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Genetic diversity of Ardi goat based on microsatellite analysis

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The aim of this study was to analyze the genetic variability of Ardi goats found in the central regions of the kingdom of Saudi Arabia using 14 microsatellite markers. Allelic richness was considerably high in this population indicating high genetic polymorphism as expected heterozygozity was 0.675. Furthermore, the population showed deviation from Hardy-Weinberg equilibrium in seven loci. Mean polymorphic information content value was found to be 0.553. Inbreeding coefficient was 0.183 suggesting moderate level of inbreeding. There was also no-significant heterozygote excess on basis of different models of infinite allele. These tests along with the mode-shift test of Ardi goat indicated no bottleneck recently. Thus, it can be recommended that the Ardi genetic variability should be maintained for its unique genetic resources, and there is a scope for further improvement in productivity through an appropriate management and breeding program. In general, results of this study can be used to establish a base of national conservation strategy of Ardi goat population in Saudi Arabia.

Key words: Ardi goat, genetic diversity, microsatellite markers, inbreeding, bottleneck.

INTRODUCTION

Goat (*Capra hircus*) is considered the most prolific ruminant among all domesticated ruminants especially under harsh climatic conditions (Yadav and Yadav, 2008). This is due to their ability of adapting to different environmental conditions, nutritional fluctuations, disease resistance and capability to survive under low input systems (Fajemilehin and Salako, 2008; Serrano et al., 2009).

Genetic characterization is very useful and widely used to categorize animals in the world (Cardellino and Boyazoglu, 2008). It is very important in terms of conservation of genetic resources and sustainable use of

animal production, breeding objectives, survival and adaptation (Aggarwal et al., 2007; Glowatzki-Mullis et al., 2008; Kevorkian et al., 2010). Therefore, characterization attributes to quantify traits such as production traits, resistance to diseases and fertility (FAO, 2006). Microsatellites are the most common markers for genetic characterization of livestock due to their various advantages and well known success (Baumung et al., 2004).

Kingdom of Saudi Arabia is the largest country in the Arabian Peninsula (2.15 million km^2), where goat is one of the most important livestock species. According to the Ministry of Agriculture statistical report (2011), around 1.06 million goats are found in the kingdom. Adaptation of indigenous goat populations for low feed quality and quantity, harsh environmental conditions and water deprivation was reported by El-Nouty et al. (1990). The

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Figure 1. Male of Ardi goat, showing grayish elongated ears, spiral horns, and black coat color (A). Female of Ardi goat, showing grayish elongated ears, semi-circular horns, and black coat color (B).

common native goat populations in the Kingdom are Ardi, Bishi, Jabaly, Hejazy, Najrani and Tohami (Al-Khouri, 1996). Ardi goat is a dual purpose, medium-sized, black colored, well-adapted to arid conditions. The average body weight of the male and female is 51 and 40 kg, respectively (Alamer, 2003; Al-Saiady et al., 2007). Horns are presented in both sexes, where the female's horn is semi-circular while the male's horn is spiral in shape. Ardi has grayish color, elongated and well drooping ears with coarse hairs (Alamer, 2006). Alshaikh and Mogawer (2001) reported the mean birth weight of male and female as 4.29 and 3.63 kg, respectively. Unfortunately, no studies have been found regarding genotypic variability of Ardi goat population in Saudi Arabia. Consequently, studying the genetic diversity of Ardi goat would be an essential step in any conservation and breeding programs of this breed. Therefore, the objective of this study was to evaluate the genetic diversity of Ardi goat in the Central Regions of Saudi Arabia based on microsatellite analysis.

MATERIALS AND METHODS

Forty three (43) unrelated animals from eleven herds confirming typical phenotypic features of Ardi goat (Figure 1) randomly selected from different areas located in central regions of Saudi Arabia were used for this study. The blood sample was drawn out from the jugular vein with a volume of 10 ml under aseptic conditions using ethylenediaminetetraacetic acid (EDTA) anticoagulant. The collected samples were brought to the laboratory on ice box for further analysis. DNA extraction was carried out using GFX Genomic Blood DNA extraction kit according to the manufacturer protocol, and quantified using Jenway Genova spectrophotometer (Krackeler Scientific Incorporation, USA). Fourteen (14) fluorescently labeled primers, recommended by International Society for Animal Genetics (ISAG), located on nine different chromosomes were used to amplify the extracted DNA (Table 1). Only the forward primer of each primer pair was labeled with one of the four fluorescent dyes as; FAM-Blue, PET-Red, NED-Yellow and VIC-Green provided by Applied Biosystems, USA. The PCR mix was prepared in a total volume of 10 µL and amplification was performed using an Applied Biosystems GeneAmp® PCR system 9700. The amplification conditions were preceded by a five

