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GENETIC DIVERSITY OF DIFFERENT JORDAN GOAT BREEDS USING MICROSATELLITE MARKERS

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ABSTRACT

Genetic diversity of goat breeds in Jordan was investigated using six microsatellite markers (MS). The markers were *BM143*, *CSRD247*, *INRA40*, *OARAE54*, *ILSTS005* and *MCM527*. The selected goats were predefined into four breeds based on morphological and geographical considerations as Jabali, Dhaiwi, Shami and Sahrawi. The number of alleles, expected and observed heterozygosity per population and locus were (6.92, 0.703, 0.685, and 9.83, 0.728, 0.624, respectively). The average population differentiation coefficient (F_{st}) and inbreeding coefficient (F_{is}) were 0.019 and 0.048, respectively, indicating greater differentiation between pairwise breed comparisons and more inbreeding found in each breed than expected. In particular, F_{is} values were 0.064, -0.020, -0.070 and 0.123 in Jabali, Dhaiwi, Shami and Sahrawi, respectively. The F_{st} values were largest (0.078) between Shami and Sahrawi, and smallest (0.024) between Jabali and Dhaiwi. These results reflected high level of genetic diversity and differentiation in studied goat breeds as well as at the studied markers. On the other hand, genetic distances undoubtedly confirmed breed differentiation among the studied goat breeds. Distance matrix was constructed of four taxa representing the four breeds. Our current findings showed closeness of Dhaiwi to both Jabali and Sahrawi. Those three breeds were clustered together, whereas Shami was in a separate cluster. The population structure analysis inferred that four breeds would form the four predefined breeds. As a consequence, individual correspondence analysis confirmed the degree of differentiation between all individuals of the four studied goat breeds except only few individuals of Sahrawi goats that may have descended from other breeds of nearby countries. The present study provides the first microsatellite genetic diversity and differentiation of goat breeds in Jordan.

Key words: Goats, Microsatellite, Biodiversity, Phylogeny.

INTRODUCTION

Goats - as small ruminants - are important for the livelihood of poor farmers in the world. In particular, goat breeds of Mediterranean area play an effective role in transferring thousands of marginal hectares into high quality animal products for human livelihood (Boyazoglu and Flamant, 1990). In Jordan, however, goat farming received high attention lately by researchers and other stakeholders as a potentially significant resource for alleviating poverty in under represented rural areas. Unfortunately, goat farming in Jordan had been recently facing major threats to the genetic diversity resulting from persistent drought and unplanned crossbreeding (MOA, 2010). Several reports identified and characterized several native breeds (Al-Atiyat *et al.*, 2012; Zaitoun *et al.*, 2005). These breeds are Mountain Black (Jabali), Black Bedouin (Dhaiwi), Desert (Sahrawi) and Damascus (Shami) (Zaitoun *et al.*, 2005). These breeds vary in their own morphological characteristics, predominant geographical sources and production systems.

It is widely accepted that domesticated animals descended from a single ancestor, originating in Asia. It is also reported that today's world goat breeds originated from central Asia from the Stone Age (Piper and Ruvinsky, 1997). At the present time, the Mediterranean domesticated goat breeds are the most closely related to their ancestors (Magdalena *et al.,* 2009). However, there are no published data on goat genetic diversity and differentiation in Jordan at the molecular DNA level employing Microsatellite DNA markers. Indeed, MS provided wide opportunities to analyze genetic variation at the DNA level in animal breeds. Microsatellite DNA markers are widely used as they are polymorphic and are randomly distributed in the organism's genome (Karaca *et al.,* 1999; Ying-Hui *et al.,* 2014). These markers have also been successfully used to study the biodiversity and genetic relationship between and within breeds and/or populations (Rosenberg *et al.,* 2001). Additionally, they provide reliable information on allele profiles and allele

frequencies for a single DNA sample that can be extracted from blood or tissue.

In light of the limited information available on the molecular genetic variation and differentiation of goat breeds in Jordan (Galal *et al.,* 2008), the present study aimed to analyze the genetic diversity and differentiation of local goat breeds in different regions of Jordan using MS markers.

