# Genetic Differentiation between Egyptian Buffalo Populations Using Microsatellite Markers

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**Abstract:** In this study, twelve microsatellite loci were utilized to determine genetic differences between five Egyptian buffalo populations and genetic characterization of these populations. A total of 80 blood samples were collected randomly from five farms in five different governorates (Cairo, Kafr El-Sheikh, Menoufia, Ismailia, and Beni Suef). The genetic parameters like number of alleles, allelic frequencies, F parameters inbreeding coefficient ($F_{IS}$), Total inbreeding coefficient ($F_{IT}$) and Population differentiation ($F_{ST}$), deviation from Hardy-Weinberg Equilibrium (HWE), genetic distances and evolutionary relationships between pairs of studied buffalo populations were obtained using four different programs. Results revealed that all the five populations under study had significant deviations from HWE, as well all loci deviated significantly from HWE across all populations except locus BM1818. This is attributable to non-random mating and selection within these populations, presence of null alleles at some loci, and transfer of buffaloes from one place to another which led to underestimation of heterozygosity. Values of $F_{IT}$ across all populations were high, whereas, values of $F_{ST}$ were low, indicating the high degree of inbreeding in these populations and the low degree of genetic differentiation among them. The lowest genetic distance was found between Sids and Ismailia populations (0.200) while the highest genetic distance was detected between Kafr El-Sheikh and Ismailia (0.373). Genetic distances and neighbor joining showed that Sids and Ismailia populations were grouped together than the remaining groups (Cairo, Kafr El-Sheikh, and Shebeen El-Kom populations). The information obtained by this study will assist in the establishing effective breeding programs and furthermore, in conserving indigenous Egyptian buffalo breed in the future.

***Keywords:*** Egyptian buffalo, genetic differentiation, Hardy-Weinberg Equilibrium, inbreeding coefficient, microsatellites

## INTRODUCTION

The Egyptian buffalo (*Bubalus bubalis*) contributes significantly to the agricultural economy and food security in Egypt (Abou-Bakr *et al.,* 2012). Egyptian buffalo possess a great importance to Egypt due to its great ability of adaptation to various environments such as the tropical climate and resistance to diseases (Abu El-Magd *et al.,* 2015). In addition, Egyptian buffalo consider as the primary dairy animal in Egypt, and an important source of red meat (Abou-Bakr *et al.,* 2012; Attia *et al.,* 2014; Abu El-Magd *et al.,* 2015). Buffalos' production represents 44% and 36% of the whole production for milk and meat in Egypt, respectively (FAO, 2019). Buffalos' milk is preferred by the Egyptian consumer due to its white color, high quality (in rural regions that consume unpasteurized milk), and high fat percentage (Abu El-Magd *et al.,* 2015; Al-Hosary *et al.,* 2015).

Genetic characterization at the molecular level of different Egyptian buffalo populations is an essential prerequisite to any effective breeding programs. The investigation of the genetic relationships among buffalo populations will provide a useful tool in supporting conservation decisions and may contribute to the selection and preservation of genetic resources (El-Kholy *et al.,* 2007). Microsatellite markers are recommended for the analysis of genetic variation and relationships among farm animal populations because of their unique genetic properties (Erhardt and Weimann, 2007; Athe *et al.,* 2018). A considerable number of genetic diversity studies were carried out using microsatellite markers for many livestock species by several investigators throughout the world. However, only a few of these studies investigated the genetic diversity in Egyptian buffalo.

Therefore, genetic improvement of Egyptian buffalo could be achieved through the use of molecular genetic information in selection programs. Such breeding program could have the potential to increase productivity, enhance environmental adaptation, maintain genetic diversity, and allow early selection to reduce generation intervals (Naqvi, 2007; Sikka and Sethi, 2008).

The current study aimed to study genetic differences among five Egyptian buffalo populations and genetic characterization for these populations using 12 microsatellite markers.

## MATERIALS AND METHODS

### Location

This study was carried out at Biotechnology Laboratory of Animal Production and Fisheries Department, Faculty of Agriculture, Suez Canal University; Ismailia, Egypt.