minutes initial denaturation at 94°C followed by 35 cycles of 30 s each 94°C denaturation and annealing temperature of primer for 55 to 65°C (based on the primer) for 60 s; 72°C extension for 90 s and final extension at 72°C for 10 min. PCR products were run in 2% Agarose gel to confirm the amplification. The electrophoresis conditions were 150 voltages for one hour. Then it was checked using the 'Syngene Gene Genius' gel documentation system, Amplified products were separated by ABI Prism Genetic Analyzer 3130 following the manufacturer's protocol. Microsatellite fragment sizing was achieved by the GeneMapper® version 4.0 and allele sizes were scored and verified. Genetic analysis was carried out using Cervus (Kalinowski et al., 2007) version 3.0.3 from Field Genetics Limited to calculate out the expected heterozygozity (H_e), observed heterozygosity (H_o) and polymorphic information content (PIC). Wright's F-statistics was used to calculate F_{is} by GenePop® version 4.0.10 (Raymond and Rousset, 1995). The exact test for deviations from Hardy-Weinberg Equilibrium (HWE) was also performed using GenePop. In addition, Bottleneck analysis was carried out using Bottleneck software version 1.2.02 (Cornuet and Luikart, 1996). Popgene version 1.31 was utilized to calculate the effective number of alleles, polymorphic information content and Ewens-Watterson test for neutrality of the microsatellite markers (Yeh et al., 1999).

RESULTS

The results of genetic variability parameters are shown in Table 2. All 14 microsatellites were found to be polymorphic. A total of 93 alleles were detected with a minimum of three observed alleles (*SRCRSP3* and *MAF209*) and a maximum of nine observed alleles (*OarFCB20* and *OarAE54*). Observed and expected heterozygosity (H_o and H_e) are shown in Table 2. The highest observed heterozygosity (0.875) was shown by locus *SPS113*, while the lowest (0.256) was shown by locus *MAF209*. Maximum expected heterozygosity (0.831) was given by *MAF70* and the minimum (0.353) was shown by *ILSTS005*. Out of the 14 studied markers, 10 markers showed higher heterozygote alleles than the homozygote. However, all of the studied markers showed alleles within the expected sizes.

Hardy Weinberg Equilibrium (HWE) test has shown that seven loci (*ILSTS011*, *SPS113*, *ILSTS029*, *SRCRSP3*, *MAF70*, *ILSTS005* and *OarAE54*) were in HWE (*p*>0.05). All markers showed acceptable informative capacity with more than 0.5 value for PIC except *ILSTS005*. Mean F_{is} value for studied Ardi population was 0.183±0.22 by Weir and Cockerham approach (Table 2). Bottleneck analysis under the Sign test, standardized difference test and Wilcoxon rank test for Ardi goat indicated no bottleneck (Table 3). The mode shift indicator like qualitative method of bottleneck estimation showed a normal L-shaped curve in graphical representation of alleles proportion verses allele frequency distribution (Figure 2).

DISCUSSION

This study is considered to be the first about the genetic diversity of Ardi goat and the result explicated essential

Table 1. Primers, sequence, annealing temperature, chromosome number, and their expected sizes.

