# ASSESSMENT OF GENETIC DIVERSITY AMONG TWO EGYPTIAN CATTLE POPULATIONS (*Bos taurus*) BASED ON AUTOSOMAL MICROSATELLITE MARKERS

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## SUMMARY

*Two native cattle populations raised in Farafra and Siwa oases, located in the Western Desert of Egypt, were genotyped using eight microsatellite molecular markers (MM8, INRA063, BM1818, ILSTS054, ILSTS005, HEL5, ILSTS006 and ILSTS011). Blood samples, collected from 38 individual cattle (19 from Farafra and 19 from Siwa), were subjected to DNA extraction and subsequently to SSR-PCR amplification. Heterozygosity and Wrights F-statistics (FIS, FST and FIT) were calculated to assess the genetic variation in these populations.*

*In the present study, high values of FIS were detected in Siwa (83%) and Farafra (69%) cattle with moderate genetic differentiation (13%) between the two populations. A total number of 22 and 29 alleles with means 2.75±0.71 and 3.63±0.74 were observed in Siwa and Farafra cattle, respectively. Alleles observed per locus ranged between 2 (loci ILSTS054, ILSTS005 and ILSTS011) to 4 (locus HEL5) in Siwa cattle and between 3 (loci ILSTS005, HEL5, ILSTS006 and ILSTS011) to 5 (locus BM1818) in Farafra cattle populations. Mean values of observed and expected heterozygosities were 0.09± 0.27 and0.46 ± 0.21 for Siwa cattle, meanwhile it ranged from 0.20± 0.35to 0.66 ± 0.07 in Farafra cattle. Polymorphic information content value (PIC) ranged from 0.10 to 0.71 for marker ILSTS005 and HEL5, respectively, with a mean value of 0.45 for all loci in Siwa cattle. Moreover, its range was 0.52 (ILSTS054) to 0.74 (BM1818) for all loci with a mean of 0.64 in Farfra cattle. Population fixation indices traced about 0.653 variation referring to differences among individuals versus total variance (FIT), where it was the lowest among populations differences versus total variance (FST= 0.237) indicating low level of population differentiation. A pair-wise difference amongst Siwa and Farafra cattle populations was recorded (0.546) among populations (F index (FIS).Moreover, 4 and 11private alleles were observed in Siwa and Farafra cattle populations, respectively. Following that we are suggested the use of these alleles as population fingerprint and they could be used to differentiate these two populations.*

***Keywords: DNA microsatellite, genetic diversity, cattle, indigenous population***

## INTRODUCTION

In the recent years, rapid advanced development in molecular genetic techniques have made it possible to identify differences between individuals at the DNA level and using genomic variation for the genetic improvement of livestock. Molecular methods have provided new markers for the estimate of genetic variation, even to the level of analysis at the DNA sequences itself. Molecular markers have been widely used to assess this variability since they provide information on every region of the genome. Microsatellites (highly polymorphic simple sequences repeats) are the most widely used molecular markers. Genetic variability within and among populations is important and may contribute to the selection and preservation of genetic resources (Hassanane *et al.* 2006).

According to Food and Agriculture Organization (FAO, 1993) the Egyptian cattle consisted of eight breeds: two of them were disappeared or diminished (African aurochs and Hamitic longhorn) while the other six cattle breeds are Baladi, Domiette, Egyptian, Maryuti, Menufi, and Saidi, are still present (www.Fao.org). Local populations may have different names, but without apparent differences in phenotype; a change in phenotype may occur without change in name, or all populations may have just one name and be phenotypically similar. The Egyptian Baladi cattle are draft medium sized animals. Its color ranges from brown to black or pied. Due to the low milk productivity of these animals, it is neglected for a long time and its genetic improvement took place only through hybridization with exotic breeds (Morsy, 1980). Egypt is predominantly desert and arid or semi-arid rangelands which can be divided into four major physical regions; the Nile Valley and Delta, Western Desert, Eastern Desert and Sinai Peninsula (Figure 1). The new valley (El-Kharga, El-Dakhla and El-Farafra) and Siwa oases located in the Western Deseret are especially for being geographically isolated regions. Accordingly, those

oases have specific farm animal genetic resources (cattle- sheep- goat- camels) able to survive efficiently in the specific condition of these areas. Unique individuals of these animals need to be screened, evaluated and collected to maintain them as nucleus herds and to benefit them in sire centers. So, this study was aimed to assess the genetic diversity in the Egyptian native cattle raised in Siwa and El-Farafra Oasis (Figure 2 and 3) located in the Western desert by using microsatellite markers.

