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GENETIC POTENTIAL OF VARIOUS CHROMOSOMES IN EGYPTIAN BUFFALO

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The present study was aimed to verify the genetic characteristics of twenty microsatellite markers -distributed on six chromosomes- that related to milk performance traits in Egyptian buffalo. Nineteen markers from Egyptian buffalo were successfully amplified, out of which fourteen were polymorphic. Sixty-seven alleles had been detected for all nineteen loci. Eight loci reflected high polymorphic level. The average number of alleles per polymorphic locus was 4.43, and the allele frequencies ranged from 1.22 to 93.75. The expected heterozygosity (H_{exp}) values prolonged from 0.12 to 0.86. Hardy-Weinberg (HWE) Chi-square (X^2) statistical test of the genotype frequencies was calculated by using GENEPOP software. The global results of Wright's F-statistics (F_{IS}) were near zero with a mean of 0.13. The results pointed to a number of polymorphic loci that might be relied upon to study diversity within Egyptian buffalo. Moreover, the diversity level within the native Egyptian buffalo indicated possibilities of genetic improvement.
Keywords: Genetic characteristics, microsatellites, Egyptian buffalo**.**

INTRODUCTION

Currently, livestock systems have both positive and negative impacts on the natural resource base, common health, social justice and economic development (World Bank, 2009). Animal agriculture has been a significant element in the integrated farming systems in the crop-livestock farming systems in developing countries. It serves in an essential diversified role in providing animal protein source, manure, fibre for clothes and draft power as well as financial instruments and enriching livelihood (Swanepoel *et al.,* 2010).

Buffaloes have a prime importance within the farmer societies and therefore in the economies of many developing countries worldwide (Hussain *et al.,* 2017). A majority of buffalo population all-over the world raised is under low input and low output system and they can adapt to harsh environmental conditions and live on poor quality forage (Zaidi and Anwar, 2018). The number of buffaloes all-over the world has expanded quickly over the past few decades. The world buffalo population is estimated to be approximately 195 million, spreading in some 42 countries; of which 97% of them are found in Asia, while approximately 3% are found in the rest of the world (FAO, 2011). The countries with the majority population of dairy buffaloes are India, Pakistan, China, Egypt and Nepal. In Pakistan, Egypt and Nepal, there are more dairy buffaloes than dairy cows (Borghese, 2005). Moioli (2005) classified buffalo production system in Egypt based on the number of animals per farmer; the majority of buffalo farmers in Egypt maintain these animals under small holder production system (holding one to five animals).

In recent decades, commercial buffalo farms have significantly grown in Egypt. Buffalo numbers have increased to be about 3.98 million heads in 2011 (Satoh and Aboulroos, 2017) and are expected to rise and reach 7.4 million head in 2050(Fahim *et al.*, 2014). In Egypt, buffalo is ranked as the second main source of milk and meat. They contributed to about two million and half tons of milk in 2011 (Satoh and Aboulroos, 2017) and about 33.2 percentage of local meat production in 2005 (El-Nahrawy, 2011). Consumers in Egypt prefer products that are derived from buffalo milk rather than those derived from cow's milk, the price of buffalo milk is twice that of cows' milk. This may be due to their excess fat content, colour, and flavour. In recent times, breeding programmers – especially in Bulgaria, China, Egypt, India and Pakistan – have attempted to improve the milk yield of buffalo. Genetic characterization and diversity studies on Egyptian buffalo populations are essential to facilitate the breeding program's makeup. Techniques based on molecular genetics such as microsatellite markers offer opportunities to study diversity. Microsatellites are helpful markers because of the low assay cost, distribution throughout genetic materials, highly polymorphic, co-dominant inheritance, and easy to analyse (Cañon *et al.*, 2006).