S/N	Marker name	Primer sequence (5′ to 3′)	Annealing temperature (°C)	Chromosome number	Size (bp)
1	*SPS113*	F- CCTCCACACAGGCTTCTCTGACTT R-CCTAACTTGCTTGAGTTATTGCCC	58	10	134-158
2	*ILSTS029*	F-TGTTTTGATGGAACACAGCC R-TGGATTTAGACCAGGGTTGG	55	3	135-185
3	*OarFCB48*	F-GTTAGTACAAGGATGACAAGAGGCAC R-GACTCTAGAGGATCGCAAAGAACCAG	58	17	149-173
4	*OARFCB20*	F- GGAAAACCCCCATATATACCTATAC R-AAATGTGTTTAAGATTCCATACATGTG	58	2	93-112
5	*SRCRSP3*	F- CGGGGATCTGTTCTATGAAC R- TGATTAGCTGGCTGAATGTCC	55	10	95-135
6	*MAF209*	F-ATCACAAAAAGTTGGATACAACCGTG R-CATGCACTTAAGTATGTAGGATGCTG	55	17	100-104
7	*MAF70*	F-CACGGAGTCACAAAGAGTCAGACC R- GCAGGACTCTACGGGGCCTTTGC	65	4	120-190
8	*OarAE54*	F-TACTAAAGAAACATGAAGCTCCCA R-GGAAACATTTATTCTTATTCCTCAGTG	58	25	105-145
9	*ETH10*	F-GTTCAGGACTGGCCCTGCTAACA R-CCTCCAGCCCACTTTCTCTTCTC	55	5	190-220
10	*ILSTS005*	F-GGAAGCAATTGAAATCTATAGCC R-TGTTCTGTGAGTTTGTAAGC	55	10	160-230
11	*ILSTS011*	F- GCTTGCTACATGGAAAGTG R- CTAAAATGCAGAGCCCTACC	58	14	250-300
12	*BM6444*	F-CTCTGGGTACAACACTGAGTCC R-TAGAGAGTTTCCCTGTCCATCC	65	2	118-200
13	*TGLA53*	F- GCTTTCAGAAATAGTTTGCATTCA R- ATCTTCACATGATATTACAGCAGA	55	16	142–166
14	*INRA023*	F-GAGTAGAGCTACAAGATAAACTTC R-TAACTACAGGGTGTTAGATGAACT	58	3	196-215

bp, Base pair.

level of genetic variability and polymorphism. All measures of genetic diversity variations, such as observed number of alleles, effective number of alleles and PIC, showed high polymorphism across studied loci, thus proving suitability of these microsatellite markers for genetic diversity studies. Average of observed and expected heterozygosity was 0.553 and 0.675, respectively, concluding that the Ardi goat has substantial amount of genetic diversity, when compared to some other goat breeds around the world. Ardi goat showed higher expected genetic diversity (H_e=0.68) when compared with goat of Sri Lanka South (H_e=0.48), Sri Lanka N-Central (H_e=0.49) and Australian goat (H_e=0.45)

(Barker et al., 2001), some Indian goat breeds such as, Jamunapari (H_e=0.54), Marwari (H_e=0.63), Zalawadi (H_e=0.58), Gohilwadi (H_e=0.67), and Surti (H_e=0.64) (Fatima et al., 2008; Kumar et al., 2005; Gour et al., 2006), Swiss goat breeds (H_e=0.66; Glowatzki-Mullis et al., 2008), Canary Island goats (H_e=0.62; Martı´nez et al., 2004), Kalahari Red goats (H_e=0.63; Kotze et al., 2004), Sub-Saharan breeds (H_e=0.54; Muema et al., 2009) and some Korean goats (H_e=0.38;Kim et al., 2002). On the other hand, Ardi has showed less genetic diversity when compared to some Indian goat breeds such as, Kutchi (H_e=0.80), Sirohi (H_e=0.79) and Chegu (H_e=0.81), some Iranian goat breeds (H_e=0.74-0.80), Sardinian goat breed

Table 2. Genetic variability parameters of Ardi goat.

Primer	n_a	n_e	H_o	H_e	PIC	F_{is}	HWE
ILSTS011	8	2.4272	0.5833	0.5880	0.5460	0.0081	0.6626
OarFCB20	9	2.4402	0.4878	0.5902	0.5590	0.1753	0.0135
SPS113	7	4.0833	0.8750	0.7551	0.7080	-0.1612	0.4955
ILSTS029	7	4.4077	0.6905	0.7547	0.7120	0.0487	0.0000
MAF209	3	1.9928	0.2558	0.4982	0.4310	0.4895	0.4782
OarFCB48	8	4.3103	0.3953	0.7680	0.7280	0.4882	0.0000
SRCRSP3	3	2.3776	0.6111	0.5794	0.5000	-0.0555	1.0000
ETH10	7	4.3140	0.5000	0.7682	0.7190	-0.0555	0.0000
MAF70	8	5.9207	0.8667	0.8311	0.7930	-0.0436	0.5423
ILSTS005	5	1.5458	0.2667	0.3531	0.3310	0.0248	0.0496
OarAE54	9	3.1446	0.6562	0.6820	0.6440	0.0348	0.5605
BM6444	4	3.1949	0.5769	0.6870	0.6050	0.1629	0.0003
INRA023	8	5.4705	0.6341	0.8172	0.7810	0.2262	0.0001
TGLA53	7	4.3975	0.3488	0.7726	0.7300	0000	0.0002
Mean	6.643	3.550	0.553	0.675	0.626	0.183	
SD	2.061	1.311	0.194	0.138	0.138	0.212	