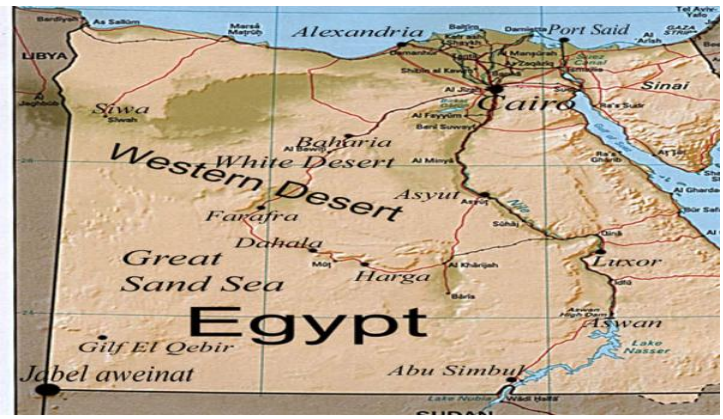
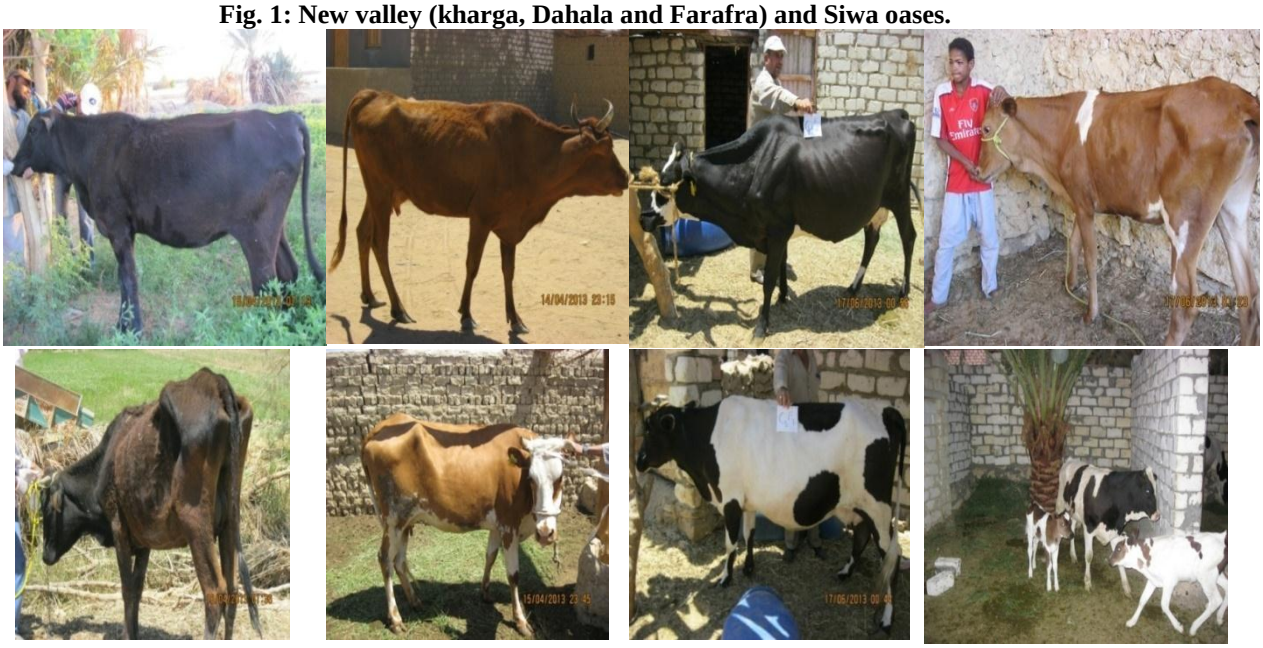



**Fig. 1: New valley (kharga, Dahala and Farafra) and Siwa oases.**



## MATERIALS AND METHODS

According to the framework of the strategy of sustainable agriculture development (2030), this work was done throughout a project focused on sustainable utilization of agriculture biodiversity, development of agriculture products and improvement of rural livelihood, based on Integration of Genetic Resources Management (BIGRM) in Western Desert communities. The project targets four oases namely El-Kharga, El-Dakhla, El-Farafra and Siwa which have specific farm animal genetic resources (AnGR), adapted cultivars of alfalfa and a huge quantity of unused byproducts of dates palm, olive and other agriculture byproducts.

Cattle raised in Siwa and El- Farafra oases were genotyped using eight cattle microsatellites markers (MM8, INRA063, BM1818, ILSTS054, ILSTS005, HEL5, ILSTS006 and ILSTS011) as recommended by (FAO, 2004) and published papers (Pandey *et al.* 2006, Karthickeyan *et al.* 2008 and Sharma *et al.* 2015). The marker set distributed on 7 different autosomal chromosomes (2, 18, 23, 21, 10, 7 and 14, respectively). Microsatellite loci, accession number, Map, flanking sequences, chromosome number, repeat type, annealing temperature and allele range in base pairs are shown in table (1).

### *Blood sample collection and DNA extraction*

Blood samples were collected from38cattle from different regions in Siwa (n=19) and Farafra oases (n=19). Genomic DNA extraction was carried out using the salting out method (Sambrook *et al.*1989).

### *Microsatellite genotyping*

A set of eight microsatellite markers (Table 1), which have been recommended for cattle in FAO's Measurement of Domestic Animals Diversity (MoDAD) program(FAO,2004), were selected according to their degree of polymorphism and genome coverage (Pandey *et al.*,2006; Karthickeyan *et al.*, 2008 and Sharma *et al.*, 2015).

