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Characterization of some Egyptian Sheep Populations Using Microsatellite and Protein Markers

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ABSTRACT

Three Egyptian sheep populations, collected from three geographically isolated regions namely Siwa, El-Dakhla and El-Farafra oases of the Egyptian western desert, were investigated using six polymorphic microsatellite markers and electrophoretic protein by SDS-PAGE. Six polymorphic microsatellite markers and electrophoretic protein were used to reveal that the genetic diversity, conduct genetic structure and assignment of microsatellite. The results indicated that; one hundred and five alleles were detected; 34 are common alleles and 71 specific alleles across six loci (67.62%). Thirteen specific alleles for Siwa sheep population ranged from 1 to 5. Also, for Dakhla sheep population 21 alleles ranged from 1 to 8. While 37 ones were obtained in the case of Farafra sheep population ranging from 1 to 14. When the heterozygotsity is high we will have the highest effective number of alleles (ENA) and the expected heterozygosity (gene diversity). The highest ENA was 10.29 for BM1314 when HE was 0.94, while the lowest ENA was 2.22 for BM8125 when HE was 0.58 with El-Dakhla sheep population. The protein profile for 33 sheep population samples collected from three different regions, Siwa had 20 protein bands that MW ranged from 17 to 269KDa. At the same concentration of protein and molecular weight, the band's volume or intensity values changed from sample to other. The homogeneity of individual Siwa samples was 30%. El-Dakhla population had 23 protein bands that ranged from17 to 283KDa. The heterogeneity's El -Dakhla population was 74%. While, El-farfra populations varied in electrophoretic protein pattern, it had 21 bands that ranged from 17 to 252 KDa. The homogeneity percentage was 24%. Finally, protein profile can be used as a marker depending on the presence or absence bands, band intensity, molecular weight and relative front values.

Keywords: Characterization, Microsatellite, Protein, Markers, Sheep populations.

INTRODUCTION

In recent years, the genomic studies of Egyptian genetic resources of livestock became one of the urgent issues, for maintaining about the rights royalty of Egyptian local breeds through using molecular level analysis.

Sheep are distributed in different regions of the world; in Egypt, it is considered one of the most important resources of red meat. Sheep represent an income source in many countries (Anous, *et al*., 2008). Sheep breed in Egypt had many types, which were divided into Rahmani, Ossimi, and Barki depending on some features such as the production of meat, dairy and wool, and the number of sheep exceeded about 4 million heads (Galal *et al*., 2005). Simple sequences repeat (SSR) markers are useful method to determine the genetics relationships and morphological traits within and between the native sheep breads (Chen *et al*., 2009; Ibrahim, 2010; Visser *et al*., 2011 and El-Sayed *et al*., 2016). Simple sequence repeats and proteins electrophoretic techniques have been increasingly used to as markers to characterize the genetic variability between and within populations and allowed to these methods to determine the genetic diversity (kayali *et al*., 2012 and Thiruvenkadan *et al*., 2014). Microsatellite markers were used to show the genetic variability and interbreed difference of individuals. Also, studying genetic diversity of local livestock genetics resources help for providing genetic enhancement and increasing diseases tolerance (Ozerov *et al*., 2008 and Glowatzki-Mullis *et al*., 2009). Polymorphic microsatellite as a marker was used to study the diversity of genetic relationships and population structure between Bulgarian local sheep breeds (Hristova *et al*., 2014). Electrophoretic techniques can be used to differentiate and identify between some treated animals meat samples, which was taken some factors into consideration like physical and chemical properties (Montowska and Pospiech, 2007). SDS-PAGE methods as comparison tools between sheep and goat species, salivary proteins was analyzed into 12.5% of liner polyacrylamide gel to understand the biological function of salivary proteins and its role in feeding behavior (Houle, 1989 and Lamy *et al*. 2008).

The main target of this study was aim to characterize and identify a number of molecular markers that influence on local Egyptian sheep, through determining the degree of polymorphism and genetic diversity between three Egyptian sheep populations, which was gotten from El-Farafra, El-Dakhla and Siwa oases. This objective is projected to employ the detected markers in marker assisted selection in sheep flocks to result in an increase in sheep population numbers and to aid in the national economy of Egypt.

MATERIALS AND METHODS

Sample collections:

Blood samples were randomly collected from fifty-two sheep belonging to three different regions in Egypt. The number of samples for each population were 21 El Farfra, 16 El Dakhla and 15 from Siwa oases. Five ml of venous blood were collected from each animal in sterile tubes having Na_2 EDTA as anticoagulant. Plasma samples were gotten from all fresh blood samples, through cooled centrifugation for 20 min at 4000 rpm and stored in separated test tubes at -20 ^oC until analysis, as recommended by Mwacharo, (2002).

DNA Extraction and Microsatellite Markers.

Fifty-two samples of blood Egyptian sheep were used in the study. DNA isolation by the salting out method according to Sambrook *et al.* (1989). Six microsatellite primers pairs were used in this study and detailed information on these markers are presented in Table (1). The microsatellite primers were chosen based on the polymorphism degree and the covering of genome as that recommended by the Food and Agriculture Organization

(FAO, 2004 and 2011).The PCR reaction was done according to El-Sayed *et al*., (2017).

