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Genetic Variation within and between Three Iranian Goat Populations Using Nine Microsatellite Markers

Research Article

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ABSTRACT

Genetic diversity is essential and mirrors for diversity pattern and population evolution. With this motivation, the aim here is to investigate genetic similarities of three indigenous goats of Iran: Azerbaijan (n=50), Sarbisheh (n=50), Busher (n=29) using 9 microsatellites markers. We extracted genomic DNA and then employed routine polymerase chain reaction (PCR) protocol for amplification of nine specific microsatellite markers. The experimental raw genotype was applied to generate summary of statistics for molecular diversity criteria. From the results, 8 microsatellites visualized satisfactory electrophoresis pattern and reasonable degree of polymorphism. The ILSTS004 (0.42) and BM415 (1.8) microsatellite markers indicated highest and lowest value for Shannon index. We obtain 57-67% range for observed heterozygosity value regarding to Azerbaijan and Sarbisheh goat, respectively. From these results it is clear the high genetic similarity was found between the Sarbisheh and Busher populations. Overall, bottleneck based evidence using IAM and SMM models demonstrated normal 'L'-shaped distribution in investigated Azerbaijan and Sarbisheh goat. In conclusion, this analysis leads evidence for sufficient genetic variation within investigated goat.

KEY WORDS conservation, genetic diversity, indigenous goat, microsatellite markers.

INTRODUCTION

Iran is in particular geographical area with wealth biodiversity animal resource and claimed as center of domestication of animal genetic resources (Vahidi *et al.* 2014). Domestic goat (*Capra aegagrus hircus*) is storehouse of genetic materials claimed as the first small ruminants and assumed as undoubted strategic ancient livestock particularly, for rural families' economy and with has large distribution in worldwide (Zheng *et al.* 2020) A summary of previous literatures demonstrated this species first time domesticated in high Zagros mountain and arid lowland plains of Iraq and Iran. The reported FAO statistics indicated that approximately 674 million goat exist globally and developing countries contributed almost 94% of this value for themselves (Miller and Lu, 2019). Iranian indigenous goats have been classified into 20 ecotype and Azerbaijan, Sarbisheh, Busher are three goats with limited genetic information in the country (Tavakolian, 2000). The spectrum of genetic diversity existing among livestock breed is assay for initiated and designing any conservation plan for maintain this criteria (Zhang *et al.* 2018). Various factors such as reproductive fragmentation, and anthropogenically factors, various breeding plans and physical isolation for a different period contributed for loss of diversity and increase of inbreeding and genetic bottleneck phenomena. Over the time horizon, indigenous specific livestock diversity always experienced loss of diversity forever and if conservation plan not exist then maintaining of this diversity may not be replaced even in future (Askari *et al.* 2011; Zhang *et al.*

2018). Monitoring of genetic diversity indicators is pre-required to understand factors reduced chances of breed survival due to decreased fitness through inbreeding depression. Therefore, molecular genetics offers accurate tools to conserver and access to knowledge about current situation of animal genetic resources for sustainable agriculture (Kevorkian *et al*. 2010). Microsatellites markers are high polymorphic chromosomal regions with one to six base pairs long specific variable motif with CA common pattern in mammalian genome (Amos *et al.*1996; Goldstein and Schlotterer, 1999). Mutation rate in the genomic region of microsatellites is very high rate (Abdul-Muneer, 2014). These markers have several advantages such as relative ease of acquisition, high polymorphism, neutrality, and easy to sample preparation (Crowford *et al.* 1997).

It also detects changes in simple nucleotide repetitions, random and abundant distribution in the genome and their alignment which used for several types of studies (Abdul-Muneer, 2014). The summary of previous reports have been listed specific identified microsatellites in different livestock and poultry genomes such as camelids, cattle, buffaloes, goats, sheep, horses and donkeys, chickens, and ducks (Abdelkader *et al*. 2017; Cherifi *et al*. 2017; Tefiel *et al*. 2018; Abdelkader *et al*. 2020; Rahal *et al*. 2020). In goat linkage map spans 2737 cM with 307 loci linkage map, there are 504 SSR markers assigned for 60 chromosomes (De-Gortari *et al*. 1998), and there is a list of suitable STR markers for genetic study recommend by FAO and genetic society for international comparison of obtained outputs (FAOSTAT, 2020). Therefore, the aim of the study of intra and inter-population genetic relationships three Iranian Azerbaijan, Sarbisheh, Busher indigenous goat population using microsatellite markers.