Polymerase Chain Reaction (PCR) was performed using 50-100 ng genomic DNA in a 25 µl reaction volume containing 10µl Master Mix (Emerald AMP GT PCR Master Mix, Takara Bio. Inc. composed of 10 pmol of each primer, DNA polymerase, optimized reaction buffer, dNTPs and a density reagent). The premix also contained a vivid green dye which is separate into blue and yellow dye fronts. The PCR reactions was carried out under the following conditions: an initial denaturation step (for 2 min at 95°C), followed by 35 cycles of denaturation (for 30sat 95°C); annealing (at 45-58$^{o}$Cfor 60 s) at optimized primer annealing temperature (Table 1). Then, extension (for 60 s at 72°C) and final extension (for 60 sat 72°C). Amplified fragments were analyzed on 10% polyacrylamide gel and stained with Ethidium bromide. The resultedgels were photographed and images were analyzed using Gel Documentation System (Alphaimager $^{TM}$ 2200, Cell Biosciences).

**Table1. All information about the eight microsatellite markers used in this study, including locus name, accession number, Map, flanking sequences, chromosome number, repeat type, annealing temperature and allele range in base pairs**

| Locus Name | Accession No[1] | Map[1] | Forward primer[1,2] | Reverse primer[1,2] | Chr.No2 | Repeat type[1] | Ta[2] | Allele range[2,3] |
|---|---|---|---|---|---|---|---|---|
| MM8 | -- | -- | CCCAAGGACAGAAAAGACT | CTCAAGATAAGACCACACC | 2 | (GT)11 | 55°C | 114-144 |
| INRA063 | X71507 | M1 | ATTTGCACAAGCTAAATCTAACC | AAACCACAGAAATGCTTGGAAG | 18 | (AC)13 | 55-58°C | 167-189 |
| BM1818 | G18391 | M19 | AGCTGGGAATATAACCAAAGG | AGTGCTTTCAAGGTCCATGC | 23 | (TG)18 | 56-60°C | 248-278 |
| ILSTS054 | -- | -- | GAGGATCTTGATTTTGATGTCC | AGGGCCACTATGGTACTTCC | 21 | (TC)19 | 55°C | 132-148 |
| ILSTS005 | L23481 | M4 | GGAAGCAATGAAATCTATAGCC | TGTTCTGTGAGTTTGTAAGC | 10 | (AG)16 | 54-58°C | 176-194 |
| HEL5 | X65204 | M5 | GCAGGATCACTTGTTAGGGA | AGACGTTAGTGTACATTAAC | 21 | (CT)22 | 52-57°C | 145-171 |
| ILSTS006 | L23482 | M20 | TGTCTGTATTTCTGCTGTGG | ACACGGAAGCGATCTAAACG | 7 | (GT)23 | 55°C | 277-309 |
| ILSTS011 | -- | -- | GCTTGCTACATGGAAAGTGC | CTAAAATGCAGAGCCCTACC | 14 | (CT)15 | 58°C | 249-273 |

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)
3. Published papers (Pandey *et al*., 2006, Karthickeyan *et al*., 2008 and Sharma *et al*., 2015)

***Statistical analysis***

All gels were visualized and scored with Alphaimages2200 software version 4.0.1 (Cell Biosciences). A Tandem Repeat Analyzer software package was adopted according to Benson (1999), to correct data for estimating the allele sizes according to 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 Arlequin 3.11 software package after data conversion using CONVERT program (Glaubitz, 2004). Number of alleles per locus (no), effective number of alleles (ENA), observed heterozygosity (Ho) and expected heterozygosity (He) were calculated to evaluate genetic variation within–breed. Moreover, within–population inbreeding estimate (Wright's, 1978), known as, fixation index (FIS) at each microsatellite locus was estimated using the POPGENE, version 1.31(Yeh *et al.,* 1999). The polymorphic information content (PIC) for each locus was also calculated according to Botstein *et al.* (1980).

## RESULTS AND DISCUSSION

Allele size of the eight markers (MM8, INRA063, BM1818, ILSTS054, ILSTS005, HEL5, ILSTS006 and ILSTS011) ranged from 125-169bp, 163-215bp, 213-285bp, 110-167bp, 160-192bp, 127-193bp, 260-329bp and 240-270bp, respectively in Siwa and Farafra cattle populations (Table 2). Results agree with the selective standard of the microsatellite loci (the Secondary Guidelines for Development of National Farm Animal Genetic Resources using reference Microsatellite given by FAO, 2004).In Gaolao and Kenkatha cattle, Chaudhari *et al.* (2009) found that allele size ranged from 118-150bp, 170-188bp, 258-280bp, 176-190bp, 279-301bp and260-272bp with MM8, INRA063, BM1818, ILSTS005, ILSTS006 and ILSTS011, respectively. Gralak *et al.* (2004) reported that the size ranged from 262-264bp, 282bp with BM1818 and ILSTS006, respectively in European bison bovine. Meanwhile, Russell *et al.* (2000) recorded size range from 178-186bp and 149-169bp with INRA063 and HEL5, respectively in Criollo cattle. Karthickeyan *et al.* (2008) found size range from 138-146bp, 180-186bp, 262-278bp, 132-148bp, 182-194bp, 150-166bp, 290-300bp and 262-274bp with MM8, INRA063, BM1818, ILSTS054, ILSTS005, HEL5, ILSTS006 and ILSTS011, respectively in Ongole cattle. In Indian cattle Kale *et al.* (2010) recorded size range from 151-167bp and 288-300bp with HEL5 and ILSTS006, respectively in Gir, Deoni and Kankrej breeds, while ranged from 114-144bp, 162-190bp, 137-195bp, 275-303bpand 249-273bp with MM8, INRA063, HEL5, ILSTS006 and ILSTS011, respectively in others Indian breed(Sharma *et al.* 2015). Ndiaye *et al.* (2015) reported size range from 194-206bp and 274-292bp with INRA063 and BM1818, respectively in Senegalese cattle breeds. Agung *et al.* (2016) observed size range from 248-278bp and 277-309bp with BM1818 and ILSTS006in Indonesian cattle breeds.