As given in table (1), the details and characteristics of microsatellite markers used for the genetic analysis in our study are shown. As a locus name, flanking sequences, chromosome number, annealing temperature and allele range in base pairs.

Table 1. Information about the six microsatellite markers that used in this study, including locus name, flanking sequences, chromosome number, annealing temperature and allele range in base pairs.

Locus Name	Forward primer	Reverse primer	Chr. No	Ta[1] °C	Allele range
BM757	TGGAAACAATGTAAACCTGGG	TTGAGCCACCAAGGAACC	9	47	182-230bp
BM827	GGGCTGGTCGTATGCTGAG	GTTGGACTTGCTGAAGTGACC	3	47	150-294bp
BM1314	TTCCTCCTCTTCTCTCCAAAC	ATCTCAAACGCCAGTGTGG	22	48.4	103-184bp
BM8125	CTCTATCTGTGGAAAAGGTGGG	GGGGGTTAGACTTCAACATACG	17	46	50-84bp
OarHH47	TTTATTGACAAACTCTCTTCC TAACTCCACC	GTAGTTATTTAAAAAAATATCATA CCTCTTAAGG	18	58	43-148bp
OarVH72	CTCTAGAGGATCTGGAATGC AAAGCTC	GGCCTCTCAAGGGGCAAGAGCAGG	25	56	64-148bp

1.Annealing temperature, (FAO, 2004)

Electrophoretic analysis of SDS-PAGE

Constant concentration of 5 µl of thirty-three plasma samples of three selected sheep populations were mixed well with 20 µl of sample buffer (0.125 M Tris–HCl pH 6.8, 10% SDS, 2-mercaptoethanol, 50% glycerol with 1% of bromophenol blue) and prior to pouring into 4% stacking gel and 12% of separating polyacrylamide gel wells.

The samples were run at 60 v until entered the separation gel, then the voltage was raised to 110 v. Molecular mass protein standards from 5 to 250 kDa; PageRuler Broad Rang Unstained Protein Ladder, #26630, Thermo Scientific.

Preparation of polyacrylamide gel, electrophoretic conditions, staining with Coomassie blue and destaining gels were done according to Laemmli (1970).

Statistical analysis:

Gels were visualized and scored with Alphaimager 2200 software Version 4.0.1. All scored microsatellite data employing the Arlequin 3.11 software package after data conversion using CONVERT program. POPGENE software package (Yeh *et al*., 1999) calculation of the allele frequencies; observed the expected number and an effective number of alleles (Kimura and Crow, 1964). Phoretix-nonlinear dynamics software analysis was used to calculate the proteins on gel electrophoresis depend on the staining, measure the distance of migration as well as that of the tracking dye. The intensity value for each protein band was calculated depending on the sum of the peak height/intensity levels of the band and represented as a relative comparison. According to Sneath and Sokal (1973), gel images were analyzed by constructed tree depending on Unweighted Pair Group with Arithmetic Mean (UPGMA) phylogenetic method.

RESULTS AND DISCUSSION

One hundred and five alleles were detected, the allele numbers were higher than those reported by El-Sayed *et al*., (2016) who a found a total number of alleles was 42 across the 10 microsatellites markers in two goat populations which, located in El-Farafra and Siwa Oases.

Also, El-Sayed *et al*., (2017) reported that the total number of alleles was 32 a set of six microsatellite markers with three breeds and one population; Baladi, Zaraibi, Damascus and Farafra genotypes. While, the allele numbers were lower than those reported by ozerov *et al*., (2008) out line that in all, 233 alleles were found across the 20 microsatellite loci and the number of alleles at the locus varied from 6 (OarCP34) to 17 (BM4621, CSSM31, and OarFCB304) with an average value of 12 in sheep of the four Kazakh breeds. Ibrahim *et al*., (2010) reported that number of 119 alleles across 31 SSR loci in three sheep populations was identified from three breeds Balkhi, Hashtnagri and Michni.

Results revealed thirty four are common alleles and seventy one specific alleles across six loci in three sheep populations (Siwa, Dakhla and Farafra). Ibrahim *et al*. (2010) reported that three sheep populations shared a considerable number (76%) of alleles. A total number of 91 alleles were participate among the three breeds;o Balkhi, Hashtnagri and Michni sheep with a mean of 2.9, ranging from 0 (OarCP38) to 6 (OarFCB304). Also, El-Sayed *et al*., (2017) reported that total of 27 common alleles were detected versus six microsatellite loci overall genotypes for Baladi, Zaraibi, Damascus and Farafra.