MATERIALS AND METHODS

Animals and sampling

To perform this research, the sampling process from unrelated animals carried out in three provinces of Iran, namely Azerbaijan (n=50) in northwest and Busher (n=50) in southern region and Sarbisheh (n=29) in both sex goat from Birjand in south Khorasan. To ensure that sampled animals were not closely related, different herds were identified within the districts within each province.

The Azerbaijan goat expressing different coat colors and predominant coat color is black with white spots. This breed indicated reasonable resistant to diseases and harsh weather conditions and mean mature body weight for buck and does is 60 and 52.5 kg in this breed. The Black Busher goat is raising for three system: rural, farm and nomadic.

This breed expressing pure black color long coat fiber and highlights for good adaptation to environmental condition.

Mean mature body weight for buck and does is 35 and 31 kg in this breed. The Sarbishe cashmere goat is a local dual-purpose breed (cashmere and meat) that is well-adapted to dry and semi dry ecological condition of south Khorasan, which can creates employment opportunities in the region and empower rural and nomadic people. Main geographical distribution of this breed is South Khorasan and Sarbisheh (kilometer of 70th of Birjand-Zahedan road), the main breeding center for this breed (Figure 1). In laboratory process, 10 mL of whole blood was collected from the jugular-vein from each animal by using syringes and needles and vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and instantly stored in 4 ˚C and then stored at -20 ˚C for future use. According to the FAO and the International Association of Animal Genetics (ISAG) (FAO, 2004), we selected 9 STR markers (Barker, 1994) with different chromosomal positions (Table 1).

Molecular analysis

The genomic DNA was purified from blood according to the Samadi Shams conventional protocol (Samadi Shams *et al.* 2011). Gel monitoring and NanoDrop 2000/2000c spectrophotometer methods (Thermo Fisher Scientific, USA) were used for quality and quantity measurement test. PCR amplification was performed in a total volume of 15 µL containing master mix kit (Ampliqon, Denmark) 7.5 µL master mix 2X, 1 pmol of each primer (Forward and Reverse), and 4.9 µL ddH_2O, and 1.5 µL of genomic DNA (all these steps were done on ice). Model of PCR Machin for amplification of fragment was Biometra company and the PCR program was used in the PCR machine to replicate the STR loci of the Touch-down PCR specific program was designed to simultaneously amplify the locus and minimize nonspecific and starter bands. The "touchdown" PCR protocol used with initial denaturation of 95 ˚C for 8 min, followed by the first stage of amplification of 12 cycles involving a denaturation step at 94 ˚C for 1min, annealing based on reducing the temperature from 68 ˚C to 52 ˚C for 40 sec, and extension at 72 ˚C for 30 sec, and finally, the final reproduction temperature was set at 72 ˚C for 8 min. The PCR products were determined on 6% agarose gels stained with metaphor in 1X TAE buffer. Allele sizes were estimated using the 11 lines (25-755-bp) ladder (Life science company).

Statistical analysis

Individual genotypes are measured at each location or allele size using UVdoc 99.02 analysis software (UVI Tech, Cambridge, UK), then for preparation of input files for each specific package, CONVERT version 1.31 (Glaubitz, 2004), and CREAT version 1.1 packages (Coombs *et al.* 2008) were employed.

Figure 1 Overview of breed external appearance and main geographical distribution

Table 1 Characteristics of selected loci of SSR markers for this study

Loci	Primer sequence	Chr.	Allele range	Motif
BM4307	ATAACACAAAAAGTGGAAAACACTC ATTTTATCTCAGGTCCCTTTTTATC	1	187-203	(CA)12(TA)6
CSSM004	ATGCGTCCTAGAAACTTGAGATTG GAAATCATCTGGTCATTATCAGTG	1	196-220	(GT)10(TA)5
TEXAN-6	AGGCAGTTACCATGAACCTACC ATTCCTGGTGGGCTACAGTCTAC	1	157-175	(AC)12
CSSM032	TTATTTTCAGTGTTTCTAGAAAAC TATAATATTGCTATCTGGAAATCC	1	200-206	(CA)19
BMC1009	ACCGGCTATTGTCCATCTTG GCACCAGCAGAGAGGACATT	5	265-289	(AC)15
BM2830	AATGGGCGTATAAACACAGATG TGAGTCCTGTCACCATCAGC	5	147-203	-
BM415	CAAATTGACTTATCCTTGGCTG TGTAACATCTGGGCTGCATC	6	137-157	(TG)18
ILSTS004	CTTAAAATCTGTCTTTCTTCC TAGTGTGTATTAGGTTTCTCC	1	100-110	(CA)16
CSSM019	TTGTCAGCAACTTCTTGTATCTTT TGTTTTAAGCCACCCAATTATTTG	1	131-161	-