### Animals and blood samples collection

Samples were collected from 80 unrelated lactating buffalo females in five different governorates over the period from November 2016 through April 2017. These farms were, Agriculture Faculty farm at Cairo University (20 sample), Agricultural Research Station located in Kafr El-Sheikh governorate (29 sample), Shebeen El Kom Agriculture Faculty farm located in Menoufia governorate (10 samples), Agricultural Research Station located in Ismailia

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governorate (11 sample), and Sids Research Station located in Beni Suef governorate (10 samples). The blood samples were collected form each animal with a volume of 5 ml in $K_3$EDTA (as anticoagulant) vacutainer tubes, placed promptly on a cooling gel in ice box until reaching the Lab, and stored at -20˚C until DNA extraction later.

**DNA extraction**

DNA was extracted from the whole blood using Quick-gDNATM MiniPrep (50 Preps.) kit, Catalog No. D3024 (Sigma Co.), according to the method described by manufacture.

The quality of DNA yield was evaluated by running in 1% agarose gel through horizontal gel electrophoreses system. The concentration and purity of DNA for all the samples were quantified using Nano Drop1000 spectrophotometer. Concentration of DNA ranged between 20 and 30 ng/µl and purity of DNA ranged from 1.7 to 1.9, indicating high quality DNA.

**Selection of microsatellites markers and PCR amplification**

A total of 15 microsatellite markers were chosen for this study from the cattle genome based on their high polymorphism, polymorphic information content (PIC) with good heterozygosity and information available from previous studies in buffalo (Moore *et al.,* 1995; Barker *et al.,* 1997; Moioli *et al.,* 2001; Tantia *et al.,* 2006; Elbeltagy *et al.,* 2008; Zhang *et al.,* 2008; Bhuyan *et al.,* 2010; Jakhesara *et al.,* 2010; Marques *et al.,* 2011; Vieira *et al.,* 2011; Acosta *et al.,* 2014; Ünal *et al.,* 2014). These microsatellites were BMC1013, CSSM019, CSSM022, CSSM029, CSSM036, CSSM038, CSSM041, CSSM043, CSSM045, CSSM046, CSSM047, ETH3 (D19S2), BM1818, ILSTS005, and ILSTS33.

The optimum annealing temperature for each marker was determined by using gradient PCR thermal cycler (with varied range of annealing temperatures for each marker under the same conditions and for the same samples). Then, the PCR products were tested in 3% agarose gel through horizontal gel electrophoreses system to determine the best annealing temperature for each marker. Microsatellite markers were tested for amplification using PCR thermal cycler. All microsatellites were successfully amplified except three (CSSM041, ETH3 [D19S2], and ILSTS33). Thus, aggregate of 12 microsatellites were used for analysis of the Egyptian buffalo genome. The description of microsatellites used (chromosome assignment, primers sequence, the optimum annealing temperatures, and allelic size range) are given in Table (1).

PCR amplification was carried out in 10 µl reaction mixture. PCR components are shown in Table (2) and Table (3). The PCR protocol was as follows: initial denaturation at 95˚C for 10 min followed by 30 cycles of denaturation at 95˚C for 15 sec, annealing temperature which was determined for each marker (Table 1) for 1 min and extension at 72℃ for 1 min, followed by final extension at 72℃ for 10 min and hold at 4˚C for 5 min (Elbeltagy *et al.,* 2008).

**Table (1):** The description of microsatellites used (chromosome assignment, primers sequence, the optimum annealing temperatures (Tm) and allelic size range)

