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Najdi, Harri and Aradi Saudi Goat Breeds Possess Genetic Variation Required for Genetic Improvement

[1]Jamal S.M. Sabir, [2,3]Ayman M. Sabry, [2]Nabil S. Awad,
[2]Alaa A. Mohamed and [1]Mohammed H.Z. Mutawakil

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, KSA
[2]Biotechnology and Genetic Engineering Unit,
Scientific Research Deanship, Taif University, Taif, KSA
[3]Department of Cell Biology, National Research Center, Dokki, Giza, Egypt

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Abstract: Seven Inter-Simple Sequence Repeats (ISSR) primers were used to assess and characterize genetic diversity of Najdi, Harri and Aradi Saudi goat breeds. Thirty animals were selected randomly (5 males and 5 females per breed). These seven primers produced 138 ISSR loci (287 fragments). Amplified PCR fragments size ranged from 150 bp to 1250 bp. Most fragments were common in the three breeds. Across breeds percentage of polymorphic loci was 97.8%, where Harri breed had the largest percentage of polymorphic loci 51.03% followed by Najdi (42.03%) where Aradi had the smallest percentage (23.9%). All estimated parameters of genetic diversity such as observed number of alleles, effective number of alleles and polymorphism information index (PIC) showed high polymorphism across the studied loci and breeds. The mean coefficient of gene differentiation (Gst) was 0.59 indicating 29% of the total genetic diversity within breeds (Ht). Cluster analysis was carried out using unweighed pair group method with arithmetic averages (UPGMA) and dendrogram illustrated that Harri and Aradi goat breeds were grouped to gather where Najdi breed was group apart. Thus ISSR markers provide good insight into the genetic diversity available across and within Saudi goat breeds. It also could be concluded that Najdi, Harri and Aradi Saudi goat breeds possess the needed amount of genetic variation required for further genetic improvement of these breeds.

Key words: ISSR Markers · Saudi Goats · Polymorphism Information Content · UPGMA Dendrogram

INTRODUCTION

In Saudi Arabia (SA) goats are one of the prim animal genetic resources. Goat's milk and meat are especially popular in many parts of SA. There are around 1.06 million goats found in SA [1]. Najdi, Harri and Aradi goat breeds are among the most widely spread goat breeds in SA. Najdi breed is locally known for its mutton, where Harri goats are kept for its high milk productivity, nevertheless, Aradi goats produce less milk but it produce milk persistently, therefore, are greatly appreciated by desert dwellers, where it is widely spread [2]. Although, goat is considered the most prolific ruminant among all domesticated ruminants especially under harsh climatic condition [3], with some exceptions, little attention is given to genetic improvement of goat breeds. This abandons may be partially due to that the farmers involved with goat production are frequently small holders with little political influence. This abandons may represent risk factor towards loss of such genetic resources.

Genetic improvement utilizes genetic diversity between and within breeds to improve the efficiency of production. Traditional methods of assessing genetic diversity are now being complemented by the tools of molecular markers, enabling breeders to make better decisions when choosing the germplasm used in breeding programs [4]. The information on molecular genetic

Corresponding Author: Ayman Sabry, Biotechnology and Genetic Engineering Unit, Scientific Research Deanship, Taif University, Taif, KSA., Biology Department, National Research Center, Dokki, Giza, Egypt.

variation and estimation of parameters of genetic diversity (e.g. genetic identity and genetic distance) could help in understanding the association and diversity across and within breeds in addition to quantifying genetic similarities between breeds and even to proxy the expected heterosis gained in crossing the breeds [5, 6].

Inter-simple sequence repeat (ISSR) which is a DNA markers that can be used without knowing the sequence information for genomic DNA [7]. 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 [8, 9]. ISSR markers are especially attractive given their hypervariable nature, the vast number of loci that can be examined and the small amount of fresh deride material used per sample and not to mention, high statistical power for determining differentiation between groups (due to large number of alleles). Only recently, attention has been paid to the use of DNA markers in studying genetic characterization in Saudi goats e.g. [2, 10, 11]. These studies were based on either using Random Amplified polymorphic DNA (RAPD) or microsatellites to characterize genetic diversity in goat breeds. However, genetic characterization should be a continuous process of surveying and monitoring different breeds [12].

