African Journal of Biotechnology Vol. 10(67), pp. 15109-15116, 31 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2689
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Assessing genetic diversity of Hamdani sheep breed in Kurdistan region of Iraq using microsatellite markers

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Accepted 29 July, 2011

The genetic variation in Hamdani sheep in the Kurdistan region of Iraq was studied using 35 microsatellite markers. All 35 markers were found to be highly polymorphic. The mean number of observed alleles ranged from four at BMS1494 to twenty five at BM6444 and INRABERN185 loci. The polymorphic information content (PIC) values ranged from 0.208 for BMS1494 loci to 0.935 for INRABERN185 with an average value of 0.744. The overall observed and expected heterozygosity values were 0.407 and 0.764, respectively. Test of genotype frequencies for deviation from the Hardy-Weinberg equilibrium (HWE) at each locus, revealed a significant departure from HWE due to loss in heterozygotes by high level of inbreeding. The average inbreeding value for the 35 markers investigated was 0.469. The high observed heterozygosity is an indication of genetic variability that could be used for developing efficient utilization and genetic improvement strategies for Hamdani sheep.

Key words: Hamdani sheep, microsatellites, polymorphism, genetic diversity, inbreeding, heterozygosity.

INTRODUCTION

Animal genetic diversity is important to meet current and future production needs in various environments, to allow a sustained genetic improvement and facilitate rapid adaptation to changing breeding objective (Crawford and Littlejohn, 1998; Kumar et al., 2006).

In recent years, several studies have investigated the genetic diversity in sheep using microsatellite markers (Diez-tascon et al., 2000; Hassan et al., 2003; Arora and Bhatia, 2004; Elfawal, 2006; Gutierrez-Gil et al., 2006; Gizaw et al., 2007; Peter et al., 2007; Cinculov et al., 2008). Microsatellites have become the marker of choice for many applications. Their abundance, high level of repeat-number polymorphism, manifested as the occurrence of a large number of alleles per locus, and co-dominant inheritance has facilitated their extensive use in genome mapping, phylogenetic inference and population genetics in farm animals (Crawford and Littlejohn, 1998; Jouquand et al., 2000; Moioli et al., 2001; Kumar et al., 2006).

While the genetic diversity of European, Indian and other countries sheep breeds have been well researched, the genetic diversity of the indigenous sheep in the Kurdistan region of northern Iraq has not been studied before. The Hamdani breed, a fat-tailed sheep breed, is found extensively in the Kurdistan region. It is the largest of all Iraqi sheep breeds and well known for its meat, milk and wool production and adaptability to the local agro-ecological conditions (Magid et al., 2003; Alkass and Juma, 2005). Hamdani sheep produce, on average, 78.3 kg of milk per lactation (Raaof, 2005) and 2.1 kg of coarse wool per year (Al-Barzinji, 2009), and gain 148 g body weight per day (Al-Barzinji, 2003). Until now, no organized Hamdani sheep breeding program exists in Iraq. The breed is kept at smallholder and commercial farms and agricultural research stations without applying any genetic improvement strategies. Hence, this study was undertaken with the objective to characterize the genetic diversity of the Hamdani sheep breed in Kurdistan using microsatellite markers as basis for

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designing effective utilization and genetic improvement programs, and designing breeding programs strategies.

MATERIALS AND METHODS

The samples were taken from 64 adult ewes of two flocks of Hamdani sheep at an age ranging from 2 to 5 years. One flock was kept at the experimental station of the College of Agriculture in Erbil and the other flock at a commercial farm in Klkan village of Duhok, Iraq.

Blood sample collection and DNA extraction

Jugular blood samples were collected in 10 ml vacutainer tubes containing K3 EDTA (as anticoagulant.) The samples were diluted (1:1) and extracted using Magic Buffer method (David and Olivier, 2005) which was obtained from FAO/IAEA Agriculture and Biotechnology Laboratory, Vienna, Austria. DNA concentration was determined using a UV spectrophotometer (Pharmacia LKB-Ultraspec III) at optical density of 260 nm in the Biotechnology Laboratory, International Centre for Agriculture Research in Dry Areas (ICARDA), Aleppo, Syria during November 2007 and May 2008.