n_a, Observed number of alleles; n_e, effective number of alleles; H_o, observed heterozygosity; H_e, expected number of alleles; F_{is}, inbreeding coefficient; HWE, represented in possibility values.

Table 3. Bottleneck Analysis of Ardi goat.

Test	Models of microsatellite evolution		
	IAM	TPM	SMM
Sign Test			
Expected No. of loci with heterozygosity excess	8.130	8.210	8.350
Observed No. of loci with heterozygosity deficiency	4	4	8
Probability	0.232	0.245	0.156
Standardized Differences Test			
T_2 values	1.663	-0.791	-4.942
Probability	0.049	0.214	0.000
Wilcoxon Rank Test			
Probability (one tail for Heterozygosity deficiency)	0.914	0.572	0.134
Probability (one tail for Heterozygosity excess)	0.097	0.452	0.879
Probability (two tail for Heterozygosity deficiency & excess)	0.194	0.903	0.268

IAM, Infinite allele model; TPM, two phase model; SMM, stepwise mutation model Parameters for TPM: variance= 30.00, proportion of SMM=70%, estimation is based on 1000 replications.

of Italy (H_e=0.74), Spanish Guadrrama (H_e=0.81), Croatian spotted breed (H_e=0.77) and Chinese goat breeds (H_e=0.78-82) (Behl et al., 2003; Dixit et al., 2008; Guo-hong et al., 2010; Jelena et al., 2011; Mahmoudi et al., 2010; Sechi et al., 2005; Serrano et al., 2009; Verma et al., 2007). Another measure of genetic variability is observed heterozygosity (H_o=0.55) which showed high genetic variability. This might be due to low selection pressure, large population size and immigration of new genetic materials. Average value of H_o of Ardi goat was similar to some breeds such as Gohilwari (H_o=0.51; Kumar et al., 2009), Sirohi (H_o=0.50; Verma et al., 2007), and Sub-Saharan breeds (H_o=0.56; Muema et al., 2009) and higher than Korian goat (H_o=0.36; Kim et al., 2002) and Jamunapari of India (H_o=0.42; Gour et al., 2006), but lower than Gohilwadi breed (H_o=0.63; Fatima et al., 2008),
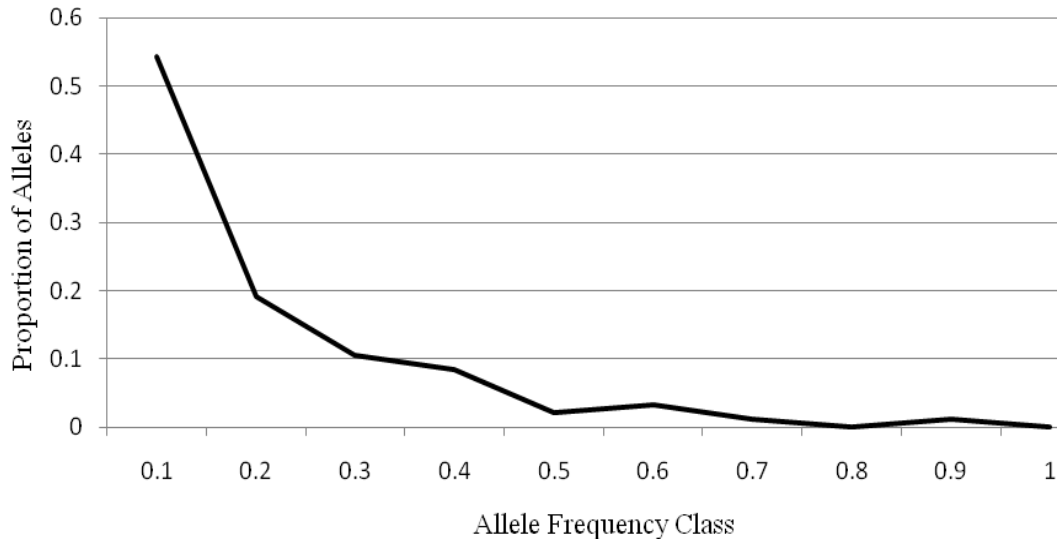
Figure 2. Mode shift analysis depicting absence of genetic bottleneck in Ardi goat, suggesting no bottleneck in Ardi.

