Full Length Research Paper

Genetic variation of *Mehraban* sheep using two inter-simple sequence repeat (ISSR) markers

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Genetic diversity within *Mehraban* sheep populations, as one of the main breeds of Iranian sheep, was studied using $(AG)_9C$ and $(GA)_9C$ as two inter-simple sequence repeat (ISSR) markers. Blood samples were collected from 210 animals in 6 flocks, 35 heads each, in different parts of Hamedan province. In the polymerase chain reaction (PCR) products, $(AG)_9C$ and $(GA)_9C$ primers amplified 28 and 36 fragments, respectively, which ranged from 100 to more than 3100 bp. Percentages of polymorphic bands in the different populations ranged from 69 to 77%. In the pooled population, all inter-simple sequence repeat (ISSR) fragments were polymorphic. Shannon and Nei gene diversity indices were 0.2256 and 0.1258, respectively, which indicated low genetic diversity of *Mehraban* sheep. The population studied was at Hardy-Weinberg equilibrium for most of the ISSR-loci. Analysis of molecular variance (AMOVA) partitioned the ISSR variation into inter and intra population components, where inter-populations and intra-populations accounted for 9 and 91% of the total variation, respectively. The results of this study showed that the *Mehraban* sheep is a pure native breed that has a low genetic diversity between subpopulations and could be noticed for its potentials in response to selection or crossing with other breeds.

Key words: Inter-simple sequence repeat (ISSR) markers, *Mehraban* sheep, genetic diversity.

INTRODUCTION

There are more than 50 million heads of sheep in Iran, including 27 breeds and ecotypes (Vatankhah et al., 2004). One of the most important breeds of Iranian sheep is *Mehraban* sheep which is reared in Hamedan province, in western parts of Iran. The *Mehraban* sheep is a fat-tailed carpet wool sheep and usually has a light body, dark face and brown neck and is adapted to harsh and rocky environments (Figure 1). *Mehraban* sheep is primarily used for meat production. Mean birth weight and body weight at 90 days of age, and average pre-weaning daily gain are 3.88, 21.58 and 0.2 kg, respectively (Zamani and Mohammadi, 2008). Average litter size in *Mehraban* sheep is 1.1 which is similar to other Iranian

breeds of sheep (Pezhman, 2009). The approximated population size of *Mehraban* sheep is 700,000 heads (Anonymous, 2009), which is much smaller than 2.2 million heads as a previous approximation (Bathaei and Leroy, 1998). This means that *Mehraban* sheep may be in danger of extinction and needs conservation. The severe decline in number of *Mehraban* sheep population is largely due to lack of a society for this breed. Moreover, genetic structure of this breed has been rarely studied. The previous researches on *Mehraban* sheep were estimation of genetic parameters for body weight traits at different ages (Zamani and Mohammadi, 2008).

Molecular markers are increasingly used for the study of genetic diversity of populations in recent years. Inter-simple sequence repeat (ISSR) marker is similar to random amplification of polymorphic DNA (RAPD) and can be used without knowing the sequence information for genomic DNA (Zietkiewicz et al., 1994). ISSR has mild

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Figure 1. Ewe (left) and ram (right) *Mehraban* sheep.

technical difficulty, good reproducibility and reasonable cost, permitting its use for genetic studies of population in different species (Dogan et al., 2007; Hakki et al., 2010; Wang et al., 2008).

This study was conducted to evaluate the genetic variation within and between different geographical subdivided populations of *Mehraban* sheep, using inter-simple sequence repeat (ISSR) markers.

MATERIALS AND METHODS

Sample collection

Blood samples were collected from 210 two to four years old *Mehraban* sheep in six flocks, located in three geographical regions of Hamedan province. The studied flocks were located in Ovj-Tapeh (K_O) and Gol-Tapeh (K_G) villages in Kabood-Rahang area, Abbas-Abad (M_A) and Bahareh (M_B) villages in Malayer area, and Haji-Abad (A_H) and Vender-Abad (A_V) villages in Asad-Abad area. Kabood-Rahang, Malayer and Asad-Abad areas are located in the northeastern, southern and western parts of Hamedan province, respectively, with an average distance of 200 km each to others.