Selection of microsatellite markers and PCR preparation and amplification

A total of 35 fluorescently labelled microsatellite markers (Table 1) were chosen based on the degree of polymorphism reported in the literature (FAO, 2004). They were further optimized and tested for polymorphism using genomic DNA extracted from individual animals. Only forward primers of each pair were labelled with one of the three fluorophore, that is, pentachlororo-6-arboxyflouroscien (NED) and 6 carboxyflouroscien (FAM) and (VIC) dye phosphoramidites which were synthesized and supplied by Applied Biosystems (ABI). Each polymerase chain reaction (PCR) mixture with the final volume of 10 µl contained 1.5 mM $MgCl_2$, 2 µM dNTPs, 5 pmol forward primer, 5 pmol reverse primer, 0.5 U Taq DNA polymerase and 20 ng genomic DNA. The following program was run for amplification: 1 min initial denaturation at 95°C followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s and an extension step of 10 min at 72°C using a Thermo-Hybrid PX2 thermal cycler. Thereafter, a mixture of 1 µl of PCR products, 0.20 µl of standard size florescent dye GS 350 ROXTM (Applied Biosystems) and 6 µl of Hi-Di formamide was made, heat denatured at 95°C for about 5 min and snap chilled on ice for 5 min. Each sample was analyzed on POP-4 polymer using a 36 cm capillary at 15 KV and run for 20 min on ABI 3100R genetic analyzer following the Applied Biosystems user manual version 3.1.0. Microsatellite fragment sizes were calculated using Gene Mapper program version 3.7 and the size standard peaks were defined by the user (Applied Biosystems).

Statistical analysis

The 35 microsatellites loci described earlier were used for the assessment of genetic diversity, hence; the PIC values, observed and expected heterozygosities were calculated using GENEPOP version 3.3 (Raymond and Rousset, 1995) and PowerMarker version 3.25 (Liu and Muse, 2005) software. The observed and effective numbers of alleles (Kimura and Crow, 1964) were also calculated using GENEPOP software. F-statistics were determined using F-Stat software (Goudet, 2002) with a Jackknifing procedure applied on the loci by deriving their significance levels. The tests for deviation from Hardy-Weinberg equilibrium were based on 'p' values of the F_{IS} statistics obtained after permuting the alleles among individuals within sample.

RESULTS AND DISCUSSION

Allelic variation

The mean number of alleles (MNA) observed for all markers was 14.17, and varied from 4 (BMS1494) to 25 (BM6444 and INRABERN185) alleles (Table 2). As this is the first report on evaluation and selection of polymorphic microsatellite markers for the genetic characterization of Hamdani sheep or related breeds in the Kurdistan region of Iraq, no comparative values are available in literature. However, the MNA obtained in this study was higher than that reported for various other sheep breeds (Forbes et al., 1995; Worley et al., 2004; Mukesh et al., 2006; El Nahas et al., 2008).

The MNA and heterozygosity values to determine the extent and distribution of the genetic diversity within the Hamdani breed were available (Figure 1). The MNA detected in each genotype and the expected heterozygosities are critical indicators of the genetic polymorphism within the sample of Hamdani breed under this investigation. MNA is the average number of alleles observed in a breed, while the expected heterozygosities are the proportion of heterozygotes observed in a breed (Nei, 1978). The highest number observed per genotype and major allele's frequencies for single locus were 43 for BM6444 and 0.87 for BMS1494, respectively (Table 2).

Results show that Hamdani sheep breed is highly polymorphic, the highest polymorphism at loci BM6444 and INRABERN185 were 25 alleles (Table 2). The found value is higher than that of seven alleles at BM1818 locus reported in Soay sheep (Paterson et al., 1998), nine at MAF65 and eight at MAF209 locus in Karayaka sheep (Koban, 2004), twelve alleles at OarFCB020 and eleven at OarFCB048 locus in the Kivircik sheep (Cerut et al., 2004). However, the number of alleles for single locus in this study was lower than that reported in merino at CSRD247 and MAF065 (Buduram, 2004) and MAF065, ILSTS087 and CSRD247 in Austrian sheep breeds (Baumung et al., 2006). The least polymorphic locus in our study was BMS1494 with four alleles.

Heterozygosity

The observed (Ho) and expected heterozygosity (He) (gene diversity) values for each locus are indicated in Table 2. The mean number of observed and expected heterozygosity values for the 35 loci was 0.407 and 0.764,

Table 1. Characteristics for 35 microsatellite markers reported for genetic variability analysis.

