MICROSATELLITE MARKERS POLYMORPHISM BETWEEN TWO EGYPTIAN GOAT POPULATIONS (*Capra hircus*)

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Goats are considered one of the important economic sources of meat in Egypt. The majority of Egyptian goats belong to the Baladi breed, which is distributed all over the Nile valley and Delta region, while Barki goats are found throughout the coastal zone of the Western Desert. Zaraibi goats are a prolific breed originated in Nubia with high dairy potential and they have shown encouraging response to selection for dairy production while maintaining their high prolificacy. Other breeds, Saidi and Wahati goats are concentrated in the Southern region, while, Black Sinai goats can be found only in the Negev and Sinai deserts (Elbeltagy, 2012). While, Galal *et al.* (2005) had reported that goats are distributed across the country, especially dense in the Nile valley and Delta and with lower concentration in the North-Western coastal region and at the Oases. They further pointed out that in the Nile valley goats are usually found in small holdings as mixed flocks with sheep and other farm animals like cattle and buffaloes, while in the North-Western Mediterranean coast they are in large herds mixed with sheep. Egypt is predominantly desert and arid and semi-arid rangelands and can be divided into four major physical regions; the Nile Valley and Delta, Western Desert, Eastern Desert and Sinai Peninsula (Fig. 1). The New Valley Oases (El-Kharga, El-Dakhla, El-Farafra and Siwa) in the western desert are geographically isolated regions. The climate is hot and the temperature is cold at night in certain seasons. Accordingly, these oases have farm animal genetic resources (cattle, sheep, goat and camels) that are able to survive and are adapted to the prevailing conditions in these areas. Unique individuals of these animal species need to be screened, evaluated, collected and maintained as nucleus herds to benefit from them in sire centers.

Genetic diversity studies based on molecular genetic markers would help in understanding the genetic relationship and diversity within and among local Egyptian breeds and populations. Many researchers employed the random amplified polymorphic DNA markers technique (RAPD) to characterize and estimate genetic distances among different genotypes (Williams *et al*., 1990; Welsh and McClelland, 1990; Nyamsamba *et al*., 2002; Ouafi *et al*., 2002). With the further development and application of various DNA marker techniques, microsatellites and/or simple sequence markers were found to be useful in the study of genetic diversity within and among goat populations and breeds (Li *et*

Egypt. J. Genet. Cytol., 45: 89-103, January, 2016
Web Site (*www.esg.net.eg*)

al., 1999; Meng-Hua *et al*., 2002; El-Seoudy *et al*., 2005; Agha *et al*., 2008; Takahashi *et al*., 2008; Mahmoudi *et al*., 2010; Siwek and Knol, 2010; Visser *et al*., 2011; Marini *et al*., 2014; Thiruvenkadan *et al*., 2014).

The determination of genetic variation based on DNA markers provides accurate information for genetic distance analysis that allows a ranking of populations according to the level of phylogenetic distinction (May *et al.*, 1995). Liu *et al*. (2008) mentioned that the inbreeding coefficient (FIS) measures the relative heterozygote deficiencies and non-random mating in populations and breeds. Its value ranges between -1 (all individuals heterozygote), 0 (random association of alleles) and 1 (all individuals homozygote). If inbreeding is avoided, F=0; negative F indices result from selection in favor of the heterozygotes, while positive values indicate that the considered population has an inbreeding system of mating.

Therefore, the aim of this study is to determine the degree of polymorphism and genetic diversity between two Egyptian goat populations found in the western desert oases (El-Farafra and Siwa) using microsatellite markers, to assess their inbreeding coefficients and to identify SSR markers that could be useful in goat breeding programs.

MATERIALS AND METHODS

This work was implemented as part of a project focused on sustainable utilization of agriculture biodiversity, development of agricultural products and improvement of rural livelihood in the western desert of Egypt namely, in the oases where farm animal genetic resources (AnGR), are adapted to harsh environments.

1. Animal sampling and DNA extraction

A total of 34 blood samples were randomly collected from two goat populations located in El-Farafra and Siwa Oases (14 and 20 samples, respectively). The volume of blood sample was 15 ml from each individual. DNA isolation was carried out according to the salting out method by Sambrook *et al*. (1989).

2. Microsatellite- PCR genotyping

Ten microsatellite markers (ILSTS28, ILSTS029, SRCRSP5, MAF70, SRCRSP23, OarFCB48, SRCRSP9, OarAE54, SRCRSP8 and SPS113) are shown in Table (1). The microsatellite primers were selected based on the degree of polymorphism and genome coverage that have been recommended by the Measurement of Domestic Animals Diversity (MoDAD) (FAO, 2004 and 2011) and published on the FAO website (www.dad.fao.org/en/refer/library/guideli n/marker.pdf). The PCR reaction included: 50 ng template DNA, 10 pmol from each primer, 10 µl Master Mix (Emerald AMP GT PCR Master Mix, Takara Bio.Inc. composed of DNA polymerase, optimized reaction buffer, dNTPs, and a density reagent. The premix also contains a vivid green dye that separates into blue and yellow dye fronts. The PCR reactions

was carried out under the following conditions: an initial denaturation step for 2 minute at 95°C, followed by 35 cycles of denaturation for 30 seconds at 95°C; annealing temperature (53-65°C) for 60 seconds at optimized primer annealing temperature as shown in Table (1). Extension for 60 seconds at 72°C and final extension step at 72°C for 60 seconds. Amplified fragments were analyzed on a 10% polyacrylamide gel and stained with Ethidium bromide. The gel was photographed and the image analyzed using Gel Documentation System (Alphaimager™ 2200, Cell Biosciences).

