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Genetic Diversity Analysis of Five Egyptian Buffalo Populations Using Microsatellite Markers

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Abstract

For assessing the genetic diversity and genetic characterization of five Egyptian buffalo populations a total of 12 microsatellite markers were used. The total number of buffaloes sampled was 80, collected at random from five farms in five different governorates; Cairo, Kafr El-Sheikh, Shebeen El-Kom, Menoufia, and Beni Suef. The genetic parameters (allelic diversity, allelic frequencies, observed heterozygosity, unbiased expected heterozygosity, and polymorphic information content) were calculated using three different programs. All used microsatellites were polymorphic and ranged from four alleles (Loci; CSSM029, CSSM036, CSSM038, CSSM043, CSSM046, and ILSTS005) to nine alleles (Loci; BM1818 and CSSM047) with a total of 64 alleles in the whole population. Allelic richness for the whole population ranged between 3.297 (in locus CSSM029) and 6.806 (in locus CSSM047) with overall mean 4.574. Within populations, Kafr El-Sheikh population had the highest average of allelic richness (4.384). This indicates the potential of this population to adapt with environmental changes in future compared with other populations. BMC1013, BM1818, CSSM019, and CSSM047 showed the highest allelic richness. PIC estimates were high and ranged between 0.65 (in locus CSSM029) and 0.92 (in locus CSSM022) with an average of 0.82. Values of H_o were lower than values of H_{Nb} for all populations, which denoting depression of heterozygotes in these populations and may be attributable to existence of null alleles and inbreeding. This study as well proves the usefulness of heterologous bovine microsatellite markers in evaluation of the genetic variability in Egyptian buffalo populations due to high polymorphism, informativeness of these markers which can be used to develop future breeding strategies and conservation decisions on our indigenous breed.

Keywords: observed heterozygosity, polymorphic information content, unbiased expected heterozygosity

1. Introduction

Egyptian buffalo possess a great importance to Egypt due to its great ability of adaptation to various environments such as the tropical climate, excellent nutritional benefits, and resistance to the diseases (Abu El-Magd et al., 2015). In addition, Egyptian buffalo consider as the primary dairy animal in Egypt, and being an important source of red meat (Abou-Bakr et al., 2012; Attia et al., 2014a; Abu El-Magd et al., 2015). Buffalos' production represents 44.26% 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, and high fat percentage (6-8%) (Ibrahim, 2012; Al-Hosary et al., 2015; Abu El-Magd et al., 2015).

Developments in DNA technologies have made it possible to uncover a large number of genetic polymorphisms at the DNA sequence level, and to use them as markers for the evaluation of the genetic basis for the observed phenotypic variability (Jakhesara et al., 2010; Othman et al., 2012). As well, genetic markers provide information about allelic variation at a given locus. The increasing of molecular markers availability in farm animals allows the detailed analyses and evaluation of genetic diversity and furthermore the detection of genes influencing economically important traits. The majority of molecular markers used nowadays with high-throughput systems are microsatellite markers (Erhardt & Weimann, 2007). The use of microsatellites has become a standard method to estimate genetic diversity in livestock, because of their unique genetic properties

(Erhardt & Weimann, 2007; Athe et al., 2018). There are a considerable number of genetic diversity studies for many livestock species were carried out by several investigators from all over the world. However, only a few of these studies (El-Kholy et al., 2007; Abou-Bakr et al., 2012) investigate the genetic diversity using microsatellites analysis in Egyptian buffalo. Genetic characterization of this breed is still incomplete which is very important for conservation decisions and designing future breeding policies.

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

The present study was designed to assessing the genetic diversity and genetic characterization of five Egyptian buffalo populations using 12 microsatellite markers.

2. Materials and Methods

2.1 Location

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

2.2 Selection of Animals and Blood Samples Collection

Data were collected in agreements with ethical standards, and safety guide procedures. Samples were collected from 80 unrelated lactating buffalo females belonging to five farms 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 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_3EDTA (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.