Marker	Chromosome location	Number of allele	Allele size range (bp)	Ho	He	PIC	Annealing temperature (°C)	Reference
BM1258	20	8	108–120	0.727	0.704	0.772	58	Hoda et al. (2009); Mukesh et al. (2006); Peter et al. (2007); Cinculov et al. (2008)
BM1818	20	8	258–284	0.657	0.678	0.629	53	Beraldi et al. (2006)
BM6444	2	6	128–165	0.758	0.775	0.713	TD 50-65	Beraldi et al. (2006)
BMS1494	18	3	227–237	-	0.490	-	58	Rahman et al. (2006)
CSRD247	14	8	211–264	0.691	0.644	0.593	63	Baumung et al. (2006); Visser and Marle-Koster (2009)
DRBP1	20	8	105–147	0.675	0.673	0.619	54	Sechi et al. (2005); Visser and Marle-Koster (2009)
ILSTS005	7	6	144–158	0.400	0.450	0.730	55	Fatima (2006)
ILSTS011	9	3	268–282	0.465	0.449	0.401	TD 50-65	Beraldi et al. (2006)
ILSTS019	21	7	128–172	0.958	0.645	0.580	50	
ILSTS029	1	8	164–180	0.911	0.850	0.834	55	Ramamoorthi et al. (2009)
ILSTS044	1	8	142–170	0.913	0.752	0.716	55	
ILSTS087	6	6	110–120	0.883	0.681	0.766	55	
INRA005	10	8	120–180	0.695	0.679	0.635	TD 50-65	Beraldi et al. (2006)
INRA023	1	16	198–223	0.805	0.872	0.790	55	Baumung et al. (2006)
INRA063	14	9	133–236	0.990	0.850	0.820	54	Seidani et al. (2009)
INRA132	20	9	152–178	-	-	-	58	de Gortari et al. (1997)
INRABERN185	18	4	256–284	-	0.502	0.510	55	Walkden-Brown et al. (2008)
MAF035	23	5	104–122	0.503	0.625	0.610	60	Kusza et al. (2009)
MAF065	15	4	118–140	0.518	0.512	0.453	TD 50-65	Beraldi et al. (2006)
MAF070	4	6	124–166	0.743	0.785	0.749	63	
MAF209	17	7	109–135	0.756	0.738	0.692	63	Beraldi et al. (2006)
MCM527	5	6	151–205	1.000	0.750	0.690	50	Seidani et al. (2009)
OarAE054	25	14	122–148	0.715	0.814	0.820	63	
OarAE129	5	15	136–167	0.419	0.666	0.580	52	Baumung et al. (2006)
OarFCB020	2	13	92–118	0.400	0.658	0.800	60	Kusza et al. (2009)
OarFCB048	17	4	143–167	0.450	0.457	0.405	55	Beraldi et al. (2006)
OarFCB304	19	6	151–214	1.000	0.720	0.660	63	
SPS113	10	6	133–172	0.980	0.780	0.730	55	Seidani et al. (2009)
SRCRSP03	10	8	-	0.430	0.670	-	55	Sechi et al. (2005)
SRCRSP07	16	5	-	0.470	0.620	-	55	Sechi et al. (2005)
SRCRSP09	12	9	112-156	0.737	0.660	0.604	60	Visser and Marle-Koster (2009)
SRCRSP15	12	4	184-196	-	0.355	0.780	48	Maudet et al. (2002)
SRCRSP24	2	6	150-170	0.735	0.692	0.658	55	Maudet et al. (2002); Visser and Marle-Koster (2009)

Ho, Heterozygosity; He, heterozygosity; PIC, polymorphic information content.

Table 2. Genetic parameters measured in the Hamdani sheep breed using 35 microsatellite loci.