The molecular measurements indexes such as genotype and allele frequencies, the observed number of alleles (Na), effective number of alleles (Ne), observed (Ho), expected (He) heterozygosity's, Shannon index (I), polymorphic information content (PIC), inbreeding coefficient (F_{IS}), F-statistics (F_{IS}, F_{IT} and F_{ST}), genetic distance, allele admixture and population structure, clustering, analysis of molecular variance (AMOVA), and bottleneck index were calculated. From used different software and the computational package include POPGENE version 1.31 (Yeh *et al.* 1999), ARLEQUIN version 3.5.2.2 (Excoffier and Lischer, 2010), GenAlEx version 6.5 (Peakall and Smouse, 2012), STRUCTURE version 2.3.4 (Pritchard *et al.* 2000), BOTTLENECK version 1.2.02 (Piry *et al.* 1999).

RESULTS AND DISCUSSION

Consideration of molecular genetic diversity criteria is important to understand level of genetic variability of DNA variability to ensure about breed survivability and natural response against harsh condition and environmental stressors such as and feed shortage, drought and heat and disease. Today, the wide diversity of livestock breeds and strains accessible is part of our cultural legacy and it merits protec-

ttion. Wide applicability of SSR in different area of research indicated this marker able to work on genetic diversity, research on genetic bottleneck, quantitative trait locus (QTL) mapping, parentage control, fraud identification and discovery of pedigree errors and sex determination and association study for fecundity and resistance to disease.

The outcomes of this report have been divided into two parts. First part deals the assessment of suitability and polymorphism on selected SSR loci for this investigated goat breed and second part as main goal of research to determine genetic diversity in two level of intra and inter-population genetic relationships three Iranian Azerbaijan, Sarbisheh, Busher indigenous goat population using microsatellite markers

Characteristics of selected microsatellite loci

From the results, all chosen microsatellites indicated satisfactory gel pattern in investigated goat population. Figure 2 shows the actual estimated amplified band per investigated loci through whole investigated population. As we can concludes range of allele size was variable approximately 100 bp 280 bp which is accordance to FAO recommended loci list and most pervious similar literatures (FAO, 2011).

For suitability of chosen microsatellites, loci have to indicated high number of observed allele, observed hetrozygosity and PIC index as well as Shannon index, in this regards, outcomes of our analysis showed: The highest and lowest Shannon index was ranged ILSTS004 (0.42) and BM415 (1.8). Also, the highest and lowest observed hetrozygosity was ranged CSSM32 (0.92) and CSSM004 (0.10). Furthermore, the highest and lowest observed hetrozygosity was ranged CSSM32 (0.92) and CSSM004 (0.10).

As interpretation of outcome is this part, heterozygosity index of loci an appropriate indirectly tells about combination of observed alleles within each loci and as SSR express high number of alleles per loci therefore this heterozygosity highly depend on sample size and number of SSR loci. Our results in this area show in accordance and accordance of some similar reports with this logical justification. Our results showed that the mean of observed and expected heterozygosity in Sarbisheh, Azerbaijan, Busher and total were 0.75 and 0.69, 0.57 and 0.61, 0.69 and 0.64 and 0.67 and 0.66, respectively.

The mean of observed and effective number of alleles were obtained 4.88 and 3.84, 6.88 and 3.16, 5.44 and 3.27 and 8.55 and 3.76 for Sarbisheh, Azerbaijan, Busher and total respectively. The results observed hertozygosity in this research was lowest than for four Tunisian sheep breeds with 30 STR markers (Kdidi *et al*. 2015), in three local sheep breeds in Saudi Arabia, using 17 STR markers (Mahmoud *et al*. 2017) and in four Romania native sheep breeds with 18 STR markers (Dudu *et al*. 2020). These results of present study for Shandon index index was highest then (Greguła-Kania *et al*. 2015) in Poland, while lowest than (Jyotsana *et al*. 2010) in India, (Musthafa *et al*. 2012) in Saudi Arabia and (Hussain *et al*. 2019) in Pakistan.

