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Using Inter Simple Sequence Repeat Multi-Loci Markers for Studying Genetic Diversity in Kermani Sheep

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Abstract

The Kermani sheep has adapted to a tropical region and is an important meat producing animal in Iran and its economic efficiency is mainly dependent on its growth and reproduction ability. The ability of a population to respond adaptively to environmental changes depends on its level of genetic variability or diversity. Thus, genetic diversity in indigenous breeds is a major concern considering the necessity of preserving what may be a precious and irreplaceable richness, regarding new productive demands. Inter simple sequence repeat (ISSR) is the genome region between microsatellite loci. The aim of this study was to evaluate the diversity of Kermani sheep using ISSR markers. DNA was extracted from 100 blood samples of five populations using optimized and modified salting out method. Polymerase chain reaction (PCR) was performed using two ISSR primers (GA)9C and (AG)9C. The amplified PCR fragment sizes ranged from 100 to 3100 bp and primers amplified 28 (A1 to A28) and 36 (G1 to G36) fragments, respectively. Nei's gene diversity was 0.57 and 0.55 for (AG)9C and (GA)9C respectively. Shannon's index detected by (AG)9C (0.91) was higher than that of (GA)9C (0.89). Results showed that this breed has high genetic diversity. Given the economic importance and good genetic diversity of this breed, special consideration should be taken to prevent the breed from extinction. Thorough studies should be carried out to help understand the relationship between meat production or wool quality and markers at different loci to ensure these traits are maintained or improved.

Keywords: Kermani sheep; Genetic diversity; Meat production; Wool quality; ISSR

Introduction

There are more than 50 million heads of sheep in Iran, of 27 breeds and ecotypes [1]. One of the most important breeds of Iranian sheep is Kermani sheep [2]. This local breed lives in the south-eastern of Iran and is a fat-tail breed and well adapted to a wide range of harsh environmental conditions in Kerman province. The ability to adapt to different environmental circumstances is a desirable characteristic of this breed. Using molecular genetics methods similar to DNA markers is one of the best choices for faster and better accomplishment of animal breeding programs [3].

A species without enough genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites [3]. In addition, the ability of a population to respond adaptively to environmental changes depends on its level of genetic variability or diversity [4]. Thus, genetic diversity in indigenous breeds is a major concern considering the necessity of preserving what may be a precious and irreplaceable richness, regarding new productive demands. Conservation should be based on a deep knowledge of the genetic resources of the specific breed. Therefore, it is important to try to genetically characterize indigenous breeds [5] and the applications of molecular genetics have many important advantages [6].

Inter simple sequence repeat (ISSR) is the genome region between microsatellite loci. The ISSR is a molecular marker method which does not need the genome sequence information and leads to multi-loci and highly polymorphic patterns [7]. Each ISSR band corresponds to a DNA sequence delimited by two inverted microsatellites. The ISSR loci are dominant markers with the assumption of only two alleles per locus. It has been shown that the ISSR markers are universal, quick, easy to apply, highly reproducible and polymorphous [8]. The ISSR method has been used in genetic diversity studies in several species such as cattle [9,10], cattle, goat and sheep [4], sheep [1], fish [11], silkworm *Bombyx mori* [12], mouse [13] and so on. It seems that the ISSR markers could be used to find markers associated with major and minor genes controlling important traits. Although many molecular studies have been performed on Iranian Kermani sheep [14-29], but until now, there is a very little data about role of ISSR markers in evaluating animal biodiversity, thus the present study was conducted to study the genetic diversity of Kermani sheep using ISSR multi-loci markers. Monitoring of genetic polymorphisms within populations of Kermani sheep is an important component of breed maintenance and reproductive programs. Characterization of the population genetic structure of Kermani sheep can become the first step toward breed preservation and restoration, and contributes to advancing breeding programs. This is particularly important in Iran, where a method of validating sheep identification through genetic testing has not been completely resolved. Hence, results of this research can help to achieve valuable information for above mentioned purposes.