3. Statistical analyses

Gels were visualized and scored with Alphaimager 2200 software Version 4.0.1. All scored microsatellite data was first adjusted using a Tandem Repeat Analyzer software package to estimate each allele size according to its number of repeats for each marker. Then, a spread sheet program (Microsoft Excel) was used to arrange the data for each population regarding each locus. Data was analyzed employing the Arlequin 3.11 software package after data conversion using CONVERT program. POPGENE software package (Yeh *et al*., 1999) was used to calculate allele frequencies; observed expected number of alleles and effective number of alleles (Kimura and Crow, 1964).

RESULTS AND DISCUSSION

Ten microsatellite polymorphic SSR-PCR markers used, including locus name, accession number, genome location, primer sequences, repeat type, annealing temperature and sequence tagged site (STS) size in base pairs are shown in Table (1). The microsatellite ILSTS28, ILSTS029, SRCRSP5, MAF70, SRCRSP23, OarFCB48, SRCRSP9, OarAE54, SRCRSP8 and SPS113 gave a total number of alleles per locus 4, 3, 3, 2, 4, 7, 4, 5, 7 and 3 (Table 2) with a size range from 142-187, 135-171, 144-184, 132-171, 70-121, 97-181, 96-147, 88-158, 170-266 and 127-165 bp, respectively (Table 5). This is in agreement with the selective standard of microsatellite loci given by the Secondary Guidelines for Development of National Farm Animal Genetic Resources using reference Microsatellite (FAO, 2004 and 2011). In a study conducted by Luikart *et al*. (1999) they reported that the number of alleles for locus ILSTS029 was 5, 5, 2 and 7 with a size range from 153-163, 153-167, 153-163 and 153-169 bp in Mongolian, Angora, Saanen and Murciana-Grenadina goat populations, respectively. While in our study (Table 2) only 3 alleles were present in Farafra and Siwa goats for the same locus (ILSTS029) with a size range 135-171 bp (Table 5). In a similar study on Baladi and Zaraibi Egyptian goat breeds, Hassanane *et al*. (2010) using three different microsatellite loci (INRA005, ILSTS005 and ILSTS087), the microsatellite INRA005 gave 6 alleles with a size range from 114-126 bp, ILSTS005 gave 5 alleles with a size range between 164-188 bp, while the microsatellite ILSTS087 gave 8 alleles with a size range between

136-158 bp.

A total number of 42 alleles were detected across the 10 microsatellites markers. These results were lower than those reported for the Girgentana goat breed with 21 microsatellite markers (Siwek and Knol, 2010), Baladi and Zaraibi breeds with three microsatellite markers (Hassanane *et al*., 2010), Ardi goat breed with 14 microsatellite markers (Riyadh *et al*., 2012), Salem Black goat breed with 25 microsatellite markers (Thiruvenkadan *et al*., 2014), and Malaysian Goat Breeds with 30 microsatellite markers (Marini *et al*., 2013).

The number of alleles per population ranged from 2 (loci MAF70, SRCRSP8 and SPS113) to 4 (loci ILSTS028, OarFCB48 and SRCRSP9) in Farafra goats, and 2 (loci MAF70 and SRCRSP23) to 7 (locus SRCRSP8) in Siwa goat population (Table 2). The numbers of alleles of the two goat populations were lower than those reported for the Mongolian Goat Populations (Takahashi *et al*., 2008), Girgentana goat breed (Siwek and Knol, 2010), South African Angora goats (Visser *et al*., 2011), Malaysian Goat Breeds (Marini *et al*., 2013) and Salem Black goat breed (Thiruvenkadan *et al*., 2014). The mean number of observed alleles per locus in Farafra (3.00) and Siwa (3.4) goats were also low compared to the mean number of alleles per locus reported for the Katjang, Jamnapari, Boer and Savanna goat breeds (5.43, 5.70, 5.90 and 5.70, respectively) by Marini *et al.* (2014). Barker (1994) suggested that loci with at least four alleles should be used in diversity studies to reduce the standard error of the estimated distance. In the present study only four loci, ILSTS029, SRCRSP5, MAF70 and SPS113 were found to have less than four alleles. The low number of alleles observed could be due to the small sample size, low number of polymorphic loci or the effect of inbreeding (Pandey *et al*., 2006; Maletsanake *et al*., 2013).