Marker	Number of sample	Genotype number	Allele number	Allele size range (bp)	Range of frequency	H_e	H_o	PIC	HWE (significance)	Inbreeding value
BM1258	64	28	14	106–144	0.016–0.164	0.895	0.359	0.886	0.001	0.603
BM1818	64	27	15	230–282	0.008–0.438	0.773	0.813	0.758	0.001	-0.043
BM6444	64	43	25	108–208	0.008–0.188	0.919	0.594	0.915	0.001	0.361
BMS1494	64	5	4	221–231	0.008–0.875	0.224	0.125	0.208	0.001	0.448
CSRD247	64	24	12	207–245	0.008–0.242	0.852	0.344	0.835	0.001	0.601
DRBP1	64	22	20	86–162	0.008–0.438	0.760	0.203	0.740	0.001	0.736
ILSTS005	64	18	13	166–242	0.008–0.313	0.784	0.313	0.754	0.001	0.626
ILSTS011	64	22	16	205–327	0.008–0.352	0.815	0.453	0.798	0.001	0.450
ILSTS019	64	15	10	150–184	0.008–0.375	0.743	0.547	0.705	0.001	0.271
ILSTS029	64	18	12	131–187	0.008–0.523	0.667	0.203	0.633	0.001	0.699
ILSTS044	64	28	17	131–213	0.008–0.266	0.839	0.375	0.821	0.001	0.558
ILSTS087	64	31	16	138–188	0.008–0.305	0.845	0.625	0.830	0.001	0.267
INRA005	64	30	14	110–140	0.016–0.195	0.901	0.516	0.893	0.001	0.434
INRA023	64	32	23	165–237	0.008–0.195	0.914	0.203	0.908	0.001	0.781
INRA063	64	23	15	145–195	0.008–0.258	0.805	0.797	0.778	0.001	0.018
INRA132	64	30	15	138–176	0.008–0.359	0.818	0.531	0.803	0.001	0.358
INRABERN185	64	34	25	201–375	0.008–0.125	0.938	0.266	0.935	0.001	0.721
MAF035	64	16	9	94–114	0.008–0.344	0.756	0.688	0.720	0.001	0.099
MAF065	64	14	10	103–185	0.008–0.813	0.334	0.141	0.326	0.001	0.584
MAF070	64	32	15	126–204	0.008–0.188	0.880	0.422	0.869	0.001	0.526
MAF209	64	18	10	94–126	0.008–0.273	0.807	0.172	0.781	0.001	0.790
McM527	64	27	15	137–223	0.008–0.258	0.848	0.313	0.831	0.001	0.636
OarAE054	64	19	11	106–146	0.008–0.547	0.665	0.328	0.643	0.001	0.513
OarAE129	64	14	13	85–177	0.008–0.633	0.579	0.156	0.562	0.001	0.734
OarFCB020	64	35	18	78–130	0.008–0.195	0.889	0.469	0.880	0.001	0.479
OarFCB048	64	25	15	137–199	0.008–0.266	0.814	0.281	0.793	0.001	0.659
OarFCB304	64	23	13	146–190	0.008–0.445	0.760	0.531	0.719	0.001	0.286
P19(DYA)	64	20	14	158–196	0.008–0.383	0.783	0.219	0.760	0.001	0.724
SPS113	64	12	8	124–170	0.008–0.602	0.575	0.438	0.528	0.001	0.247
SRCRSP03	64	17	11	154–222	0.008–0.469	0.729	0.422	0.705	0.001	0.428
SRCRSP07	64	31	18	106–184	0.008–0.195	0.883	0.313	0.873	0.001	0.651
SRCRSP09	64	16	10	92–154	0.008–0.469	0.683	0.563	0.638	0.001	0.210
SRCRSP15	64	32	18	141–223	0.008–0.211	0.876	0.453	0.866	0.001	0.490

Table 2. cont.

SRCRSP24	64	10	7	98–156	0.016–0.641	0.550	0.547	0.514	0.001	0.011
TCRVB6	64	29	15	222–268	0.008–0.242	0.846	0.531	0.829	0.001	0.378
Mean	64	23.4	14.2	-	-	0.764	0.407	0.744	-	0.469

Ho, Heterozygosity; He, heterozygosity;m HWE, Hardy-Weinberg equilibrium; PIC, polymorphic information content.

respectively. The highest and lowest ob-served heterozygosities for a single locus were 0.813 for BM1818 and 0.125 at BMS1494, respectively. The mean observed heterozygosity for the 35 loci was smaller than the expected heterozygosity (except for BM1818) which is an evidence of the presence of overall loss in hetero-zygosity within breed (De Araujo et al., 2006).

Heterozygote deficiency analysis revealed that the loci exhibited significant deviations from Hardy-Weinberg equilibrium (HWE) ($P<0.01$).