2.3 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.

2.4 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 only three markers (CSSM041, ETH3 [D19S2], and ILSTS33). And thus, aggregate of 12 microsatellites were used for analysis of the Egyptian buffalo genome. Chromosome assignment, primers sequence, and the optimum detected annealing temperatures for the analyzed microsatellites are given in Table 1.

PCR amplification was carried out in 10 µl reaction mixture. PCR components are shown in Tables 2 and 3. The PCR protocol was as follows: initial denaturation at 95 ^{o}C for 10 min followed by 30 cycles of denaturation at 95 ^{o}C for 15 sec, annealing temperature which was determined for each marker (Table 1) for 1 min and extension at

72 °C for 1 min, followed by final extension at 72 °C for 10 min and hold at 4 °C for 5 min (Elbeltagy et al., 2008).

Table 1. Chromosome assignment, primers sequence, and the optimum detected annealing temperatures for the analyzed microsatellites

No.	Locus	Chromosome assignment	Primer sequences (5′-3′)	Temp. (ºC)*	Reference
1	BMC1013	3p	F: AAAAATGATGCCAACCAAATT R: TAGGTAGTGTTCCTTATTTCTCTGG	59.4	FAO (2011)
2	CSSM019	1q	F: TTGTCAGCAACTTCTTGTATCTTT R: TGTTTTAAGCCACCCAATTATTTG	57	Moore et al. (1994); FAO (2011)
3	CSSM022	4q	F: TCTCTCTAATGGAGTTGGTTTTTG R: ATATCCCACTGAGGATAAGAATTC	60	Moore et al. (1994); FAO (2011)
4	CSSM029	9	F: GCTCCATTATGCACATGCCATGCT R: CGTGAGAACCGAAAGCACACATTC	59	Moore et al. (1994); FAO (2011)
5	CSSM036	1p	F: GGATAACTCAACCACACGTCTCTG R: AAGAAGTACTGGTTGCCAATCGTG	59	Moore et al. (1994); FAO (2011)
6	CSSM038	11	F: TTCATATAAGCAGTTTATAAACGC R: ATAGGATCTGGTAACTTACAGATG	59	Moore et al. (1994); FAO (2011)
7	CSSM043	1p	F: AAAACTCTGGGAACTTGAAAACTA R: GTTACAAATTTAAGAGACAGAGTT	56	Moore et al. (1994); FAO (2011)
8	CSSM045	2q	F: TAGAGGCACAAGCAAACCTAACAC R: TTGGAAAGATGCAGTAGAACTCAT	53	Moore et al. (1994); FAO (2011)
9	CSSM046	11	F: TGCACAATCGGAACCTAGAATATT R: GGCTATTAACTGTTTTCTAGGAAT	59	Moore et al. (1994) and FAO (2011)
10	CSSM047	3q	F: TCTCTGTCTCTATCACTATATGGC R: CTGGGCACCTGAAACTATCATCAT	56	Moore et al. (1994); FAO (2011)
11	BM1818	23**	F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	59	Tantia et al. (2006); Rad et al. (2013)
12	ILSTS005	11	F: GGAAGCAATGAAATCTATAGCC R: TGTTCTGTGAGTTTGTAAGC	56	Tantia et al. (2006); FAO (2011)

Note. * Temp.: Annealing temperatures in the current study, **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**

2.5 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. 50 bp and 100 bp 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.

2.6 Statistical Analysis

Three different programs were used to calculate the genetic parameters. The software of GENETIX 4.05 (Belkhir et al., 1996-2004) was used to estimate allelic frequencies per locus in each population and heterozygozity level (H_{Nb} = unbiased expected heterozygosity (Nei, 1978) and H_o = observed heterozygosity). Heterozygosity is defined as the probability that a given individual randomly sampled from a population will be heterozygous at a given locus (Tantia et al., 2006). Number of observed alleles and allelic richness based on a minimum sample size of 9 diploid individuals at each locus within each population were calculated with the FSTAT v.2.9.3.2 program (Goudet, 2002). Number of observed alleles is defined as the actual number of alleles which was detected in the population, whereas, allelic richness is the average number of alleles for each locus. Both of two measures are also referred to as allelic diversity. PIC was computed for each locus using MolKin software version 3.0 (Gutiérrez et al., 2005).