Spanish Guadarrama (H_o=0.78; Serrano et al., 2009) and Croatian spotted breed (H_o=0.76; Jelena et al., 2011).

Mean observed allele for all loci found to be 6.64 explains high level of polymorphism of the studied microsatellites. Similar observed numbers of alleles were reported for Barbari goat from India (n_a=6.33; Ramamoorthi et al., 2009), Italian goat breeds (n_a=6.5; Agha et al., 2008) and Taleshi from Iran (n_a=6.7 Mahmoudi and Babayev, 2009). However, this average values was lower than the Croatian spotted breed (n_a=8.1; Jelena et al., 2011) and the average value of seven Indian goat breeds (n_a=8.1-9.7; Rout et al., 2008). The mean number of alleles and expected heterozygosities were very accurate indicators of the genetic polymorphism within the breed. Normally average number of alleles depends on sample size and generally, number of observed alleles tends to increase with increase in population size.

The polymorphic information content (PIC) values depict the suitability of the markers and their primers used in this study for analyzing the genetic variability of Ardi goats. Qi et al. (2009) reported that the mean PIC value of 10 Chinese goat breeds was 0.79. Additionally, Kumar et al. (2009) reported that the mean PIC value of Gohilwari goat breed of India was 0.647. Mahmoudi et al. (2010) also reported mean PIC of three native goat breeds of Iranian goat was 0.72. In fact, the PIC is determined by heterozygosity and number of alleles. This fact makes microsatellite markers the choice in genetic characterization and diversity studies. In particular, the high *PIC* values of the particular marker suggest its usefulness for genetic polymorphism and linkage mapping studies in goats. To sum up, these results show that Ardi goat has considerable high amount of genetic

polymorphism. Based on HWE test, it is difficult to pinpoint the exact reason for the deviations. However, it could be due to reasons such as non-natural population, mutations, migration, non-random mating and genetic drift.

Inbreeding coefficients F_{is} were positive for 11 loci and negative for the rest of the studied loci (*SPS113*, *SRCRSP3* and *MAF70*) with an average of 0.18. Ardi showed a close inbreeding values in comparison with Mehsana (F_{is}=0.16; Aggarwal et al., 2007) and Jamunapari (F_{is}=0.19; Gour et al., 2006) breeds of India, Low inbreeding values were also reported within 45 rare breeds of 15 European and Middle Eastern countries (Caňón et al., 2006). On the other hand some of the Indian breeds showed significant inbreeding such as Marwari (F_{is}=0.26; Kumar et al., 2005) and Kutchi (F_{is}=0.23; Dixit et al., 2008) breeds. Overall, F_{is} has showed moderate level of inbreeding within the Ardi goat population. This level of inbreeding may be a result of moderate levels of mating between closely related individuals under field conditions.

Results from Mode shift indicator and Sign test, Standardized Differences test and Wilcoxon rank test showed no bottleneck in Ardi goat. The results of these tests are on the following assumptions; no immigration and emigration, sample was representative of a defined population, no population substructure and the loci were selectively neutral which was proved by the Ewens-Watterson test.

Conclusions

This study can be considered as the first attempt to study

the genetic diversity of Ardi goat in the Kingdom of Saudi Arabia. Ardi goat population has high genetic variability in comparison with other goat breeds of the world. The marker panel used in this study were good enough for genetic diversity studies. However, some of the tested loci were not following the HWE roles, since they were non-random mating population. In addition, there is no selection that was acting on any of the markers used in this study. Therefore, no genetic hitchhiking was found in Ardi goats neither bottleneck. On the other hand, inbreeding within the Ardi population was moderate depicting lack of proper management plans, so it's necessary to consider a national plan for conserving the unique genetic recourses of Ardi breed.

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