MATERIALS AND METHODS

Goat populations: Goats individuals were sampled from scatted herds of small and large size. The goats were differentiated into breeds based on morphological features as Jabali (Mountain Black), Dhaiwi (Black Bedouin), Shami (Damascus) and Sahrawi (Desert); crossbred goats were not considered.

Sampling and DNA extraction: An overall of 318 goat individuals were tissue sampled and genotyped as 72 Jabali, 114 Dhaiwi, 90 Shami and 42 Sahrawi goats (Table 1). The tissue samples were collected from does and bucks of each breed. Each sample consisted of 0.5 cm ear tissue using an ear puncher. Samples were placed in microcentrifuge tubes and stored at -18 °C until analyzed. The DNA extraction was performed using a commercially available protocol (E.Z.N.A® MicroElute Genomic DNA extraction Kit, (OMEGA-Bio-Teck, 2010). Subsequently, DNA concentrations were estimated by Nano-DNA spectrophotometer (AlphaSpec®, 2010) in which the quality of DNA was evaluated using the ratio of A260/A280.

DNA Genotyping: Six microsatellite markers located on different chromosomes were employed for genotyping experiments using Silver Sequence™ DNA System of Promega®. Marker selection was based upon their close linkage. On the other hand, the associated primers were selected for ease of use in PCR reaction with special regard to the annealing temperature and $MgCl_2$ concentration. Primer sequences were taken from the Australian Sheep Gene Mapping website (Maddox *et al.*, 2001) and synthesized by BioEngland® (Table 2). The PCR reaction utilized 10µl volume of DNA and reagents for genotyping, in which DNA samples were liquated into a 48 well PCR plate. Thermal cycling was then performed on an MJ Research PTC-100 thermal-cycler. The Amplified PCR products were resolved on a 5% polyacrylamide gel electrophoresis using a Sequi-Gen GT gel rig for Silver staining (Sambrook *et al.*, 1989). Sequencing ladders were prepared using fmol® DNA Cycle Sequencing system (Sambrook *et al.*, 1989) and 3µl of each of the four reactions loaded onto the gel, so that the size of the microsatellite alleles determined. When the electrophoresis run was completed, the gel was recovered and developed. Then, the gel was dried and viewed by APC Film Development method (Sambrook *et al.*, 1989). The film was developed as a photo picture to be ready for scoring the genotypes. Allele sizes were scored by visual comparison with the sequencing ladder; pGEM®-3Zf(-) Vector.

Genetic Analysis: Population genetics parameters of the four studied populations were investigated. Average allele number and polymorphism under Hardy-Weinberg equilibrium were measured The parameters were observed heterozygosity (H_o) and expected heterozygosity (H_e) (Nei, 1987), measured by Genetic Data Analysis (GDA) software (Lewis and Zaykin, 2001; Weir, 1996). Furthermore, third parameter of measuring polymorphism was polymorphic information content (*PIC*) estimated using CERVUS software (Botstein *et al.*, 1980). The hierarchical genetic structure of the samples, analysis of molecular variance (AMOVA), *F*-statistics, pairwise difference (F_{st}) and inbreeding coefficients (F_{is}), were computed using ARLEQUIN (Excoffier and Lischer, 2010). On the other hand genetic distances matrix between populations were measured using GDA software (Lewis and Zaykin, 2001) which utilizes the most widely used measure of genetic distances (Nei, 1972). Evolutionary distance phylogeny was drawn using MEGA software (Tamura *et al.*, 2011).

The population structure was analyzed using STRUCTURE software (Pritchard *et al.,* 2000) considering an admixture model and correlated allele frequencies between studied populations. The length of the burn-in Monte Carlo Markov chain (MCMC) were 1000 and 10000 in 100 runs for possible number of clusters (breeds/population) (K) from 2 to 8. For each K value, logarithmic probability of data (L[K]) and F_{st} values for each cluster were estimated. In addition, GENETIX software was used to predefine and identify the studied goat breeds (Belkhir *et al.*, 1996-2004).