As genetic diversity is the primary requisite for genetic improvement of livestock. The objectives of the present study were to investigate the polymorphism in a set of ISSR, to quantify the genetic diversity of Najdi, Harri and Aradi Saudi goat breeds.

MATERIALS AND METHODS

Animals: Thirty animals were randomly selected from three Saudi goat breeds, namely, Najdi, Harri and Aradi. 10 animals per breeds were selected (5 males and 5 females).

Genomic DNA Extraction: Blood samples were collected from 30 animals into vacutainers with EDTA as anticoagulant. Samples were kept at -20°C until use. Genomic DNA was extracted from peripheral blood lymphocytes according to instructions of blood DNA preparation kit (Jena Bioscience, Germany).

PCR Amplification: The PCR amplification was performed in a 25 µl reaction volume, using Promega PCR Master Mix according to the instructions by the manufacturer with 30 Pmol of each of the 7 primers. Initial denaturation at 94°C for 2 minutes, followed by 35 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 2 min and a final extension at 72°C for 2 minutes (Table 1). Amplification products were electrophoresis on 1.5% agarose gel at constant voltage and 1X TBE for approximately 1 hour. There were visualized by staining with ethidium bromide and photographed under ultraviolet light and molecular weights were estimated using DNA 100 bp ladder.

Table 1: List of primers used for ISSR amplification and annealing temperature

Primer	Sequences (5^1- 3^1)	Annealing Temperature °C
A7	$(AG)_8T$	51
A8	$(AG)_8C$	55
A9	$(AG)_8G$	46.6
A11	$(GA)_8C$	51.8
A15	$(CT)_8G$	51.8
P01	$(AG)_9C$	55
P02	$(GA)_9C$	55

Data Analysis: ISSR amplified fragments were scored for band presence (1) or absence (0) and a binary qualitative data matrix was constructed. Data analysis were performed using the NTSYS PC version 2.02 computer package program [13]. The similarity values were used to generate a dendrogram via the un- weighted pair group method with arithmetic average (UPGMA). Measurement of diversity including gene diversity (H), observed number of alleles (Ne), gene flow and Shannons information index were estimated by POP- GEN 3.2 software [14].

RESULTS

Seven ISSR primers were used to screen 30 randomly selected animals from Najdi, Harri and Aradi Saudi goat breeds. These seven primers produced 138 ISSR loci (287 fragments). Amplified PCR fragments size ranged from 150 bp to 1250 bp. Across breeds percentage of polymorphic loci was 97.8%, where Harri breed had the largest percentage of polymorphic loci 51.03% followed by Najdi (42.03%) where Aradi had the smallest percentage (23.9%) Table 2, however this order slightly changed based on the number of polymorphic loci. Table 2 also, shows the estimated parameters of the genetic variation in the three breeds and across breeds as well. For all the estimated genetic variation parameters the Najdi breed scored highest values where Aradi breed had the lowest values. It is also could be observed that

Table 2: Summary of estimated genetic variation parameters for all loci in three Saudi goat breeds

Population	Na[a]	Ne[b]	H[c]	I[d]	No. of polymorphic loci	Percentage of Polymorphic loci
Najdi	1.4±0.5	1.3±0.4	0.15±0.2	0.22±0.3	58	42.03
Harri	1.4±0.5	1.2±0.4	0.13±0.2	0.20±0.3	57	51.03
Aradi	1.2±0.4	1.1±0.3	0.08±0.2	0.13±0.2	33	23.9
Across breeds	2.0±0.2	1.5±0.4	0.29±0.2	0.44±0.2	135	97.8

