Analysis of genetic diversity in Iranian mohair goat and its color types using Inter Simple Sequence Repeat (ISSR) markers.

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ABSTRACT

Iran is one of the most important mohair-producing countries in the world. Markhoz goat is the only breed producing mohair in Iran that is showing endangerment trend in its native breeding region. The aim of this study was to evaluate the genetic diversity of Markhoz goat and its color types using ISSR markers. Four primers were designed and two of them were selected based on their number of bands and polymorphic properties. These primers generated a total of 28 amplification products, from which 23 bands were polymorphic, revealing 82.14% polymorphism. The average (± standard error) of gene diversity (h) in Markhoz goat was 0.34 ± 0.18. The study of genetic diversity in Markhoz color types (Saddle Brown, Sandy Brown and Black) determined that total heterozygosity (Ht), heterozygosity within color types (HS) and the diversity between color types (Dst) were 0.33, 0.30 and 0.03, respectively. The coefficient of population differentiation (Gst) was 0.91. These results indicate that the genetic variation between color types is a small part of the total heterozygosity. The genetic distance between colors types using Nei standard genetic distance and Nei unbiased genetic distance methods was calculated. Phylogenetic danderogram of color types grouped Saddle Brown and Sandy Brown types in one cluster and Black types in another cluster that in agreement with their phenotypic similarities. The results obtained from genetic diversity of color types indicated that the highest and lowest genetic diversity exist in Saddle Brown and Black color types, respectively. Then it seems, to conserve all color types, the breeding programs should be conducted to improve genetic diversity in Black color type.

Keywords: Genetic distance, Markhoz goats, molecular marker, polymorphism, Shannon index.

Abbreviations:

Dst: diversity between color types; Gst: coefficient of population differentiation; h: average heterozygosity; Hs: heterozygosity within color types; Ht: total heterozygosity; I: Shannon’s information index; Na: observed number of alleles; Ne: effective number of alleles; PPB: percentage of polymorphic bands.

INTRODUCTION

The archaeozoological evidence suggests that Iran is one of the most important origins of sheep and goat domestication (Zeder, 1999; Moradi et al., 2012). The beginning of Markhoz goat rising in Kurdistan goes back to 10,000 years ago. The bones of a goat (Capra hircus) found at Ganj Dareh in Kermanshah province of Iran, shows the earliest secure evidence of goat domestication in Kurdistan (Zeder and Hesse, 2000). Markhoz goat is only mohair goat in Iran and their mohair sales is the main source of income for producer whereas kids sale and milk production of this animal are of secondary importance (Rashidi et al., 2006). The coated color of this goat is White, Sandy Brown, Saddle Brown, Grey, and Black (Rashidi et al., 2008). The mohair obtained from these animals has also an important cultural role in making of local clothes in Kurdistan (Fig. 1).

In recent years, Iran has been one of the most important mohair producing countries in the world while the only mohair goat in this country, namely Markhoz goat, has been showing endangerment trend in its native breeding region. The size of population was dramatically reduced over the last decade from 25000 (Rashidi et al., 2008) to less than 5000 heads (unpublished data). This event makes it further vulnerable, to the various forces of genetic change and thus, modifying the foundation genetic structure of the breed (Khalldari et al.,...
2013). Therefore, any immediate conservation program for improvement of this breed would be very crucial. Such plan firstly includes a genetic analysis in inter and intra-population levels in this breed. Therefore, the present study of genetic variation within Markhoz goat population and their color type's relationship may provide genetic information to be used for conservation and improvement of this wonderful goat of Iran.

Recent advances in DNA molecular technology have provided new opportunities to assess genetic diversity at the DNA level (Vostry et al., 2011). ISSR markers has currently been one of the most useful marker of choice for a wide range of molecular genetic studies such as establishing population structure, population differentiation and reconstruction of phylogenetic relationships among populations specially in plants (Wang et al., 2008). In this method, microsatellite sequences of DNA are used as primers for comparison the length of the sequences amplified between them. These markers allow moving from traditional studies of single loci polymorphism (structural genes, microsatellite loci) to an analysis of multiloci spectra representing polymorphism of many genomic fragments. The use of microsatellite loci as primers in PCR to reveal polymorphism of various sites of genomic DNA is based on the act that microsatellites present in a genome with very high frequency and ISSR method marking such polymorphisms lead to reveal multiloci and polymorphic spectra of genomic fragments (Glazko et al., 2009).