MATERIALS AND METHODS

***Animals and samples collection*:** Totally, eighty-two random blood samples were collected from nonrelated

lactating Egyptian buffalo females from three different commercial farms. Animals were well restrained in mechanical restraints. When buffalos became calm and comfortable, samples were collected under aseptic condition using the smallest possible diameter (highest possible gauge) vacutainer's needle and tubes. All the animals handling process was approved via animal ethical committee of veterinary medicine college, Suez Canal University, and was in accordance with the "guide for the care and use of laboratory animals". Blood samples (10ml) were collected via the jugular vein in vacuum tubes containing anticoagulation (K3EDTA). DNA was isolated from the whole blood using the ABIOpure™ Genomic DNA Kit (Alliance Bio *Co.).*

***Microsatellite analysis*:** Twenty microsatellite markers distributed across 6 chromosomes (1, 3, 6, 12, 14, and 20) according to cattle genome linkage map were used in the present study (Table 1). Fitting markers annealing

Table 1. Markers, location, primer sequences, identified annealing temperatures and detected fragment size.

Locus	Chromosome		Primer sequences (5` ⟶ 3`)	Annealing temp.℃	Fragment size (bp)
BM6438	1	F:	TTGAGCACAGACACAGACTGG	56	118 -122
		R:	ACTGAATGCCTCCTTTGTGC		
HUJII77	3	F:	TCCATCAAGTATTTGAGTGCAA	59	102-110
		R:	ATAGCCCTACCCACTGTTTCTG		
BL41		F:	CCTCTGCCATCTTTATTCCG	59	116-130
		R:	AAGATCAACTTATTCCTCACAGTGG		
ILSTS096		F:	GTGACCTGGAGAAGTTTTCC	---	---
		R:	ACCACGCTCTGACTTGTAGC		
FBN13	6	F:	ACTTTCATTAGATTGCTGCAAATAG	56	85
		R:	AAATATGGAAACGACCTGTGG		
ILSTS97		F:	AAGAATTCCCGCTCAAGAGC	58	108-124
		R:	GTCATTTCACCTCTACCTGG		
BM143		F:	ACCTGGGAAGCCTCCATATC	58	72-84
		R:	CTGCAGGCAGATTCTTTATCG		
BM415		F:	GCTACAGCCCTTCTGGTTTG	54	84-92
		R:	GAGCTAATCACCAACAGCAAG		
CSN3		F:	CCAACATATAAACCCAGGAATCC	56	118-128
		R:	GACATACAATACACAAGCATAC		
BM6404	12	F:	TCCCTAATGTTGAATGGACTTC	58	80-86
		R:	CGAAAAGAGTCAGACACCAGC		
CSSM066	14	F:	ACACAAATCCTTTCTGCCAGCTGA	58	92-104
		R:	AATTTAATGCACTGAGGAGCTTGG		
BM1508		F:	CAGGTGTACAGCAAACTGAATC	56	72-79
		R:	CGTCAAAACATTCGTTCAGG		
ILSTS039		F:	CCTAATGACTACCAACAGGG	48	102
		R:	TCCATGGAATCACAAAGAGC		
AGLA29	20	F:	AGGAAGCCGAGTGAGATATGTAAGC	58	88-98
		R:	TTACAGCCTGTGTGAATGTCCTCTA		
ILSTS72		F:	ATGAATGTGAAAGCCAAGGG	46	86-90
		R:	CTTCCGTAAATAATTGTGGG		
BM5004		F:	TCTGGAGTGAATGTTTCTGAGG	57	75
		R:	TTGTGATGAGCACCTGAAGG		
BM3517		F:	GTGTGTTGGCATCTGGACTG	57	76-82
		R:	TGTCAAATTCTATGCAGGATGG		
TGLA304		F:	GATCTGTCAACCTTTCAATTGATTC	56	65
		R:	CTAGGTGTAGAACTGAGGAGGGT		
TGLA443		F:	CAATGGATACAGGTTGAGATAATCC	61.5	102-132
		R:	TTGCAAAGAGTCAGATGTGACTGAA		
TGLA153		F:	GGAGTGGGAGAAAGGCTCAAA	48	72
		R:	TGCTTTACAGTGTTGTGTTAGTTT		