RESULTS AND DISCUSSION

Genetic diversity within goat breeds: The AMOVA analysis revealed 4.84% and 95.16% of genetic variation within and between studied goat breeds, respectively. Tables 1 and 2 showed the genetic diversity parameters resulted from genotyping the six studied markers. The genetic diversity was based on measuring the number of alleles per locus, H_e, H_o, and *PIC*. Average allele numbers were 7.33, 7.67, 6.83 and 5.83 for Jabali, Dhaiwi, Shami and Sahrawi goats, respectively, with an overall average of 6.92 (Table 1). The H_e and H_o were 0.679, 0.696, 0.712 and 0.734 (average: 0.705) and 0.601, 0.617, 0.722 and 0.579 (average: 0.630) for Jabali, Dhaiwi, Shami and Sahrawi goats, respectively (Table 1). On the other hand, average allele numbers were 13, 10, 6, 12, 8 and 10 with average of 10 for *BM143*, *CSRD247*, *INRA40*, *OARAE54*, *ILSTS005* and *MCM527*, respectively (Table 2). Furthermore, H_e and H_o were

0.863, 0.766, 0.561, 0.860, 0.465 and 0.829 and 0.776, 0.783, 0.462, 0.693, 0.509 and 0.882 for *BM143*, *CSRD247*, *INRA440*, *OARAE54*, *ILSTS005* and *MCM527*, respectively (Table 2). The average of H_e, H_o, *PIC* and fixation index (*f*) were 0.724, 0.685, 0.699 and 0.048, respectively. In general, the results of these genetic parameters were considered high reflecting high genetic diversity in studied goat breeds as well as at studied markers. A comparative description on results genetic diversity parameters in studied breeds with other similar studies in Asia, Africa and Europe using MS markers showed similar results. For close example, genetic diversity parameters were reported for Ardi, Black Bedouin (Dhaiwi), and Damascus (Shami) the most common native goat breeds of Kingdom of Saudi Arabia, Jordan, and Syria, respectively. Ardi, Black Bedouin, and Damascus goats exhibited very slightly high average allele number (8.25, 9, and 7.25, and H_e of and 0.750, 0.804, and 0.779, respectively) (Al-Atiyat and Aljumaah, 2014). Furthermore, Muema *et al.* (2009) found that the average range of allelic number, H_o and H_e were, respectively, 4.9-5.6, 0.57 and 0.58 for Somali goat and 4.88-5.67, 0.55 and 0.58 for Ethiopian goat. On the other hand, H_e and H_o varied from 0.61 to 0.78 and 0.60 to 0.78, respectively in Chinese indigenous goats (Yang *et al.*, 2002). Canon (2006) studied 1426 goats from 45 goat breeds of the Middle East and Europe where H_o was 0.68 and H_e was 0.63 in four French goat breeds and that H_o of 0.64 and H_e of 0.60 for five goat breeds of Switzerland. In addition, H_e was 0.70 for Al-Bishi and 0.712 for Nagrani goat breeds from Kingdom of Saudi Arabia. Furthermore, Aljumaah *et al.* (2012) reported that the mean number of alleles, H_e, H_o and *PIC* was 6.64, 0.553, 0.665 and 0.628 for Ardi goats of Saudi Arabia. Mahmoudi *et al.* (2011) found that average number of alleles, H_e and *PIC* of Iranian goats was 7.57, 0.80 and 0.76. Finally, Globaldiv project of two large got microsatellite datasets has generated a picture of goat diversity across continents indicating a gradient of decreasing diversity from the domestication center towards Europe and Asia (Ajmone-Marsan *et al.*, 2014).

Genetic differentiation between goat breeds: *F*-statistics were estimated for statistically differentiated goat breeds and presented in Table 3. The first parameter, inbreeding coefficient (F_{is}), was positive in two breeds; Jabali and Shami goat breeds. The F_{is} positive values indicate more inbreeding than expected in those breeds (Table 3). In other words, inbreeding in those breeds was more heterozygosity-deficient. The resulted inbreeding values were noticeably variable; 0.064, -0.020, -0.067 and 0.213 in Jabali, Dhaiwi, Shami and Sahrawi, respectively. The second parameter, (F_{st}), on the other hand, showed the high differentiation between pairwise breed comparisons (Table 3). For example, the highest F_{st} value (0.078) was between Shami breed with Sahrawi breed. The value of F_{st} between Jabali and Dhaiwi was, however, smallest 0.024. Furthermore, it was 0.042 between Dhaiwi with Sahrawi. Finally, the F_{st} values between Jabali with Shami and Sahrawi were 0.056, and 0.040, respectively, and between Dhaiwi with Shami was 0.033 (Table 3).

