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Molecular Analysis of Microsatellites Associated with Milk Yield and Composition in Egyptian Buffalo

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ABSTRACT

Milk production traits are economically important for dairy industry. The aim of the present study was to detect QTL responsible for milk yield and composition in the Egyptian buffalo. A total number of 106 purebred Egyptian buffalo cows were genotyped for a set of ten microsatellite markers. Daily milk records analyzed were 33,299; including only records corresponding to the first five lactations with days in milk prolonged from 5 to 290. All markers tested were successfully amplified, showing a considerable degree of polymorphism. Estimates of observed heterozygosity varied from 0.5 for BM6438 to 0.950 for CSSM061. Expected heterozygosity ranged between 0.505 and 0.855 for the same two loci, respectively. Microsatellite BM143 showed significant ($P<0.05$) effect on average daily milk yield deviation (ADMYD) and protein percent. Also, the last trait was influenced significantly ($P<0.05$) by the locus CSSM047. Fat yield (kg) was affected by microsatellites BM1443 ($P<0.05$) and CSSM061 ($P<0.01$). Marker ETH3 showed marginally significant effects on fat percent and protein yield (kg). The findings obtained may be useful in increasing accuracy of selection and rate of genetic gain in Egyptian buffalo.

Keywords: polymorphism, microsatellites, association, milk, protein, fat, Egyptian buffalo

INTRODUCTION

Availability of dense genetic maps composed of highly polymorphic markers, especially microsatellites and single nucleotide polymorphism (SNP), enabled mapping of quantitative trait loci (QTL) affecting genetic variation of economically important traits (Navani *et al*., 2002; Ihara *et al*., 2004; Hu *et al*., 2010; Venturini *et al*., 2014; Iamartino *et al*., 2017). Subsequently, this would provide an opportunity for the implementation of marker-assisted selection (MAS) and recently genomic selection (GS) that could accelerate the rate of genetic improvement (Meuwissen *et al*., 2016).

Phenotype such as milk production presents a unique chance for the application of GS for many reasons. First, this trait is solely expressed in females, whereas non-expressing males account for the greatest portion of genetic gain achieved as a result of the commonly-used artificial insemination especially in dairy cattle rather than buffalo. Second, GS provides a perfect alternative to progeny testing of young dairy bulls, which requires a lot of time, work and expenditure. Therefore, progeny testing makes dairy cattle and buffalo breeding programs too expensive and also limits the achievable annual genetic progress due to long generation intervals.

Three cornerstones are required for a feasible implementation of GS in livestock, which involves a dense marker map, a cost-effective technology to genotype animals for high density SNP chips and an adequate data analysis method. Fortunately, all these conditions were met primarily in dairy cattle breeding (Meuwissen *et al*., 2016). At the beginning of the second decade of the 21st century, GS became reality and genomically-selected young bulls were used in breeding programs for dairy cattle (Meredith *et al*., 2012) and buffalo (Venturini *et al*., 2014; Iamartino *et al*., 2017). Application of GS in dairy cattle accelerated genetic gain in production and non-production traits. However, further work is needed to widespread the use of GS in major farm animal species.

Compared to dairy cattle, no organized genome mapping efforts have been devoted to water buffalo for QTL detection and also no systematic studies have been undertaken to develop specific polymorphic DNA markers and genetic maps in this economically important livestock species. For fortune, comparative genomics suggest that primers for most cattle-derived microsatellites do amplify buffalo sequences in homologous regions of the respective genomes (Michelizzi *et al*., 2011). If sufficient numbers of these markers are polymorphic in buffalo, they will facilitate the development of a linkage map when pedigreed families are properly identified and DNA is made available to the growing buffalo mapping community. Knowledge on the buffalo genome has benefited widely from structural and functional genetic studies with bovine genome (Navani *et al*., 2002; Iamartino *et al*., 2017; Colli *et al*., 2018). These data provide new insights to unravel the genetic determinism involved in the variation of some traits of breeding interest.

To date; however, information on QTL affecting phenotypic variation associated with traits of economic importance in buffalo is still scarce. Therefore, the overall objective of this study was to identify genetic variation at DNA level associated with milk yield and composition in the Egyptian buffalo based on the inter-species transferability of microsatellites from cattle to buffalo.