3. Results and Discussion

3.1 Number of Observed Alleles (n_o) and Allelic Richness (n_a)

Number of observed alleles is one of the most important measures of genetic diversity. Estimates of n_o at each locus within and over all populations are presented in Table 4. The results of current study showed that all the analyzed microsatellites were polymorphic as shown from the values of n_o per locus in the whole population which ranged from 4 for loci CSSM029, CSSM036, CSSM038, CSSM043, CSSM046, and ILSTS005 to 9 for loci BM1818 and CSSM047. In comparison of these findings with other studies on river buffalo for some similar loci, Moioli et al. (2001) and Elbeltagy et al. (2008) concluded similar number of alleles to that obtained for microsatellites CSSM036, CSSM038, and CSSM043 in Egyptian, Italian, and Greek buffalo breeds. Arora et al. (2004) and Kathiravan et al. (2012) clarified comparable number to that observed for the locus CSSM045 in some Indian buffalo breeds. Additionally, analogical allelic number to that mentioned in this study was reported by Kumar et al. (2006) and Soysal et al. (2007) for the markers BM1818 and CSSM047 in some Indian buffalo breeds. Moreover, results of some other studies on different breeds of river buffalo showed as well identical number of alleles to that observed in the present study for some loci but it was higher for others. Ünal et al. (2014) worked on Turkish buffalo and concluded 4 alleles for each of CSSM036, CSSM038, CSSM046, and ILSTS005 loci, and higher number of alleles for CSSM029 and CSSM043 loci (5 and 8 alleles, respectively). Tantia et al. (2006) worked on Indian buffalo and reported 4 alleles for each of CSSM029 and ILSTS005 loci, and higher number of alleles for CSSM043 locus (5 alleles).

As a result of increasing the number of alleles for all analyzed loci (12 microsatellites) over three alleles, these microsatellites could be a useful tool for the estimation of genetic diversity in the Egyptian buffalo, according to one of the most important criteria used to select the appropriate microsatellites for diversity studies which had been suggested by Barker (1994). This denotes presence of high degree of allelic polymorphism in the Egyptian buffalo population. The total number of detected alleles over all loci in the whole population was 64 alleles with overall mean of 5.33. Kafr El-Sheikh population showed the highest number of detected alleles (59 allele with average = 4.92), whereas Sids population showed the lowest number of detected alleles (44 allele with average = 3.67). Aminafshar et al. (2008) demonstrated a slightly lower number of alleles across 14 microsatellites for Guilan buffalo population compared to that found in this study. However, Elbeltagy et al. (2008) observed a higher number of alleles across 15 microsatellites for Nile-Delta and Southern-Egypt buffalo populations (86 and 84 alleles, respectively). A comparable number of alleles were reported for Banni and Cuban buffalo breeds by Mishra et al. (2009) and Acosta et al. (2014). Other researchers detected a considerably higher number of alleles for both Indian and Egyptian buffalo breeds (Jakhesara et al., 2010; Abou-Bakr et al., 2012; Singh et al., 2017).

Allelic richness (n_a) is one of the most common measures of genetic variability (Leberg, 2002). This measure indicates the potential of a population on long-term for adaptation and persistence (Greenbaum et al., 2014). Increasing the allelic richness of a population can lead to raising its ability for adaptation with environmental changes in future (Caballero & García-Dorado, 2013). Estimates of allelic richness per locus within and over all populations are given in Table 4. Allelic richness for the whole population ranged between 3.297 for locus CSSM029 and 6.806 for locus CSSM047 with overall mean 4.574. Buffalo population from Kafr El-Sheikh

region possessed the largest allelic richness (7.331 for CSSM047), whereas, Sids population possessed the least allelic richness (1 for CSSM029). Findings of the current study generally revealed that the highest allelic richness loci were BMC1013, BM1818, CSSM019, and CSSM047.