To date a relatively small number of examples have successfully evaluated the genetic diversity in different domestic animals using ISSR marker. Glazko (2003) studied relationship among domestic and wild Artiodactyls and Perissodactyla (14 and 7 species, respectively) with ISSR-PCR marker. Triapitsyna and Glazko (2005) studied the genetic structure changes and polymorphism peculiarities of Holstein cattle that reproduced under influence of low-dose irradiation of Chernobyl accident. Some other similar studies have been taken on cattle and sheep spices in recent years (such as Zamani et al., 2009; Stolpovsky et al., 2010), however, to our best knowledge, the studies of ISSR marker in Goat specie is very limited. Yong et al. (2010) and Askari et al. (2011) studied the genetic diversity of Ritu Tibetan Goats and Rayini goat using this marker, although the sequence of primers used in these studies are different to our research.

The objective of this study was to evaluate the genetic diversity in Markhoze goat and their color types using ISSR markers and it seems that this is one of the first attempts to study genetic variation in goats, using ISSR marker technology. The experimental results of this study will provide evidence for the reliability and usefulness of ISSR markers, to estimate genetic diversity within and between goat populations.

**MATERIAL AND METHODS**

**ISSR Analysis:**

Blood samples were collected from 60 unrelated animals on Sanandaj Markhoz goat Performance Testing Station in Kurdistan province, I.R. of Iran (the only place that there are available pedigree and phenotypic records of this breed).

Total nuclear DNA was isolated from the blood cells using Salting out method (Sambrook and Russel, 2001). The DNA was quantified by comparing with known concentration of λ DNA and goat genomic DNA by electrophoresis. The DNA from blood cell was stored at 4°C for future analysis.

Genomic DNA was PCR amplified using ISSR primers obtained from Microsatellite sequences reported on goat genome (Kumar et al., 2005; Gour et al., 2006). ISSR-PCR analysis was carried out with primers containing P1:(AC)3T, P2:(CA)3T, P3:(TG)3CAC and P4:(GT)3C, and finally P1 and P2 were selected owing to the existence of polymorphic properties in the gel. The PCR was performed in a reaction volume of 25µl containing around 150ng DNA template, 0.1-1 µM of primer, 12.5 µl Master mix kit (Sinnagene Co., Iran) and dH2O (variable). Amplification was carried out under the following conditions: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 2 min. An additional extension step at 72°C for 10 min was included at the end of 35th cycle.

The PCR products were separated electrophoretically on 2% agarose gel (for (AC)3T primer) and 8% denaturing polyacrylamide gel (for (CA)3T primer) and visualized over the UV light after Ethidium bromide staining to detect the
amplification. 1Kb DNA Ladder (Fermentas, Germany) was used as a size standard for sizing PCR products. Bands considered manually by at least two persons independently for greater accuracy.

Statistical Analysis:

Only bands were included in the analyses that were unambiguously scored across all samples. The ISSR profiles were scored for each individual as present (1) or absent (0) on the basis of size comparison with 1 Kb DNA Ladder (Fermentas, Germany). Data were analyzed using NTSYSpc software, version 2.02 (Rohlf, 1992). Similarities between individuals were estimated using Jaccard coefficients similarity (Sneath and Sokal, 1973) and the resulting pairwise similarities were expressed as dendrograms. Inter population genetic variation: observed number of alleles (N_a), effective number of alleles (N_e), Nei’s gene diversity (h) and Shannon’s information index (I), were calculated using the PopGene program (Population Genetic Analysis) version 1.31 (Yeh et al., 1999).

The genetic structure of Markhoz color types was further investigated using Nei’s gene diversity statistics, including the total genetic diversity (H_T), genetic diversity within populations (H_S) and the relative magnitude of genetic differentiation among populations (G_ST = (H_T-H_S)/H_T) (Nei, 1978).

Nei’s genetic distances (Nei 1972, 1978) between color types were calculated using the PopGene32 program. All cluster analyses were conducted using UPGMA (unweighted pair group method with arithmetic mean) and the resulting clusters were expressed as dendrograms.

RESULTS

ISSR Variation:

In the preliminary study, the repeatability of the PCR products was initially examined by the four primers on both agarose and polyacrylamide gels for 10 samples twice. The test indicated that the patterns of ISSRs are highly polymorphic and reproducible where using (AC)_9T and (CA)_9T primers. Thus, these two primers were selected for ISSR-PCR analysis. The two selected primers generated altogether 28 unambiguous bands of which 23 (82.14%) were polymorphic (Table 1). The sizes ranged from 200 to 1000 bp. The numbers of bands varied from 13 to 15 for (AC)_9T and (CA)_9T primers, respectively (Fig. 2 and 3).

![Fig. 2. Amplification product patterns obtained using primer (AC)_9T (ISSR-PCR) on 2% agarose gel. Note: Lane 35-43 represent goat individual. M is DNA ladder marker.](image)

Table 1. The nucleotide sequences of the two selected primers, the numbers of bands scored, percentage of polymorphic bands, observed number of alleles, effective number of alleles, average heterozygosity and Shannon index.