In addition, in present study, the range in values for He and observed Ho was higher than similar previously published data (Kusza *et al*. 2010) in Bulgaria, (Ocampo *et al*. 2016) in Colombia and (Dossybayev *et al*. 2019) in Kazakhstan.

Moreover, PIC is a parameter indicative of the degree of informativeness of a marker. The PIC value may range from zero to one. Therefore, loci with many alleles and a PIC value of one are most desirable.

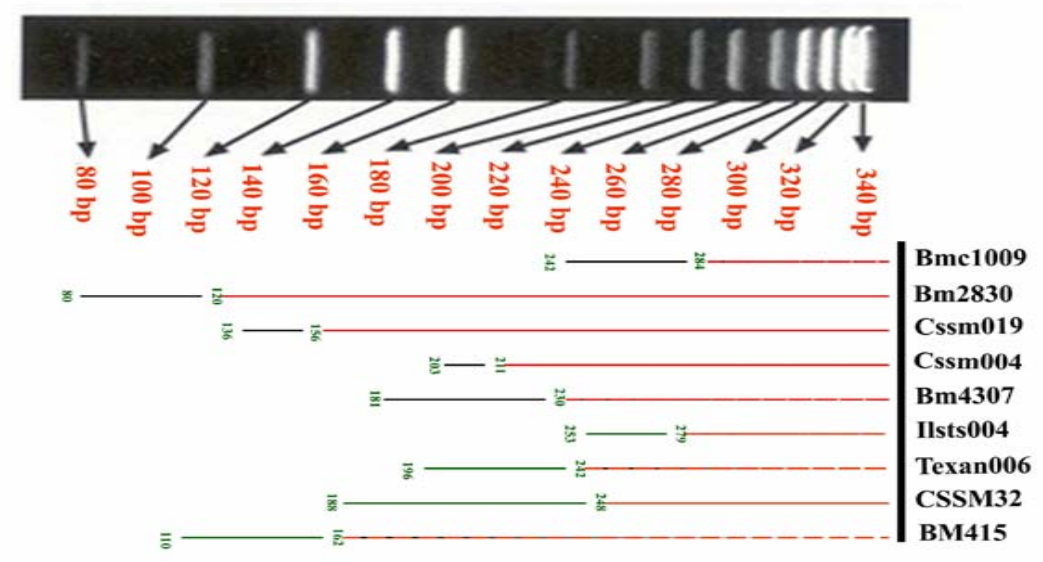

Figure 2 Summary of various parameters measures estimated for each locus in Iranian whole investigated breeds

The result of PIC in present study was in same line with China local sheep breeds (Zenga *et al*. 2010), sheep breeds in South Africa (Qwabe, 2011), and (Greguła-Kania *et al*. 2015) in Poland, and lower from Jordanian (Jawasreh *et al*. 2018) and Iranian (Ebrahimi *et al*. 2017) sheep breeds. Some factors, such as mutations and selection, affect the amount of polymorphism in STR loci.

Figure 3 and 4 summarizes various parameters measures estimated for each locus in Iranian whole investigated breeds.

Following the criteria of Botstein *et al*. (1980), the investigated markers in indigenous sheep breeds of India were observed to be highly informative (PIC>0.5), reasonably informative (0.25<PIC<0.5) and slightly informative, less than 0.25 (Jyotsana *et al*. 2010).

Hence, the degree of informativeness of a marker reveals its usefulness in diversity analysis of a breed. A higher value of PIC means more alleles and greater polymorphism at that locus.

Inter-population genetic relationships of studied breeds

To the best of our knowledge, deficiency of knowledge is existed about the genetic relationship for these three indigenous and locally developed goat breeds using microsatellite markers. Considering Ho, He and F-statistics parameters within and between investigated population can show situation of genetic structure of each specific breed in response to reproductive mating system, natural and artificial selection, immigration and fragmentation evens and genetic bottleneck. F-statistics parameter able to measure the population differentiation or variation within and between populations. The result of present study showed Azerbaijan and Sarbisheh has negative F_{IS} value (reasonable inbreeding) in compare of Sarbisheh goat (experiencing inbreeding).