Table 4. Number of observed alleles (n_o) and allelic richness (n_a) at each locus within and over all populations:

Observed alleles (n_o)						
Locus	**Cairo**	**Ismailia**	**Kafr El-Sheikh**	**Shebeen El-Kom**	**Sids**	**Overall Location**
BMC1013	6	5	5	4	4	6
BM1818	5	4	7	6	5	9
CSSM019	6	5	6	5	5	6
CSSM022	4	4	5	3	4	5
CSSM029	4	3	4	3	1	4
CSSM036	4	3	4	4	2	4
CSSM038	4	2	4	3	4	4
CSSM043	4	3	4	3	4	4
CSSM045	4	5	4	3	4	5
CSSM046	2	2	3	3	2	4
CSSM047	6	6	9	6	6	9
ILSTS005	4	4	4	4	3	4
Overall Loci	**53**	**46**	**59**	**47**	**44**	**64**
Mean	**4.42**	**3.83**	**4.92**	**3.92**	**3.67**	**5.33**
Allelic richness (n_a)*						
Locus	**Cairo**	**Ismailia**	**Kafr El-Sheikh**	**Shebeen El-Kom**	**Sids**	**Overall Location**
BMC1013	5.543	4.984	4.935	3.989	4.000	5.457
BM1818	4.430	3.818	5.310	5.995	4.895	6.327
CSSM019	5.273	4.974	5.226	4.895	4.800	5.281
CSSM022	3.848	3.974	4.910	3.000	4.000	4.701
CSSM029	3.695	2.948	3.393	2.995	1.000	3.297
CSSM036	3.990	3.000	3.472	3.989	2.000	3.812
CSSM038	3.848	2.000	3.990	3.000	3.995	3.922
CSSM043	3.944	3.000	3.837	2.995	3.895	3.731
CSSM045	3.696	4.945	3.913	3.000	3.995	4.318
CSSM046	1.998	1.974	2.782	2.995	2.000	3.505
CSSM047	5.525	5.922	7.331	5.795	5.700	6.806
ILSTS005	3.871	3.984	3.512	4.000	2.995	3.735
Mean	**4.138**	**3.794**	**4.384**	**3.887**	**3.606**	**4.574**

Note. *based on a minimum sample size of 9 diploid individuals.

3.2 Allele Sizes and Frequencies

Allele sizes and frequencies for each microsatellite within and over all populations are shown in Tables 5 and 6. The values of allelic frequency for all the studied loci within the populations ranged from 0.017 for locus BM1818 (in Kafr El-Sheikh population) to 1.000 for locus CSSM029 (in Sids population) whilst values of allele frequency in the whole population ranged from 0.010 for locus BM1818 to 0.782 for locus CSSM029. Results demonstrated the presence of four private alleles at three microsatellite loci with frequencies varied from very low (0.052) to high (0.690). These alleles were the allele 224 bp at locus BMC1013 in Cairo population, the alleles 254 and 272 bp at locus BM1818 in Shebeen El-Kom and Kafr El-Sheikh populations, the allele 164 bp at locus CSSM046 in Kafr El-Sheikh population. In addition, eight rare alleles were observed in six loci (frequency < 0.05; Tables 5 and 6). These alleles were 256 and 276 bp at locus BM1818 in Kafr El-Sheikh and Cairo populations, the allele 154 bp at locus CSSM019 in Cairo and Kafr El-Sheikh populations, the alleles 195 and 172 bp in Kafr El-Sheikh population at loci CSSM029 and CSSM036 respectively, the alleles 144 and 148 bp at locus CSSM047 in Kafr El-Sheikh population, the allele 190 bp at locus ILSTS005 in Kafr El-Sheikh population.

