two private alleles with low frequencies in the Sary-arkinsskaya Kazakh sheep breed. Yilmaz et al. (2015) designated 7 alleles in the Karacabey Merino Turkish sheep breed.

Means of effective number of alleles implied by the three Saudi sheep populations, SW, BR and NJ, were 4.893, 4.192 and 3.781, respectively, with a grand mean of 4.289. Turkish sheep breed displayed higher mean effective number of alleles of 7.040 (Yilmaz et al., 2015). Balochi and Rakhshani sheep breed in Pakistan displayed the lowest average effective number of alleles of 2.969 (Wajid et al., 2014). The mean observed heterozygosity values in the present study were 0.895, 0.938 and 0.719 for SW, BR and NJ sheep breeds, respectively, with a grand mean of 0.851. This value is higher than that reported by other studies of Indonesian sheep (0.574), Coimbatore sheep in India (0.625) Turkish sheep (0.66) and Colombian sheep (0.680) (Jakaria et al., 2012; Hepsibha et al., 2014; Yilmaz et al., 2015; Ricardo et al., 2016, respectively). On the other hand, the expected heterozygosity (He), the best estimator of genetic diversity in a population (Kim et al, 2002), was 0.782, 0.741 and 0.716 for SW, BR and NJ sheep, respectively, with a grand mean of 0.746. It was found to be higher than that reported by other studies in Indonesian sheep (0.687) but was lower than that in Turkish sheep (0.870) and Colombian sheep (0.770)

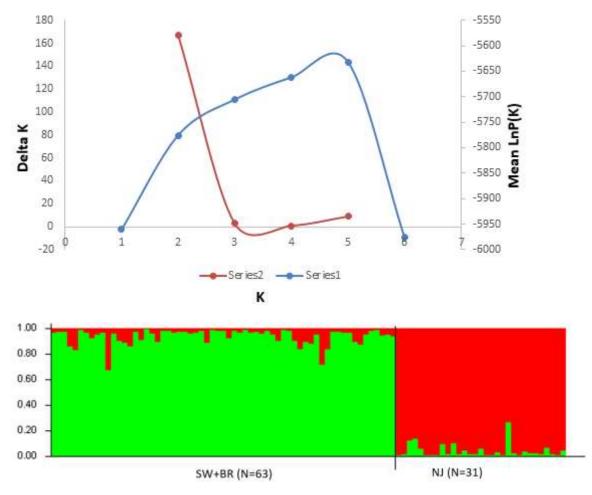


Figure 1. (a) The approximate number of genetic clusters (K) within the three different sheep populations, SW, BR and NJ based on results from the software structure. The "estimated log probability of the data", Ln Pr(X/K), (Pritchard et al., 2000), and ΔK (Evanno et al., 2005) are shown for each value of K from one to six. (b) The most likely value of K inferred by structure was two. Bar plot of the estimated membership coefficient, Q, for each of the 94 individuals in each of two genetic clusters (K). Each individual is represented by a thin vertical line, which is partitioned into K (2) colored segments that represent the individual's estimated membership fractions in K clusters. Black lines separate individuals of different clusters based on structure analysis, SW+BR and NJ.

(Jakaria et al., 2012; Yilmaz et al., 2015; Ricardo et al., 2016, respectively). Interestingly, the lowest Ho was observed in SW sheep (0.400) in the OarFCB128 marker and the lowest value of He was in the MAF214 marker of NJ sheep breed (0.497). In general, all breeds showed high genetic diversity for all loci analyzed. A breed with constant gene and genotype frequencies is said to be in HWE (Falconer and Mackay, 1996). Of the most important steps in this study was to verify whether the genotypes studied were in HWE. Results indicated that there were some genotypes with several loci that followed HWE (P>0.05); 4, 8 and 12 loci in SW, BR and NJ sheep breeds, respectively. The deviation from HWE may have resulted from reasons which we were unable to specify. It may have probably resulted from genetic drift or from both artificial and natural selection as well as it

could have resulted from the mutations, migration or nonrandom mating. Gene flow was high in some populations but lower in others. F_{IS} value for all loci was 0.145, which indicates that some moderate inbreeding has likely occurred within each population, although it does not explain the genetic variation among the three sheep populations under investigation. Outbreeding is limited due to isolation of breeding groups to specific geographical regions or even farms. In addition, F_{ST} value of 0.042 indicates little genetic differentiation has occurred. A previous study by Radha et al. (2011) using 25 microsatellite markers in Indian sheep indicated that seven out of 25 loci in sheep populations were in HWE. The least PIC value was 0.627 (OarAE129) indicating that all microsatellite markers were highly polymorphic. The high PIC values and also the average number of

alleles per each locus indicate the appropriateness of using the 17 microsatellite markers in investigating the genetic diversity within Saudi sheep.