temperatures were detected by using grading PCR thermal cycle (Table 1). The PCR was carried out for each locus in a total volume 10µl consisted of 2µl of Genomic DNA (20ng), 5µl 2X PCR AmpliTag gold PCR Master mix (applied biosystems), 0.4 µl primer mix (50 pmoles), and 2.6 µl nuclease-free H2O. The PCR protocol was displayed in Table 2. The PCR protocol was the same for all primers except for annealing temperature that was examined as pervious described (Table 1).

Table 2.PCR protocol.

Steps	Temperature	Time
Initial denaturation	95ºC	10min.
Denaturation	95ºC	30sec.
Annealing	As determined	30sec.
Extension	72ºC	30sec.
steps 2 to 4 were to be repeated for 35 cycles		
Final extension	72ºC	10min.
Maintenance	4ºC	∞

An equal amount (3µl) of each reaction product was added to 1µl of 6X loading dye and run on vertical 8% polyacrylamide gel. Molecular weight of each band in a gel (bp) was identified by comparison with the 50 bp DNA ladder.

***Statistical analysis*:** Bio-Rad Quantity One software package (version 4.6.3) was used to analyse electrophoresis gels. Genotypes were appointed for each animal based on allele size data. FSTAT software (version 2.9.3.2) was used to calculate the number of alleles (N), allele frequency, (H_{ob}) and (H_{exp}) per each locus (Nei, 1987; Goudet, 2002). Wright's F-statistics F_{IS} were computed by using GENEPOP software (version 3.4) (Nei and Kumar, 2000). Also, Hardy-Weinberg equilibrium (HWE) was tested over loci using pervious software. According to Botstein (Botstein *et al*., 1980) the PIC values were calculated.

RESULTS

A set of twenty cattle microsatellite markers distributed across 6 chromosomes (1, 3, 6, 12, 14, and 20) was tested for amplification on Egyptian buffalo genomic DNA (Table 1). Nineteen Markers (95%) were successfully amplified. Among those amplified microsatellites fourteen loci (73.7%) were polymorphic (Table 3 & Fig. 1). The remaining five markers (26.3%) were monomorphic (FBN13, ILSTS039, BM5004, TGLA304 and TGLA153) with a single allele. For

Table 3. Allele size (bp) and genotype frequencies (Freq) for each polymorphic locus.

BM6438		HUJII77		BL41		ILSTS97		BM143	
bp	**Freq.**	**bp**	**Freq.**	**bp**	**Freq.**	**bp**	**Freq.**	**bp**	**Freq.**
118	68.29	102	87.80	116	86.25	108	10.98	72	1.22
120	26.83	106	7.32	130	13.75	110	32.93	74	1.22
122	4.88	108	1.22			112	3.66	76	37.80
		110	3.66			114	36.59	78	36.59
						116	6.10	79	1.22
						118	1.22	80	4.88
						122	3.66	82	12.20
						124	4.88	84	4.88
BM415		**CN3**		**BM6404**		**CSSM066**		**BM1508**	
bp	**Freq.**	**bp**	**Freq.**	**bp**	**Freq.**	**bp**	**Freq.**	**bp**	**Freq.**
84	64.63	118	93.75	80	23.75	92	15.85	72	26.83
86	12.20	128	6.25	82	22.50	94	58.54	77	6.10
88	2.44			84	2.50	96	6.10	79	67.07
90	13.41			86	51.25	102	1.22		
92	7.32					104	18.29		
AGLA29		**ILSTS72**		**BM3517**		**TG443**			
bp	**Freq.**	**bp**	**Freq.**	**bp**	**Freq.**	**bp**	**Freq.**		
88	10.00	86	86.25	76	50.00	102	14.63		
90	48.75	90	13.75	78	12.50	108	7.32		
92	36.25			80	21.25	120	6.10		
98	5.00			82	16.25	122	15.85		
						126	7.32		
						128	8.54		
						130	25.61		
						132	14.63		