Materials and Methods

Blood samples were obtained from 100 animals of five populations. DNA was extracted from the whole blood using an optimized and modified salting-out method [30]. Polymerase chain reaction (PCR) was performed using two ISSR primers (GA)9C and (AG)9C, as described in Table 1. The PCR products were electrophoresed on 1% agarose gel

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with 1×TAE buffer at 80 V for 2 h along with 0.1 kb ladder (CinnaGen Co., Iran). The gels were stained with ethidium bromide and visualized under UV light (BTS-20.M, UVItec Ltd., UK). The sizes of amplified fragments were determined using ONE-Dscan software (Scanalytics, Inc., Fairfax, VA). Based on the presence or absence of the bands, the ISSR profiles were scored as 1 or 0, respectively, assuming that each ISSR band is produced by a dominant allele at a given locus. The loci with allelic frequencies between 1 and 99% or frequency of dominant genotype (presence of the band) higher than 2% were considered as the polymorphic loci. Measurement of diversity including gene diversity (H), observed number of alleles (Ne), Shannon's information index etc., were estimated by POPGEN 3.2 software [31]. The ISSR marker data were collected and used to analyze genetic diversity through cluster analysis. A UPGMA tree was prepared using the NTSYS-PC sub-program "Simqual" which used "Sham" coefficient to establish genetic relationships at the molecular level. Polymorphic Information Content (PIC) was estimated by using HET software.

Results

The extracted DNA had good quality (Figure 1). The tested DNA of Kermani sheep used in the present study was amplified using the specific primers and yielded PCR products (Figures 2 and 3).

Primer	Primer sequence (5'-3')	Annealing Temperature (°C)
(AG)9C	5'-AGA GAG AGA GAG AGA GAG C-3'	55
(GA)9C	5'-GAG AGA GAG AGA GAG AGA C-3'	55

Table 1: Characterization of used ISSR primers for studied population of Kermani sheep.

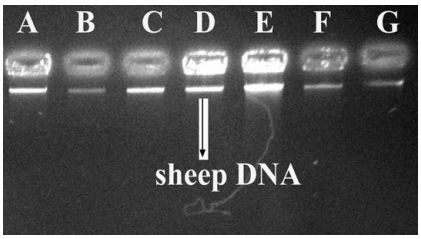
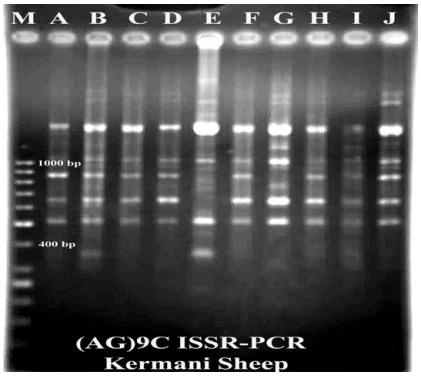
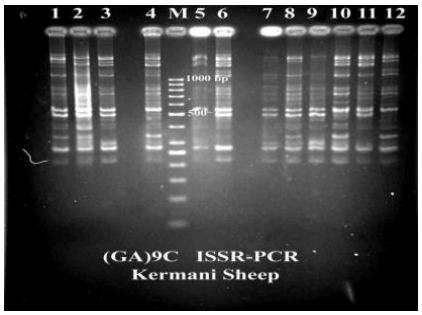


Figure 1: Some samples of extracted DNA from studied animals on 1% agarose gel.



Note: Locus name and its length are: A_1 (100-210), A_2 (310-340), A_3 (350-380), A_4 (420-440), A_5 (459-490), A_6 (500-540), A_7 (550-600), A_8 (610-650), A_9 (660-700), A_{10} (710-780), A_{11} (790-840), A_{12} (850-900), A_{13} (910-1000), A_{14} (1010-1040), A_{15} (1050-1100), A_{16} (1110-1140), A_{17} (1150-1200), A_{18} (1210-1300), A_{19} (1310-1400), A_{20} (1410-1500), A_{21} (1510-1600), A_{22} (1610-1700), A_{23} (1710-1800), A_{24} (1810-1900), A_{25} (1910-2100), A_{26} (2110-2200), A_{27} (2210-2300) and A_{28} (>3100)

Figure 2: ISSR marker profiles of 10 sheep generated by (AG)9C primer in 2% agarose gel. Size marker is M100.