| Locus | Chromosome assignment | Primer sequences (5´- 3´) Forward (F) Reverse (R) | Tm (ºC) | Allelic size range (bp) | Reference |
|---|---|---|---|---|---|
| **BMC1013** | 3p | F: AAAAATGATGCCAACCAAATT<br>R: TAGGTAGTGTTCCTTATTTCTCTGG | 59.4 | 224 – 238 | FAO (2011) |
| **CSSM019** | 1q | F: TTGTCAGCAACTTCTTGTATCTTT<br>R: TGTTTTAAGCCACCCAATTATTTG | 57 | 144 – 154 | Moore *et al.* (1994) and FAO (2011) |
| **CSSM022** | 4q | F: TCTCTCTAATGGAGTTGGTTTTTG<br>R: ATATCCCACTGAGGATAAGAATTC | 60 | 208 – 216 | |
| **CSSM029** | 9 | F: GCTCCATTATGCACATGCCATGCT<br>R: CGTGAGAACCGAAAGCACACATTC | 59 | 195 – 201 | |
| **CSSM036** | 1p | F: GGATAACTCAACCACACGTCTCTG<br>R: AAGAAGTACTGGTTGCCAATCGTG | 59 | 170 – 176 | |
| **CSSM038** | 11 | F: TTCATATAAGCAGTTTATAAACGC<br>R: ATAGGATCTGGTAACTTACAGATG | 59 | 179 – 187 | |
| **CSSM043** | 1p | F: AAAACTCTGGGAACTTGAAAACTA<br>R: GTTACAAATTTAAGAGACAGAGTT | 56 | 248 – 256 | |
| **CSSM045** | 2q | F: TAGAGGCACAAGCAAACCTAACAC<br>R: TTGGAAAGATGCAGTAGAACTCAT | 53 | 106 – 120 | |
| **CSSM046** | 11 | F: TGCACAATCGGAACCTAGAATATT<br>R: GGCTATTAACTGTTTTCTAGGAAT | 59 | 160 – 168 | |
| **CSSM047** | 3q | F: TCTCTGTCTCTATCACTATATGGC<br>R: CTGGGCACCTGAAACTATCATCAT | 56 | 142 – 158 | |
| **BM1818** | 23* | F: AGCTGGGAATATAACCAAAGG<br>R: AGTGCTTTCAAGGTCCATGC | 59 | 252 – 278 | Tantia *et al.* (2006) and Rad *et al.* (2013) |
| **ILSTS005** | 11 | F: GGAAGCAATGAAATCTATAGCC<br>R: TGTTCTGTGAGTTTGTAAGC | 56 | 188 – 194 | *Tantia et al.* (2006) and FAO (2011) |

*chromosome assignment in cattle.

**Table (2):** PCR components for markers "BMC1013, CSSM019, CSSM022, CSSM043, CSSM045, and CSSM046"

| Component | Volume / sample |
|---|---|
| **Master mix (1x)** | 5 µl |
| **Upstream primer (F)** | 0.1 µl |
| **Downstream primer (R)** | 0.1 µl |
| **DNA template** | 2 µl |
| **DNase free water** | 2.8 µl |
| **Total volume** | 10 µl |

**Table (3):** PCR components for markers "CSSM029, CSSM036, CSSM038, CSSM047, BM1818, and ILSTS005"

| Component | Volume / sample |
|---|---|
| **Master mix (1x)** | 5 µl |
| **Upstream primer (F)** | 0.2 µl |
| **Downstream primer (R)** | 0.2 µl |
| **DNA template** | 2 µl |
| **DNase free water** | 2.6 µl |
| **Total volume** | 10 µl |

**Electrophoresis of PCR products for determining the alleles for each marker**

Alleles for each marker were determined by running horizontally 6 µl of the PCR product mixed with 1 µl of gel loading dye on 3% agarose gel electrophoresis and stained by 0.5 µl ethidium bromide with concentration of 10 mg / ml at 100 V and 40 mA for 120 min. 50bp and 100bp DNA ladders were used to estimate alleles size in base pairs (bp). A constant control sample as animal reference which was amplified by "CSSM029" marker was used in all gels to ensure an accurate estimate of allelic size.

**Statistical analysis**

Four different programs were used to calculate the genetic parameters. The software of GENETIX 4.05 (Belkhir *et al.,* 1996 and 2004) was used to estimate allelic frequencies per locus in each population, heterozygosity level ($H_{Nb}$ = unbiased expected heterozygosity (Nei, 1978) and $H_O$ = observed heterozygosity), F parameters according to Weir and Cockerham (1984), and genetic distances between populations according to Nei (1972). F parameters include $F_{IS}$ (inbreeding coefficient), $F_{IT}$ (total inbreeding coefficient), and $F_{ST}$ (population differentiation). $F_{IS}$ is an estimate of variation within populations that measures the reduction in heterozygosity in an individual due to nonrandom mating within sub populations. $F_{ST}$ is an estimate of the variation due to differentiation among populations, which is the reduction in heterozygosity of a population due to genetic drift. $F_{IT}$ is the overall inbreeding coefficient of an individual relative to the total population. This includes the contribution due to nonrandom mating within subpopulations $F_{IS}$ and that due to population subdivision $F_{ST}$ (Tantia *et al.,* 2006). Genetic distance is the degree of gene difference (genomic difference) between species or populations that is measured by some numerical methods. Genetic distance is measure of overall evolutionary divergence, i.e. genetic similarities and dissimilarities between two populations groups such as between species, breeds, strains and populations (Naqvi, 2007).