Elbeltagy et al. (2008) also observed the existence of exclusive alleles in the Nile-Delta, Southern-Egypt, and Italian buffalo populations but with low frequencies (between 0.01 and 0.17). Ángel-Marín et al. (2010) detected many rare alleles (50 alleles) in all the analyzed microsatellites (8 loci) for Colombian buffalo herds.

Table 5. Allele sizes and frequencies at loci BMC1013, BM1818, CSSM019, CSSM022, CSSM029, and CSSM036 in all the studied populations

Allele size (bp)	Cairo	Ismailia	Kafr El-Sheikh	Shebeen El-Kom	Sids	Overall
Locus BMC1013						
224	0.200	0.000	0.000	0.000	0.000	0.041
226	0.067	0.350	0.172	0.100	0.200	0.178
232	0.067	0.100	0.172	0.500	0.400	0.248
234	0.333	0.100	0.172	0.000	0.200	0.164
236	0.067	0.100	0.310	0.300	0.000	0.155
238	0.267	0.350	0.172	0.100	0.200	0.218
Locus BM1818						
252	0.400	0.500	0.448	0.000	0.400	0.349
254	0.000	0.000	0.000	0.250	0.000	0.050
256	0.000	0.000	0.017	0.150	0.000	0.033
268	0.067	0.000	0.086	0.100	0.000	0.051
270	0.133	0.227	0.069	0.000	0.200	0.126
272	0.000	0.000	0.052	0.000	0.000	0.010
274	0.367	0.000	0.276	0.200	0.050	0.178
276	0.033	0.227	0.052	0.150	0.100	0.112
278	0.000	0.045	0.000	0.150	0.250	0.089
Locus CSSM019						
144	0.133	0.273	0.207	0.000	0.400	0.203
146	0.167	0.091	0.069	0.250	0.050	0.125
148	0.533	0.182	0.414	0.400	0.000	0.306
150	0.067	0.273	0.138	0.100	0.300	0.176
152	0.067	0.182	0.138	0.200	0.200	0.157
154	0.033	0.000	0.034	0.050	0.050	0.034
Locus CSSM022						
208	0.067	0.273	0.138	0.000	0.556	0.207
210	0.267	0.364	0.276	0.300	0.222	0.286
212	0.333	0.091	0.241	0.400	0.000	0.213
214	0.333	0.273	0.207	0.300	0.111	0.245
216	0.000	0.000	0.138	0.000	0.111	0.050
Locus CSSM029						
195	0.067	0.091	0.034	0.100	0.000	0.058
197	0.067	0.091	0.103	0.000	0.000	0.052
199	0.667	0.818	0.724	0.700	1.000	0.782
201	0.200	0.000	0.138	0.200	0.000	0.108
Locus CSSM036						
170	0.286	0.500	0.172	0.300	0.700	0.392
172	0.143	0.300	0.034	0.100	0.000	0.116
174	0.286	0.200	0.138	0.100	0.000	0.145
176	0.286	0.000	0.655	0.500	0.300	0.348

Table 6. Allele sizes and frequencies at loci CSSM038, CSSM043, CSSM045, CSSM046, CSSM047, and ILSTS005 in all the studied populations