Mean F_{ST} values among the three sheep populations ranged between 0.029 (between SW and BR populations), 0.029 (between SW and NJ populations) and 0.038 (between BR and NJ populations), indicating little genetic differentiation among Saudi sheep populations. Ferrando et al. (2014) also found close F_{ST} value in six breeds located in the eastern Pyrenees ranging from 0.011 to 0.053. When Saudi sheep populations were compared with other populations from different countries, the F_{ST} values were lower than those found in this study (Sassi-Zaidy et al., 2014; Álvarez et al., 2012).

Structure analysis demonstrated that the studied sheep breeds studied formed two well defined clusters. SW and BR breeds belong to the first and NJ to the second. Both clusters contained few individuals with admixed profiles. Ninety-six percent of the 63 SW-BR samples had Q>0.90 for the first cluster. Within the second cluster, only one sample had Q<0.80. These data confirm the isolation between the two groups. Najdi is of Saudi Arabia origin while the other two native sheep breeds, SW and BR, were introduced form Soudan and Somalia, respectively. Therefore, and despite the morphological differences, this result expressed the high genetic similarity between the two introduced breeds. This may be explained by several generations of admixture between SW and BR. Also, it highlighted the importance of adopting molecular markers as criteria to differentiate between these breeds.

Conclusions

In conclusion, 17 microsatellites were genotyped to investigate the genetic structure of 3 sheep breeds in Saudi Arabia. It also inferred genetic diversity within breeds and strong gene flow exchange between the breeds under investigation. The results of the present study represent baseline information of genetic pattern and diversity in these Saudi sheep which are commonly raised in Saudi Arabia. Hence, studying additional microsatellite markers may reveal more information on the population structure. Furthermore, larger numbers of animals from different breeds are required to establish a robust genetic analysis for genotyping and characterizing the sheep population in Saudi Arabia.

Conflicts of Interests

The authors have not declared any conflict of interests.

REFERENCES

Abouheif MA, Abdo GM, Basmaeil SM, Alsobayel AA (1989).

Identification of the preference patterns of different breeds of sheep for consumption in Saudi Arabia. Asian Australas. J. Anim. Sci. 2:129-132.

- Álvarez IJ, Capote A, Traoré N, Fonseca K, Pérez M, Cuervo M (2012). Genetic relationships of the Cuban hair sheep inferred from microsatellite polymorphism. Small Rumin. Res. 104:89-93.
- Arora R, Bhatia S (2004). Genetic structure of Muzzafarnagri sheep based on microsatellite analysis. Small Rumin. Res. 54:227-230.
- Ayadi M, Maatar AM, Aljumaah RS, Alshaikh MA, Abouheif MA (2014). Factors affecting milk yield composition and udder health of Najdi ewes. J. Anim. Vet. Adv. 6:28-33.
- Blackburn HD, Toishibekov Y, Toishibekov M, Welsh CS, Spiller SF, Brown M, Paiva SR (2011). Genetic diversity of *Ovis aries* populations near domestication centers and in the New World. Genetica 139(9):1169-1178.
- 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.
- do Amaral Crispima B, de Oliveira Senob L, Alves do Egitoc A, Miranda de Vargas Juniorb F (2014). Application of microsatellite markers for breeding and genetic conservation of herds of Pantaneiro sheep. Electron. J. Biotechnol. 17(6):317-321.
- El-fawal MA (2006). Molecular genetic diversity in characteristics of some Egyptian sheep breeds. Master Thesis, Animal Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.
- Erlich HA (1991). PCR Technology: principles and applications for DNA amplification. WH Freeman and Company New York.
- Evanno G, Regnaut S, Goudet J (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. Mol. Ecol. 14:2611-2620.
- Falconer DS, Mackay TFC (1996). Introduction to Quantitative Genetics Longman Essex UK 4th edition.
- FAO (2011). Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines nr 9. FAO, Rome, Italy. 100 p.
- Ferrando A, Goyache F, Parés Casanova PM, Carrión C, Miró J, Jordanai Vidal J (2014). Genetic relationships between six eastern Pyrenean sheep breeds assessed using microsatellites Spanish. J. Agric. Res. 12(4):1029-1037.
- Geng R. Chang HÝ, Ang ZP, Sun WW, Ang LP, Lu SX, Tsunoda Km Ren ZJ (2003). Study on origin and phylogeny status of Hu sheep. Asian Australas. J. Anim. Sci. 16:743-747.
- Groenen MAM, Cheng H, Bumstead N, Benkel BF, Briles WE, Burke T, Burt DW, Crittenden LB, Dodgson J, Hillel J, Lamont S, Ponce de Leon A, Soller M, Takahashi H, Vignal A (2000). A consensus map of the chicken genome. Genome Res. 10(1):137-147.
- Gutiérrez-Gil B, Uzun M, Arranz J, Primitivo FS, Yildiz S, Cenesiz M, Bayon Y (2006). Genetic diversity in Turkish sheep. Acta Agric. Scand. A. 56:1-7.
- Hassan AA, Abou Mosallam HA, Oraby HA, de Hondt El-Nahas SM (2003). Genetic Diversity of Three Sheep Breeds in Egypt Based on Microsatellites Analysis. J. Gen. Engin. Biotechnol. (NRC) 1:141-150.
- Hepsibha P, Karthickeyan SMK, Guru V (2014). Microsatellite marker based assessment of genetic structure of Coimbatore breed of sheep (*Ovis aries*) in Tamil Nadu. Ind. J. Biotechnol. 13:203-206.
- Jakaria J, Żein MSA, Sulandari S, Subandriyo S, Muladno M (2012). The use of Microsatellite Markers to Study Genetic Diversity in Indonesian sheep. J. Indones. Trop. Anim. Agric. 37(1):1-9.
- Jianlin H, Mburu D, Ochieng J, Kaufmann H, Rege J, Hanotte O (2000). Application of New World Camelidae microsatellite primers for amplification of polymorphic loci in Old World camelids. Anim. Genet. 31:404-406.
- Kalinowski ST, Taper ML, Marshall TC (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16:1099-1006.
- Kevorkian SEM, Georgescu SE, Manea MA, Zaulet M, Hermenean AO, Costache M (2010). Genetic diversity using microsatellite markers in four Romanian autochthonous sheep breeds. Rom. Biotechnol. Lett. 15(1):5059-5065.
- Kim KS, Yeo JS, Choi CB (2002). Genetic diversity of north-east Asian cattle based on microsatellite data. Anim. Genet. 33(3):201-204.

