Genetic Diversity of Iranian Mehraban Sheep Using Two Inter Simple Sequence Repeat (ISSR) Markers

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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 Iran is Mehraban sheep. The origin of Mehraban sheep is Hamedan province in the western parts of Iran. This breed is adapted to harsh and rocky environments. The Mehraban sheep is a fat-tailed carpet wool sheep and usually has a light body and dark face and neck brown colour. Mehraban sheep is primarily used for meat production. Mean birth weight and body weight at 90 das of age, and average pre-weaning daily gain are 3.88 kg, 21.58 kg 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 3 million heads as a previous approximation (Bathaei, 1993). This means that Mehraban sheep is in danger of extinction and needs to conservation.

On the other hand, genetic variation is a basic requirement for animal breeding, whereas a high genetic variation is needed for genetic improvement of domestic animals. In recent years, genetic markers are increasingly used for study of genetic diversity. Moreover, the polymorphism determined by these markers is one of the valuable parameters for study of populations and understanding of their genetic differences. Inter-simple sequence repeat (ISSR) marker is a DNA marker and can be used without knowing the sequence information for genomic DNA (Zietkiewicz et al., 1994). The ISSR marker technique involves polymerase chain reaction (PCR) amplification of DNA using a single primer composed of a microsatellite sequence, The ISSR has mild technical difficulty, good reproducibility and reasonable cost, permitting its use for genetic studies of population (González et al. 2005; Wang et al. 2008).

The aim of this research was to assay two ISSR primers and determine the level of genetic diversity and differentiation of Mehraban sheep in different areas of its origin.

Material and Methods

Two hundred ten (210) Mehraban sheep in 6 flocks, 35 heads each, in Hamedan province were used for this study. The studied flocks were located in Ovj-Tapeh and Gol-Tapeh villages in Kabood-Rahang area (K_o and K_G, respectively), Abbas-Abad and Bahareh

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villages in Malayer area (M_A and M_B, respectively) and Haji-Abad and Vender-Abad villages in Asad-Abad area (A_H and A_V, respectively).

Blood samples were collected in EDTA contained tubes. DNA was extracted using DIAtom DNA Prep 100 kit. Polymerase chain reaction (PCR) was performed using PCR Master Mix kit. Two different ISSR primers including $(AG)_9C$ and $(GA)_9C$ were used for PCR.

Amplification was performed in a 96-well My Cycler thermal cycler, under the following conditions: 2 min at 94 ^oC for 1 cycle followed 30 s at 94 ^oC, 30 s at 55 ^oC, and 2 min at 72 ^oC for 35 cycles, and 2 min at 72 ^oC for final extension.

The PCR products were electrophoresed in 1% agarose gel and photography was performed by a gel documentation system. Alleles were scored and band sizes were determined using ONE-Dscan software. The data were analyzed using POPGENE and NTSYS.

Results and Discussion

In overall, by use of $(AG)_9C$ and $(GA)_9C$ primers, 28 fragments (from 100 to >3100bp) and 36 fragments (from 100 to >3100bp) were identified in PCR products, respectively.

Both of ISSR primers showed polymorphic fragments, which approve their efficacy for analysis of genetic diversity.

The results of estimated genetic distances of studied populations are illustrated in figure 1. As it could be shown in this figure, the studied populations in different areas had low genetic distances, whereas 5 flocks had a similarity more than 0.8. The studied populations were finely grouped according to the area. However, A_H was an exception. After seeking to find an answer for this problem, we found that Mehraban sheep in A_H flock is mixed with the Afshari sheep (another Iranian breed, closed to the Mehraban). These results mean that $(AG)_9C$ and $(GA)_9C$ ISSR primers finely show the genetic distance and are useful for genetic study of populations.

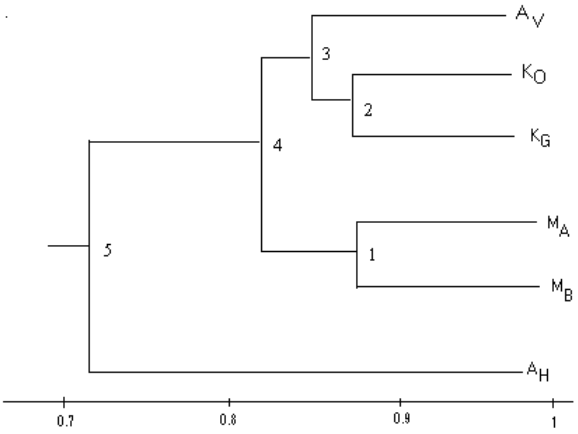

Figure 1: Estimated genetic similarities of studied populations, using Nei's Index

Shannon's information (Lewontin, 1972) and Nei's gene diversity (Nei, 1973) indices and number of effective alleles were 0.2526, 0.1444, 1.1940 for $(AG)_9C$ primer and 0.2045, 0.1114, and 1.1465 for $(GA)_9C$ primer, respectively.
In Kermani sheep (a native breed of Iran), Esfandyarpour et al. (2008) reported 0.9107 and 0.8940 as Shannon's information indices and 0.5699 and 0.5540 as Nei's gene diversity indices for $(AG)_9C$ and $(GA)_9C$ markers, respectively. In Holstein cattle, using $(GA)_9C$ ISSR marker, Shannon and Nei indices were reported as 0.11 and 0.07, respectively (Pashaei et al., 2009). This means that the genetic diversity of Mehraban sheep is less than Kermani sheep and slightly higher than Holsteins.

The studied population was at Hardy-Weinberg equilibrium for most of ISSR fragments. The equilibrium of the studied population along with a low genetic diversity of animals reveal that Mehraban sheep is a limited breed, without any selection and immigration. Moreover, this breed probably has a high level of inbreeding. This means that Mehraban sheep is a pure breed and must be noticed for its potential ability to cross with other breeds.

Conclusion

The studied ISSR primers in this study, $(AG)_9C$ and $(GA)_9C$ are useful for genetic study of populations.

The studied populations in different areas were similar and had low genetic distances. Mehraban sheep is a pure breed and has a low genetic diversity. This breed probably has a noticeable potential to cross with other breeds.

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