Regarding specific alleles, a total number of 71 out of 105 alleles (67.62%) were noticed overall loci for the three sheep populations. While El-Sayed *et al*., (2016) detected that a total of 20 out of 42 alleles (47.62%) were detected overall loci (10 microsatellite loci) in two goat populations located in El-Farafra and Siwa Oases. El-Sayed *et al*., (2017) also reported that a total of 5 out of 32 alleles (15.63 %) were noticed in overall loci for Baladi and Farafra goats, 2 specific alleles were observed in Baladi breed while 3 ones were obtained in the case of Farafra goat population. For Siwa sheep population 13 alleles ranged from one in BM757 to 5 in BM827 and 21 ranged from one in BM757 to 8 in BM827 for Dakhla sheep population specific alleles were observed, while 37 ones were obtained in the case of Farafra sheep population ranged from one in BM8125 to 14 in OarHH47. Ibrahim *et al*. (2010) reported that Michni carried 12 unique alleles, whereas Balkhi (BM1329) and Hashtnagri (OarFCB304) carried one each across all loci, BM1329 was the most mutated locus, yielded three unique alleles in Michni population, Among others loci, BM8125, HUJ616 yielded two unique alleles, BM1824, MAF65, OarCP38, OarFCB20 and YMS1 yielded one unique allele each in Michni population. El-Sayed *et al*.,

(2016) detected that Farafra goats 8 specific alleles with a mean value of 0.8 while 12 were obtained in Siwa goat populations with a mean of 1.2. El-Sayed *et al*., (2017) reported that the number of alleles ranged from 3 (loci TGLA53, McM527 and CSRD247) to 6 (locus BM6444) in Baladi goat breed. While, Zaraibi goat breed ranged from 2 (locus TGLA53) to 5 (locus TCRVB6) and from 1 (locus CSRD247) to 4 (locus ETH10) in Damascus goat breeds and from 2 (loci BM6444 and TGLA53) to 6 (loci TCRVB6 and ETH10) in Farafra goat population.

Consequently, these specific alleles would be utilized as population fingerprint even one allele for one locus. The obtained results showed that all specific alleles detected in Siwa, El-Dakhla and El- Farafra sheep populations were observed in all markers with the exception of BM1314 and OraHH47 in Siwa and El-Dakhla sheep populations, respectively.

Allele frequencies in Siwa sheep population per locus ranged from 0.067 (for OarHH47 in allele 64) to 0.400 (for OarVH72 in allele 60). While, in El-Dakhla sheep population ranged from 0.039 (for BM1314 in allele 133 and 172) to 0.385 (for BM8125 in allele 78), Also, in El-Farafra sheep population ranged from 0.024 (for BM757 in allele 210) to 0.175 (for OarHH47 in allele 49) as shown in table 2. In Hashtnagri sheep the frequency of null alleles ranged from 0.017 (BM1329) to.959 OarCP38 and in Michni population null alleles were found at highest frequency at six loci ranged from 0.001 (BM8125) to 0.830 (ILSTS5) by (Ibrahim *et al*., 2010). El-Sayed *et al*., (2017) reported that the highest allele frequency for overall loci was 1.00 for allele 244 at locus CSRD247 in the case of Damascus goat breed and the lowest one was 0.0500 associated with Farafra goat population at locus TCRVB6 (for alleles 210 and 240).

Table 2. Common and specific alleles, their frequencies for each allele as observed for analyzed Siwa, El Dakhla and El Farafra sheep populations.

Locus	Common alleles bp	Specific alleles		
		Siwa	Dakhla	Farafra
BM757	186, 190, 194, 198, 202, 206, 218, 222	230(0.031)	182(0.267)	210(0.024), 226(0.048)
BM827	216, 222, 225, 243	207(0.071), 210(0.143), 213(0.071), 237(0.071), 249(0.143)	150(0.083), 195(0.083), 255(0.083), 261(0.167), 270(0.250), 273(0.083), 288(0.083), 294(0.167)	219(0.033), 228(0.067), 231(0.067), 240(0.133), 252(0.033)
BM1314	118, 139, 142, 145, 148, 151, 160, 166, 178	---	133(0.039), 136(0.077), 169(0.154), 172(0.039)	100(0.050), 103(0.050), 115(0.050), 121(0.050), 124(0.050), 157(0.050), 163(0.050), 181(0.050), 184(0.100)
BM8125	52, 54, 56, 58,	60(0.400), 62(0.133)	74(0.077), 76(0.231), 78(0.385), 80(0.154), 82(0.077), 84(0.077)	50(0.056)
OarHH47	43, 55, 58	61(0.200), 64(0.067)	---	46(0.050), 49(0.175), 52(0.025), 67(0.075), 73(0.075), 79(0.025), 118(0.025), 121(0.025), 124(0.075), 130(0.025), 133(0.050), 139(0.100), 145(0.025), 148(0.025)
OarVH72	104, 108, 112, 116, 120, 124	88(0.188), 96(0.188), 100(0.313)	64(0.267), 68(0.200)	128(0.100), 132(0.050), 136(0.100), 140(0.050), 144(0.100), 148(0.050)
Total	34	13	21	37
Specific alleles			71	
Total no. of alleles			105	