[a]Na*: Observed number of alleles

[b]Effective number of alleles

[c]Nei's genetic diversity

[d]Shannon's Information index

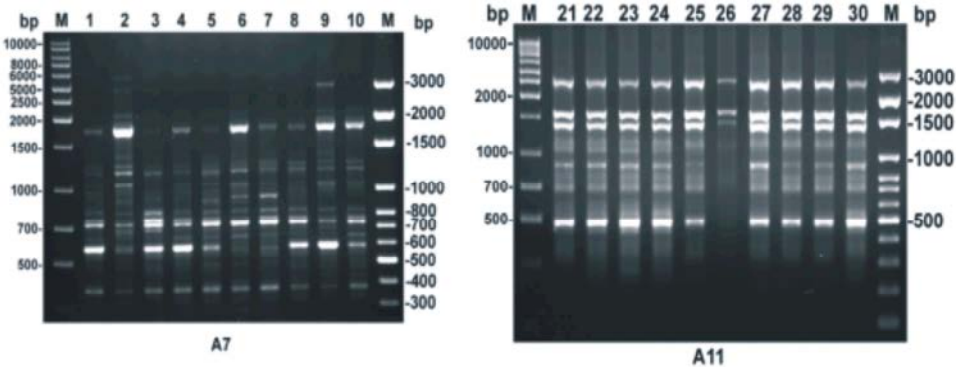

Fig. 1: Electrophoretic band patterns of ISSR, which obtained with primers A7 and A11 in 1.5% agarose gel. Ladders are shown in both sides of the gel

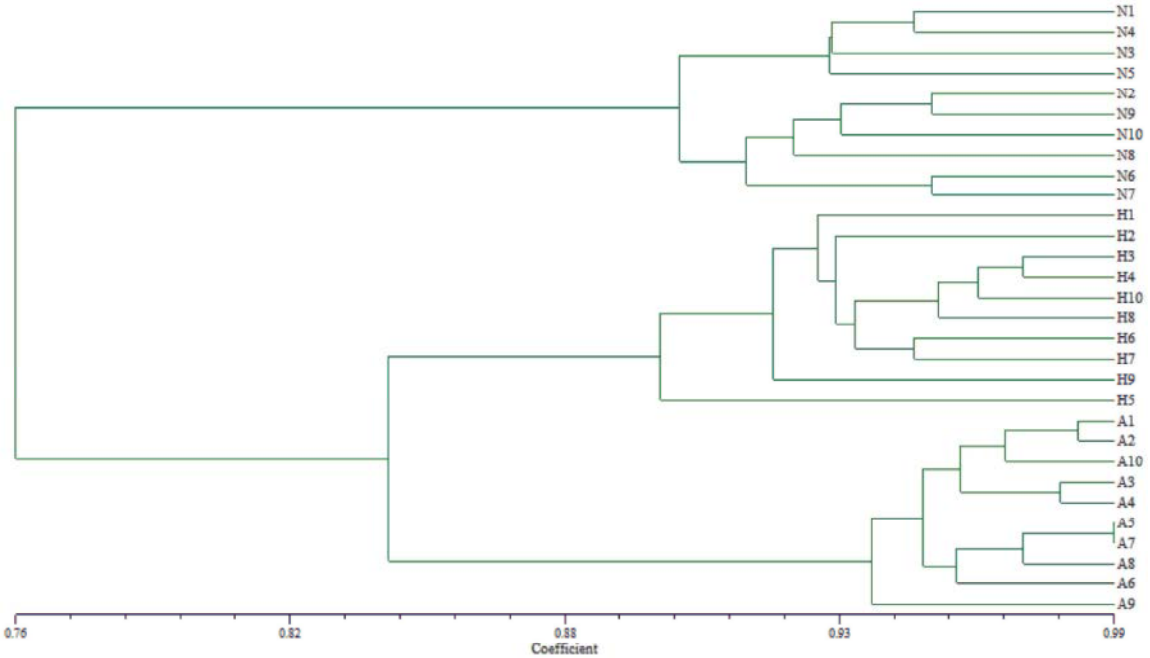

Fig. 2: Dendrogram illustrating genetic relationships among 3 Saudi goat breeds in genetic diversity study generated by UPGMA Cluster analysis from 278 ISSR bands produced by 7 primers

estimated parameters for both Najdi and Harri breeds were higher than Aradi breed. This is also associated with the higher number of polymorphic loci for both Najdi and Harri breed compared to Aradi (Table 2). The mean of observed number of alleles (Na) ranged from 1.4 ± 0.5 in Najdi to 1.2 ±0.4 in Aradi where, across breeds was 2±0.2. The mean Nei's gene diversity (H) ranged from 0.08±0.2 in Aradi to 0.15±0.2 for Najdi breed and the across breeds