Allele size (bp)	Cairo	Ismailia	Kafr El-Sheikh	Shebeen El-Kom	Sids	Overall
Locus CSSM038						
179	0.300	0.545	0.259	0.500	0.300	0.381
181	0.233	0.000	0.207	0.200	0.100	0.148
185	0.067	0.000	0.276	0.000	0.200	0.109
187	0.400	0.455	0.259	0.300	0.400	0.363
Locus CSSM043						
248	0.200	0.111	0.103	0.150	0.050	0.123
252	0.100	0.056	0.121	0.000	0.100	0.075
254	0.267	0.000	0.207	0.100	0.200	0.155
256	0.433	0.833	0.569	0.750	0.650	0.647
Locus CSSM045						
106	0.067	0.091	0.000	0.000	0.100	0.052
110	0.233	0.227	0.207	0.400	0.350	0.284
112	0.000	0.455	0.138	0.300	0.200	0.219
118	0.633	0.136	0.517	0.300	0.350	0.387
120	0.067	0.091	0.138	0.000	0.000	0.059
Locus CSSM046						
160	0.000	0.091	0.000	0.200	0.000	0.058
164	0.000	0.000	0.690	0.000	0.000	0.138
166	0.800	0.909	0.241	0.700	0.400	0.610
168	0.200	0.000	0.069	0.100	0.600	0.194
Locus CSSM047						
142	0.067	0.091	0.107	0.000	0.000	0.053
144	0.000	0.000	0.018	0.050	0.050	0.024
146	0.133	0.000	0.107	0.100	0.000	0.068
148	0.067	0.000	0.036	0.000	0.050	0.031
150	0.467	0.182	0.179	0.200	0.500	0.305
152	0.200	0.364	0.214	0.200	0.200	0.236
154	0.000	0.091	0.179	0.400	0.000	0.134
156	0.000	0.091	0.089	0.050	0.050	0.056
158	0.067	0.182	0.071	0.000	0.150	0.094
Locus ILSTS005						
188	0.286	0.700	0.310	0.333	0.600	0.446
190	0.143	0.100	0.034	0.222	0.000	0.099
192	0.500	0.100	0.483	0.333	0.300	0.343
194	0.071	0.100	0.172	0.111	0.100	0.111

Figures (from 1 to 4) represent some results of the amplification after running the PCR products of each microsatellite stained by ethidium bromide on 3% agarose gel.

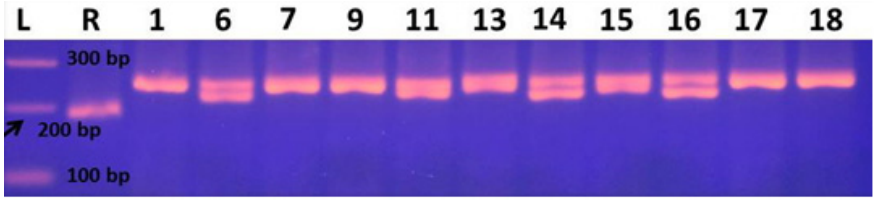

Figure 1. Results of the amplification after running the PCR products of microsatellite BMC1013 stained by ethidium bromide on 3% agarose gel

Note. L: 100 bp DNA ladder, R: reference animal, and lanes 1, 6, 7, 9, 11, 13, 14, 15, 16, 17, 18 = PCR products of BMC1013 lying between range of 224-238 bp.

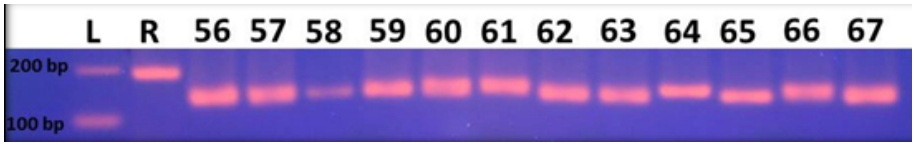

Figure 2. Results of the amplification after running the PCR products of microsatellite CSSM019 stained by ethidium bromide on 3% agarose gel

Note. L: 100 bp DNA ladder, R: reference animal, and lanes from 56 to 67 = PCR products of CSSM019 lying between range of 144-154 bp.

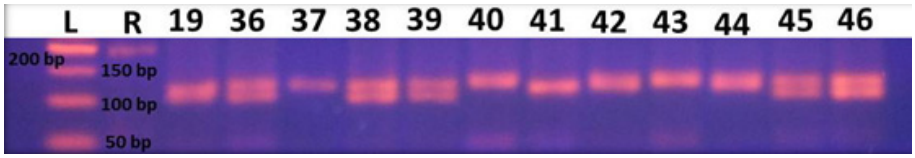

Figure 3. Results of the amplification after running the PCR products of microsatellite CSSM045 stained by ethidium bromide on 3% agarose gel

Note. L: 50 bp DNA ladder, R: reference animal, and lanes 19, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46 = PCR products of CSSM045 lying between range of 106-120 bp.