It was reported that limited genetic differentiation among local Asian goat breeds was noticed in a global goat genetic differentiation study (Ajmone-Marsan *et al.*, 2014). Similar results were found in the literature indicating convergence and divergence between studied populations of close geographical regions. For example, in three geographically close Arabian countries, Shami goat of Syria was more differentiated from Dhaiwi goats of Jordan than Ardi goats of Saudi Arabia (Al-Atiyat and Aljumaah, 2014). The F_{st} was low (5.3%) among African goat populations (Muema *et al.*, 2009); 0.078 and 0.086 for Al Bishi and Nagrani, respectively (Canon *et al.*, 2006) and 0.07 for Iranian goats (Mahmoudi *et al.*, 2011). In addition, F_{is} as inbreeding indicator was 0.10 for Iranian goat (Mahmoudi *et al.*, 2011); 0.20 for Indian goats (Dixit *et al.*, 2010) and 0.183 in Saudi Ardi goat (Aljumaah *et al.*, 2012).

Genetic Distance and Phylogeny: Distance matrix constructed from allele frequencies of six loci in which four taxa has been found (Table 4), representing the four predefined breeds. Table 4 also shows the genetic distance measures below diagonal, whereas identity genetic measures above diagonal. Both measures were estimated based on Nei's (1972) genetic distance measurements. Thus, phylogeny analysis was performed utilizing these results to formulate UPGMA phenogram as seen in Figure (1). The phenogram shows three nodes created as Node 1 (level = 0.070), Node 2 (level = 0.126) and Node 3 (level = 0.214) (Figure 1). Therefore, the results undoubtedly confirm breed differentiation among the investigated goat breeds. The finding showed closeness of Dhaiwi to both Jabali in one separated cluster and then to Sahrawi. Three of them, in particular, were in one cluster, whereas Shami goat was separated in another. These results provided the first molecular genetics evidence of breed differentiation of the studied goat breeds in Jordan. Recent study provided strong evidence of admixture between Ardi and Dhiawi goat resulting shortest genetic distance between Ardi and Black Bedouin goats longer between Ardi and Shami goats (Al-Atiyat and Aljumaah, 2014). A report based on DNA sequencing indicated same results of reasonable degree of differentiation between Jordan goat breeds (Al-Atiyat *et al.*, 2012). On the other hand, these findings confirm the earlier results reported based on morphological traits (Zaitoun *et al.*, 2005), production traits (Tabbaa and Al-Atiyat, 2009) and thermo-tolerance response traits (Al-Tamimi *et al.*, 2013). Therefore, these

findings undoubtedly confirmed the breed similarity and differentiation among the studied goat breeds. In addition, such evolutionary distances would be fundamental to molecular evolution and useful for phylogenetic reconstructions. On the other hand, for example, Dixit *et al.* (2010) reported that genetic distance tended to be least (0.22) between Ganjam and Malabari goat breeds and the widest (0.83) between Kanniadu and Malabari breeds.

Admixture of individual from predefined breeds: The clustering analysis of allele frequencies and multivariate analysis revealed at least five discrete cluster [K]/breeds. The presumed number of breeds ranged from K=2 to K= 8. The values of likelihood (ln Pr(X|K) and Alpha for a predefined number of Ks were plotted in Figure 2. The best values of ln Pr(X|K and Alpha were obtained for K = 5 (-4360.16 and 1.431196, respectively) (Figure 2).