Number of observed alleles at each locus within each population were calculated with the FSTAT v.2.9.3.2 program (Goudet, 2002). The GENEPOP Program version 4.2 (Raymond and Rousset, 1995; Rousset, 2008) was used to compute an exact test for deviation from Hardy-Weinberg equilibrium (HWE) and null allele frequencies per locus within each population. POPTREE2 Program version 2.0 (Takezaki *et al.,* 2010) was used to perform evolutionary analyses of allele frequency data. Different types of genetic distance and construct phylogenetic trees of populations was calculated using the neighbor-joining (NJ) method (Saitou and Nei, 1987).

## RESULTS AND DISCUSSION

**Hardy-Weinberg Equilibrium (HWE)**

HWE is a useful indicator of genotype frequencies within a population and whether they are based on a valid definition of alleles and a randomly mating sample. HWE assumes a stable population of adequate size without selective pressures and is used to compare observed genotype frequencies to those expected within a population (Short *et al.,* 2007). Therefore, excess of heterozygote individuals than homozygote individuals, association of loci with some genes of economics importance, could be a result of migration and high mutation rate of microsatellite (Aminafshar *et al.,* 2008).

All the five populations illustrated significant deviations ($P<0.05$) from HWE, as well as all loci deviated significantly ($P<0.05$) from HWE across all populations except one locus only (BM1818) was in HWE. This is attributable to non-random mating and selection within these populations, presence of null alleles at some loci, and transfer of buffaloes from one place to another which led to underestimation of heterozygosity. Elbeltagy *et al.* (2008) and Attia *et al.* (2014) deduced that Nile-Delta and Italian buffalo populations deviated significantly from HWE (inbred populations) whilst the Southern-Egypt buffalo did not deviate from HWE (outbred population).

The results in the literature on several different breeds of buffalo (Arora *et al.,* 2004; Ángel-Marín *et al.,* 2010; Bhuyan *et al.,* 2010) showed significant departures from HWE at some loci (CSSM036,

CSSM043, and CSSM045) and non-significant at others (CSSM19, CSSM022, CSSM038, ILSTS005, and BM1818). This disagrees with the findings of other authors. Mishra *et al.* (2008) and Rad *et al.* (2013) mentioned that BM1818 deviated considerably from HWE in Indian and khouzestan buffalo breeds. Marques *et al.* (2011) declared that the loci CSSM022 and CSSM036 were in HWE but the loci BM1818 and CSSM019 deviated from HWE in some Brazilian buffalo populations and did not deviate in others. Jakhesara *et al.* (2010) found both the loci CSSM022 and CSSM043 had significant departures from HWE in Mehsana breed of Indian buffalo.

**Inbreeding coefficient ($F_{IS}$)**

Values of $F_{IS}$ for each locus within and over all populations are demonstrated in Table (4). The microsatellites CSSM022, CSSM029, CSSM036, CSSM046, and ILSTS005 had the highest values (1.000) in all populations. In contrast, the microsatellite BM1818 had the lowest values in all studied populations (between -0.259 in Shebeen El-Kom population and -0.146 in Kafr El-Sheikh Population). These findings indicated that the absence of heterozygosity at the loci CSSM022, CSSM029, CSSM036, CSSM046, and ILSTS005 and excess of heterozygosity at the locus BM1818.

**Table (4):** Inbreeding coefficient ($F_{IS}$) for each locus within and over all populations

| Locus | Inbreeding coefficient ($F_{IS}$) | | | | | |
|---|---|---|---|---|---|---|
| | **Cairo** | **Ismailia** | **Kafr El-Sheikh** | **Shebeen El-Kom** | **Sids** | **Overall** |
| **BMC1013** | 0.499 | 0.522 | 0.390 | 0.431 | 0.462 | 0.481 |
| **BM1818** | -0.224 | -0.161 | -0.146 | -0.259 | -0.232 | -0.220 |
| **CSSM019** | 0.916 | 0.897 | 0.916 | 0.920 | 0.920 | 0.914 |
| **CSSM022** | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| **CSSM029** | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| **CSSM036** | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| **CSSM038** | 0.457 | 0.387 | 0.284 | 0.420 | 0.319 | 0.401 |
| **CSSM043** | 0.617 | 0.592 | 0.498 | 0.603 | 0.533 | 0.592 |
| **CSSM045** | 0.481 | 0.387 | 0.394 | 0.428 | 0.445 | 0.436 |
| **CSSM046** | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| **CSSM047** | 0.880 | 0.846 | 0.863 | 0.868 | 0.888 | 0.871 |
| **ILSTS005** | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| **Mean** | **0.719** | **0.706** | **0.683** | **0.701** | **0.695** | **0.706** |