Regarding specific alleles, a total of 20 out of 42 alleles (47.62%) were detected overall loci (10 microsatellite loci) in the two populations. For Farafra goats 8 specific alleles were observed one in each of loci ILSTS028 and SRCRSP9 and to 2 in loci SRCRSP23, OarFCB48 and OarAE54 with a mean value of 0.8 while 12 were obtained in Siwa goat populations one in each loci SRCRSP23 and SPS113 and to 5 in locus SRCRSP8) with a mean of 1.2. In addition, the total number of common alleles was 22 as shown in Table (2). While the Barki and Zaraibi breeds had smaller numbers of alleles (Agha *et al*., 2008). The mean number of alleles shared between Barki and Ardi was 3.1, between Barki and Zaraibi 4.0 and between Ardi and Zaraibi 3.5, whereas the mean number of the alleles shared by the three breeds was 2.3 (Mahrous *et al*., 2013).

Heterozygosity reflects the degree of genetic variation of the microsatellite loci in livestock. The mean observed and expected heterozygosities were found to

be 0.21, 0.24 and 0.56, 0.59 in Farafra and Siwa goat populations, respectively. The values were lower than the mean expected heterozygosity for the two goat populations studied. The Farafra goats showed the lowest observed heterozygosity suggesting higher level of inbreeding in this population compared to the Siwa population. The high level of inbreeding in both populations studied was confirmed by the inbreeding coefficient (IC) which was estimated as (0.62) and (0.59) in Farafra and Siwa goat populations, respectively, as shown in Table (3). Similar to the present study, the observed heterozygosities of Malaysian goat breeds were lower than the expected heterozygosities (Marini *et al*., 2014). High heterozygosity values indicate high genetic diversity as well as a high degree of genetic variation. Araújo (2006) reported that the (H*E*) values were 0.6952 for Alpine, 0.7043 for Saanen and 0.4984 for Moxotó goat populations. While, Visser *et al*. (2004) reported that the heterozygosity (Hz) values per population were high except for the Boer goats (0.49), which showed the lowest (Hz) value compared to the other populations.

The effective number of alleles (Table 3) 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 which was lower than the Kannaiadu (4.22), Osmanabadi (3.25) and Sangamneri (3.30) breeds studied by Dixit *et al.* (2010) and from Sangamneri (4.04) by Verma *et al.* (2011) and from Katjang (3.56), Jamnapari (3.81), Boer (3.96) and Savanna (3.92) by Marini *et al*. (2013).

Analysis of molecular variance AMOVA (Table 4) of Farafra and Siwa goat populations based on microsatellite DNA variation showed that the majority of the genetic diversity obtained in the current study is presented by among individuals within populations (46.93%) and within individuals (29.37%). Population fixation indices give an idea about the population structure in terms of inbreeding coefficient and population differentiation. Population Fixation indices revealed a 0.7063 of variation referring to differences among individuals versus total variance (Fit). While, among populations differences versus total variance was the lowest fixation index (Fst = 0.2371) indicating low level of population differentiation. A pair wise difference among Frafra and Siwa goat populations was 0.6151 based on among breeds F index (Fis) as shown in Table (4). Our results are higher than the Fis values 0.044, 0.066, 0.032, 0.082, 0.064, 0.034, 0.045 and 0.069 obtained with Zavkhan Buural, Ulgii Red, Bayandelger, Zalaajinst White, Sumber, Erchim Black, Dorgon and Gobi Gurvan Saikhan Mongolian Goat Populations, respectively as reported by Takahashi *et al*. (2008). Also, Thiruvenkadan *et al*. (2014) reported the value of Fis was 0.233 for Salem Black goat population. While, Li *et al*. (2002) reported the mean Fst value (0.105) for Chinese indigenous goat populations demonstrated that only about 10.5% of the total genetic variation attrib-

utes to the differentiation between populations and 89.5% to individuals within the populations. Pairwise genetic differentiations quantified by Fst estimates ranged from 0.042 between Egyptian Baladi and Barki breeds to 0.149 between Zaraibi and Montefalcone and that Genetic differentiation (Fst) between Egyptian breeds was reported by Agha *et al*. (2008) . Recently, Marini *et al*. (2014) reported the mean Fit and Fst values of 0.46 and 0.06, respectively was estimated in Malaysian Goat Breeds. To help interpreting (Fst), Wright (1978) divided the value of Fst into four intervals: (1) from 0 to 0.05, indicating little genetic differentiation; (2) from 0.05 to 0.15, indicating moderate genetic differentiation; (3) from 0.15 to 0.25, indicating great genetic differentiation; (4) from 0.25 to 1, indicating very great genetic differentiation.