These five inferred breeds would correspond to the four predefined studied population and probably one crossbreeding and/or upgrading breed. Therefore, it was surprising that best presumed clustering number did not match the four predefined breeds. When two clusters, ancestral breeds (K=2) were assumed, a clear division was observed between the native Jordanian goat breeds (Jabali, Dhaiwi and Sahrawi) and Shami goats. Shami goat individuals had no shared proportion with individuals of other breeds, indicating no common ancestors. Clusters of three (K=3) grouped individuals was formed from of Jabali and Dhaiwi in one separated line away from Shami and Sahrawi individuals, showing that these two breed groups had a particular genetic background that was possible to differentiate. The fourth inferred population, supposed to be most probable inferred population number, was formed by most of the four populations and a similar proportion of genetic background of K=3. The fifth inferred cluster (K=5) was basically considered most possible to reality (Figure 3). The statistic Alpha peaked at K = 5 (Figure 3 B) indicating support for 5 breeds. More explanation, the fifth goat breed need more morphological, geographical and historical information gathered about each individuals of the that unknown breed. These unknown breed individuals were basically assigned as Sahrawi individuals. As a consequence, individual correspondence analysis was performed to confirm the degree of differentiation between all individuals of the four studied goat breeds (Figure 4). Figure 4 shows a graphical representation of the estimated membership coefficients to the clusters for each individual for *K*= 5. Each individual is represented by a single vertical line, broken into 5 colored segments, whose lengths are proportional to each of the inferred clusters. The graphical representation of this analysis based on allele frequencies of MS markers in a three-dimensional space was indicating three factorial dimensions. The first and second factors (axes 1 and 2) accounted for 45.60% and 31.81% of total variation, respectively and clearly distinguished Shami goat individuals from other goat breeds individuals. However, few individuals shared reasonable correspond to be possibly assigned to Shami breed. In general, Sahrawi individuals were inter-mixed with Jabali individuals (Figure 4). The individuals presented groups belonging to one separate population rather than being mixed population. Only few individuals of Sahrawi goats were clearly distinguished from other Sahrawi individuals. These individuals may belong to the four resulted breed in the Structure analysis, but an individual was excluded as otherwise based on this molecular data analysis. This individual may belong to other breeds of nearby countries such as Syria or Iraq, which are geographically closer to the sampling area of Sahrawi. Limited reports regarding goat breed-individual assignments are available. A popular report by Canon *et al.* (2006) stated that most northern and central European goat breed individuals (71%) were assigned to their breeds with a success rate of more than 80% in which least four discrete clusters were revealed. They were eastern Mediterranean (Middle East), central Mediterranean, western Mediterranean and central/ northern Europe. They assumed that about 41% of the genetic variability among the breeds could be explained by their geographical origin.

Table 1. Descriptive statistics of allele number (*A*), expect (*He*) and observed (*Ho*) heterozygosity for each breed under Hardy-Weinberg (*HW*) equilibrium.

Breed	*n*	*A*	*He*	*Ho*
Jabali	57	7.33	0.677	0.635
Dhaiwi	67	7.67	0.676	0.689
Shami	48	6.83	0.721	0.771
Sahrawi	40	5.83	0.735	0.645
Mean		**6.92**	**0.703**	**0.685**

Table 2. Descriptive statistics of allele number (*A*), expect (*He*) and observed (*Ho*) heterozygosity, Polymorphic information content (*PIC*) and fixation index (*f*) for each locus under Hardy-Weinberg (*HW*) equilibrium.

Locus	*A*	*He*	*Ho*	*PIC*	*HW*	*f*
BM143	13	0.863	0.778	0.848	NS	0.098
CSRD247	10	0.766	0.783	0.735	NS	-0.0216
INRA40	6	0.561	0.462	0.526	NS	0.176
OARAE54	12	0.86	0.693	0.843	***	0.196
ILSTS005	8	0.465	0.509	0.435	NS	-0.094
MCM527	10	0.829	0.882	0.808	*	-0.064
Mean	**9.83**	**0.724**	**0.685**	**0.699**		**0.048**

Table 3. Comparison of populations based on F_{st} * and population specific F_{is} *.