**Table2. Allele size range (bp) observed, number of alleles (no, observed; ENA effective) and heterozygosis (HO, observed; He, expected) for eight microsatellite loci in Siwa and Farafra cattle populations**

| | | | Number of alleles | | | | Heterozygosis | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Allele size range | | Siwa | | Farafra | | Siwa | | Farafra | |
| locus | Siwa | Farafra | no | ENA | no | ENA | HO | He | HO | He |
| MM8 | 147-169 | 125-158 | 3 | 2.65 | 4 | 3.25 | 0.00 | 0.64 | 0.00 | 0.71 |
| INRA063 | 163-189 | 176-215 | 3 | 1.97 | 4 | 2.26 | 0.00 | 0.51 | 0.11 | 0.57 |
| BM1818 | 249-285 | 213-285 | 3 | 2.11 | 5 | 3.72 | 0.00 | 0.54 | 0.00 | 0.75 |
| ILSTS054 | 148-167 | 110-167 | 2 | 1.44 | 4 | 2.05 | 0.00 | 0.31 | 0.00 | 0.53 |
| ILSTS005 | 160-176 | 160-192 | 2 | 1.11 | 3 | 2.89 | 0.00 | 0.10 | 0.68 | 0.67 |
| HEL5 | 127-193 | 127-171 | 4 | 3.46 | 3 | 2.98 | 0.00 | 0.73 | 0.00 | 0.68 |
| ILSTS006 | 283-329 | 260-306 | 3 | 2.16 | 3 | 2.88 | 0.75 | 0.55 | 0.84 | 0.67 |
| ILSTS011 | 255-270 | 240-270 | 2 | 1.38 | 3 | 2.77 | 0.00 | 0.29 | 0.00 | 0.65 |
| **Mean** | | | 2.75 | 2.04 | 3.63 | 2.85 | 0.09 | 0.46 | 0.20 | 0.66 |
| **SD** | | | 0.71 | 0.76 | 0.74 | 0.52 | 0.27 | 0.21 | 0.35 | 0.07 |
| **IC** | | | | | | | 0.80 | | 0.70 | |

no: Observed number of alleles ENA : Effective number of alleles [Kimura and Crow (1964)]
IC: inbreeding coefficient. (IC= (HE- HO)/HE)

**Table3. Polymorphic information content (PIC) values, FIS values and F-Statistics analysis for the 8 microsatellite loci in Siwa and Farafra cattle populations**

| | PIC | | | FIS | | F-Statistics | | |
|---|---|---|---|---|---|---|---|---|
| **locus** | **Siwa** | **Farafra** | **Mean** | **Siwa** | **Farafra** | **FIS** | **FIT** | **FST** |
| MM8 | 0.62 | 0.69 | 0.66 | 1.00 | 1.00 | 1.00 | 1.00 | 0.16 |
| INRA063 | 0.49 | 0.55 | 0.52 | 1.00 | 0.82 | 0.90 | 0.91 | 0.08 |
| BM1818 | 0.53 | 0.74 | 0.64 | 1.00 | 1.00 | 1.00 | 1.00 | 0.17 |
| ILSTS054 | 0.31 | 0.52 | 0.42 | 1.00 | 1.00 | 1.00 | 1.00 | 0.05 |
| ILSTS005 | 0.10 | 0.65 | 0.38 | 1.00 | -0.02 | 0.09 | 0.38 | 0.32 |
| HEL5 | 0.71 | 0.66 | 0.69 | 1.00 | 1.00 | 1.00 | 1.00 | 0.03 |
| ILSTS006 | 0.53 | 0.65 | 0.59 | -0.37 | -0.27 | -0.34 | -0.17 | 0.13 |
| ILSTS011 | 0.28 | 0.63 | 0.46 | 1.00 | 1.00 | 1.00 | 1.00 | 0.10 |
| **Mean** | 0.45 | 0.64 | 0.55 | 0.83 | 0.69 | 0.73 | 0.76 | 0.13 |

Population specific FIS indices per polymorphic locus (absolute values)