Allele size in base pair, their frequencies for each locus, average of allele frequencies and polymorphism information content (PIC) values for each locus per population as observed in the present study are given in Table (5). The highest allele frequency overall loci was 0.750 for allele 87 at locus SRCRSP23 in the case of Siwa goat population. While, the lowest one was 0.053 associated with Siwa goat population at two loci ILSTS029 (for allele 135) and SRCRSP8 (for alleles, 170 and 266), respectively. The highest average of allele frequency estimated was (0.500) for Farafra population at loci MAF70, SRCRSP8 and SPS113 and for Siwa goat population (0.500) at loci MAF70 and SRCRSP23. On the other hand, the lowest one was 0.143 in the case of locus SRCRSP8 for Siwa goat population. Visser *et al*. (2011) reported that allele frequency ranged from -0.017 (BM 1818) to 0.150 (SRCRSP 8) with an average of 0.043 in South African Angora goats. The highest frequency of a specific allele was 0.178 for ILSTS087 (155 bp) in Maltese goats (Agha *et al*., 2008). However, Thiruvenkadan *et al*. (2014) found that the alleles with the lowest frequency (0.001-0.1) were found to be abundant in Salem Black goat breed.

The polymorphic information content (PIC) was an ideal indicator for the measurement of allele polymorphism. Loci were considered highly polymorphic when PIC>0.5; moderately polymorphic when PIC ranged from 0.25 to <0.5 and low polymorphic when PIC<0.25 (Botstein *et al*., 1980). Table (5) shows the highest PIC was (0.791) for SRCRSP8 and the lowest PIC was (0.375) for SRCRSP23. All markers exceeded 0.5 except MAF70 and SPS113 in both Siwa and Farafra goat populations. Meanwhile, PIC value was lower than 0.5 in Farafra goats for loci OarFCB48 and SRCRSP8 and also for Siwa goats for locus SRCRSP23. The overall average PIC per population of the ten markers were 0.530 and 0.570 for Farafra and Siwa goat populations, respectively, which indicated that the ten microsatellite markers contained high polymorphic loci in both Egyptian goat populations. In the genetic diversity analysis, microsatellite markers with PIC>0.7 were taken as the most ideal selected markers. From the selected mi-

crosatellite markers in the present study, the PIC of SRCRSP8 and OarFCB48 for Siwa goats exceeded 0.7 which indicated that these loci could be used as genetic markers for genetic diversity analysis of Siwa goat population. Hassanane *et al*. (2010) reported that the PIC of (ILSTS087) exceeded 0.7 in Egyptian Zaraibi breed. The markers SRCRSP8 and OarFCB48 could be used in marker assisted selection (MAS) to improve the performance of Egyptian goat populations.

A further conclusion is that the microsatellites analyzed in this study were informative and should be used in future genetic diversity studies of goats in Egypt. Also, the importance of the conservation of genetic diversity should be considered by goat breeders in the interest of the long-term improvement of Egyptian breeds.

In conclusion, the present investigation proved the usefulness of the ten microsatellite markers used herein in discriminating between the two Farafra and Siwa goat populations. Among 42 alleles across the two populations a total of 20 unique alleles were obtained (8 alleles for Farafra and 12 alleles for Siwa goat populations). In this regard, SRCRSP8 produced the highest number (5) of unique alleles compared to the other markers. This result could be utilized as a molecular tool in fingerprint analysis of Siwa goat population. The present work suggests using wide genome scan analysis based on more recommended microsatellites covering goat genome which could be further employed in MAS (marker assisted selection) and QTL (Quantitative Trait Loci) programs. A further studies with increasing the population sample size is recommended to characterize and measure genetic diversity in the New Valley oases.

SUMMARY

Two native goat populations present in El-Farafra and Siwa oases, located in the Western Desert of Egypt were genotyped using ten microsatellite molecular markers (SSR). Blood samples taken from a total of 34 individual goats, 14 from Farafra and 20 from Siwa, were subjected to DNA extraction and subsequently to SSR-PCR amplification. The number of alleles ranged from two for MAF70 marker to seven for OarFCB48 and SRCRSP8 loci, the average number per population for Farafra goats was 3.0 and 3.4 for Siwa goats with a total number of a 42 alleles for both populations. The mean observed heterozygosity (*H*o) and expected heterozygosity (*H*E) for both populations varied from 0.21 to 0.24 and 0.56 to 0.59, respectively. Fixation indices revealed a 0.7063 variation referring to differences among individuals versus total variance (Fit). While, among populations differences versus total variance had a lower fixation index (Fst = 0.2371) indicating low level of genetic differentiation between Farafra and Siwa populations. A pair wise difference between Farafra and Siwa goat populations was (0.6151) based on among breeds F index (Fis).The highest PIC was observed for SRCRSP8 mi-

crosatellite marker (0.791) and the lowest PIC was 0.375 for SRCRSP23. The average PIC of the ten markers was 0.530 and 0.570 for Farafra and Siwa goat populations, respectively, which indicated that the ten microsatellite markers contained highly polymorphic loci in both Egyptian goat populations. In the genetic diversity analysis, microsatellite markers with PIC>0.7 were taken as the most ideal selected markers. From the selected microsatellite markers in the present study, the PIC of OarFCB48 and SRCRSP8 (Siwa) exceeded 0.7 which indicated that these loci could be used as genetic markers for genetic diversity analysis of Siwa goat population. The markers generated by OarFCB48 and SRCRSP8 loci could be utilized in marker assisted selection (MAS) to improve the performance of Egyptian goat populations.