	Jabali	Dhaiwi	Shami	Sahrawi
Jabali	**0.064**			
Dhaiwi	0.024	**-0.020**		
Shami	0.056	0.064	**-0.067**	
Sahrawi	0.040	0.042	0.078	**0.123**

* *FST* below the diagonal and *FIS** on diagonal.

Table 4. The genetic matrix, genetic identity measures (above diagonal) and genetic distances measures (below diagonal).

Breeds	Measures		
Jabali	0.932	0.845	0.882
Dhaiwi	0.070	0.828	0.880
Shami	0.168	0.188	0.751
Sahrawi	0.125	0.128	0.286

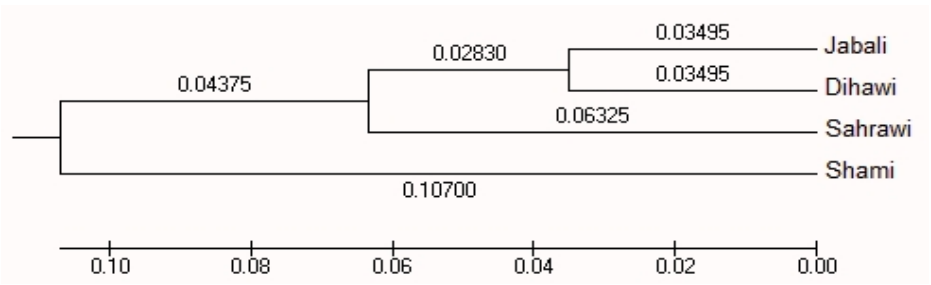
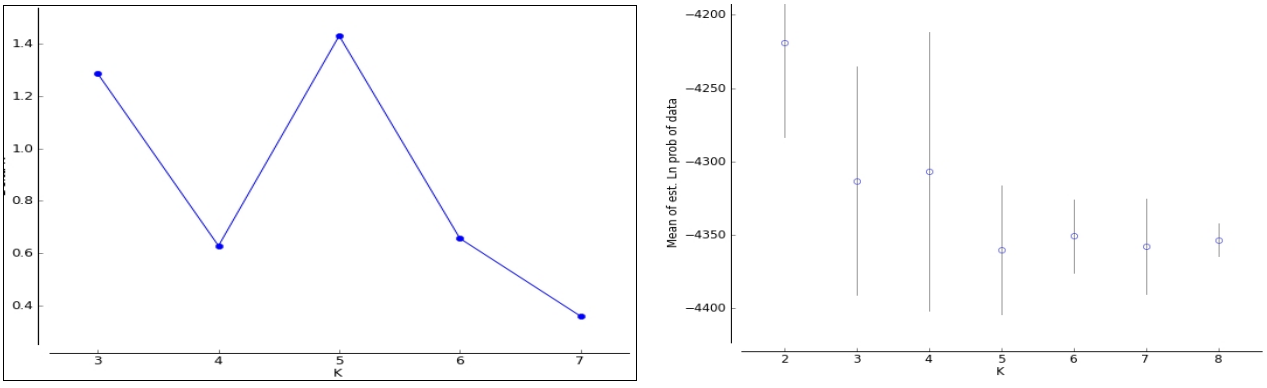


Figure 1. Cluster analysis among the studied goat breeds according to the UPGMA phenogram.



A. **B.**

Figure 2. Plot of data likelihoods for several values of mean Alpha values (A) and K (from 2 to 8) (B).