**Table4. AMOVA analysis of Farafra and Siwa cattle populations based on microsatellite DNA variation.**

| **Source of variation** | **d.f.** | **S .S.** | **Percentage of variation** | **Fixation indices** |
|---|---|---|---|---|
| Among populations | 1 | 16.58 | 23.65 | FIS= 0.5455 |
| Among individuals within populations | 36 | 69.26 | 41.65 | FST= 0.2365 |
| Within individuals | 38 | 21.50 | 34.70 | FIT= 0.6530 |
| **Total** | 75 | 107.34 | ---- | ---- |

FIS: Fixation indices (Among populations)
FST: Fixation indices (Among individuals within populations)
FIT: Fixation indices (Within individuals)

**Table5. Allele size in base pairs, their frequencies for each locus and population and average of allele frequencies as observed in the present study**

| Locus | Alleles (bp) | Frequencies | | Locus | Alleles (bp) | Frequencies | |
|---|---|---|---|---|---|---|---|
| | | **Siwa** | **Farafra** | | | **Siwa** | **Farafra** |
| MM8 | 125 | 0.00 | 0.35 | INRA063 | 163 | 0.29 | 0.00 |
| | 136 | 0.00 | 0.24 | | 176 | 0.06 | 0.08 |
| | 147 | 0.18 | 0.35 | | 189 | 0.65 | 0.58 |
| | 158 | 0.47 | 0.06 | | 202 | 0.00 | 0.32 |
| | 169 | 0.35 | 0.00 | | 215 | 0.00 | 0.02 |
| **Average** | -- | **0.33** | **0.25** | **Average** | -- | **0.33** | **0.25** |
| | 213 | 0.00 | 0.36 | ILSTS054 | 110 | 0.00 | 0.11 |
| | 231 | 0.00 | 0.31 | | 129 | 0.00 | 0.17 |
| BM1818 | 249 | 0.06 | 0.11 | | 148 | 0.81 | 0.66 |
| | 267 | 0.59 | 0.11 | | 167 | 0.19 | 0.06 |
| | 285 | 0.35 | 0.11 | | | | |
| **Average** | -- | **0.33** | **0.20** | **Average** | -- | **0.50** | **0.25** |
| ILSTS005 | 160 | 0.05 | 0.42 | HEL5 | 127 | 0.31 | 0.29 |
| | 176 | 0.95 | 0.26 | | 149 | 0.13 | 0.36 |
| | 192 | 0.00 | 0.32 | | 171 | 0.37 | 0.35 |
| | | | | | 193 | 0.19 | 0.00 |
| **Average** | -- | **0.50** | **0.33** | **Average** | -- | **0.25** | **0.33** |
| ILSTS006 | 260 | 0.00 | 0.37 | ILSTS011 | 240 | 0.00 | 0.26 |
| | 283 | 0.63 | 0.24 | | 255 | 0.17 | 0.26 |
| | 306 | 0.22 | 0.39 | | 270 | 0.83 | 0.48 |
| | 329 | 0.15 | 0.00 | | | | |
| **Average** | -- | **0.33** | **0.33** | | | **0.50** | **0.33** |

A total number of 22 and 29 alleles with mean values of 2.75±0.71 and 3.63±0.74were observed in Siwa and Farafra cattle, respectively (Table 2). Alleles observed per locus ranged between 2 (loci ILSTS054, ILSTS005 and ILSTS011) to4 (locus HEL5) in Siwa cattle and between 3(loci ILSTS005, HEL5, ILSTS006 and ILSTS011) to5 (locus BM1818) in Farafra cattle population (Table 2). The mean numbers of alleles observed in the investigated populations was slightly lower than those reported by other authors. Pandey *et al.* (2006) recorded that the number of observed alleles varied between 3 (ILSTS011) to 10 (ILSTS034) with an overall mean number of 5.95±1.9 alleles per locus. Chaudhari *et al.* (2009) observed a total of 239 and 197 distinct alleles with mean values of 9.52 and 7.92 in Gaolao and Kenkatha cattle, respectively. They also, reported that alleles observed per locus ranged between 5 (loci ILSTS006 and ILSTS030) and 15 (locus ILSTS034) and between 4 (locus ILSTS006) and 14 (loci BM1824 and ILSTS006) in Gaolao and Kenkatha cattle, respectively. A total of 74 alleles were detected across the 9 loci with an average of 7, 5 and 5 alleles per locus in Fuga, Butana and Kenana breeds, respectively (Hussein *et al.* 2014). Moreover, they recorded a total of 359 alleles with (ILSTS34), which represent the highest number of alleles per locus (37), while (CSSM08) was the lowest polymorphic alleles (8). Sharma *et al.* (2015) reported an average number of alleles per locus to be 6.571 ± 0.732 and 10.619 ± 0.824 in Hariana and Shahabadi cattle, respectively with a mean number of allele across all the loci 8.784 ± 0.25 and average number 10.45 allele per locus. The observed number of alleles per locus (Na) varied from 6 in INRA063 to 16 in TGLA53 in Senegal cattle (Ndiaye *et al.* 2015). Agung *et al* (2016) detect 317, 143 and 91 alleles in Simmental purebred, Simmental Cross Ongole Grade (PO), respectively using 12 microsatellites. They suggested that the lower values of expected number of alleles compared to the observed may be due to several low allele frequencies in the populations.