When the heterozygotsity is high will have the highest of the effective number of alleles (ENA) and the expected heterozygosity (gene diversity). The highest ENA was 7.54 for BM827 when HE was 0.90, while the lowest ENA was 3.38 for OarHH47 when HE was 0.73 with Siwa sheep population. Also, the highest ENA was 10.29 for BM1314 when HE was 0.94, while the lowest ENA was 2.22 for BM8125 when HE was 0.58 with El-Dakhla sheep population. However, the highest ENA was 7.11 for OarHH47 when HE was 0.92, while the lowest ENA was 2.46 for BM8125 when HE was 0.63 with El-Farafra sheep population. this means that the highest in heterozygotsity with El-Dakhla sheep while, the lowest hetergygosity in Farafra sheep populations as shown in Table (3). El-Sayed *et al*., (2016) detected that the effective number of alleles varied from 1.65 (SRCRSP8) to 3.24 (SRCRSP9) with a mean value of 2.19 in Farafra goats and varied from 1.60 (SRCRSP23) to 3.85 (OarFCB48) with a mean value of 2.60 in Siwa goats.

Means of observed heterozygosities were 0.09, 0.16 and 0.17 in Siwa, Dakhla and Farafra sheep populations, respectively. While, means of expected heterozygosities were 0.81, 0.79 and 0.80 in Siwa, Dakhla and Farafra sheep populations respectively. Values of the observed heterozygosity were lower than that reported by OZEROV *et al*. (2008) while, higher than the mean of expected heterozygosity for four sheep breeds: Degeres Mutton-Wool, Kazakh Arkhar Merino, Kazakh Finewool, and Edilbaev. The values were lower than the mean of observed heterozygosity, while it was higher than the mean of expected heterozygosity for the four goat genotypes (Baladi, Zaraibi, Damascus and Farafra) by El-Sayed *et al*., (2017). These positive values indicate heterozygote deficit with a mean Fis value of 0.817. Marini *et al*., 2014 reported that a positive Fis value indicates an excess of homozygotes, while a negative value shows deficit in homozygotes.

When the observed heterozygosity was highest the FIS and FIT were lowest. The highest Ho was 0.47, 0.45 and 0.63, while the lowest FIS and FIT was 0.3542 and 0.4374 for the same locus OarHH47 with Siwa, El-Dakhla and El-Farafra sheep populations, respectively. While, the lowest in the observed heterozygosity the highest in FIS and FIT was 1.000 in BM1314 locus.

FST ranged from 0.0796 in BM1314 to 0.2378 in BM8125 with mean of 0.1330 which was higher than *Gyr* breed. (Fst of 0.02) in *fehr* and *fekr* breeds are moderately differentiated Fst of 0.078 and 0.112 respectively, Zackel breeds showed relatively little genetic differentiation compared to the mean Fst value of 0.097 as reported by Neubauer *et al*., (2015). According to Hartl and Clark (2007), moderate differentiation Fst ranged from 0.05 to 0.15.

The mean Fit and Fst values of 0.841 and 0.133, respectively were measured by the degree of differentiations within and among breeds. The mean of Fit was higher than those of Baladi, Zaraibi, Damascus and Farafra goat genotypes with the mean Fit of 0.767, while, the mean of Fit was lowest than that reported by El-Sayed *et al*., (2017).

Table 3. Heterozygosis (HO, observed; He, expected; ENA effective) and F-Statics for six microsatellite loci in Siwa, Dakhla and Farafra sheep populations

locus	Siwa			Dakhla			Farafra			F-Statics		
	Ho	He	ENA	Ho	He	ENA	Ho	He	ENA	FIS	FIT	FST
BM757	0.06	0.83	5.07	0.42	0.68	2.91	0.25	0.73	3.20	0.660	0.705	0.132
BM827	0.00	0.90	7.54	0.00	0.79	3.77	0.14	0.80	3.92	0.939	0.945	0.095
BM1314	0.00	0.83	5.00	0.00	0.94	10.29	0.00	0.88	5.44	1.000	1.000	0.080
BM8125	0.00	0.78	4.09	0.11	0.58	2.22	0.00	0.63	2.46	0.942	0.955	0.238
OarHH47	0.47	0.73	3.38	0.45	0.87	5.90	0.63	0.92	7.11	0.354	0.437	0.129
OarVH72	0.00	0.81	4.57	0.00	0.87	5.76	0.00	0.83	4.57	1.000	1.000	0.133
Mean	0.09	0.81	4.94	0.16	0.79	5.14	0.17	0.80	4.45	0.817	0.841	0.133

ENA : Effective number of alleles (Kimura and Crow, 1964)