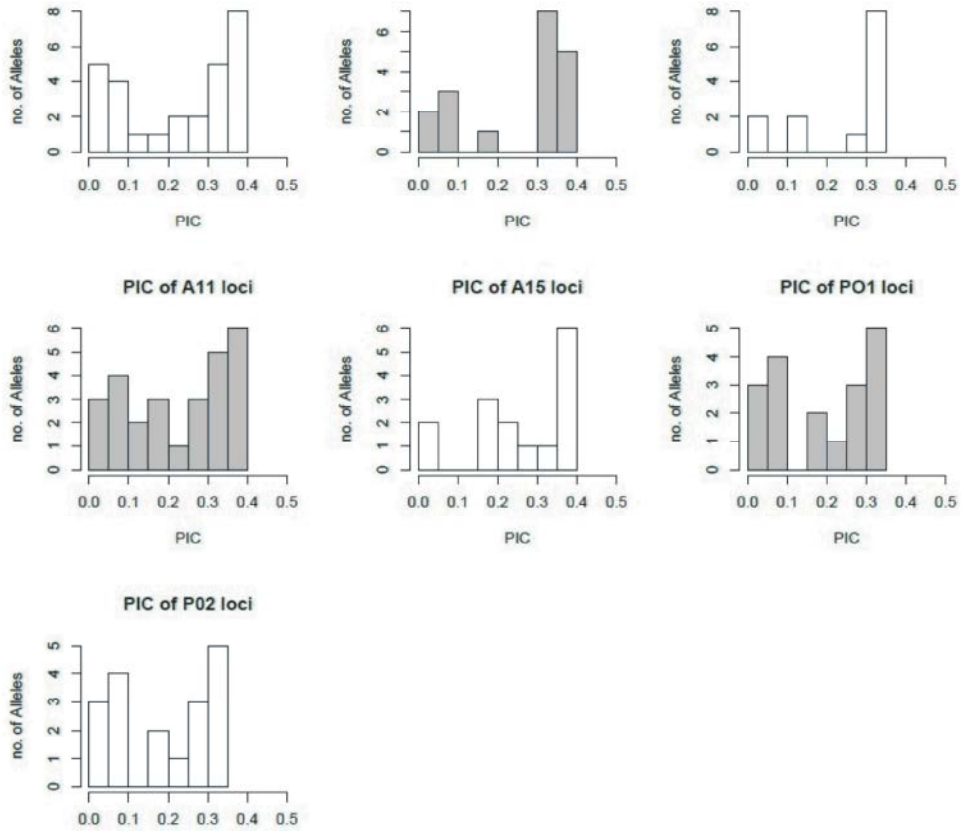

Fig. 3: Polymorphism information content (PIC) of the loci of the 7 primers

Table 3: Overall genetic variation across three Saudi goat breeds

Ht	Hs	Gst	Nm
0.29±0.03	0.12±0.01	0.59	0.35

Ht = Total genetic diversity,
Hs = Neis genetic diversity within subpopulations,
Gst = Mean coefficient of gene differentiation,
Nm =Mean estimated number of gene flow

Table 4: Nei's Original genetic identity (above diagonal) and genetic distance (below diagonal).

Population	Najdi	Harri	Aradi
Najdi		0.6747	0.6543
Harri	0.3935		0.7889
Aradi	0.4243	0.2371	

Table 5: Specific bands observed in one breed

Primer-(pb)	Najdi	Harri	Harri
A7- (180)	√		
A9- (450)			√
A9- (600-800)	√		
A11- (480)		√	
A11- (1000)			√
P01- (350)	√		
P01- (1150)			√
P02- (1100)	√		
P02- (1180)			√

was 0.29±0.02. The range of polymorphic loci ranged from 33% in Aradi breed to 58% in Najdi breed. The Shannon's indices (I) ranged from 0.13±0.2 to 0.22±0.3 where Aradi breed was the lowest index, the mean of Shannon index across family was 0.44±0.2. Among the 3 goat breeds the mean coefficient of gene differentiation (Gst) was 0.59 (Table 3), indicating 29% of the total genetic diversity within the three breeds. Based on the Gst value the mean estimated number of gene flow (Nm) between populations was found to be 0.35 (Table 3).

The Nei [15] measures of genetic distance and identity between each pair of breeds are given in table 4 and indicate that the genetic identity between Harri and Aradi breeds was (0.7889) higher than that between Najdi and each of Harri (0.6747) and Najdi and Aradi (0.6543). The higher value of genetic identity the higher the proportion of genes that identical between the two populations [15]. This result is also supported by the estimates of genetic distances between paris of breeds where the genetic distance between Harri and Aradi was 0.2371 vs. 0.3935 and 0.4243 between Najdi and each Harri and Aradi.