Effective number of alleles (ENA) is a reciprocal of gene homozygosity (Hartl and Clark, 1997) and it is used to corollary the HE (when heterozygosity is high ENA will be the highest). In Siwa cattle, the lowest ENA was 1.11 for (ILSTS005) when HE was 0.10, while the highest ENA was 3.46 for (HEL5) when HE was 0.73(Table 2).Moreover, the lowest ENA was 2.05 for (ILSTS054) when HE was 0.53, while the highest ENA was 3.72 for (BM1818) when HE was 0.75 in Farafra cattle. The mean effective number of alleles varied from 2.04 ± 0.76 to 2.85 ± 0.52in Siwa and Farafra population (Table 2).Averages of effective number of alleles in the present study was lower than that reported by Sharma *et al.* (2015) whose observed that averages effective number of alleles within one population varied from 3.374 ±0.329 to 4.745 ± 0.532 in Hariana and Shahabadi cattle, respectively. Also, Ndiaye *et al* (2015) reported a mean effective number of alleles per population of 4.48±0.21 in four local cattle breeds (Gobra zebu, Maure zebu, Djakoré, and N'Dama) of Senegal.

Results showed that the observed heterozygosity (Ho) in Siwa cattle ranged between 0.00(loci MM8,

INRA063, BM1818, ILSTS054, ILSTS005, HEL5 and ILSTS011) to 0.75 (locus ILSTS006), while it was between 0.00 (loci MM8, BM1818, ILSTS054, HEL5 and ILSTS011) to 0.84 (ILSTS006) in Farafra cattle population(Table 2).Moreover, expected heterozygosity (He) ranged between 0.10 (ILSTS005) to 0.73 (HEL5) in Siwa and between 0.53 (ILSTS054) to 0.75 (BM1818) in Farafra cattle population. The mean values of the expected heterozygosity were 0.46 ± 0.21and 0.66 ± 0.07 in Siwaand Farafra cattle populations, respectively (Table 2). Gralak *et al.* (2004) recordedan expected heterozygosity (He) ranged from 0.13 (HEL9) to 0.53 (ETH3). Pandey *et al.* (2006) reported that the averaged of observed heterozygosity was 0.540±0.171 over the 21 loci, while the average of expected heterozygosity ranged from 0.530 (ETH225) to 0.825 (INRA035) with an overall mean of 0.685±0.100 in Kenkatha population. Moreover, Chaudhari *et al* (2009) recorded that the observed heterozygosity (Ho) ranged between 0.014 (ILSTS006) to 0.7800 (ILSTS034) in Kenkatha and between 0.1000 (ILSTS006) to 0.7500 (ILSTS030) in Gaolao cattle, while the expected heterozygosity (He) ranged between 0.1523 (ETH3) to 0.8881 (ILSTS034) in Kenkatha and between 0.3330 (ETH152) to 0.9029 (ILSIS034) in Gaolao cattle. They also reported that the means values of observed and expected heterozygosities were 0.47 ± 0.24 and 0.62 ± 0.21, respectively in Kenkatha and 0.53 ± 0.17 and 0.68 ± 0.14, respectively in Gaolao cattle.Hussein *et al* (2014) reported an observed mean (Ho) and expected heterozygosity (He) of 0.778 and 0.725 in Fuga vs. 0.737; 0.695 in Butana and 0.693; 0.651 in Kenana cattle, respectively. Among populations, observed heterozygosity ranged from 0.459 ± 0.07 to 0.724 ± 0.036 with the lowest value found in Ongole cattle and the highest in Kenkatha cattle, while the observed heterozygosity was lower than the expected heterozygosity in Bachaur, Ponwar, Shahabadi, Purnea, Mewati, Gaolao, Hariana and Ongole cattle populations (Sharma *et al.,* 2015). Ndiaye *et al.* (2015) reported that all loci showed high levels of heterozygosity (>0.60), except for INRA063, which generated Ho and He values of 0.44 and 0.60, respectively. The slight difference between the mean observed compared to the expected heterozygosity detected in the present study may reflect slight inbreeding/ and or crossbreed and selection against heterozygotes. Moreover, the nature of the markers used may also contribute to the observed level of heterozygosity as a result of non-detection of homozygotes from heterozygotes due to presence of null alleles.

The mean value of FIS obtained for Siwa cattle (0.83) indicate high level of inbreeding within this population, which confirmed by IC (0.80), while the mean value for Farafra cattle (0.69)reflect high variability in these population confirmed by inbreeding coefficient 0.70 (Table 2 and 3). These findings might be due to more recent divergence of Farafra cattle than Siwa cattle one.