Cluster analysis based on Nei's genetic distance indicated that the studied populations formed two main groups. The 1st group included El-Dakhla and Siwa and the 2nd group harbored El-Farafra as illustrated in diagram (1).

```
+---------------------------------------------------- El-Farafra
--2
 !                          +---------------------  El-Dakhla
 +-------------------------------1
                            +------------------------- Siwa
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Diagram 1. Dendrogram Based Nei's Genetic distance of three sheep populations produced by UPGMA clustering based on Nei's genetic distance using 6 microsatellite loci.

Protein Electrophoretic

Protein electrophoretic profiles SDS-PAGE of whole sheep samples were collected from three geographically isolated regions namely Siwa, El-Dakhla and El-Farafra oases in the Egyptian western desert. Comparative study was performed between thirty-three samples of three sheep populations under study, the experiments were carried out by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique as shown in figures (1,2and 3). The intensity value for each protein band was calculated depending on the sum of the peak height/intensity levels of the band and represented as a relative comparison.

1- Siwa population

Twenty protein bands with molecular weights ranging from 17 to 269 kDa were observed in the protein profile of Siwa as shown in Tables (4 and 7) and Fig (1).

These bands were distributed on the gel in a range of Rf: from 0.056 to 0.934. The results revealed that the protein pattern showed 6 monomorphic bands by 30% homogeneity. Table (4) exhibited the presence and absence of the protein bands in all of Siwa population samples. Also, table (4) had the level of bands intensity, which was useful to differentiate within and between samples. At the same concentration of protein and molecular weight, the band's volume or intensity values were changed from sample to other. For instance, at the molecular weight of 180 kDa. the highest value of intensity was 1694 in lane 1 while the lowest value was 568 in lane 10. The heterogeneity within Siwa population samples was 70%.

2- El-Dakhla population.

The electrophoretic protein pattern of individuals of El-Dakhla population is showed in tables (5 and 7) and Fig. (2). Total number of protein bands were 23, which distributed along of gel in ranging from 283 KDa at Rf 0.037 to 17 KDa at Rf 0.850. Protein profile has17 polymorphic and 6 monomorphic bands by 74% of heterogeneity. Intensity bands were calculated and the highest value was 5920 in lane 11 and the lowest value was 3881in lane 8 at the same molecular weight 76 KDa. Also, El-Dakhla,s protein profile has some specific bands which may be used to distinguish this population, for instance, protein bands at molecular weights 196, 164, 127,96, and 17 KDa were presented in all of El-dakhla samples. The results also revealed that molecular weight at 214 KDa was vanished in all of ElDakhla samples, except one sample (L3). Molecular weights at 108 KDa were appeared in two samples while disappeared in the rest of samples. Electrophoretic profiles of sheep population's proteins have been reported by Montowska and Pospiech, (2007). Also, Lamy *et al*. (2008). reported that the difference between two species (sheep and goats) by protein electrophoresis

Table 4. Molecular weight (MW) of SDS- PAGE plasma proteins for Siwa sheep population

Samples Bands	MW	RF	L 1	L 2	L 3	L 4	L 5	L 6	L 7	L8	L 9	L 10	L 11
1	269	0.056	615	932	844	1235	0	609	872	557	490	539	485
2	252	0.086	0	0	0	0	1122	0	0	0	0	0	0