<table>
<thead>
<tr>
<th>Primer sequence (5’→3’)</th>
<th>Number of bands</th>
<th>Polymorphic bands</th>
<th>PPB^†</th>
<th>N_a</th>
<th>N_e</th>
<th>h (S.D.)</th>
<th>I (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(AC)_9T</td>
<td>13</td>
<td>9</td>
<td>69.23</td>
<td>1.69 ± 0.48</td>
<td>1.54 ± 0.49</td>
<td>0.29 ± 0.22</td>
<td>0.42 ± 0.31</td>
</tr>
<tr>
<td>(CA)_9T</td>
<td>15</td>
<td>14</td>
<td>93.33</td>
<td>1.93 ± 0.25</td>
<td>1.70 ± 0.29</td>
<td>0.39 ± 0.13</td>
<td>0.56 ± 0.17</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>23</td>
<td>82.14</td>
<td>1.82 ± 0.39</td>
<td>1.62 ± 0.36</td>
<td>0.34 ± 0.18</td>
<td>0.50 ± 0.25</td>
</tr>
</tbody>
</table>

*PPB: Percentage of polymorphic bands; N_a: observed number of alleles; N_e: effective number of alleles; h: average heterozygosity; I: Shannon index.

Table 2. The sample size, numbers of bands scored, percentage of polymorphic bands, average heterozygosity and Shannon index of Markhoz color types.

<table>
<thead>
<tr>
<th>Color type</th>
<th>Sample size</th>
<th>No. of scorable bands</th>
<th>No. of polymorphic bands</th>
<th>PPB^†</th>
<th>h (S.D.)</th>
<th>I (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saddle Brown</td>
<td>38</td>
<td>28</td>
<td>23</td>
<td>82.14</td>
<td>0.34 ± 0.18</td>
<td>0.49 ± 0.25</td>
</tr>
<tr>
<td>Sandy Brown</td>
<td>15</td>
<td>28</td>
<td>20</td>
<td>71.43</td>
<td>0.29 ± 0.20</td>
<td>0.42 ± 0.29</td>
</tr>
<tr>
<td>Black</td>
<td>7</td>
<td>28</td>
<td>18</td>
<td>64.29</td>
<td>0.27 ± 0.21</td>
<td>0.39 ± 0.30</td>
</tr>
</tbody>
</table>

*PPB: Percentage of polymorphic bands; h: average heterozygosity; I: Shannon index.
Fig. 3. Amplification product patterns obtained using primer (CA)_T (ISSR-PCR) on 8% denaturing polyacrylamide gel. Note: Lane 45-58 represents goat individuals. M is DNA ladder marker.

Table 3. Nei’s genetic distances from ISSR data, using the PopGene program. The standard genetic distances are below the diagonal and corrected distances, above the diagonal.

<table>
<thead>
<tr>
<th>Population</th>
<th>Saddle Brown</th>
<th>Sandy Brown</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saddle Brown</td>
<td>---</td>
<td>0.0055</td>
<td>0.0709</td>
</tr>
<tr>
<td>Sandy Brown</td>
<td>0.0162</td>
<td>---</td>
<td>0.0778</td>
</tr>
<tr>
<td>Black</td>
<td>0.0894</td>
<td>0.1000</td>
<td>---</td>
</tr>
</tbody>
</table>

Genetic Variation:
Genetic characterization of Markhoz goat intra-population has been shown in Table 1. The total percentage of polymorphic bands (PPB) was 82.14, the mean observed number of alleles (N_a) was 1.82, while the mean effective number of alleles (N_e) was 1.62. Total Nei’s gene diversity (h) and Shannon’s information index (i) were 0.34 and 0.5, respectively.

In a phylogenetic analysis, the UPGMA individual dendogram, in 70% Jaccard’s similarity coefficients, indicated that all animals could be distinguished in main six separated clusters by ISSR markers (Fig. 4).

Markhoz Color Type:
The information of ISSR markers in assessing genetic diversity of Markhoz color types was summarized in Table 2. The results obtained from color types indicated that the highest and lowest polymorphism, genetic diversity and Shannon index were existed in Saddle Brown and Black color types, respectively. Among the three forms of Markhoz color types, total heterozygosity (H_t), the average heterozygosity within populations (H_s) and diversity between the three populations (D_ST) were 0.33 ± 0.03, 0.30 ± 0.03 and 0.03, respectively. The mean G_st value was 0.091, indicating that only 9.1% of the total gene diversity was observed between color types, while the remaining 90.9% was accounted for the within color types component of variation.