The value of the Polymorphic information content (PIC) for Siwa cattle ranged from 0.10 to 0.71 inILSTS005and HEL5, respectively with a mean of 0.45 for all loci (Table 3). Mean while, it ranged from 0.52 to 0.74 for inILSTS054and BM1818, respectively with a mean of 0.64for Farafra cattle (Table 3). These differences reflect high genetic variability within Farafra population. The majority of the microsatellite loci used in this study was highly revealing (Table 3). According to classification of Botstein *et al.* (1980), the highly informative markers have PIC values >0.50, the reasonably informative markers have PIC value between 0.25-0.50 and the slightly informative markers have PIC value <0.25. In the present study, three markers (ILSTS054, ILSTS005 and ILSTS011) had reasonably informative PIC values of 0.42, 0.38 and 0.46, respectively, while the majority of the loci were highly informative. Similarly, Chaudhari *et al.* (2009)found that all other loci possessed a high PIC value (> 0.5) except six loci (BM1824, ETH3, ETH152, HEL51, ILSTS005 and ILSTS006) in Kenkatha and five loci (BM1824, CSRM60, ETH152, ILSTS005 and ILSTS006) in Gaolao cattle.PIC values varied from 0.304 (ETH225) to 0.793 (BMC3113) in Kenana and Fuga breed with a mean of 0.664, 0.630 and 0.596 in Fuga, Butana and Kenana cattle, respectively (Hussein *et al.,* 2014). Moreover, Ndiaye *et al.* (2015) reported that Gobra zebu had the highest PIC value (0.75), while N'Dama had the lowest value (0.66). Results of the present study showed that the markers used are highly informative for characterization of both cattle populations.

The within–population inbreeding estimate (FIS) was 0.83and 0.69in Siwa and Farafra cattle, respectively (Table 2).The FIS used to obtain a deeper insight to appraise the degree of inbreeding and endangerment potentiality, it is considered as an important tool to judge the conservation priority (Simon and Bchenauer, 1993). Accordingly, when FIS is less than 0.05, the breeds are not in danger; between 0.05 – 0.15, they are potentially endangered; between 0.15 – 0.25, they are minimally endangered; between 0.25-0.40, they are endangered; and more than 0.40, they are critically endangered. Pandey *et al.* (2006) found that (FIS) estimates ranged between -0.179- 0.572 with an average of 0.214 and an average deficiency (21.4%) of heterozygote existed in the Kenkatha population. The within–population inbreeding estimate (FIS) was 0.2121 and 0.2248in Gaolao and Kenkatha cattle, respectively (Chaudhari *et al.,* 2009).Hussein *et al*. (2014) observed that the lowest value of FIS in Butana (-0.830) as compared with Kenana and Fuga cattle (-0.195 and -0.317, respectively).Moreover, Sharma *et al.* (2015) observed significant heterozygote deficit in eight out of 12 investigated breeds being highest in Ongole (0.221).

Mean values of FIS, FIT and FST were 0.73, 0.76 and 0.13, respectively (Table 2). High values of heterozygosities were observed in Siwa and Farafra

populations (83 and 69%, respectively), while a moderate genetic differentiation (13%) was observed between the two populations. Chaudhari *et al*. (2009) reported that the mean FIS, FIT and FST values were 0.2318, 0.2487 and 0.0219 with a substantial deficit of heterozygotes 21.21% and 22.48%, in Gaolao and Kenkatha cattle populations, respectively. They also, recorded a little genetic differentiation (2.19%) between the two breeds. Moreover, Sharma *et al.* (2015) observed a substantial deficit (4.9 % and 17.5 %) of heterozygotes in cattle populations with a moderate genetic differentiation (13.3 %) between the two breeds of Indian cattle.

Results of the AMOVA analysis are represented in Table (4). Results showed that the majority of the genetic diversity obtained in the current study is presented by among individuals within populations (41.65%) and within individuals (34.70%). Population fixation indices give an idea about the population structure in terms of inbreeding coefficient and population differentiation. Population Fixation indices revealed a 0.653 of variation referring to differences among individuals versus total variance (Fit). While, among populations differences versus total variance was the lowest fixation index (Fst= 0.237) indicating low level of population differentiation. A pair wise difference among Frafra and Siwa cattle populations was 0.546 based on among breeds F index (Fis) as shown in Table (4). Sharma *et al.* (2015) reported that AMOVA revealed that percent of variation among populations was 24 %, while it was 76% within populations.

In Siwa cattle, fourout of the 22 alleles were private alleles (locus MM8, ILSTS006, INRA063 and HEL5); while in Farafra cattle 11out of the 29 alleles were detected as private alleles (locus MM8, INRA063, BM1818, ILSTS054, ILSTS005, ILSTS006 and ILSTS011). Regarding specific alleles, a total number of 15 out of 51 alleles (29.41%) were noticed overall loci for the two studied populations as shown in Table (5). Consequently, those private alleles would be utilized as population fingerprint (even one allele for one locus) and could be used to differentiate between the two populations. Russell *et al.* (2000) detected the 145-bp allele in Chinipas cattle, which was absent in cattle raised in others regions. Gralak *et al.* (2004) observed two alleles (74 bp at the monomorphic locus TGLA227 and 89 bp at locus CSRM60), which seem to be specific for the European bison. Moreover, Chaudhari *et al.* (2009) reported that 14 out of the 239 alleles were private alleles (locus ETH10, ETH152, HEL51, ILSTS005, ILSTS006, ILSTS0554, INRA005, MM8, HAUT24) in Gaolao cattle, while 6 out of 197 alleles were recorded (locus CSRM60, ETH185, HAUT27, INRA063 in Kenkatha cattle.