MATERIALS AND METHODS

Animals and phenotypes:

A total number of 153 purebred lactating Egyptian buffalo originated from two herds located in the Governorate of Giza, Egypt were used. Both herds were owned by Faculty of Agriculture, Cairo University; however, they were managed independently. Animals were maintained in a semi-intensive production system, fed with Egyptian clover (*Trifolium alexandrinum*), hay and commercial concentrate, and were supplemented with minerals and vitamins. Daily milk yield was collected during the period from 2010 to 2015, resulting in a total number of 59,969 records. To make sure that the dataset was homogenous, some editing steps were carried out. For instance, phenotypic records from individuals without certainly known birth dates and calving dates were removed. Likewise, the records shorter than 5 days and longer than 290 days in milk (DIM) were excluded. Only data belonging to the first five lactations were involved. The remaining data set included 106 lactating buffalo with 33,299 daily milk records. Traits studied included average daily milk yield deviation, fat percent, protein percent, fat

yield and protein yield. The numbers of records per lactation and their corresponding mean, standard deviation as well as minimum and maximum estimates are illustrated in Table 1.

Table 1. Basic statistics for milk records analyzed*

Lactation	No.	M	SD	Min	Max
1	6,090	6.90	2.55	0.5	18
2	7,012	7.71	3.18	0.5	16
3	7,382	7.43	3.19	0.5	17
4	7,502	7.94	3.43	0.5	18.5
5	5,309	6.93	3.17	0.5	18.5
1 - 5	33,295	7.43	3.16	0.5	18.5

*No: number of records, M: mean (kg), SD: standard deviation, Min: minimum, Max: maximum

Sampling, DNA isolation and microsatellite genotyping:

The genetic material used in the present study was composed of 40 randomly selected buffalo cows belonging to two herds (20 animals per herd). The chemical analysis of milk constituents was performed by Bentley 150 Infrared Milk Analyzer (Bentley Instruments), based on samples collected during morning milking from each buffalo.

Blood samples were taken from jugular vein in vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA), as anticoagulant. Genomic DNA was extracted from blood samples using phenol chloroform-extraction procedure (Sambrook *et al*., 1989). Quality and molecular weight of DNA were measured electrophoretically using 0.8% agarose gel. The extracted DNA was kept frozen at -20°C till further use.

Based on the previously published data related to mapping results and association of marker loci and milk production traits in bovine species, ten microsatellites located on different bovine autosomes were chosen for analysis (Table 2).

Table 2. Characteristics of microsatellites analyzed in the current study*

Locus	Chr.	All.	Primer sequence 5`- 3`	°C	Ref.
BMS711	1 (1)	9	F: AGCTTCTTATGGCAACACCTG R: TGAAATCGCAGAGTTGTACATG	58	Ihara *et al.* (2004)
BM143	6 (7)	12	F: ACCTGGGAAGCCTCCATATC R: CTGCAGGCAGATTCTTTATCG	58	Ihara *et al.* (2004)
BM6438	1 (1)	6	F: TTGAGCACAGACACAGACTGG R: ACTGAATGCCTCCTTTGTGC	56	Ihara *et al.* (2004)
BM1443	23 (2)	11	F: AATAAAGAGACATGGTCACCGG R: TCGAGGTGTGGGAGGAAG	56	Ihara *et al.* (2004)
ETH2	5 (4)	6	F: CCCACAGGTGCTGGCATGGCC R: CCATGGGATTTGCCCTGCTAGCT	56	Ihara *et al.* (2004)
CSSM061	Unknown	8	F: AGGCCATATAGGAGGCAAGCTTAC R:TTCAGAAGAGGGCAGAGAATACAC	60	FAO (2011)
ETH225	9 (9)	7	F: GATCACCTTGCCACTATTTCCT R: ACATGACAGCCAGCTGCTACT	61	FAO (2011)
ETH3	19 (3)	5	F: GAACCTGCCTCTCCTGCATTGG R: ACTCTGCCTGTGGCCAAGTAGG	65	FAO (2011)
BMC1013	19 (3)	7	F: AAAAATGATGCCAACCAAATT R: TAGGTAGTGTTCCTTATTTCTCTGG	54	FAO (2011)
CSSM047	8 (3)	5	F: TCTCTGTCTCTATCACTATATGGC R: CTGGGCACCTGAAACTATCATCAT	55	FAO (2011)

*Chr: cattle chromosome assignments (buffalo chromosome assignments in parentheses), All: number of alleles in cattle, F: forward, R: reverse, °C: annealing temperature, Ref: reference

The polymerase chain reaction (PCR) assays were conducted based on 50 ng of genomic DNA in 10 µl reaction using TaqMan Universal PCR Master Mix (Applied Biosystems). The PCR protocol was as follows: primary denaturation at 95°C for 5 min, then 35 cycles consisted of (a) denaturation at 95°C for 30 sec, (b) annealing temperature corresponding to each individual marker for 1 min, and (c) an extension at 72°C for 1 min. A final extension cycle at 72°C for 5 min was applied; and then PCR products were stored at 4°C until subsequent use.