Approximately, 5 ml of blood samples were obtained from jugular vein and collected in EDTA contained tubes.

DNA extraction and polymerase chain reaction (PCR) amplification

Genomic DNA was extracted using DIAtom DNA Prep 100 kit (GenFanavaran Co, Iran). Polymerase chain reaction (PCR) was performed in a final volume of 25 µl that contained 2 µl (20 ng) of template DNA, 12.5 µl (1X) of PCR Master Mix kit (CinnaGen Co, Iran), 9.5 µl H_2O and 1 µl (100 µM) of each ISSR primers. Two ISSR primers including $(AG)_9C$ and $(GA)_9C$ (GenFanavaran Co, Iran) were used for PCR amplification.

Reactions were run on a MyCycler thermal cycler (Bio-Rad Co., USA) under the following thermal conditions: Initial denaturation at 94°C for 2 min, followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 2 min and a final extension at 72°C for 2 min. The samples were stored at -20°C.

The PCR products were electrophoresed on 1% agarose gel with 1× TAE buffer at 80 V for 2 h along with 0.1 Kb ladder (CinnaGen Co, Iran). Gels were stained with ethidium bromide and photographed under UV light (BTS-20.M model, UVltec Ltd, UK).

Data analysis

ISSR profiles were scored based on the presence or absence of bands as 1 or 0, respectively. Sizes of the amplified fragments were determined using ONE-Dscan software (Scanalytics, Inc., Fairfax, VA).

Frequency of polymorphic ISSR fragments, Nei's genetic diversity (Nei, 1973) and Shannon index (Lewontin, 1972) were used to calculate the genetic diversity of the studied populations, using POPGENE 1.31 software (Yeh et al., 1999).

POPGENE 1.31 software was also employed to determine Nei's unbiased genetic distances (Nei, 1978) and Hardy-Weinberg equilibrium was assayed in the studied populations. A dendrogram based on Nei's unbiased genetic distances, with unweighted paired-group method using an arithmetic average (UPGMA) was generated using MEGA version 4 (Tamura et al., 2007) to show genetic distances of the different populations.

Analysis of molecular variance (AMOVA), using GenAlEx 6.4 (Peakall and Smouse, 2006), was performed to estimate the variation existing within and between populations. Unlike Nei's analysis, AMOVA was not based on the assumption of Hardy–Weinberg equilibrium (Wang et al., 2008).