3	235	0.100	1473	1023	1270	1140	454	0	920	1753	727	0	0
4	214	0.150	497	0	0	545	798	0	0	0	1050	0	0
5	196	0.171	0	0	362	0	0	0	0	0	0	0	0
6	180	0.200	1694	1094	1004	1542	866	707	828	1392	640	568	607
7	144	0.271	2576	2036	1658	1502	0	1127	1080	1218	1028	1794	0
8	108	0.35	5969	3555	2411	5513	3463	1897	1898	0	2123	2180	1599
9	96	0.384	0	1220	1009	0	0	1037	0	2172	1300	1115	0
10	82	0.419	0	1544	0	0	0	864	625	1011	0	0	908
11	76	0.439	1344	0	1138	510	837	0	1307	797	876	825	0
12	53	0.517	9701	8928	7983	9782	7338	6206	7615	7286	7020	7313	6642
13	41	0.566	1821	1225	1433	1527	1147	1054	987	1092	1328	1329	1189
14	34	0.605	624	1086	693	1151	732	376	628	507	851	330	458
15	28	0.643	0	0	0	1433	0	0	0	0	0	0	0
16	27	0.654	0	0	0	0	0	0	1180	1060	0	0	0
17	21	0.709	1328	968	1026	1727	1044	805	1130	946	851	700	845
18	19	0.741	823	849	1058	0	0	0	0	0	0	0	0
19	18	0.772	0	0	0	861	570	373	543	588	581	273	589
20	17	0.934	1069	498	1210	1160	472	1004	962	310	589	973	1109
Total bands	-	-	13	13	13	14	12	11	14	14	14	11	9

(0) means absence of band, (intensity value) means presence, Rf means relative front, MW = molecular weight

Table 5. Molecular weight (MW) of SDS- PAGE of plasma proteins for El- dakhla sheep population.

Samples Bands	MW	RF	L 1	L 2	L 3	L 4	L 5	L 6	L 7	L 8	L 9	L10	L11
1	283	0.037	0	0	0	0	568	323	194	161	164	635	0
2	269	0.055	0	528	615	0	0	0	0	0	422	523	707
3	252	0.084	0	272	349	0	319	252	217	0	0	1206	625
4	235	0.106	423	517	523	383	602	583	0	545	437	0	0
5	214	0.145	0	0	305	0	0	0	0	0	0	0	0
6	196	0.177	332	655	825	388	516	548	601	492	516	1666	1062
7	164	0.232	1082	1379	1468	537	1181	1387	1101	1200	1255	1579	1466
8	127	0.300	1223	3296	2891	1369	2315	1475	1814	1669	1527	3385	2398
9	108	0.353	0	0	1002	566	0	0	0	0	0	0	0
10	96	0.384	816	848	904	716	1316	732	666	715	886	1871	1165
11	76	0.440	4321	0	0	4497	0	4385	4384	3881	0	0	5920
12	67	0.47	0	6844	7034	0	5776	0	0	0	5714	8437	0
13	53	0.507	0	0	0	748	0	1567	1171	1202	944	1092	1683
14	47	0.541	1135	1676	1910	707	1555	0	0	0	394	1837	0
15	34	0.605	0	653	0	0	0	0	551	385	1093	573	2113
16	31	0.623	0	0	314	0	824	463	0	0	0	437	0
17	28	0.640	0	0	303	0	0	0	0	0	0	499	0
18	27	0.655	0	427	0	0	0	734	910	307	0	653	1291
19	25	0.671	0	219	714	0	525	0	0	573	664	0	0
20	21	0.720	644	398	814	2525	596	555	735	736	1222	871	715
21	19	0.750	0	0	0	0	0	0	350	457	0	916	0
22	18	0.776	387	225	443	514	296	507	0	0	0	446	0
23	17	0.850	682	458	436	903	669	336	616	616	745	1474	2386
Total bands	-	-	10	15	17	12	14	14	13	14	14	18	11

(0) means absence of band, (intensity value) means presence, Rf means relative front, MW = molecular weight

3- El-Frafra population

El-Frafra population varied for electrophoretic protein pattern. Total bands of protein were 21 those bands ranged from 252 KDa at Rf 0.040 to 17 KDa at Rf 0.906. The results showed that the monomorphic bands was 5 with Homogeneity percentage 24% and polymorphic bands was 16 bands with heterogeneity by 76%. The molecular weights 243 KDa with Rf 0.059 and 223 KDa with Rf 0.093 were present only in Lane 6 and Lane 7 respectively. Also, the molecular weights 89, 41, 37, 28 and 25 with Rf 0.390, 0.574, 602, 664 and 690 respectively, were absent in all of samples of El-frafra populations. On the other hand, the band of molecular weight 33 KDa at Rf 0.620 was presented in all of samples except lane 6. Also, band 17 KDa at Rf

0.906 was exist in all samples except two samples lane 8 and 10. At the same amount of injected protein concentration, the intensity of bands was the highest compared with the other populations. So, the highest value of intensity was 7549 at 33 KDa. while the lowest value was 22 at 18 KDa. as shown in Table (6) and Fig. (3). this agrees with El-hamamsy and Behairy (2015) and Carvajal-Serna *et al.* (2018).