F-statistics [total inbreeding coefficient ($F_{IT}$) and population differentiation ($F_{ST}$)]

Across all loci, the estimates of means of $F_{IS}$ were high for all populations (from 0.683 for Kafr El-Sheikh population to 0.719 for Cairo population) with overall mean of 0.706. This means that reduction of heterozygosity because of nonrandom mating within these populations. Severe inbreeding can be explained by using very small number of the same bulls from neighboring areas for all the five populations. Some authors (Mishra *et al.,* 2008; Mishra *et al.,* 2009; Jakhesara *et al.,* 2010) concluded similar findings in some breeds of Indian buffalo but with values of $F_{IS}$ less than those reported in the present study.

Conversely, Tantia *et al.* (2006) and El-Kholy *et al.* (2007) illustrated negative values of $F_{IS}$ in Indian and Egyptian buffalo populations, denoting the outbreeding or mating with migrants. In addition, Soysal *et al.* (2007) deduced high level of genetic diversity in the Anatolian water buffalo population; they reported varied values of $F_{IS}$ from -0.10 to 0.21 across all loci with a total $F_{IS}$ of 0.04.

**F-statistics [Total inbreeding coefficient ($F_{IT}$) and Population differentiation ($F_{ST}$)]**

Table (5) presents the estimates of $F_{IT}$ and $F_{ST}$ per locus across all populations, whilst Table (6) clarifies the estimated pairwise $F_{ST}$ among pairs of all studied populations. Values of $F_{IT}$ across all populations were high and ranged between 0.672 for CSSM022 locus and 0.785 for BM1818 locus, whereas, values of $F_{ST}$ were low and ranged between 0.042 for CSSM046 locus and 0.077 for CSSM047 locus with overall values of 0.702 and 0.071 for $F_{IT}$ and $F_{ST}$, respectively (Table 5).

**Table (5):** Estimates of total inbreeding coefficient ($F_{IT}$) and population differentiation ($F_{ST}$) per locus across all populations

| Locus | $F_{IT}$ | $F_{ST}$ |
|---|---|---|
| **BMC1013** | 0.725 | 0.072 |
| **BM1818** | 0.785 | 0.069 |
| **CSSM019** | 0.681 | 0.075 |
| **CSSM022** | 0.672 | 0.076 |
| **CSSM029** | 0.688 | 0.074 |
| **CSSM036** | 0.675 | 0.065 |
| **CSSM038** | 0.731 | 0.075 |
| **CSSM043** | 0.712 | 0.075 |
| **CSSM045** | 0.724 | 0.072 |
| **CSSM046** | 0.676 | 0.042 |
| **CSSM047** | 0.684 | 0.077 |
| **ILSTS005** | 0.677 | 0.074 |
| **Overall** | 0.702 | 0.071 |

The high values of $F_{IT}$ and the low values of $F_{ST}$ reflect the high degree of inbreeding in these populations and the low degree of genetic differentiation among them. These results are also confirmed by the high estimates of $F_{IS}$ as illustrated in Table (4). The lowest value of $F_{ST}$ was between Cairo and Shebeen El-Kom populations (0.033) but the highest value of $F_{ST}$ was between Ismailia and Kafr El-Sheikh populations (0.116) as observed in Table (6).

Elbeltagy *et al.* (2008) and Abou-Bakr *et al.* (2012) inferred also low estimates of $F_{ST}$ in Egyptian buffalo populations (0.014 and 0.05, respectively). Attia *et al.* (2014) obtained as well low values of the means of $F_{IT}$ and $F_{ST}$ in populations of the Delta buffalos showed low level of inbreeding within and among populations and low genetic differentiation among them. Moioli *et al.* (2001) reported a low mean of $F_{IT}$ in Egyptian buffalo population but it was much less than that found in the current study (0.146). Furthermore, some other studies (Arora et al., 2004; Kumar *et al.,* 2006; Tantia *et al.,* 2006) observed low values of $F_{ST}$ among many breeds of Indian buffalo.