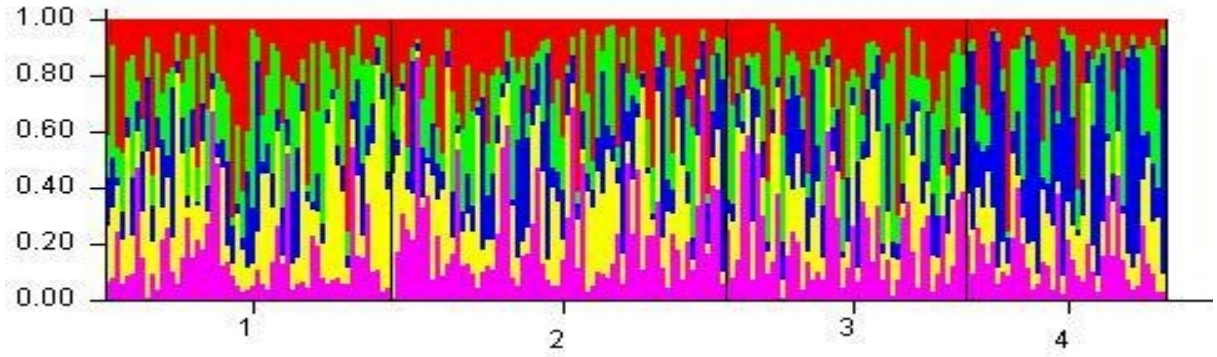


Figure 3. Population structure obtained by STRUCTURE analyses for the individuals of (the four breeds; 1: Jabali, 2: Dhaiwi, 3: Shami, 4: Sahrawi. Each individual represented by a single vertical line broken into K colored segments, with lengths proportional to each predefined breed of K from 2 to 8.

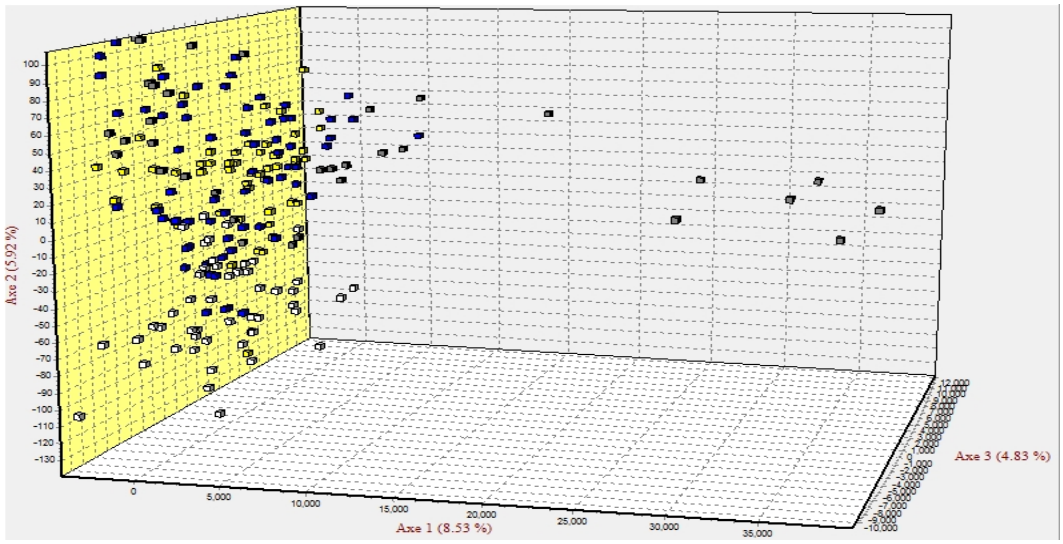


Figure 4. Correspondence individual analysis of the individuals' genotypes of the four goat breeds. Blue, yellow, white and grey squares are representing individuals of Jabali, Dhaiwi, Shami and Sahrawi, respectively.

Conclusion: The genetic parameters reflected a high level of genetic diversity in the studied goat breeds as well as selected markers. F_{is} values were positive in all breeds, indicating more inbreeding than expected in each breed. F_{st}, on the other hand, showed greater differentiation between pairwise breed comparisons. Genetic distances undoubtedly confirmed the breed differentiation among the studied goat breeds. Distance matrix constructed of breeds as matrix has 4 taxa. The finding showed closeness of Dhaiwi to both Jabali and Sahrawi. Three of them, in particular, were in one cluster, but Shami in another. The population structure analysis inferred that five breeds would form the four predefined studied breeds. As a consequence, individual correspondence analysis confirmed the degree of differentiation between all individuals of the four studied goat breeds except only one individual of Sahrawi goats that might come from other breeds of nearby countries. The present study provided the first microsatellite based genetics diversity and breed differentiation of goat breeds in Jordan. The current findings can be potentially utilized for future conservation of goat genetic resources under current drought climates and typical crossbreeding practices.

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