ACKNOWLEDGEMENTS

This work was part of a project (No: 176-A) funded by the Agricultural Development Program (ADP). Special thanks are due to Prof. Dr. Fawzy El-Fiki for his useful comments on the earlier version of the manuscript.

REFERENCES

Agha, S. H., F. Pilla, S. Galal, I. Shaat, M. D. Andrea, S. Reale, A. Z. A. Abdelsalam and M. H. Li (2008). Genetic diversity in Egyptian and Italian goat breeds measured with microsatellite polymorphism. J. Anim. Breed. Genet., 125: 194-200.

Araújo, A. M. (2006). Genetic diversity between herds of Alpine and Saanen dairy goats and the naturalized Brazilian Moxotó breed. Genetics and Molecular Biology, 29: 67-74.

Barker, J. S. F. (1994). A global protocol for determining genetic distances among domestic livestock breeds. In Proc. 5th World Cong. Genet Appl. Livestock Prod., Guelph, Canada, 21: 501-508.

Botstein, D., R. L. White, M. Skolnick and R. W. Davis (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

Dixit, E., S. Boulant, Y. Zhang, A. S. Lee, C. Odendall, B. Shum, N. Hacohen, Z. J. Chen, S. P. Whelan, M. Fransen, M. L. Nibert, G. Superti-Furga and J. C. Kagan (2010). Peroxisomes are signaling platforms for antiviral innate immunity. Cell, 141: 668-681.

Elbeltagy, A. R. (2012). Characterization and value addition to local breeds and their products in the Near East and North Africa. Regional Workshop Rabat, Morocco 19-21.

El-Seoudy, A. A., N. M. Abdel Gawad, A. M. Abu-Shady and A. Z. E. Abdelsalam (2005). Biochemical and molecular characterization of some Egyptian goat breeds. Egypt. J. Genet. Cytol., 34: 63-79.

FAO, (2004). Secondary guidelines for development of national farm animal genetic resources management plans. Measurement of domestic animal diversity (MoDAD): Recommended microsatellite markers, Rome, Italy.

FAO, (2011). Commission on Genetic Resources for Food and Agriculture thirteenth Regular Session, Draft Guidelines on Molecular Genetic Characterization of Animal Genetic Resources, Rome.

Galal, S., F. Abdel Rasoul, M. R. Anous and I. Shaat (2005). Small ruminant breeds of Egypt. In "Characterization of small ruminant breeds in West Asia and North Africa, Vol.2. North Africa.", Ed. Luis Iñiguez, International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, p. 141-193.

Hassanane, M. S., A. F. El-Kholy, A. R. Abd El-Rahman and Rasha M. Somida (2010). Genetic variations of two Egyptian goat breeds using microsatellite markers. Egyptian J. Anim. Prod., 47: 93-105.

Kimura, M. and J. F. Crow (1964).The number of alleles that can be maintained in a finite population. Genetic, 68:47.

Li, M. H., S. H. Zhao, C. Bian, H. Sheng Wang, H. Wei, B. Liu, M. Yu, B. Fan, S. L. Chen, M. J. Zhu, S. J. Li, T. A. Xiong and K. Li (2002). Genetic relationships among twelve Chinese indigenous goat populations based on microsatellite analysis. Genet. Sel. Evol., 34: 729-744.

Li, Y., C. Shilin, M. Ning, Z. Shuhhing, C. Yongxin and S. Ciwandobuj (1999). Random amplified polymorphic DNA study of Tibetan Cashmere goat. Sixth world congress on genetics applied to livestock production, Armidale, NSW, Australia.

Liu, Y., J. R. Key and X. Wang (2008). The influence of changes in cloud cover on recent surface temperature trends in the Arctic. Journal of Climate, 21: 705-715.

Luikart, G., M. P. Biju-Duval, O. Ertugrul, Y. Zagdsuren, C. Maudet and P. Taberlet (1999). Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*). Anim. Genet., 30: 431-438.

Mahmoudi, B., M. Bayat R. Sadeghi, M. Babayev and H. Abdollahi (2010). Genetic diversity among three goat populations assessed by microsatellite DNA Markers in Iran. Glob. Vet., 4: 118-124.

Mahrous, K. F., Y. M. Saleha Alakilli, M. Lamiaa Salem, H. Sekena Abd El-Aziem and A. El-Hanafy (2013). Genetic diversity in Egyptian and

Saudi goat breeds using microsatellite markers. Journal of Applied Biosciences, 72: 5838-5845.

Maletsanake, D., S. J. Nsoso and P. M. Kgwatalala (2013). Genetic variations from 12 microsatellite markers in an indigenous Tswana goat flock in South-Eastern Botswana. Livestock Research for Rural Develop., 25(2): article 21.

Marini, A., A. B. Hifzan, R. M. Tan, S. G. and J. M. Panandam (2014). Assessment of genetic diversity on goat breeds in malaysia using microsatellite markers. Mal. J. Anim. Sci., 17: 19-26.