In addition Nei’s genetic distance analysis using both standard and unbiased genetic distances indicated that the closest distance was obtained between Saddle Brown and Sandy Brown while the greatest one was between Black and Sandy Brown (Table 3).
Fig. 4. Individual dendrogram constructed from Jaccard similarity coefficient using NTSYS-pc program. The individual dendogram has been separated with different colors in 70% Jaccard’s similarity coefficient.

DISCUSSION

Maintaining the genetic diversity of livestock species is one of the paramount interests for agricultural policies and serious steps are necessary to be taken in order to protect the stocks of local animals which are in danger of extinction (Askari et al., 2011; Vostry et al., 2011). The analysis of genetic diversity is a method to estimate...
the variation present in these populations (Wier, 1996). Therefore, we used ISSR marker to assess genetic variation based on the mean allelic frequency of the 28 ISSR fragment obtained in Markhoz goat using (AC) and (CA) highly polymorphic microsatellite in this study. To our best knowledge, there have not been other references regarding evaluation of genetic variation by similar ISSR primers within any goat population. It can be, however, mentioned that the genetic diversity (h) and Shannon's information index (I) obtained in this study (with 0.34 and 0.5 values, respectively) showed higher than Rayini goat (with the values of 0.065 and 0.104, respectively) (Askari et al., 2011). However, the primers used in our research were different and so it is possible the sequence of ISSR markers used in each study have an effect on the obtained results of the mentioned statistics. Esfandyarpour et al. (2008) reported 0.56 and 0.90 values for genetic diversity and Shannon's information indices in Kermani sheep (a native breed of Iran) and Pashaei et al. (2009) reported these indices as 0.07 and 0.11 in Holstein cattle, respectively. Although the primers were still different and we consider the species differences, however, these results showed that the genetic diversity of Markhoz goat was less than Kermani sheep and higher than Iranian Holstein cattle. The value of genetic diversity in our study was also higher when comparing with other dominant genetic markers such as RAPD (with 0.22 to 0.33 in Moxoto breed populations (Olivera et al., 2005) and AFLP (with 0.21 to 0.24) for Italian goat (Ajmone-Marsan et al., 2001). This may suggest using ISSR markers as a well candidate tools for such these analyses in these animals and the combination of ISSR and other markers will eventually lead to produce a better description of the variation in the stocks of local animals which are in danger of extinction.

An individual dendrogram was generated, using clustering method of UPGMA based on the Jaccard's similarity coefficients matrix. Jaccard's similarity algorithm was used because of the dominant nature of ISSR marker bands and the fact that this algorithm ignores 0/0 matches that theoretically provide less evidence of relationship (Kosaman and Leonard, 2005). This dendrogram indicated that all animals could be distinguished in main six separated clusters (in 70% level) by ISSR markers. This result may be useful for mating management on goat parturition and could suggest the individuals which have been placed in far separated clusters to be considered for mating program.

In regard to color types, sample size for Saddle Brown type were higher (n= 38) compare to others. This was because of the abundance availability of Saddle Brown goat in Sanandaj husbandry station. This type contains valuable mohair in contrast to others and so that more favor to keep them in any breeding program in this region. Besides, most local producers have also been interested to breed Brown color goat over the time, apparently owing to their ability to blend in with their habitat and resulting to protect them from hunting and smuggling in this border line region.

The coefficient of genetic differentiation (GST) was equaled 0.09, which means that 9% of the total molecular variance was existed between Markhoz color types population when using ISSR marker. This was lower than the values detected previously in Italian goats (11%) based on AFLP markers.
(Ajmone-Marsan et al., 2001) and Swissian goats (17%) based on SSR marker (Saitbekova et al., 1999). Such a low level of differentiation present here is not surprising and is attributed to the nature of these investigations and also a difference in the method used. Current study intended to measure intra-population divergence in contrast to other publications, were used SSR and AFLP molecular markers to assess inter-population differentiation.

Another reason for obtaining such low G_{ST} in the current study may be explained by the time shortage of breeding programs for Markhoz goat in Sanandaj center. Intra-breeding program, based on color type, was merely done for about 3 generations and there was no existing plan in earlier times. It is expected that the higher amount of genetic differentiation among three types of Markhoz goat will be obtained over the time in currently breeding program. To sum, the results obtained from genetic diversity of Markhoz goat color types indicated that there is low genetic diversity in Black color compare to other types, and this phenotypic character should be considered for the further breeding program.

CONCLUSION

This study showed that ISSRs marker can be successfully employed to assess the population-level of polymorphism and genetic diversity even when using 2 primers for analysis of genetic diversity in Markhoz goat. These results of study revealed that there is still almost good diversity in this Iranian mohair goat and may supply crucial data for further conservation of gene resource population and will be employed to screen other goat breeds for comparison purposes.

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