polymorphic locus, the average number of identified alleles was 4.4, ranging from 2 to eight (Fig. 1). Effective number of alleles (Ne) is a measure of allelic evenness. The effective allele values ranged from 1.13 for CSN3 to 6.44 for TGLA443 locus with a mean of 2.12 (Fig. 1). Based on the Shannon's Index (I), TGLA443 loci had the highest value (I = 1.91), and the least value (I = 0.23) was identified for CSN3 loci (Fig. 1). The allele frequencies ranged from 1.22 to 93.75 with mean 22.6 (Fig. 2 & Table 3).

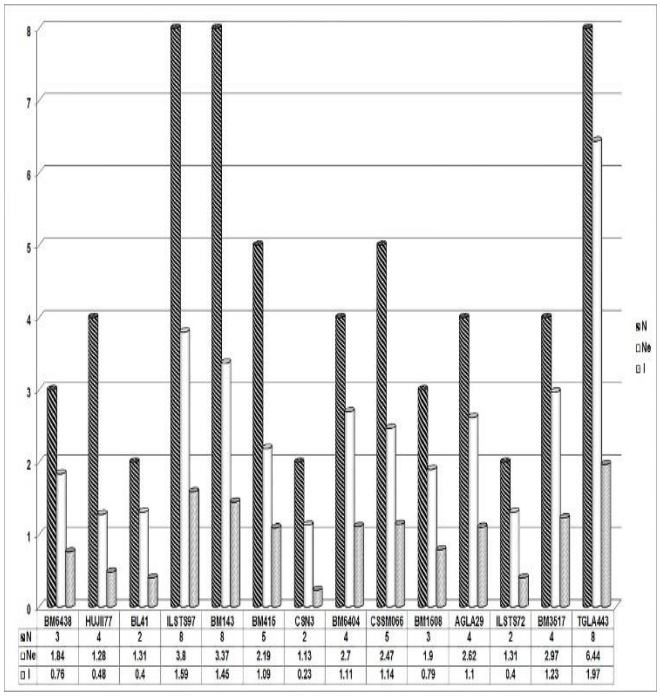

Figure 1. Observed (N), effective (Ne) number of alleles and Shannon index (I) for each locus.