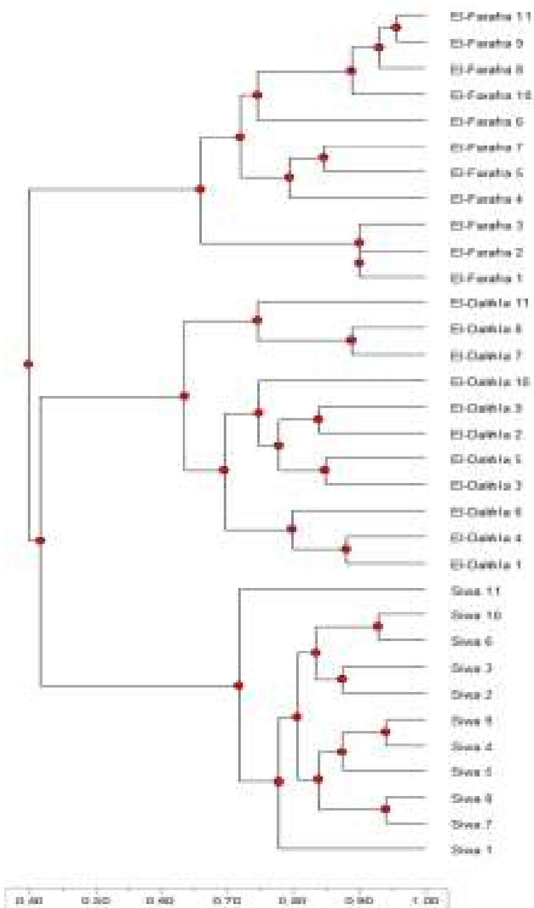

Diagram 2. UPGMA unweighted pair group method with arithmetic averages algorithm Dendrogram of genetics relationships and similarity index among the Three Egyptian populations of sheep (siwa, El dakhla and El farafra).

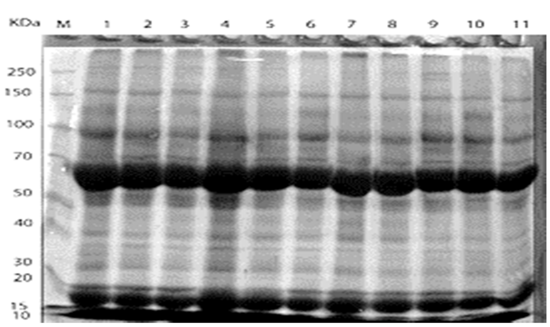

Fig. 1. SDS-PAGE profile of Siwa sheep plasma proteins and M= protein markers.

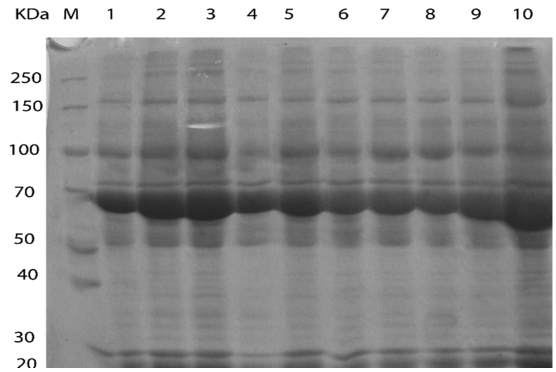

Fig. 2. SDS-PAGE profile of El dakhla sheep plasma proteins and M= protein markers.

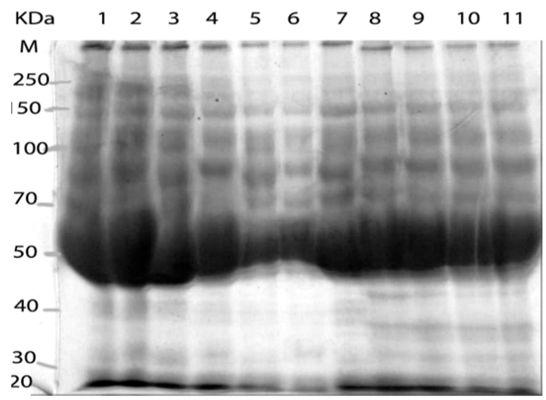

Fig. 3. SDS-PAGE profile of El-Farafra sheep plasma proteins and M= protein markers.

The Dendrogram illustrated the genetic relationship and similarity value among populations under study. average similarity between the El-farfara and (El-Dakhla and Siwa) populations was 0.40. While, the similarity index between El-Dakhla and Siwa was 0.43. The similarity index within Siwa, El Dakhla, and El-Farfra populations were 0.72, 0.63 and 0.66 respectively. This may reflected a higher degree of inbreeding in both the El-Dakhla and the Siwa populations compared to the El-farfara. The dendrogram of sheep populations was divided into two groups; the first one represented the El-farfara, while, the second represented the El-Dakhla and Siwa populations. The first one divided into two subclusters the first subcluster contained three samples of El-farfara while the second contained the rest of the samples. El-Dakhla Dendrogram divided the samples into two clusters, the first one had three samples while, the second divided into two subcluster which contained about the rest of samples. The Siwa Dendrogram divided the samples into two clusters. The first one contained about one sample while, the second one divided into two subclusters, the first had about one sample but the other one divided into sub subcluster which involved about the rest of samples. Our results are in agreement with kayali *et al.*, (2012) who stated that the Dendrogram of Similarity values could be used as a analysis tool by protein electrophoresis and molecular markers between two strains of sheep.

Table 6. Molecular weight (MW) of SDS- PAGE plasma proteins for El- farfra sheep population

Samples Bands	MW	RF	L 1	L 2	L3	L 4	L5	L6	L 7	L8	L 9	L10	L11
1	252	0.040	1307	1639	1564	1327	390	395	787	1423	1239	974	1000
2	243	0.059	0	0	0	0	0	0390	0	0	0	0	0
3	223	0.093	0	0	0	0	0	0	322	0	0	0	0
4	208	0.120	1279	490	487	172	134	637	515	231	657	498	525
5	196	0.141	2074	2144	1370	0	0	0	0	0	0	0	0
6	182	0.168	0	0	0	1322	403	0	649	890	473	484	536
7	161	021	1302	1308	1286	1019	672	1037	1051	1177	1184	950	1009
8	129	0.283	2873	3359	2833	1672	1536	1076	2249	1904	1863	1801	1672
9	95	0.378	5229	4889	3843	3498	1760	702	1903	3187	2958	1771	1956
10	89	0.390	0	0	0	0	0	0	0	0	0	0	0
11	69	0.430	0	0	0	0	803	4700	2162	0	0	1645	1105
12	41	0.574	0	0	0	0	0	0	0	0	0	0	0
13	37	0.602	0	0	0	0	0	0	0	0	0	0	0
14	33	0.620	7704	9117	6917	7019	4480	0	5748	7400	7549	6319	6976
15	28	0.664	0	0	0	0	0	0	0	0	0	0	0
16	25	0.690	0	0	0	0	0	0	0	0	0	0	0
17	23	0.714	0	0	457	76	0	0	66	595	342	0	272
18	21	0.740	779	1015	139	116	0	0	187	0	0	0	0
19	19	0.762	0	0	0	69	0	0	138	0	0	0	0
20	18	0.817	223	0	0	0	0	0	22	632	692	7046	708
21	17	0.906	637	1129	905	338	1195	926	212	0	549	0	613
Total bands	-	-	10	9	10	11	11	8	15	9	10	9	11

(0) means absence of band, (intensity value) means presence, Rf means relative front, MW = molecular weight