PCR products were visualized by running horizontally 5 µl of the PCR product on 1.5% agarose gel electrophoresis. The successful PCR products were run further on polyacrylamide vertical electrophoresis gel under denaturing conditions to determine the size of marker alleles in the presence of a specific size marker running on a special well.

Statistical analysis:

Population parameters including allele frequencies, deviation from Hardy-Weinberg Equilibrium, observed and effective number of alleles, observed and expected heterozygosity and fixation index at each locus were calculated using GenAlEx 6.5 software package (Peakall and Smouse, 2012).

Because of the limited number of individuals genotyped, primary adjustments for the environmental factors have been employed on the entire dataset containing all available contemporary animals by means of the mixed model included in SAS, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). The first order autoregressive covariance structure for repeated statement was applied, as follows:

$$Y_{ijklmno} = \mu + MF_i + LAC_j + AC_k + HYS_l + b_{m1}\,(DIM) + b_{m2}\,(\exp^{-0.05*DIM}) + pe_n + \varepsilon_{ijklmno} \quad (1)$$

Where:

$Y_{ijklmno}$ is the daily milk records; µ is the overall mean of observations; MF_i is the fixed effect of milking frequency in each day; LAC_j is the fixed effect of lactation number; AC_k is the fixed effect of age at calving; HYS_l is fixed combined effect of herd (H), year of calving (Y) and season of calving (S); b_{m1} and b_{m2} are two regression coefficients associated with the fixed lactation function, where DIM is days in milk (Wilmink, 1987); pe_n is the random permanent environmental effect, where Legendre Polynomials of order four were used (Legendre Polynomials were standardized in the period from 5 to 290 days after calving); and $\varepsilon_{ijklmno}$ is the residual term denoting the difference between observed and predicted daily milk yield for an individual buffalo at each DIM.

Then, the overall mean of adjusted daily milk yield deviations for the genotyped individuals was employed as a response variable for association analysis in a mixed model, as follows:

$$Y_{ijk} = \mu + G_i + s_j + \varepsilon_{ijk} \quad (2)$$

Where:

Y_{ijk} is the average daily milk yield deviation (ADMYD); μ is the overall mean of observations; G_i is the fixed effect of an animal's genotype for each marker; s_j is the random effect of a sire and ε_{ijk} is the residual error. For milk composition traits (fat and protein yields as well as fat and protein percentages), only the model number 2 was used. Post-hoc effects among genotype classes for each microsatellite in each trait studied were verified for significance using a Tukey-Kramer test as implemented in SAS, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Summary statistics for the ten microsatellite loci analyzed in this study are demonstrated in Table 3. All microsatellites tested were successfully amplified, showing a considerable degree of polymorphism. Taking into consideration that the set of ten loci analyzed were originally derived from cattle genome, these results point out the genetic similarity and conservation of genetic markers between cattle and buffalo. Similar results were reported by Ihara *et al.* (2004), Goldammer *et al.* (2007), Sikka and Sethi (2008), Michelizzi *et al.* (2011) and Venturini *et al.* (2014). Consequently, bovine markers can be efficiently used to identify QTL in buffalo while reducing cost and laboratory effort required for developing buffalo-specific microsatellites.

The whole observed number of alleles (Na) in herd 1 (H1) and herd 2 (H2) were 47 and 45, while their corresponding means were 4.7 and 4.5 alleles, respectively. However, the effective number of alleles (Ne) in the entire population studied ranged between 2.020 for BM6438 in H1 and 6.897 for CSSM061 in H2. As the observed number of alleles in a sample is related positively to sample size, the set of alleles detected in the current study was greater than that observed in the earlier work of Rushdi *et al.* (2017; 46 *vs* 33 alleles, respectively), due to differences in the experimental design and sampling between the two studies.

Table 3. Statistical description of the microsatellites genotyped

Marker	Na		Ne		Ho		He		F	
	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2
BMS711	3	3	2.417	2.572	0.70	0.75	0.586	0.611	-0.194	-0.227
BM143	3	3	2.417	2.532	0.70	0.70	0.586	0.605	-0.194	-0.157
BM6438	3	3	2.020	2.100	0.50	0.55	0.505	0.524	0.010	-0.050
BM1443	3	3	2.740	2.477	0.70	0.65	0.635	0.596	-0.102	-0.090
ETH2	3	3	2.787	2.740	0.65	0.70	0.641	0.635	-0.014	-0.102
CSSM061	9	8	5.970	6.897	0.85	0.95	0.833	0.855	-0.021	-0.111
ETH225	5	6	4.624	4.706	0.80	0.85	0.784	0.788	-0.021	-0.079
ETH3	6	4	5.442	3.721	0.90	0.70	0.816	0.731	-0.103	0.043
BMC1013	6	6	4.706	5.096	0.85	0.80	0.788	0.804	-0.079	0.005
CSSM047	6	6	4.420	5.594	0.85	0.85	0.774	0.821	-0.099	-0.035
Mean	4.7	4.5	3.754	3.844	0.75	0.75	0.695	0.697	-0.082	-0.080