- Krüger K, Stranzinger G, Rieders S (2002). A full genome scan panel of horse (*Equus caballus*) microsatellite markers applied to different equid species. Proc XXVIII Int Conf on Animal Genetics. August 11-15, 2002. Göttingen, Germany.
- Li C, Basarab J, Snelling WM, Benel B, Kneeland J, Murdoch B, Hansen C, Moore SS (2004). Identification and fine mapping of Quantitative trait loci for back fat on Bovine Chromosomes 2561921 and 23 in a commercial line of Bostarus. J. Anim. Sci. 82:297-972.
- Mahmoudi B, Bayat M, Sadeghi R, Babayev M, Abdollahi H (2010). Genetic Diversity among Three Goat population Assessed by Microsatellite DNA Markers in Iran. Glob. Vet. 4(2):118-124.
- Nicholas RAJ, Santini FG, Clark KM, Palmer NMA, De Santis P, Bashiruddin JB (1996). A comparison of serological tests and gross lung pathology for detecting contagious bovine pleuropneumonia in two groups of Italian cattle. Vet. Rec. 139:89-93.
- Peter C, Bruford M, Perez T, Dalamitra S, Hewwitt G, Erhardt G (2007). Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. Anim. Genet. 38:37-44.
- Pramod SP, Kumarasamy ARM, Chandra P, Sridevi P, Rahmathullah PS (2009). Molecular characterization of Vembur sheep (*Ovis aries*) of South India based on microsatellites. Indian J. Sci. Technol. 2(11):55-58.
- Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. Genetics 155:945-959.
- Radha P, Sivaselvam SN, Kumarasamy P, Kumanan K (2011). Genetic diversity and Bottleneck analysis of Kilakarsal sheep by microsatellite markers. Ind. J. Biotechnol. 10:52-55.
- Raymond M, Rousset F (1995). GENEPOP (version 4010): population genetics software for exact tests and ecumenicism. J. Hered. 86:248-249.
- Ricardo, O; Cardona H. Martínez R (2016). Genetic diversity of Colombian sheep by microsatellite markers. Chil. J. Agric. Res. 76(1):40-47.
- Sasse J, Mariasejeram M, Babu R, Kinne J, Wernery U (2000). South American Camelids microsatellite marker in the *Camelus dromedaries*. Anim. Genet. 31(1):75-76.

- Sassi-Zaidy YB, Maretto F, Charfi-Cheikrouha F, Cassandro M (2014). Genetic diversity structure and breedn relationships in Tunisian sheep. Small Rumin. Res. 119:52-56.
- Spencer PBS, Wilson KJ, Tinson A (2010). Parentage testing of racing camels (Camelus dromedarius) using microsatellite DNA typing. Anim. Genet. 41:662-665.
- Wajid A, Wasim M, Yaqub T, Firyal S, Tayyab M, Siddique S, Hussain T (2014). Assessment of genetic diversity in balochi and rakhshani sheep breeds of Baluchistan using microsatellite DNA markers. J. Anim. Plant. Sci. 24(5):1348-1354.
- Yang ZP, Chang H, Sun W, Gen RQ, Mao YJ, Tsunoda K (2004). A comparison of Two Kinds of Markers Applied in Analysis of Genetic Diversity in Sheep and Goat Populations. Asian Australas. J. Anim. Sci. 17:892-896.
- Yilmaz O, Sezenler T, Sevim S, Cemal I, Karaca O, Yaman Y, Karadağ O (2015). Genetic relationships among four Turkish sheep breeds using microsatellites. Turk. J. Vet. Anim. Sci. 39(5):576-582.