**Genetic distance**

Genetic distance serves as an useful tool for authentication of the pedigree, for characterization of different breeds or strains within a species and for evaluation of the change in variation in species over time (Naqvi, 2007).

Nei's genetic distance between the studied Egyptian buffalo populations are given in Table (7).

**Table (6):** Estimated pairwise $F_{ST}$ among pairs of all studied populations

| Population | Ismailia | Kafr El-Sheikh | Shebeen El-Kom[1] | Sids[2] |
|---|---|---|---|---|
| **Cairo** | 0.075 | 0.050 | 0.033 | 0.079 |
| **Ismailia** | ------- | 0.116 | 0.056 | 0.045 |
| **Kafr El-Sheikh** | ------- | ------- | 0.054 | 0.090 |
| **Shebeen El-Kom** | ------- | ------- | ------- | 0.081 |

(1) Menofia, (2) Benisuef governorate

**Table (7):** Nei's (1972) genetic distance between the studied Egyptian buffalo populations

| Population | Cairo | Ismailia | Kafr El-Sheikh | Shebeen El-Kom[1] | Sids[2] |
|---|---|---|---|---|---|
| **Cairo** | ------- | 0.267 | 0.203 | 0.214 | 0.290 |
| **Ismailia** | | ------- | 0.373 | 0.249 | 0.200 |
| **Kafr El-Sheikh** | | | ------- | 0.249 | 0.310 |
| **Shebeen El-Kom** | | | | ------- | 0.330 |

(1) Menofia, (2) Benisuef governorate

The lowest genetic distance was found between Sids and Ismailia populations (0.200) while the highest genetic distance was detected between Kafr El-Sheikh and Ismailia (0.373). Figure (1) showed the relationship between the studied populations. This dendrogram supports the results deduced from the genetic distance estimates which proved that Sids and Ismailia populations had the smallest genetic distance, indicating the close relationship between the two populations. This suggested that these two populations had the same origin. Cairo, Kafr El-Sheikh, and Shebeen El-Kom populations form a separate cluster, which pointed towards the close relationship between the three populations but Cairo and Kafr El-Sheikh populations were the more closely from each other compared with Shebeen El-Kom population. This is possibly due to the admixture between Beheiri and Upper-Egypt buffaloes forming Ismailia population and hence made both the Upper-Egypt and Ismailia buffalo populations separated from Beheiri buffalo.

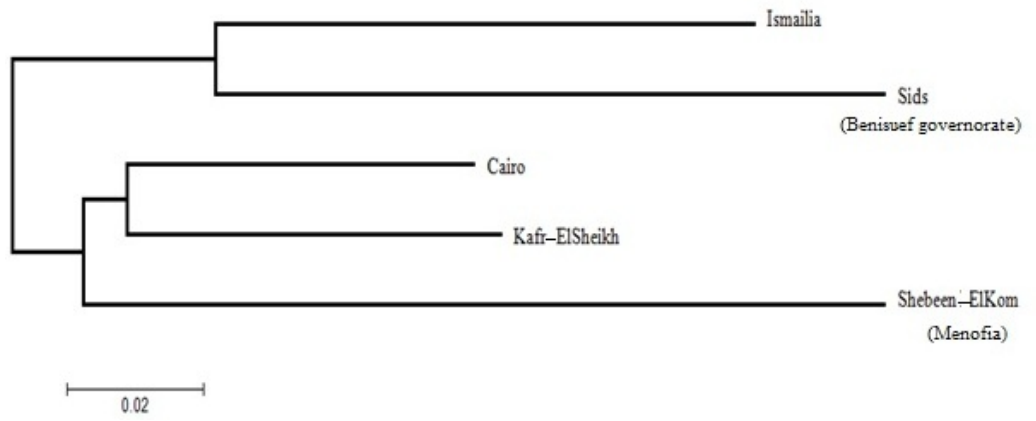


**Figure (1):** Dendrogram of neighbor joining of the five buffalo populations according to Nei's (1972) genetic distance

Similar results to those detected in this study were given by Al-Shawa (2020) in press who evaluated the genetic distance among the studied Egyptian buffalo populations using PCR-RFLP analysis data for genes polymorphism data which had also been utilized in the present study, and revealed that the maximum genetic distance was found between Kafr El-Sheikh and Ismailia populations (0.037).