Although, it is difficult to envisage the exact basis of this departure, the presence of low-frequency null alleles segregating at these loci may be the possible reason as described by Peter et al. (2005) for the marker OarAE129 used in the present study. This deviation could also be linked to high positive F_{IS} (within-population inbreeding estimate) values (Mukesh et al., 2004) observed in the investigated sheep populations (Table 2). The shortage of heterozygotes and excess of homozygotes ($F_{IS} > 0$) exhibited by the investigated populations might be attributed to a number of factors, viz. assortative mating (sample relatedness), linkage to loci under selective pressure (genetic hitchhiking), population hetero-geneity or null alleles (Nei, 1987; Peter et al., 2005). Nevertheless, we cannot disregard a certain, although slight, effect of these alleles in the observed deficit of heterozygotes. The fore-most rationale for significant F_{IS} values in these populations, however, seems to be relatedness of few samples under field conditions. The average flock size for Hamdani was observed to be 64 with 2 to 3 adult males. From the flock structure of these breeds, it is apparent that breeding is not controlled at the farmers' level: mating of rams with all the ewes in the flock, as they were housed and grazed with ewes together. Generally, few rams were left to breed with all the ewes in the flock and this factor (related individuals used for reproduction) might be responsible for high heterozygote deficiency observed in this study.

The observed heterozygosity in this study was lower than that reported (0.735) by Diez-Tascon et al. (2000) in New Zealand Merino, 0.661 by Alvarez et al. (2004) in Blond-faced Latxa sheep, 0.58 by Beraldi et al. (2006) in Soay sheep breed and 0.590 by El Nahas et al. (2008) in Barki sheep breed. The mean expected heterozygosity in this study was higher than that reported in literature (Forbes et al., 1995; Buduram, 2004; Alvarez et al., 2004; Baumung et al., 2006; Legaz et al., 2008). On the other hand, the expected heterozygosity in this study was lower than the reported 0.78 by Baumung et al. (2006) in Waldschaf sheep breed, 0.774 for Manchega and 0.789 for Merina sheep breeds (Legaz et al., 2008), 0.86 for Barki, 0.811 for Ossimi and 0.855 for Rahmani sheep breeds (El Nahas et al., 2008).

Polymorphic information content (PIC)

The PIC is a parameter indicative of the informa-tive degree of a marker. The PIC values range from 0 to 1. Loci with many alleles and PIC value of one are the most desirable (Botstein et al., 1984). PIC for all the 35 markers is shown in Table 2. Average PIC value for the 35 microsatellites was 0.744, ranged from 0.935 for INRABERN185 to 0.208 for BMS1494. All markers had PIC values higher than 0.5, except for locus BMS1494 and MAF65, indicating the Presence of genetic variability, a useful basis for developing breeding or genetic improvement strategy for Hamdani sheep. Average PIC value in this study, was in close agreement with the 0.750, 0.796, 0.795 and 0.762 reported in Sanjabi, Kordi Kordistan, Mehraban and Moghani sheep breeds, respectively (Esmaeilkhanian and Banabazi, 2006). However, the PIC values in the present study were higher than the 0.52 reported in Soay sheep (Beraldi et al., 2006) and 0.70 in Zardi, 0.72 in Kajal and 0.72 in Kolul Iranian sheep breeds (Seidani et al., 2009).

Inbreeding coefficient

Inbreeding coefficients for all markers are also given in Table 2. Average f value for overall markers of Hamdani sheep investigated was 0.469 (ranging from -0.043 at BM1818 to 0.790 in MAF209). The distinct f values for the loci reflect different levels of inbreeding. Four loci (BM1818, INRA063, MAF035 and SRCRSP24) displayed f