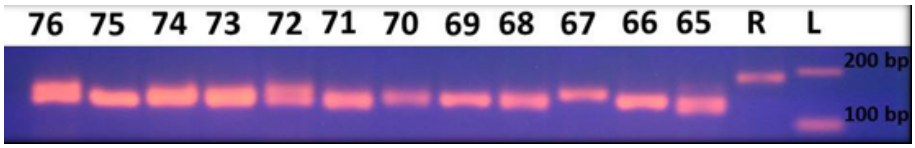

Figure 4. Results of the amplification after running the PCR products of microsatellite CSSM047 stained by ethidium bromide on 3% agarose gel

Note. L: 100 bp DNA ladder, R: reference animal, and lanes from 65 to 76 = PCR products of CSSM047 lying between range of 142-158 bp.

3.3 Polymorphic Information Content (PIC)

The PIC is a parameter indicative of the degree of informative of a marker and another important measure of DNA polymorphism (Abou-Bakr et al., 2012). The high PIC values for markers are indicative of the usefulness of microsatellites for biodiversity evaluation in breed (Jakhesara et al., 2010). Estimates of PIC at every microsatellite locus over all populations are presented in Table 7. PIC estimates were high and ranged between 0.65 (for CSSM029) and 0.92 (for CSSM022) with a mean of 0.82. These values denote high polymorphism of all microsatellites. This according to Botstein et al. (1980) who stated that the locus is highly polymorphic if PIC greater than 0.50, but it is moderately polymorphic when PIC is more than 0.25 and less than 0.50, and it is low polymorphic when PIC is lesser than 0.25. As a result of the high degree of polymorphism at these loci, these microsatellite markers have proved to be very useful in assessing the genetic variability in Egyptian buffalo populations. Results of many studies on Indian and Guilan buffalo breeds showed also high values of PIC (0.52-0.88) for most studied markers (Tantia et al., 2006; Aminafshar et al., 2008; Kataria et al., 2009; Jakhesara et al., 2010; Kathiravan et al., 2012; Singh et al., 2017). Other researchers (Arora et al., 2004; Ángel-Marín et al., 2010; Bhuyan et al., 2010; Vieira et al., 2011; Rad et al., 2013; Ünal et al., 2014) reported varied values of PIC for several markers which ranging between moderate (0.34-0.48) and high (0.51-0.82).

Table 7. Estimates of polymorphic information content (PIC) per locus across all populations

Locus	PIC
BMC1013	0.86
BM1818	0.77
CSSM019	0.88
CSSM022	0.92
CSSM029	0.65
CSSM036	0.83
CSSM038	0.80
CSSM043	0.75
CSSM045	0.79
CSSM046	0.83
CSSM047	0.91
ILSTS005	0.82
Mean	**0.82**

3.4 Heterozygosity Measure

Heterozygosity is one of the most important parameters that can give us information about diversity and even the history of a population (Emil & Marieta, 2012). It includes both observed heterozygosity (H_O) and unbiased expected heterozygosity (H_{Nb}). Values of H_o and H_{Nb} per locus for the studied populations are listed in Table 8.

Table 8. Estimates of observed heterozygosity (H_O) and unbiased expected heterozygosity (H_{Nb}) per locus for the studied populations