Marini, A., A. B. Mohd. R. Hifzan, J. A. Johari, S. G. Tan and J. M. Panandam (2013). Genetic variation of four goat breeds in Malaysia using microsatellite polymorphism markers. Mal. J. Anim. Sci., 16: 1-8.

May, B., C. C. Krueger, C. Eng and E. Paul (1995). GENES IN POPULATIONS version 2.0: A Computer Program for Analysis of Genetic Data, Cornell Laboratory for Ecological and Evolutionary Genetics, Cornell University, New York.

Meng-Hua, L., Z. Shu-Hong, B. Ci, W. Hau-Sheng, W. Hong, L. Bang, Y. Mei, F. Bin, C. Shi-Lin, Z. Meng-Jin, L. Shi-Jun, X. Tong-An and L. Kui (2002). Genetic relationships among twelve Chinese indigenous goat populations based on microsatellite analysis. Genet. Sel. Evol., 34: 729-744.

Nyamsamba, D., H. Takahashi, K. Nomura, Y. Zagclsuren, M. Minezawa and T. Amano (2002). Microsatellite analysis of Mongolian goat populations: high genetic variation within and low genetic differentiation between populations. Proceeding of the 7th world congress on genetics applied to livestock production, Montpellier, France.

Ouafi, T. A., J. M. Babilliof, C. Lcroux and P. Martin (2002). Genetic diversity of the two main Moroccan goat breeds: Phylogenetic relationships with four breeds reared in France. Small Rumin. Res., 45: 225-233.

Pandey, A. K., R. Sharma, Y. Singh, B. B. Prakash and S. P. S. Ahlawat (2006). Genetic diversity studies of Kherigarh cattle based on microsatellite markers. Genet., 85: 117-122.

Riyadh, S. A., M. M. Musthafa, M. A. Al-Shaikh, O. M. Badri and M. F. Hussein (2012). Genetic diversity of Ardi goat based on microsatellite analysis. African Journal of Biotechnology, 11: 16539-16545.

Sambrook, J., E. F. Fritsch and T. Maniatis (1989). Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory Press.

Siwek, Maria and E. F. Knol (2010). Parental reconstruction in rural goat population with microsatellite markers. Italian Journal of Animal Science, Vol. 9:e50.

Takahashi, H., D. Nyamsamba, B. Mandakh, Y. Zagdsuren, T. Amano and K. Nomura (2008). Genetic Structure of Mongolian Goat Populations Using Microsatellite Loci Analysis. Asian-Aust. J. Anim. Sci., 21: 947-953.

Thiruvenkadan, A. K., V. Jayakumar, P. Kathiravan and R. Saravanan (2014). Genetic architecture and bottleneck analyses of Salem Black goat breed based on microsatellite markers. Veterinary World, EISSN: 2231-0916.

Verma, A. K, D. K. Sinha and B. R. Singh (2011). Seroprevalence study on salmonellosis in apparently healthy dogs by enzyme linked immunosobent assay. Indian Journal of Animal Sciences, 81: 3-5.

Visser C., C. A. Hefer, E. van Marle-Köster and A. Kotze (2004). Genetic variation of three commercial and three indigenous goat populations in South Africa. South African Journal of Animal Science, 34.

Visser C., E. van Marle-Köster and H. Friedrich (2011). Parentage verification of South African Angora goats, using microsatellite markers. South African Journal of Animal Science, 41 (no. 3).

Welsh, J. and M. Mecldand (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res., 18: 7213-7218.

Williams, J. G. K, A. R. Kubclik, K. J. Livak, J. A. Rafalski and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., 18: 6531-6535.

Wright, S. (1978). Evolution and the Genetics of Population, Variability Within and Among Natural Populations. The University of Chicago Press, Chicago.

Yeh, F. C., T. Boyle, Y. Rongcai, Z. Ye and J. M. Xian (1999). POPGENE, Version 1.31. A Microsoft Window Based Freeware for Population Genetic Analysis. University of Alberta, Edmonton.

Table (1): Information on microsatellite markers used in the study.