Note: Locus name and its length are: G_1 (100-210), G_2 (220-250), G_3 (260-300), G_4 (310-340), G_5 (350-380), G_6 (390-410), G_7 (420-440), G_8 (450-490), G_9 (500-540), G_{10} (550-600), G_{11} (610-650), G_{12} (660-700), G_{13} (710-780), G_{14} (790-840), G_{15} (850-900), G_{16} (910-1000), G_{17} (1010-1040), G_{18} (1050-1100), G_{19} (1110-1140), G_{20} (1150-1200), G_{21} (1210-1300), G_{22} (1310-1400), G_{23} (1410-1500), G_{24} (1510-1600), G_{25} (1610-1700), G_{26} (1710-1800), G_{27} (1810-1900), G_{28} (1910-2100), G_{29} (2110-2200), G_{30} (2210-2300), G_{31} (2310-2500), G_{32} (2510-2600), G_{33} (2610-2700), G_{34} (2710-2800), G_{35} (2810-3100) and G_{36} (>3100)

Figure 3: ISSR marker profiles of 12 sheep generated by (GA)9C primer in 2% agarose gel. Size marker is M100.

The amplified PCR fragment sizes ranged from 100 to 3100 bp and (AG)9C and (GA)9C primers amplified 28 (A1 to A28) and 36 (G1 to G36) fragments, respectively. The (GA)9C and (AG)9C primers produced 29 (80.6%) and 24 (85.7%) polymorphic ISSR loci, respectively. Nei's gene diversity was 0.57 and 0.55 for (AG)9C and (GA)9C respectively. The highest and the lowest Nei's index for (AG)9C loci were 0.62 and 0.49 and for (GA)9C loci were 0.60 and 0.23, respectively. Mean of Nei's index for 2 markers together was 0.56 ± 0.06. Shannon's index detected by (AG)9C (0.91) was higher than that of (GA)9C (0.89). The highest and the lowest Shannon's index for (AG)9C loci were 1.03 and 0.69 and for (GA)9C loci were 0.99 and 0.39, respectively. Mean of Shannon's index for 2 markers together was 0.90 ± 0.11. Numbers of observed alleles and number of effective alleles for (AG)9C loci were 2.93 and 2.34 and for (GA)9C loci were 2.94 and 2.28, respectively. Mean of numbers of observed alleles and number of effective alleles for 2 markers together were 2.94 ± 0.23 and 2.31 ± 0.23, respectively. Expected heterozygosity and observed heterozygosity for (AG)9C loci were 0.57 and 0.99 and for (GA)9C loci were 0.56 and 0.96, respectively. Mean of expected heterozygosity and observed heterozygosity for 2 markers together were 0.56 ± 0.06 and 0.97 ± 0.12, respectively.

A cluster analysis was carried out, based on the Dice genetic similarity coefficient between individuals for ISSR markers, and an UPGMA dendrogram was generated that represented the genetic relationship among individuals (Figure 4).

Discussion

Biodiversity among domestic animals in development countries is enormous, however with the introduction of superior animal breeds with excellent performance the native animal resources with good adaptability but lower productivity are in great danger [4]. Farm animal genetic diversity is required to meet current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objectives. Production efficiency in pastoral species is closely tied to the use of diverse genetic types, but greater genetic uniformity has evolved in intensively raised species [4]. In this study, for Nei's gene diversity, Shannon indices, expected heterozygosity and observed heterozygosity, gene diversities