Table 7. Homogeneity and heterogeneity percentage within studied groups based on native-protein banding patterns.

Populations of Sheep	Total No. of bands	Polymorphic bands	Monomorphic bands	Homogeneity	Heterogeneity %
Siwa	20	14	6	30%	70%
El-Dakhla	23	17	6	26%	74%
El-Frafra	21	16	5	24%	76%

CONCLUSION

Our results revealed that using microsatellite and protein markers were approximately similar, whereas the protein reflects the gene expression. Finally, we are recommended for more studies about the new valley El-Kharga, El-Dakhla, El-Farafra and Siwa oases, which located in the Western Desert, are especially for being geographically isolated regions. Also, based on the previous results, we were concluded that the electrophoretic is one of the important and sensitive methods, which may be employed for identification and characterization of both plants and animals. So, proteins, which were separated by the SDS-PAGE method, were (i) presence of specific protein bands, (ii) disappear of protein bands, (iii) variation in the number and the level of bands intensity. In general, the specific alleles of microsatellite and specific bands of protein profiles can be utilized as fingerprinting markers for sheep populations. We suggest, apply the present work at a wide genome to scan and analysis using more recommended microsatellites covering sheep genome, which could be utilized in further work on biodiversity within breeds and species.

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توصيف بعض قطعان الأغنام المصرية باستخدام المعلمات الجزيئية والبروتينية

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تم دراسة ثلاثة أنواع من الأغنام المصرية، والتي تم جمعها من ثلاثة مناطق، وهي واحات سيوة والداخلة والفرافرة الواقعة فى الصحراء الغربية المصرية، وذلك باستخدام ستة واسمات جزيئية وكذلك تم التفريد الكهربي للبروتينات باستخدام طريقة SDS-PAGE للكشف عن التنوع الوراثي والبنية الوراثية. أشارت النتائج إلى التالي: تم الكشف عن مائة وخمسة من الأليلات 34اليلات مشتركة و71 أليلات محددة ومعينة عبر ستة مواقع بنسبة (67.62) ثلاثة عشر من أليلات المعينة ميزت اغنام سيوه وتراوحت من 1 إلى 5 اليل. كما تم تمييز اغنام الداخلة بعدد 21 اليل تراوحت من 1 إلى 8 اليل. بينما تم الحصول على 37 من الأليلات في اغنام الفرافرة وتراوحت من 1 إلى 14اليل. كذلك فسرت النتائج الى وجود تناسب طردي بين درجة تأثير الاليلات والتباين الوراثي بمعنى انه عند الحصول على درجة مرتفعة من قيم التباين الوراثي تم الحصول على قيم مرتفعة من التأثير الاليلى (ENA).سجلت النتائج اعلى قيم من التأثير الاليلى ENA وهي 10.29 على الموقع BM1314 عندما كان التباين الوراثي 0.94فى حين سجلت اقل قيمة ENA وهي 2.22 على الموقع BM8125 عندما كان التباين الوراثي المتوقع 0.58 في اغنام الداخلة. كما أوضحت النتائج الخاصة بالتفريد الكهربي للبروتينات لـ عدد33 عينة من الثلاثة مجاميع الأغنام تحت الدراسة الى: احتوت اغنام السيوه على 20 حزمة بروتينية تراوحت بين 269 إلى 17 كيلو دالتون . كذلك عند نفس تركيز البروتين والوزن الجزيئي للحزمة البروتينية لوحظ تغير في قيم حجم وشدة الاضاءة من عينة إلى أخرى. وكانت نسبة التشابة الفردية داخل اغنام السيوة 30 ٪ . بلغ عدد الحزم البروتينية داخل اغنام الداخلة 23 حزمة بروتينيَّه تراوحت 17بين و283 كيلو دالتون. وكانت نسبة التباين داخل اغنام الداخلة 74٪ . في حين أن مجموعات الفرافرة كانت أكثر تنوع في نمط البروتين الكهربي، حيث احتوت على 21حزمة بروتينية تراوحت بين 17 إلى 252 كيلو .دالتون بنسبة تجانس كانت 24٪ .أخيراً، أمكن استخدام التفريد الكهربي للبروتينات كمعلمات وراثية اعتماداً على وجود أو غياب وكذلك حجم وشدة الاضاءة وقيم الاوزان الجزيئة (MW) ومعدل الجريان للحزم البروتينية. وبالتالي نوصى بعمل المزيد من الدراسات والاختبارات على الأغنام المصرية تمهيدا لإدخالها في برامج التربية المختلفة بحيث تكون خطوة مبدئية في التحسين الوراثي والحصول على حيوانات ذات صفات إنتاجية متميزة.