Locus	**Estimates of heterozygosity within the five populations**											
	1. Cairo		***2. Ismailia***		***3. Kafr El-Sheikh***		***4. Shebeen El-Kom***		***5. Sids***		***Mean***	
	H_O	H_{Nb}	H_O	H_{Nb}	H_O	H_{Nb}	H_O	H_{Nb}	H_O	H_{Nb}	H_O	H_{Nb}
BMC1013	0.53	0.79	0.70	0.76	0.35	0.80	0.20	0.67	0.40	0.76	0.44	0.76
BM1818	0.80	0.71	1.00	0.68	0.93	0.72	0.80	0.86	0.80	0.76	0.87	0.75
CSSM019	0.07	0.68	0.00	0.81	0.07	0.76	0.10	0.76	0.10	0.742	0.07	0.75
CSSM022	0.00	0.73	0.00	0.75	0.00	0.80	0.00	0.70	0.00	0.65	0.00	0.72
CSSM029	0.00	0.52	0.00	0.33	0.00	0.45	0.00	0.48	0.00	0.00	0.00	0.36
CSSM036	0.00	0.76	0.00	0.65	0.00	0.53	0.00	0.67	0.00	0.44	0.00	0.61
CSSM038	0.67	0.72	0.36	0.52	0.38	0.76	0.60	0.65	0.20	0.74	0.44	0.68
CSSM043	0.40	0.72	0.22	0.31	0.21	0.62	0.30	0.43	0.10	0.55	0.25	0.52
CSSM045	0.47	0.55	0.27	0.74	0.35	0.66	0.40	0.70	0.50	0.74	0.40	0.68
CSSM046	0.00	0.33	0.00	0.17	0.00	0.47	0.00	0.48	0.00	0.51	0.00	0.39
CSSM047	0.13	0.74	0.00	0.81	0.11	0.87	0.10	0.78	0.20	0.72	0.11	0.78
ILSTS005	0.00	0.67	0.00	0.51	0.00	0.65	0.00	0.76	0.00	0.57	0.00	0.63
Mean	0.26	0.66	0.21	0.59	0.20	0.67	0.21	0.66	0.19	0.60		

The average of H_o across all loci ranged between 0.19 for Sids population and 0.26 for Cairo buffalo population, whereas, the average of H_{Nb} ranged between 0.59 for Ismailia population and 0.67 for Kafr El-Sheikh population. It can be observed from these results that values of H_o are lower than values of H_{Nb} for all populations, which denoting depression of heterozygotes in these populations and may be attributable to existence of null alleles and inbreeding. This is partially accord with findings of Mishra et al. (2008, 2009) in some Indian buffalo breeds. Elbeltagy et al. (2008) also concluded similar results in both Italian and Delta buffalo populations while opposite trend in the Southern-Egypt buffalo. On the other side, other researchers (El-Kholy et al., 2007; Hassanane et al., 2007) reported overabundant of heterozygotes in Egyptian buffalo populations due to increasing the values of observed heterozygosity than expected heterozygosity. The estimates of observed heterozygosity and expected heterozygosity in the current study were lower than those obtained by some investigators (Elbeltagy et al., 2008; Jakhesara et al., 2010; Abou-Bakr et al., 2012; Attia et al., 2014b; Singh et al., 2017) in Egyptian, Indian, and

Italian buffalo populations, whereas were higher than those of Moioli et al. (2001) in Egyptian, Italian, and Greek buffaloes.

Across all populations, the average of H_o varied from 0.00 for both of CSSM022, CSSM029, CSSM036, CSSM046, and ILSTS005 loci to 0.87 for BM1818 locus. The average of H_{Nb} ranged between 0.36 for CSSM029 locus and 0.78 for CSSM047. Results in Table 8 showed heterozygosity deficiency at all loci except only one locus (BM1818) which had excess in heterozygotes ($H_O > H_{Nb}$). Likewise, Arora et al. (2004) found that the observed heterozygosity was higher than the expected heterozygosity at this locus (0.45 vs. 0.39).

4. Conclusion

The current study contributes initially to completing the genetic description of the Egyptian buffalo. Genetic characterization of this breed is still incomplete which is very important for conservation decisions and designing future breeding policies. The results indicated that all the microsatellites utilized had high degree of polymorphism and have proved to be very useful in assessing the genetic variability in Egyptian buffalo populations. The findings also revealed depression of heterozygotes in all studied populations and may be attributable to existence of null alleles and inbreeding. Inbreeding can be explained by using very small number of the same bulls from neighboring areas for all the five populations.

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