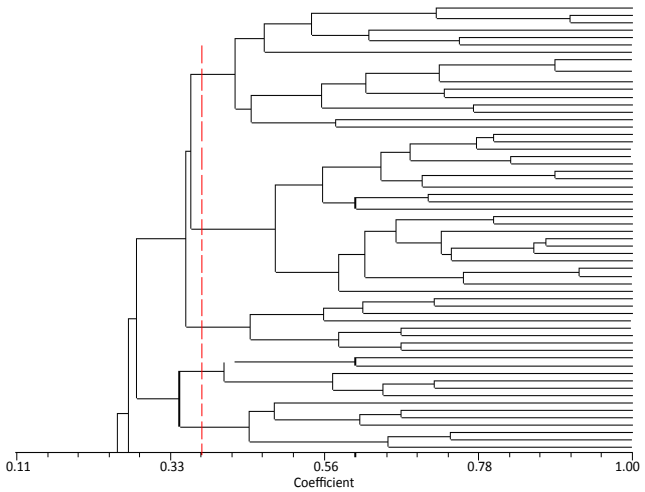


Figure 4: Dendrogram illustrating genetic relationships among animals in genetic diversity study generated by UPGMA cluster analysis from ISSR bands.

detected by (AG)9C were higher than that of (GA)9C. For other Iranian Mehraban sheep, Zamani et al. [1], reported Shannon's information indices of 0.25 and 0.20 and Nei's gene diversity indices of 0.14 and 0.11, for (AG)9C and (GA)9C markers, respectively, which are noticeably lower than the gene diversity of Kermani sheep in this study. The lower genetic diversity detected in Mehraban sheep [1] was probably due to the low geographical distances of Mehraban sheep flocks in Hamedan province. Genetic variation in Bovinae, quantity and quality of amplified DNA fragments, using ISSR-PCR method, in Mongolian yaks (Bos grunniens) and fifteen cattle breeds were evaluated. Results showed that 53 fragments out of 55 were polymorphic and there were some differences in quantity and quality of observed fragments in yaks and cattle breeds. Generally, more than 90% of the fragments were common in all investigated breeds, but differed in their frequency [32]. However, results of Askari et al. [4] showed 60 polymorphic fragments with some differences in quantity and quality of observed fragments in those three species (cattle, goat and sheep). They found that sheep had the highest polymorphic frequencies amongst the three investigated species, and their findings are consistent with our data in this paper. The haplotype analysis of ISSR markers revealed that, some of them to be significantly less frequent in each species. Furthermore, in these three species some unique haplotypes were introduced in their paper. The studied markers in the present study produced 53 polymorphic ISSR loci (83% of all detected loci). This finding agrees with previous reports on high genetic variability of ISSR loci. Zamani et al. [7] studied Iranian Mehraban sheep breed and produced 51 polymorphic ISSR loci. In a study on 19 breeds and a breeding type of cattle, the (AG)9C and (GA)9C primers detected 66 ISSR loci of which 64 (97%) were polymorphic [10]. In another study, the (GA)9C and(AC)9C primers detected 60 ISSR loci with 100–3100 bp sizes and 26.7–81.7% polymorphism, in different populations of cattle, goat and sheep [4]. Likewise, 82% of detected ISSR fragments were polymorphic in Markhoz mohair goat [33]. The ISSR method has been also applied for study of genetic variability in other species such as silkworm B. *mori* [12] and mouse [13]. Use of genetic markers to account for genetic variation of quantitative traits increases the precision of genetic selection, called marker assisted selection (MAS). The studies on MAS generally tend to focus on mapping a few quantitative trait loci (QTLs) to identify the genes of QTL. These studies often involve single nucleotide polymorphism (SNP) testing in candidate genes based on their physiological action [7]. In the recent years, genomic selection is increasingly applied in animal breeding programs. Breeding values in genomic selection are generally predicted based on SNPs or other DNA markers.

Conclusion

The ISSR loci are highly polymorphic and could be used for genetic diversity studies. Further studies should be performed in the future with a greater number of ISSR markers in order to obtain more accurate results. Genetic relationship among native livestock populations is a priority for managing farm animal genetic diversity. It is suggested that this marker system was found to be efficient in discriminating each genotype at the molecular level and can be used for genetic diversity analysis for livestock animals because a greater understanding of the potential of native species is necessary for supporting long-term genetic improvement.

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