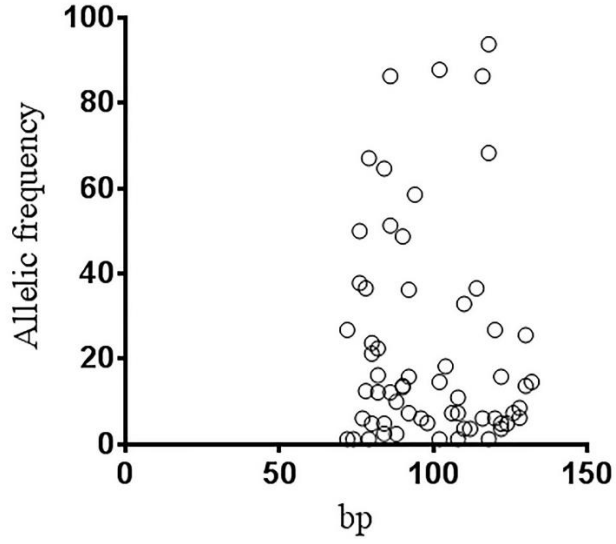

Figure 2. Allele frequency and size (base pairs) distributions

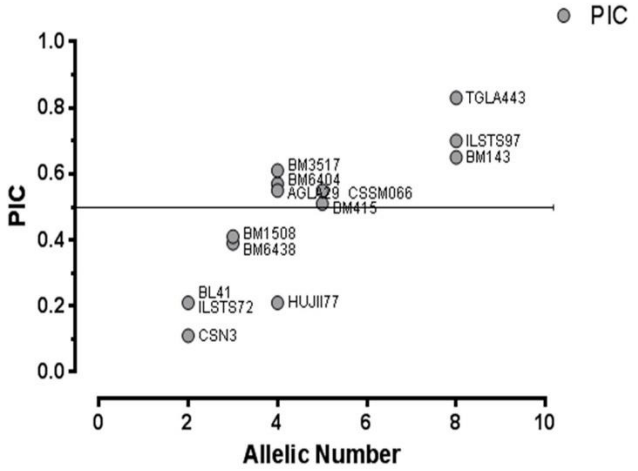

Figure 3. Polymorphic Information Content (PIC) and allelic number for each locus.

The FBN13, ILSTS039, BM5004, TGLA304 and TGLA153 loci each had one allele with a much higher frequency than the other alleles. The loci BL41, CSN3 and ILSTS72 each had two alleles with high frequencies 116 bp, 118 bp and 86 bp, respectively (Table 3).

The mean of Polymorphic Information Content (PIC) varied from 0.11 (CSN3) to 0.83 (TGLA443). Eight loci (ILSTS97, BM143, BM415, BM6404, CSSM066, AGLA29, BM3517 and TGLA443) reflected a high level of polymorphisms PIC≥0.5 (Figure 3). The H_{exp} and H_{ob} values of each locus as obtained from FSTAT software version 2.9.3.2 (Nei, 1987) are displayed in Figure 4. The results of H_{exp} for the polymorphic loci ranged from 0.12 to 0.86 against the values of H_{ob} that ranged from 0.1 to 0.88 (Figure 4).

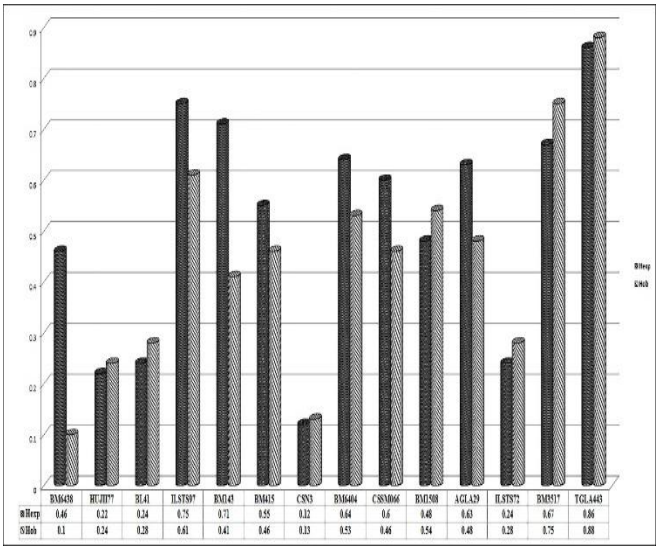

Figure 4. Expected (H_{exp}) and observation (H_{ob}) heterozygosity for each locus.

Table 4 appearances Wright's coefficient F_{IS} and P values of HWE over all polymorphic loci. The highest F_{IS} within population was observed for the locus BM6438 (0.79), whereas the lowest values were found in both loci BL41 and ILSTS72 (-0.147) (Table 4). The general values of F_{IS} were near from or to? zero with mean 0.13. Results in Table 4 displays chi-square (X^2) test for HWE ($p<0.05$) of the genotype frequencies at each locus within the samples. The results showed that Eight loci (BM6438, ILSTS97, BM143, BM415, BM6404, CSSM066, AGLA29 and BM3517) deviated significantly from HWE ($P<0.05$).

Table 4. Wright's F-statistics (F_{IS}) value and Chi-square test and P value of Hardy Weinberg equilibrium (HWE) at 14 different microsatellite loci.