F_{IS} index the deficiency of heterozygosity in an individual due to non-random mating within population and this parameter ranged from -1 to +1, but in nature it is always nearer to zero. If Fis value become 1 means closer relationship among the individuals.

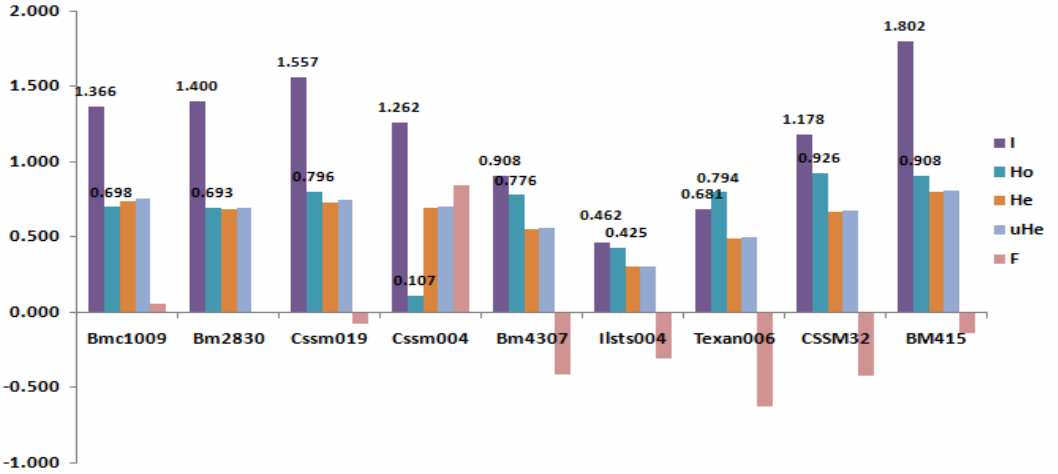

Figure 3 Summary of various parameters measures estimated for each locus in Iranian whole investigated breeds

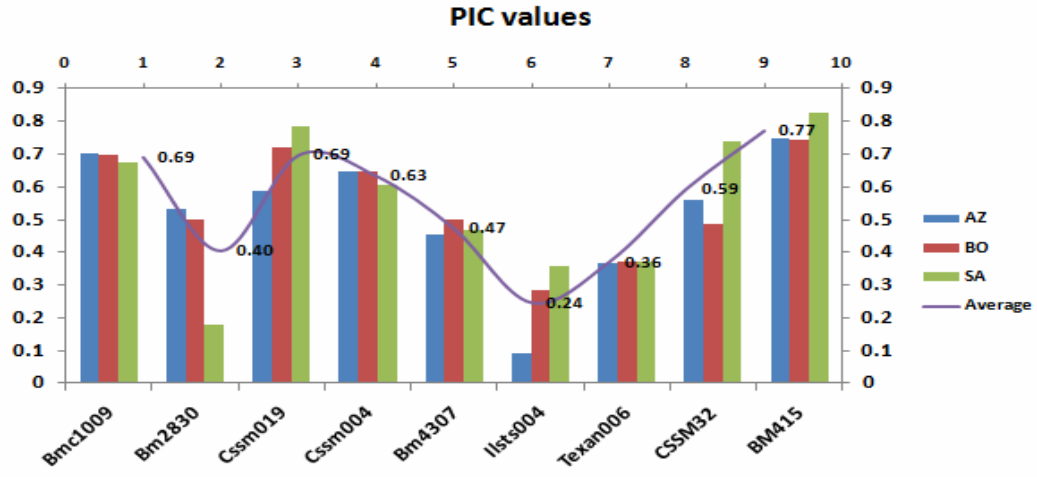

Figure 4 Summary of polymorphic information content (PIC) parameters per investigated loci in each studied goat breed

Moreover, F_{ST} parameters is the deficiency in heterozygosity due to subdivision in the population and value of this index many ranged from 0 to 1 and is classified into low (<0.15), moderate (0.15 to 0.25) and high (>0.25) genetic differentiation and The lower and high F_{ST} values indicate higher relationship and inbreeding versus out breeding between the populations respectively. Figure 5 illustrated some statistical molecular genetics parameters per investigated goat population.