RESULTS AND DISCUSSION

ISSR polymorphism

Both primers produced different bands ranging from 100 to more than 3100 base pairs. A sample for the amplified bands by $(GA)_9C$ primer is presented in Figure 2. $(AG)_9C$ and $(GA)_9C$ primers amplified 28 (A_1 to A_{28}) and 36 (G_1 to G_{36}) fragments, respectively. The percentage of polymorphic bands in different populations varied from 68 to 75% and 64 to 78% in $(AG)_9C$ and $(GA)_9C$ ISSR fragments, respectively (Table 1). Percentages of polymorphic bands in different populations were from 69% in M_B to 77% in A_H and K_O, with narrow variation among
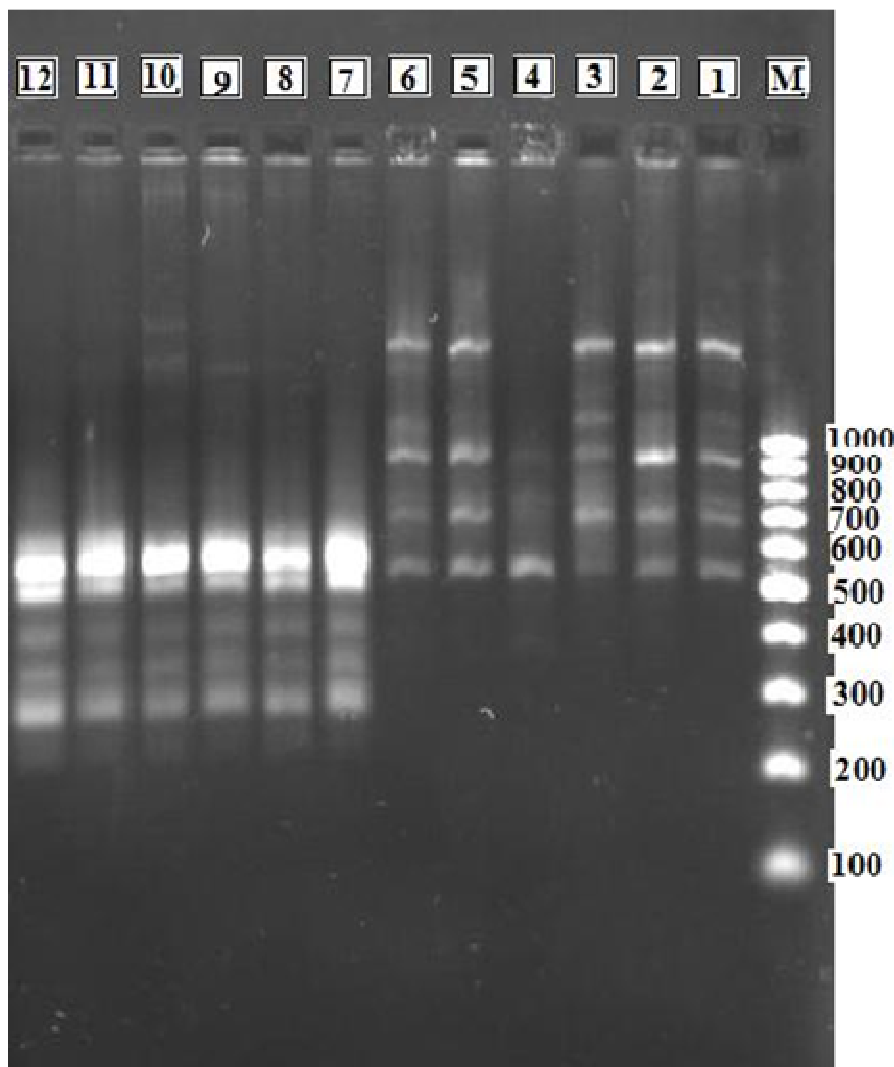
Figure 2. ISSR-PCR patterns obtained by $(AG)_9C$ and $(GA)_9C$ primers in K_G (1 to 6) and primer in A_H (7 to 12), respectively; M: 0.1 Kb ladder.

populations (Table 1). However, all ISSR fragments were 100% polymorphic in the pooled population.

Population gene diversity

Gene diversities (heterozygosity) of different populations detected by different ISSR markers are presented in Table 1. Nei's gene diversity for different populations was from 0.1194 in K_G to 0.1542 in M_A by $(AG)_9C$, from 0.0836 in M_B to 0.1238 in A_V by $(GA)_9C$ and from 0.1046 in M_B to 0.1275 in A_H by both primers. It seemed that the studied subpopulations of *Mehraban* sheep have relatively similar genetic diversities. Generally, in the pooled population, $(AG)_9C$ detected slightly more Nei's gene diversity (0.1444) in comparison to $(GA)_9C$ (0.1114). The results of gene diversity, detected by Shannon's index, were similar to the results of Nei's diversity (Table 1).

Shannon's index for different populations was from 0.2043 in K_G to 0.2556 in M_A by $(AG)_9C$, from 0.1493 in M_B to 0.2140 in A_V by $(GA)_9C$ and from 0.1815 in M_B to 0.2192 in A_V by both primers. In the pooled population, Shannon's index detected by $(AG)_9C$ (0.2526) was higher than that of $(GA)_9C$ (0.2045). In both Nei's gene diversity and Shannon indices, gene diversities detected by $(AG)_9C$ were higher than that of $(GA)_9C$. However, the results obtained from different ISSR primers, were similar to the results obtained, when they were used together.