Loci	F_{IS}	HWE	
		Chi-square	P value
BM6438	0.792	42.77	0.00
HUJII77	-0.085	0.70	0.99
BL41	-0.147	0.91	0.34
ILSTS97	0.184	69.56	0.00
BM143	0.421	66.50	0.00
BM415	0.159	54.77	0.00
CSN3	-0.054	0.14	0.71
BM6404	0.179	31.44	0.00
CSSM066	0.233	55.22	0.00
BM1508	-0.119	4.18	0.24
AGLA29	0.244	28.88	0.00
ILSTS72	-0.147	0.91	0.34
BM3517	-0.119	65.84	0.00
TGLA443	-0.027	28.26	0.45
Mean	0.134		

DISCUSSION

Studies of comparative genome have reported that: sequences of microsatellite primers are usually present across related species and can be utilized for the development of markers in linked species (Navani *et al.*, 2002). Recently, various research on domestic buffalo that used cattle microsatellite markers have been issued in Southeast Asia and China (Barker *et al.*, 1997; Flamand *et al.*, 2003; Babar *et al.*, 2009; Yang *et al.*, 2011), Africa(Van Hooft *et al.*, 2000; El-Kholy *et al.*, 2007; Elbeltagy *et al.*, 2008), and Iraq (Jaayid and Dragh, 2014). Moioli in 2001 compared buffalos from three countries, Egypt, Greece and Italy (Moioli *et al.*, 2001). In the present study, of the nineteen markers that successfully amplified, fourteen (7.3.3%) were polymorphic (Fig. 1). This proportion was slightly elevated when compared with earlier studies on water buffalo (Navani *et al.*, 2002; Khade *et al.*, 2019). Evaluated a total of 30 microsatellite markers in a Cuban water buffalo, found 93% of tested regions were amplified, 96.4% of which successful were polymorphic (Uffo *et al.*, 2017). Estimates of allelic frequency used analyse and quantify the genetic variation of population. Allele frequencies results cleared that not all markers were equally informative. The mean of allele frequencies was 22.6. The estimates of PIC display how the markers can indicate the population polymorphism depending on the number and frequency of alleles (Chesnokov and Artemyeva, 2015). Eight of the studied loci had been highly polymorphic PIC≥0.5 (Fig. 3). The high estimates of PIC further proved the suitability of the used set of markers to applications such as parentage control, programs of linkage-mapping, and genetic improvement studies. H_{exp} and H_{ob} indexes are suitable ways to measure the level of genetic variability within populations, as they depend on the distribution of allele frequencies. In the present study, the values of H_{exp} and H_{ob} for each locus was estimated using FSTAT software version 2.9.3.2 (Nei, 1987). The average H_{exp} of the studied loci was 0.38, but in general the average H_{exp} of these markers is significantly higher on cattle populations (Farnir *et al.*, 2000; Vallejo *et al.*, 2003). The most authoritative markers for breeding judgment are those with high average heterozygosity, and with greater mean numbers of observed alleles. Sewall Wright formulated the mathematical framework to characterize the genetic variation distribution in subdivided populations that used a set of inbreeding coefficients (Wright, 1931; Wright, 1951). Wright's coefficient F_{IS} is a deviation measure from HWE proportions within local subpopulations. The F_{IS} measures varied from -0.15 to 0.79 with mean 1.34. A negative measure indicates a deficit of homozygotes. Moreover, the test for HWE displayed significant deviations of 8 loci ($P<0.05$). The common reason of heterozygote deficiency is non-random mating or population substructures (Allendorf and Luikart, 2007).

***Conclusion*:** This study has gone some way towards enhancing our understanding of genetic diversity within Egyptian buffalo population. This paper has highlighted number of polymorphic loci that might be relied upon to study diversity within Egyptian buffalo. Our research confirmed that the level of diversity within native buffalo that also indicates the possibilities of genetic improvement. Our investigations into this area are still ongoing to confirm the QTL that associated to milk performance traits in Egyptian buffalo. Additionally, analysing more of microsatellites might provide beneficial information that can assist in developing genetic improvement strategy for indigenous animals.

Conflict of interest: We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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