Positive values of F_{IS} indicate loss of heterozygosity in some STR markers, similar to the results reported by (Hristova *et al*. 2014), (Vahidi *et al*. 2016), and (Karsli *et al*. 2020). The positive F_{IS} value suggested inbreeding to be one of the main causes of lack of heterozygotes in Iranian sheep. Low heterozygotes and excess of homozygotes within the studied populations may be related to several factors such as the mating system of animals, number of samples, selection (genetic hitchhiking), and null alleles (Nei, 1987).

The cluster graph resulted of Nei's genetic distance and genetic identity (Nei, 1987) and the phylogenetic relationship between breeds made by the neighbor-joining tree is shown by the genetic distance matrix. As you can concluded here the greatest genetic similarity cluster was observed between the Sarbisheh and Busher populations. Figure 6 illustrated graphical cluster between breeds made by the neighbor-joining based on Nei distance and genetic identity. Analysis of molecular variance (AMOVA) provided an estimate of the measure of population genetic differentiation within and among populations. The AMOVA showed that 97 percent of the genetic variation among three investigated breeds is attributed to among populations compared with 3 percent due to variation within and between individuals (Figure 7).

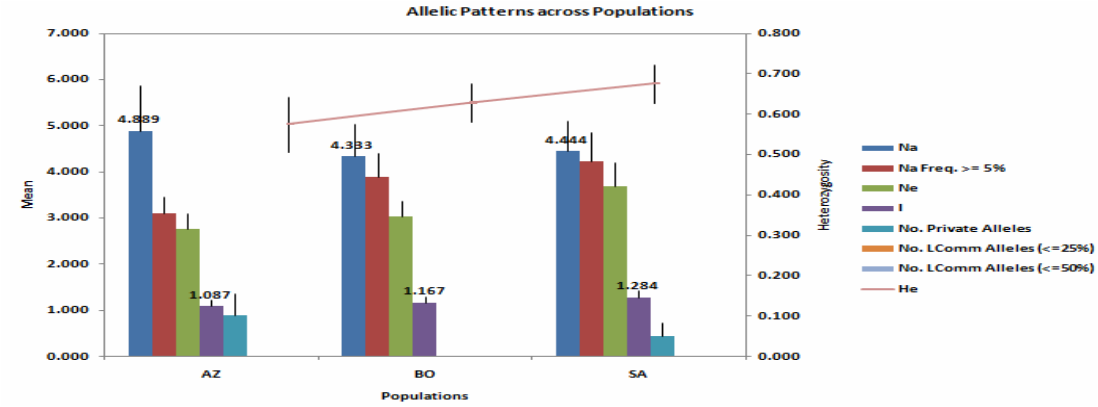

Figure 5 Summary of molecular parameters per population in this study

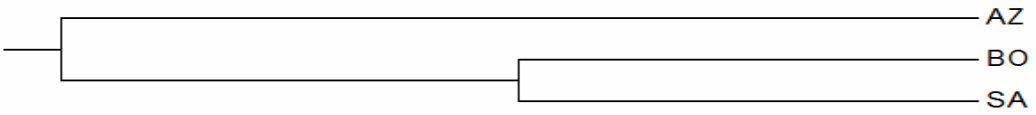

Figure 6 Phylogenetic relationship between breeds made by the neighbor-joining based on Nei distance and genetic identity

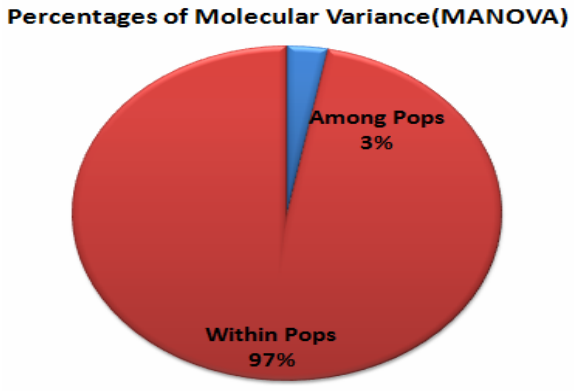

Figure 7 Results of MANOVA extracted from Arlequin and GenAlEx software

On the basis of bottleneck outcome, interpretation of IAM and SMM models demonstrated normal 'L'-shaped distribution of the mode-shift analysis test and the lack of bottleneck in the studied Azerbaijan and Sarbisheh goat populations (Table 8). Giving more explanation for this calculation, bottleneck analysis try to evaluate population decline in response to factors affecting genetic diversity of breed structure based on mode shift test, under the assumption of the two-phase model. The occurrence of a normal L-shaped curve revealed no loss of alleles in the investigated populations and hence the absence of a genetic bottleneck. The Mode-shift indicator test was also utilized to detect potential bottlenecks, as the non-bottleneck populations that are near mutation-drift equilibrium are expected to have a large proportion of alleles with low frequency. This test discriminates many bottlenecked populations from stable populations. A graphical representation utilizing allelic class and proportion of alleles showed a normal 'L'shaped distribution. The L shaped curve indicated the abundance of low frequency alleles. This finding suggested the absence of any detectably large, recent genetic bottleneck (last 40-80 generations) in declining population, where the probability of low frequency allele's loss was very high.