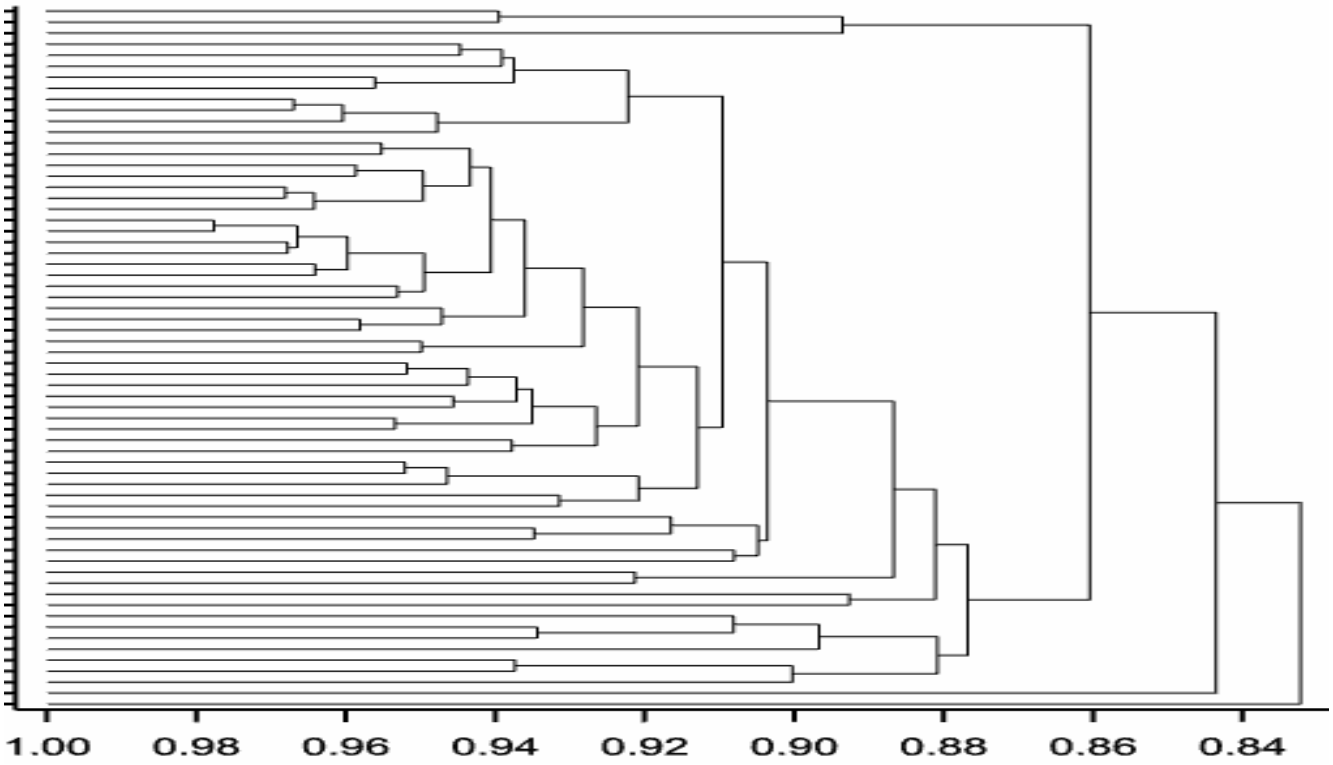

Figure 1. Neighbour-joining phylogenetic tree based on Nei's standard genetic distance values constructed using 64 individual Hamdani ewes.

values close to zero; the negative/close to zero values of f for the loci indicates that the mates were less related in comparison to the average population (Wang, 1996). Fourteen loci had f values lower than 0.5, while seventeen loci had f values higher than 0.5. The presence of higher positive inbreeding value indicate the availability of high homozygosity suggesting that the Hamdani sheep breed both at commercial and agricultural research centre levels are managed under uncontrolled mating system. Hence, care should be taken to keep inbreeding at a fairly low level by avoiding mating of brothers with sisters or parents with their offspring, which can produce random losses of desirable genes. This finding is in line with the findings of De Araujo et al. (2006), Alvarez et al. (2004), Mukesh et al. (2006) and Kusza et al. (2009). On the other hand, low inbreeding values for various sheep breeds have been reported in literature (Diez-Tascon et al., 2000; Soysal et al., 2005).

In conclusion, assessing genetic diversity should be the first step in establishing appropriate conservation programs for Hamdani sheep breed at national level. The set of microsatellite markers used in this work was generally suitable in assessing genetic diversity in the Hamdani sheep population analyzed, revealing high levels of genetic variability, assessed by both the number of alleles and heterozygosity. The 64 individual ewe samples were collected from two traditionally managed farms (commercial farm and agricultural research station), which showed signs of accumulated inbreeding that might lead to genetic bottlenecks. The results reported in this manuscript may serve as useful indicators for developing further in-depth studies by increasing the sample sizes and number of microsatellite markers for setting conservation priorities, taking into consideration within-population variability, in addition to information on traits of current or potential economic importance, including adaptations.

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