In the present study, the highest allele frequency overall loci was 0.95 for allele 176 at ILSTS005 locus in Siwa population, while the lowest one (0.05) was for allele 160 at the same locus. In addition, the highest average of allele frequency estimated was at HEL5 (0.50), ILSTS005 (0.50) and ILSTS011 (0.50). In Farafra cattle population, the lowest one (0.20) was at BM1818. Russell *et al.* (2000) detected five alleles at INRA063 in the cattle from the Temoris regionthat contained a specific allele (184 bp) with a frequency of 0.14. They also recorded another unique allele frequency (186-bp), with a higher representation among the Chinipas samples (0.42) than among samples from different regions (0.06 to 0.18) HEL5at locus. Furthermore, allele frequencies were the greatest in cattle from all regions for the 151- and 163-bp alleles, excluding cattle from Temoris, which had allele frequencies of 0.25 at 149 and 167bp alleles.

## CONCLUSION

The present investigation proved the usefulness of using eight microsatellite markers to discriminate cattle raised in Siwa and Farafra oases. Four out of twenty-two alleles were detected as private alleles (locus MM8, ILSTS006, INRA063 and HEL5) in Siwa cattle, while 11 out of 29 private alleles were observed in Farafra cattle (locus MM8, INRA063, BM1818, ILSTS054, ILSTS005, ILSTS006 and ILSTS011). Regarding the specific alleles, a total of 15 out of 51 alleles (29.41%) were noticed overall the loci for the two studied populations. Results confirmed that microsatellite markers could be strongly utilized as a molecular tool in fingerprint analysis for Farafra and Siwa cattle populations. High heterozygotes (83% and 69%) were detected in Siwa and Farafra cattle populations, respectively with a moderate genetic differentiation (13%) between the two populations. The present work suggested using wide genome scan analysis based on more recommended microsatellites covering cattle genome, which could be utilized in further work concerning MAS (marker assisted selection) and QTL (Quantitative Trait Loci) programs. Moreover, further studies are recommended for more details about Siwa and Farafra cattle population.

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**دراسة التنوع الوراثى بين مجموعتين من الأبقار المصرية بإستخدام بعض الواسمات الجزيئية الجسدية**

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استخدمت فى تلك الدراسة مجموعتين من الابقار الموجودة بواحتى (سيوة والفرافرة) والتى تقع فى الصحراء الغربية لمصر وذلك باستخدام تمانية من الواسمات الجزيئية وهم MM8 وINRA063 وBM1818 وILSTS054 وILSTS005 وHEL5 و ILSTS006اوILSTS011 حيث تم جمع عدد ٣٨ عينة دم بواقع (١٩ عينة من الفرافرة و١٩ عينة من سيوة ) واستخلصت المادة الوراثية من العينات وذلك لعمل التحليلات الوراثية اللازمة بطريقة اليمكروستلايتSSR-PCRولقد تم تحديد التباين الوراثى عن طريق حساب التباينات واختبارات كلا من (FIS,FST,FIT) .وجدفى تلك الدراسة ان نسبة التباين الوراثى الملحوظ كان (٨٣%) فى عينات سيوة ووجد انة يساوى (٦٩%) فى ابقار الفرافرة ووجدت نسبة الاختلاف فيما بين المجوعتين كانت (١٣%) فقط . ولقد وجد ان العدد الكلى للاليلات ٢٢ أليل بمتوسط قدرة 2.75±0.71 فى عينات سيوةبينما وجد ٢٩ أليل بمتوسط قدرة 3.63±0.74فى عينات الفرافرة. ووجد ان الاليلات على مستوى المواقع تراوحت مابين ٢اليل فى ILSTS005 ,ILSTS054او ILSTS011الى ٤ أليل فى HEL5 لعينات سيوة بينما تراوح من ٣ أليل فى ILSTS005, HEL5, ILSTS006اوILSTS011 الى ٥ أليل فى BM1818 فى عينات الفرافرة ووجدت قيمة كلا من الملاحظ والمتوقع للتباين الوراثى وهى 0.27 ±0.09 و٠.٢١±٠.٤٦ فى عينات السيوة بينما وجدت ٠.٣٥±٠.٢٠ و٠.٠٧±٠.٦٦ فى عينات الفرافرة . اما نسبة الـ PIC تراوحت من ٠.١ الى ٠.٧ لكلا من ILSTS005اوHEL5على التوالى بمتوسط عام قدرة ٠.٤٥ علاوة على ذلك تراوحت من ٠.٥٢ فى (ILSTS054)الى ٠.٧٤ فى (BM1818) بمتوسط عام قدرة ٠.٦٤ فى عينات الفرافرة . ولقد وجدت مقاييس FIT=0.65 وهذا يشير الى مقدار الاختلاف فيما بين العينات بينما سجلت اقل قيمة للـ FST=0.24 ذلك دليل على الاختلافات القليلة مابين المجموعتين بينما سجلت FIS= 0.55 .وجدت ٤ أليلات و١١ أليل خاص لكلا من سيوة والفرافرة على التوالى تلك الاليلات تعد كبصمة وراثية لكل نوع من تلك الأنواع من الابقار حتى ولوكان أليل واحد لكل موقع فان ذلك يعتد بة فى التفرقة مابين المجموعتين من الابقار.