Similar pattern of detections were also reported by earlier research workers (Manjari *et al*. 2018; Mohankishore *et al*. 2019). The population structure was computed using Structure (Pritchard *et al*. 2000). In structure analysis, the Ln Pr (X|K) increased clearly from K= 1 to K= 4 and reached a plateau at K= 3, and therefore K= 3 was taken as the most likely number of inferred populations.

This is also highlighted that Busher, Azari, and Sharbisheh were well-differentiated. The result of the clusters revealed the presence of admixtures which are indicative of gene flow between these breeds.

As final conclusion, we can suggest here that there is sufficient genetic variation within investigated indigenous goat breed and Azerbaijan and Sarbisheh goat can assume as really different breeds. These results will help future researchers as a key guide to better understanding the genetic relationships and breed differences in Iranian sheep breeds for making future breed policies and programs to protect any breed of goat in the country.

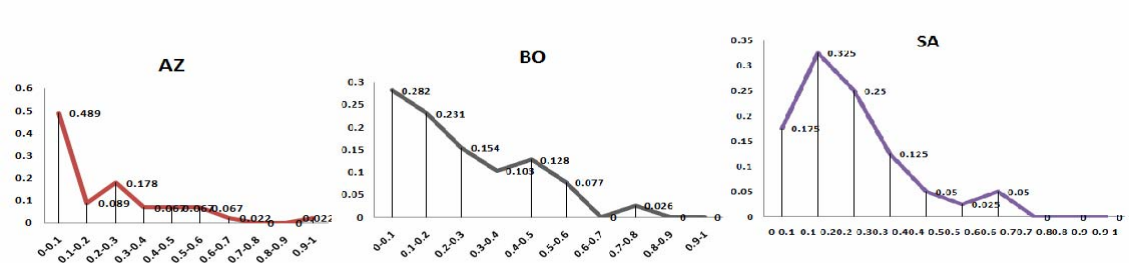

Figure 8 Results of bottleneck analysis in three investigated breed
L-shape pattern means lack of loss diversity in the studied breed

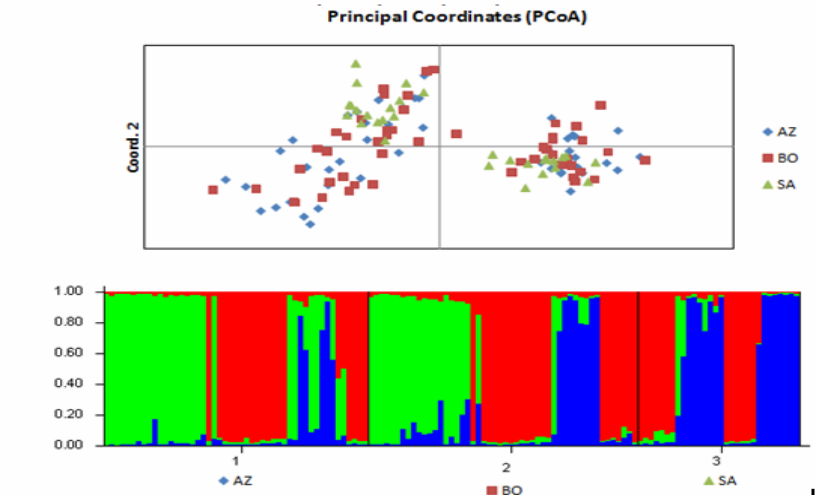

Figure 9 Results of PCOA and Bayesian clustering based on MCMC and model test in the studied breed

CONCLUSION

This present study demonstrated genetic similarity with and between individuals in four Iranian goat breeds and in summary statistical output indicated the ability of STR for estimation of genetic diversity in goat. Finally, further investigation for genetic variability of the genome in goat is needed.

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