For *Kermani* sheep (another native breed of sheep in Iran), Esfandyarpour et al. (2008), reported Shannon's information indices of 0.9107 and 0.8940 and Nei's gene diversity indices of 0.5699 and 0.5540, for $(AG)_9C$ and $(GA)_9C$ markers, respectively, which are noticeably higher than the gene diversity of *Mehraban* sheep in this study. Esmaeilkhanian and Banabazi (2006) obtained the highest average gene diversity of 0.847 in five micro-

Table 1. Genetic variability of the studied population, detected by ISSR primers.

Population	Number of polymorphic loci	Percentage of polymorphism	Nei's genetic diversity	Shannon index
Marker: $(AG)_9C$				
A_V	21	75	0.1303	0.2258
A_H	21	75	0.1440	0.2401
K_G	19	68	0.1194	0.2043
K_O	21	75	0.1384	0.2331
M_A	20	71	0.1542	0.2556
M_B	21	75	0.1317	0.2229
Total	28	100	0.1444	0.2526
Marker: $(GA)_9C$				
A_V	27	75	0.1238	0.2140
A_H	28	78	0.1147	0.1985
K_G	26	72	0.1031	0.1821
K_O	28	78	0.1155	0.2023
M_A	26	72	0.0950	0.1792
M_B	23	64	0.0836	0.1493
Total	36	100	0.1114	0.2045
Markers: $(AG)_9C$ and $(GA)_9C$				
A_V	48	75	0.1266	0.2192
A_H	49	77	0.1275	0.2167
K_G	45	70	0.1102	0.1918
K_O	49	77	0.1255	0.2158
M_A	46	72	0.1209	0.2126
M_B	44	69	0.1046	0.1815
Total	64	100	0.1258	0.2256

Table 2. Pairwise Nei's unbiased genetic distances among the populations studied.

Population	A_H	K_G	K_O	M_A	M_B
A_V	0.0124	0.0076	0.0097	0.0077	0.0088
A_H		0.0165	0.0133	0.0179	0.0181
K_G			0.0018	0.0068	0.0053
K_O				0.0039	0.0034
M_A					0.0000
M_B					

satellite markers among five studied populations of Iranian sheep. This was higher than the result found in this study. The genetic diversity reported by Esmaeilkhanian and Banabazi (2006) is an inter-breed genetic diversity, while the genetic diversity detected in this study was intra-breed diversity. The higher genetic diversity detected in *Kermani* sheep (Esfandyarpour et al., 2008) was probably due to the high geographical distances of *Kermani* sheep flocks in Kerman province.

Genetic distances of populations

The results of the estimated Nei's unbiased measures of genetic distances (Nei, 1978) for the studied populations are presented in Table 2. Accordingly, the highest genetic distance among the populations was 0.0181, which indicates a high genetic similarity between the populations studied. Genetic distances of the studied populations were in the range reported for 18 populations of Tuvinian short-fat-tailed sheep (Stolpovskii et al., 2010) and much lower than the genetic distances of 0.18 to 0.559 estimated between five Iranian breeds of *Sanjabi*, *Kurdistan Kordi*, *Khorasan Kordi*, *Mehraban* and *Moghani* sheep (Banabazi et al., 2007).