Contrary to the findings of the current study, Elbeltagy *et al.* (2008) revealed much lower genetic distances (0.06) between the Southern-Egypt and Delta buffalo populations. In addition, other investigators (Atta *et al.,* 2009; Abdel-Aziem *et al.,* 2010; Othman *et al.,* 2012) detected lower genetic distances (0.13) between Egyptian buffalo populations using PCR-RAPD Technique. El-Kholy *et al.* (2007) stated wider range of genetic distance in comparison with those reported in this study which ranged from 0.014 (between Al-Minya and Qina buffalo populations) to 0.603 (between Delta and Menofya buffalo).

Sukla *et al.* (2006) observed high genetic distances values between some Indian buffalo breeds (from 0.26 to 0.29) which were analogical to those reported between some populations in the present study. Singh *et al.* (2017) detected greatly higher estimates of the genetic distances in Indian buffalo populations which ranged between 0.76 and 0.85 with an average of 0.82. Conversely, Arora *et al.* (2004) and Kumar *et al.* (2006) showed small genetic distance between Indian buffalo breeds.

## CONCLUSION

The findings demonstrated high level of inbreeding in all studied populations and low level of genetic differentiation among them. This indicates that reduction of heterozygosity would be due to nonrandom mating and selection within these populations. Severe inbreeding can be explained by using very small number of the same bulls from neighboring areas for all the populations under study.

Results of genetic distances and neighbor joining showed that Benisuef governorate (Sids) and Ismailia populations were grouped together rather than the remaining groups [Cairo, Kafr El-Sheikh, and Menofia (Shebeen El-Kom populations)]. The genetic differences in all the studied populations were not great, nevertheless, genetic distances were great between these populations and therefore we will need more samples and a larger number of microsatellites, especially which have the ability to discover the highest polymorphism. Consequently, the information obtained by this study will assist in the establishing effective breeding programs and furthermore, in conserving indigenous Egyptian buffalo breed in the future.

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# الاختلافات الوراثية بين عشائر الجاموس المصري باستخدام واسمات المكررات المتسلسلة القصيرة

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في هذه الدراسة استخدمت اثني عشر من مواقع المكررات المتسلسلة القصيرة لتقدير الاختلافات الوراثية بين خمس عشائر من الجاموس المصري والتوصيف الوراثي لهذه العشائر. تم تجميع ثمانون عينة دم عشوائيا من خمس مزارع في خمس محافظات مختلفة (القاهرة، كفر الشيخ، المنوفية، الإسماعيلية، بنى سويف). تم الحصول على المقاييس الوراثية مثل عدد الاليلات، التكرارات الاليلية، مقاييس F (معامل التربية الداخلية، معامل التربية الداخلية الكلى، الاختلافات الوراثية بين العشائر)، الانحراف عن اتزان هاردي واينبرج، المسافات الوراثية، العلاقات التطورية بين أزواج عشائر الجاموس المدروسة وذلك باستخدام أربع برامج مختلفة. أظهرت النتائج أن جميع العشائر موضع الدراسة لها انحرافات معنوية عن اتزان هاردى واينبرج كذلك انحرفت جميع المواقع معنويا عن اتزان هاردى واينبرج خلال جميع العشائر فيما عدا موقع BM1818. يعزى ذلك إلى التزاوج الغير العشوائي والانتخاب داخل هذه العشائر، وجود أليلات لم تنجح في التضاعف في بعض المواقع، نقل الجاموس من مكان إلى آخر والذي أدى إلى نقص في التنوع الوراثي. قيم $F_{IT}$ خلال جميع العشائر كانت عالية بينما قيم $F_{ST}$ كانت منخفضة وذلك إشارة إلى الدرجة العالية من التربية الداخلية في هذه العشائر والدرجة المنخفضة من الاختلاف الوراثي بينهم. وجدت المسافة الوراثية الأقل بين عشائر الإسماعيلية والصعيد (٠,٢) بينما اكتشفت المسافة الوراثية الأعلى بين عشائر الإسماعيلية وكفر الشيخ (٠,٣٧٣). المسافة الوراثية والقرابة الوراثية أوضحت أن عشائر سدس والإسماعيلية قريبة من بعضها عن المجاميع الباقية (عشائر القاهرة وشبين الكوم وكفر الشيخ). المعلومات المتحصل عليها من خلال هذه الدراسة سوف تساعد في تأسيس برامج تربية أكثر كفاءة في المستقبل والتي تهدف إلى الحفاظ على سلالة الجاموس المصري المحلية.