Locus Name	Access No[1]	Location[1]	Primer[1]	Repeat type[1]	Ta[2]	STS Size[3]
ILSTS28	L37211	OAR 3	F:TCC AGA TTT TGT ACC AGA CC R:GTC ATG TCA TAC CTT TGA GC	(AC)15	53	105-177
ILSTS029	L37252	BTA3	F:TGTTTTGATGGAACACAG R:TGGATTTAGACCAGGGTTGG	(GT)18	55	148-170
SRCRSP5	L22197	CHI21	F:GGACTCTACCAACTGAGCTACAAG R:TGAAATGAAGCTAAAGCAATGC	(AC)20	55	156-178
MAF70	M77199	BTA4	F:CACGGAGTCACAAAGAGTCAGACC R:GCAGGACTCTACGGGGCCTTTGC	(AC)39	65	134-168
SRCRSP23	---	unknown	F:TGAACGGGTAAAGATGTG R:TGTTTTTAATGGCTGAGTAG	(CA)17	58	81-119
OarFCB48	M82875	OAR17	F:GAGTTAGTACAAGGATGACAAGAG GCAC R:GACTCTAGAGGATCGCAAAGAACC AG	(AC)14	58	149-173
SRCRSP9	L22200	CHI12	F:AGAGGATCTGGAAATGGAATC R:GCACTCTTTTCAGCCCTAATG	(GT)17	58	99-135
OarAE54	L11048	OAR25	F:TACTAAAGAAACATGAAGCTCCCA R:GGAAACATTTATTCTTATTCCTCAG TG	(CA)14	58	115-138
SRCRSP8	L22200	Unknown	F:TGCGGTCTGGTTCTGATTTCAC R:GTTTCTTCCTGCATGAGAAAGTCGA TGCTTAG	(GA)16	55	215-255
SPS113	---	BTA10	F:CCTCCACACAGGCTTCTCTGACTT R:CCTAACTTGCTTGAGTTATTGCCC	(AT)19	58	134-158

1. Gene bank accession number; www.ncbi.nlm.nih.gov/. http://www.ncbi.nlm.nih.gov/genome/sts/sts.cgi?uid=280100
2. Annealing temperature, (FAO, 2004 and 2011).
3. STS: sequence tagged site size according to NCBI database.

Table (2): Specific alleles, common alleles and total number of alleles for Farafra and Siwa goat populations.

Markers	No. of alleles per population		No. of specific alleles		Common alleles	Total no. of alleles
	Farafra	Siwa	Farafra	Siwa		
ILSTS28	4	3	1	0	3	4
ILSTS029	3	3	0	0	3	3
SRCRSP5	3	3	0	0	3	3
MAF70	2	2	0	0	2	2
SRCRSP23	3	2	2	1	1	4
OarFCB48	4	5	2	3	2	7
SRCRSP9	4	3	1	0	3	4
OarAE54	3	3	2	2	1	5
SRCRSP8	2	7	0	5	2	7
SPS113	2	3	0	1	2	3
Total	30	34	8	12	22	42
Means	3.0	3.4	0.8	1.2	2.2	4.2

Table (3): Observed heterozygosity (H_O), expected heterozygosities (H_E), effective number of alleles (Ne) and their means, standard deviation estimated for each population.

	Farafra			Siwa		
	H_O	H_E	Ne	H_O	H_E	Ne
ILSTS28	0.000	0.550	2.130	0.053	0.681	2.971
ILSTS029	0.667	0.667	2.571	0.684	0.551	2.155
SRCRSP5	0.000	0.568	2.174	0.000	0.568	2.174
MAF70	0.600	0.505	1.923	0.000	0.416	1.658
SRCRSP23	0.000	0.554	2.123	0.000	0.385	1.600
OarFCB48	0.000	0.476	1.849	0.000	0.763	3.853
SRCRSP9	0.667	0.721	3.236	0.684	0.607	2.448
OarAE54	0.000	0.582	2.279	0.000	0.628	2.522
SRCRSP8	0.000	0.416	1.658	0.526	0.812	4.782
SPS113	0.154	0.517	1.988	0.450	0.476	1.865
Mean	0.209	0.556	2.193	0.240	0.589	2.603
St. Dev	0.305	0.089	0.443	0.306	0.140	1.017
IC	Farafra	0.62				
	Siwa	0.59				

IC: inbreeding coefficient. ($IC= (H_E - H_O)/H_E$)
St. Dev: standard deviation

Table (4): AMOVA analysis of Farafra and Siwa goat populations on microsatellite DNA variation.

Source of variation	d. f.	S. S.	Percentage variation	Fixation indices
Among populations	1	14.94	23.71	F_{is}=0.6151
Among individuals within populations	32	65.16	46.93	F_{st}=0.2371
Within individuals	34	16.50	29.37	F_{it}=0.7063
Total	67	96.60	----	----

F_{IS}: Fixation indices (Among populations)
F_{ST}: Fixation indices (Among individuals within populations)
F_{IT}: Fixation indices (Within individuals)

Table (5): Allele size in base pair, their frequencies for each locus and population, average allele frequencies and polymorphic information content (PIC) as observed in the present study.