A dendrogram for genetic distances of the studied populations is illustrated in Figure 3. The studied popu-
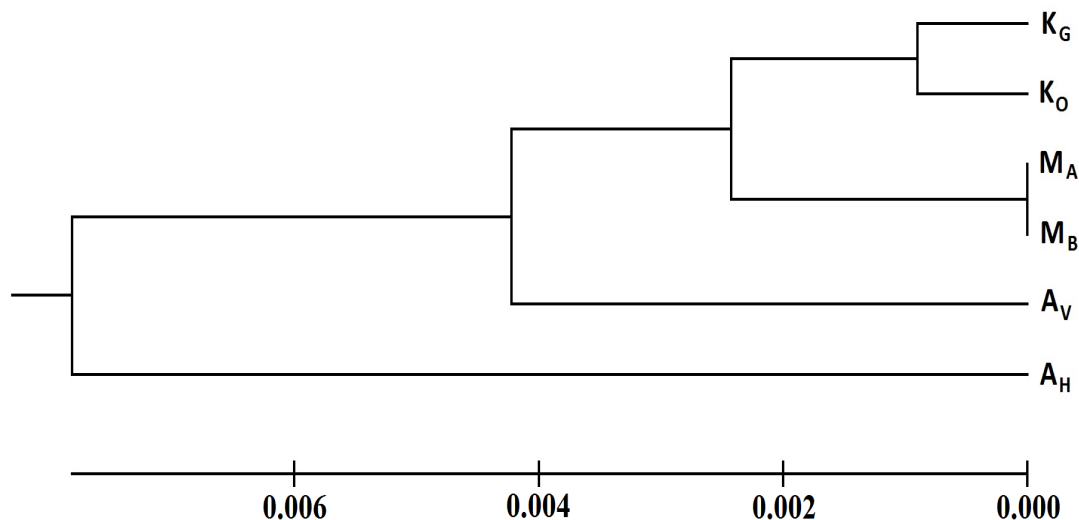
Figure 3. Estimated Nei's genetic distances (Nei, 1978) for the populations studied, using unweighted paired-group method using an arithmetic average (UPGMA) method.

Table 3. Analysis of molecular variance among six populations of *Mehraban* sheep using ISSR markers.

Source of variation	Degree of freedom	Sum of squares	Variance component	Percentage of variation (%)
Among populations	5	66.952	13.390	9
Within populations	204	613.943	3.010	91
Total	209	680.895		

lations in different areas had low genetic distances and were finely grouped according to geographical distances. However, A_H was an exception, which was found distant from other populations. After seeking to find an answer for this observation, it was found that, in the A_H population, *Mehraban* sheep, was to some extent mixed with the *Afshari* sheep (another Iranian breed, close to the *Mehraban* sheep). The Afshari sheep was determined with direct observation. These results indicated that $(AG)_9C$ and $(GA)_9C$ ISSR primers, finely show genetic distances and are useful for genetic study of populations.

Analysis of molecular variance

The result of AMOVA is illustrated in Table 3. The AMOVA of the six studied populations showed that 9% of total variation was inter-populations and 91% was related to intra-population. Low inter-population variation means that the *Mehraban* sheep in different parts of its region had a low variation.

Hardy-Weinberg equilibrium

The studied populations were at Hardy-Weinberg equili-

brium for 75% of the total ISSR fragments. Disequilibrium in 25% of the ISSR fragments could be attributed to the genetic mutation in some of ISSR fragments and the low number of the population investigated in this study (Falconer and Mackay, 1996).

Low genetic diversity of the studied populations and Hardy-Weinberg equilibrium in most of ISSR fragments indicate that there are no noticeable selection and immigration in the population of *Mehraban* sheep. This means that *Mehraban* sheep is a native pure breed and could be noticed for its potentials in response to selection and crossing with other breeds. Low genetic diversity of the *Mehraban* sheep is probably due to small population size and high frequency of inbreeding mating in *Mehraban* sheep flocks. However, the use of more ISSR primers may detect more genetic variations in the *Mehraban* sheep.

However, further researches with intensive sample collection and more markers, especially co-dominant markers, are needed for clearer understanding of the genetic structure of *Mehraban* sheep.

Conclusion

The studied ISSR primers produced polymorphic loci and

seemed to be useful for the population genetic studies of *Mehraban* sheep. Most observed variations were significantly related to within populations. In other words, the studied Mehraban's subpopulations represent characterized *Mehraban* sheep. The results of the study showed that *Mehraban* sheep is a native pure breed and could be noticed for its potentials in response to selection or crossing with other breeds. More researches are needed for more understanding of genetic structure of *Mehraban* sheep.

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