Phylogenetic analysis was carried out to detect the evolutionary relation- ship among the three goat breeds using UPGMA method [16]. The phylogenetic tree presented in Fig. 2 showed that Harri and Aradi goat breeds were grouped to gather where Najdi breed was group apart.

Generally, most fragments ware common in all three breeds, few fragments were rarely found to be breed specific (Table 5). However, primers A8 and A15 produced no specific bands.

Figure (3) showed the Polymorphism information content (PIC) (a parameter associated with the discriminating power of markers [17] values for the 7 ISSR markers. For all markers PIC values ranged from 0.0 to 0.375.

DISCUSSION

In general, farm animal genetic diversity is required to meet current production needs in various environments, to allow continuous genetic improvement and to ease rapid adaptation to changes in breeding objectives [18]. Thus, assessment of genetic diversity considered the first step in developing future of genetic evaluation for the goat breeds in Saudi Arabia. Recently, DNA markers are increasingly used for study of genetic diversity. Moreover, DNA polymorphism is an important parameter for study of populations and understanding of their genetic differences. Due to the use of longer primers and high annealing temperature ISSR markers are advantageous in terms of reproducibility. This makes ISSR markers capable of detecting more genetic loci compared to RAPD [7]. To the best of the authors' knowledge, this study is considered the first to use ISSR markers to assess genetic diversity in Saudi goat breeds. Apart from Askari *et al.* [17], studies of genetic diversity in goat breeds were carried either using microsatellites [11, 19, 20] or Random Amplified Polymorphic DNA (RAPD) [3, 10].

All parameters of genetic diversity such as observed number of alleles, effective number of alleles and PIC showed high polymorphism across the studied loci and breeds, thus providing the eligibility of ISSR markers to assess genetic diversity studies. The results of the present work provide evidence for the reliability of ISSR marker for estimation of genetic diversity within and between Saudi goat breeds. In the present study, the percentage of polymorphic markers within and across breeds indicating substantial genetic diversity at the breed level. The mean value of Ht (Nei's genetic diversity among populations) and the mean coefficient of gene differentiation (Gst) were 0.29 and 0.59 these values are higher than what reported by Askari *et al.* [17] on Iranian Rayini goat. This elevation in Ht and Gst values might be due the larger number of ISSR primers used in our study (7 primers) compared to only 2 primers used by Askari *et al.* [17]. It is also worth to mention that primers P01 and P02 used in both studies. However, the value of Hs (Nei's genetic diversity within subpopulations) on Saudi goat breeds was similar to Hs value of Iranian goat population 0.12 vs. 0.1219. Shannon Information index is a measure of approximate diversity, this measure showed that both Najdi and Harri are genetically more diverse than Aradi, this a reflection of the number of polymorphic loci, 58 and 57 vs. 33 polymorphic loci.

A UPGMA tree was prepared using the NTSYS-PC sub-program "Simqual" which used "Sham" coefficient to establish genetic relationships at the molecular level. The selected genotypes were differentiated and placed as individual entities for ISSR marker system-generated cluster trees. It could be concluded from the topology of this phylogeny tree that Harri and Aradi breeds descended from common ancestor differ from that for Najdi breed. This result is in accordance with what found by Sabir *et al.* [10] using RAPD markers.

Polymorphism information content (PIC) is a measure of a marker for detecting polymorphism within a population and depends on the number and frequency distribution of detectable alleles [21]. Due to the dominance nature of ISSR markers PIC value should be between 0 and 0.5 [22]. PIC values obtained in this study laid within the above mentioned range (0.0–0.375). This range is wider from what reported by Askari *et al.* [17] (0.0987-0.2182), this wider range could be an indication of larger genetic diversity of the three Saudi goat breeds. This difference could be also ascribed to larger number of ISSR primers used in our study.

CONCLUSION

In this work we analyzed the applicability of ISSR as molecular genetic marker to assess and characterize genetic diversity of Najdi, Harri and Aradi goat breeds. All estimated parameters of genetic diversity showed high polymorphism across the studied loci and breeds. Thus ISSR markers provide good insight into the genetic diversity available across and within Saudi goat breeds. It also could be concluded that Najdi, Harri and Aradi saudi goat breeds possess the needed amount of genetic variation required for further genetic improvement of these breeds.

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