Locus	Alleles (bp)	Frequencies		Locus	Alleles (bp)	Frequencies	
		Farafra	Siwa			Farafra	Siwa
ILSTS28	142	0.071	0.000	ILSTS029	135	0.333	0.053
	157	0.643	0.342		153	0.500	0.395
	172	0.071	0.368		171	0.167	0.553
	187	0.214	0.290				
Average	---	0.250	0.333	Average	---	0.333	0.333
PIC	---	0.531	0.663	PIC	---	0.611	0.536
SRCRSP5	144	0.300	0.300	MAF70	132	0.600	0.727
	164	0.600	0.600		171	0.400	0.273
	184	0.100	0.100				
Average	---	0.333	0.333	Average	---	0.500	0.500
PIC	---	0.540	0.540	PIC	---	0.480	0.397
SRCRSP23	70	0.182	0.250	OarFCB48	97	0.071	0.000
	87	0.000	0.750		111	0.071	0.118
	104	0.636	0.000		125	0.714	0.177
	121	0.182	0.000		139	0.143	0.000
					153	0.000	0.059
					167	0.000	0.353
					181	0.000	0.294
Average	---	0.333	0.500	Average	---	0.250	0.200
PIC	---	0.529	0.375	PIC	---	0.459	0.741
SRCRSP9	96	0.292	0.000	OarAE54	88	0.071	0.000
	113	0.417	0.263		102	0.500	0.231
	130	0.208	0.553		116	0.429	0.000
	147	0.083	0.184		144	0.000	0.231
					158	0.000	0.539
Average	---	0.250	0.333	Average	---	0.333	0.333
PIC	---	0.691	0.592	PIC	---	0.561	0.604
SRCRSP8	170	0.000	0.053	SPS113			
	186	0.000	0.263				
	202	0.000	0.316		127	0.000	0.175
	218	0.000	0.132		146	0.539	0.700
	234	0.000	0.105		165	0.462	0.125
	250	0.273	0.079				
	266	0.727	0.053				
Average	---	0.500	0.143	Average	---	0.500	0.333
PIC	---	0.397	0.791	PIC	---	0.497	0.464
(PIC)Mean	Frarafra	0.530					
	Siwa	0.570					

PIC: polymorphic information content.

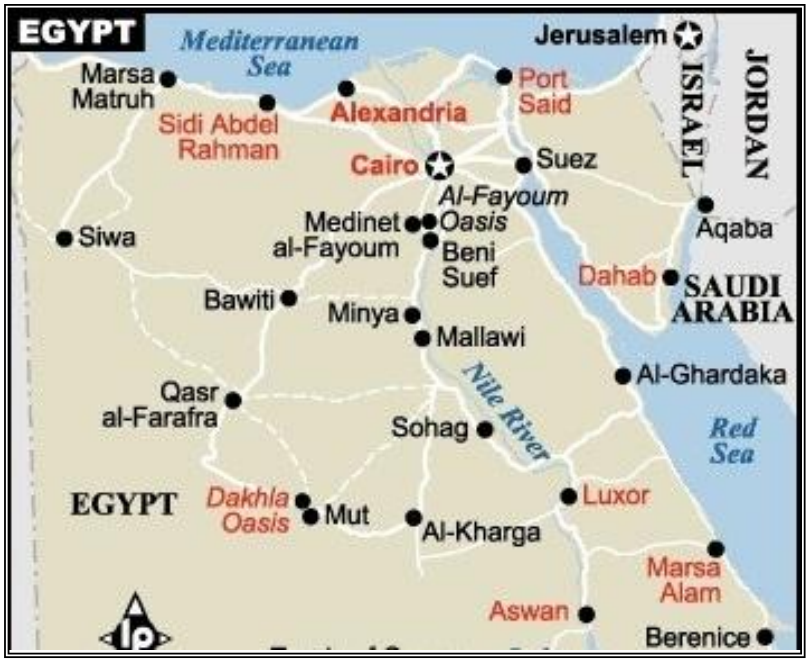

Fig. (1): New Valley Oases (El-Kharga, El-Dahala, El-Farafra and Siwa).

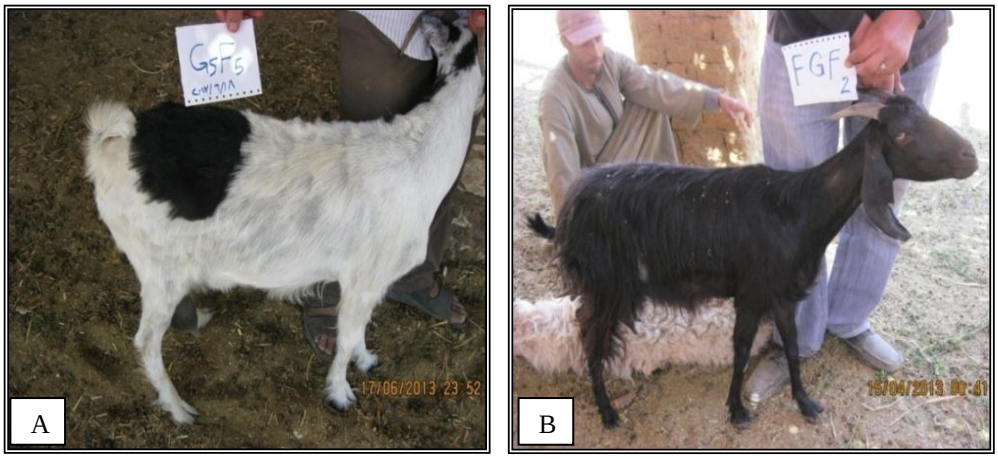

Fig. (2): A and B some phenotypic variations observed in Farafra and Siwa goats.