Na: observed number of alleles, Ne: effective number of alleles, Ho: observed heterozygosity, He: expected heterozygosity, F: fixation index, H1: herd 1, H2: herd 2

The overall mean of observed heterozygosity (Ho) was the same in both herds, while H2 had higher expected heterozygosity (He) than H1. Estimates of Ho varied from 0.5 for BM6438 in H1 to 0.95 for CSSM061 in H2. Correspondingly, the same loci showed the lowest and the highest estimates for He, respectively. In fact, contrary to the recently reported SNP-based estimate of Ho = 0.383 in river and swamp buffalo (Colli *et al.*, 2018), the average Ho estimate found in the current study is considerably higher, which is in line with that obtained by El-Kholy *et al.* (2007) in the Egyptian buffalo. The contradiction between microsatellite- and SNP-based heterozygosity estimates may be due to marker selection, since in the present study only ten microsatellite markers were genotyped.

Fixation index (F) was generally negative for all analyzed loci except BM6438, ETH3 and BMC1013. Estimates of F ranged from -0.227 for BMS711 in H2 to 0.043 for ETH3 in H2. The overall mean of F were -0.082 and -0.080 in H1 and H2, respectively. With exception of F values, all genetic diversity estimates calculated in this study were higher than those obtained in the present author's previous work (Rushdi *et al.*, 2017). These results may be explained by the increase in number of the animals genotyped and variation due to sampling individuals from two herds instead of one. In general, the estimates of population genetic diversity displayed in Table 3 are located within the range of microsatellites tested in the Egyptian buffalo reported by El-Kholy *et al.* (2007), Mekkawy *et al.* (2012) and Rushdi *et al.* (2017).

Deviation from Hardy-Weinberg Equilibrium (HWE) using chi square test was performed (Table 4). Markers per herd that exhibited significant deviation from HWE in the two studied herds were BMS711, BM143, BM6438, BM1443 and ETH2. On the other hand, only three loci (CSSM061, ETH225 and CSSM047) did not reveal significant deviation from HWE in both herds. Microsatellites ETH3 and BMC1013 deviated significantly ($P<0.05$) from HWE in H2, but not in H1.

Generally speaking, no large differences were found between the two herds with regard to all aspects of genetic variation parameters of marker loci analyzed. Geographical distance and gene flow may be considered. All genetic diversity measures calculated for the microsatellites applied in the present study can be considered of a small to medium magnitude. The limited number of animals genotyped in both herds may have consequences on the measures of genetic diversity calculated. The extent of polymorphism detected in buffalo in the present study is generally lower than that found in cattle (Georges *et al.*,

1995; Ron *et al*., 2001; Zabolewicz *et al*., 2011). Nevertheless, the results obtained could be valuable for implementation of future genetic improvement schemes of Egyptian buffalo. Our findings are in coincidence with previous reports on several buffalo breeds (Moioli *et al*., 2001; Navani *et al*., 2002; Tantia *et al*., 2006; Shokrollahi *et al*., 2009; Rushdi *et al*., 2017).

Table 4. Summary of Chi-Square Tests for Hardy-Weinberg Equilibrium

Marker	Herd	DF	ChiSq	Prob	Significance
BMS711	1	3	11.837	0.008	**
	2	3	12.909	0.005	**
BM143	1	3	11.837	0.008	**
	2	3	13.486	0.004	**
BM6438	1	3	13.373	0.004	**
	2	3	12.041	0.007	**
BM1443	1	3	17.460	0.001	***
	2	3	14.530	0.002	**
ETH2	1	3	22.526	0.000	***
	2	3	17.460	0.001	***
CSSM061	1	36	31.667	0.675	NS
	2	28	26.408	0.551	NS
ETH225	1	10	8.881	0.543	NS
	2	15	15.429	0.421	NS
ETH3	1	15	22.114	0.105	NS
	2	6	13.218	0.040	*
BMC1013	1	15	23.082	0.082	NS
	2	15	26.121	0.037	*
CSSM047	1	15	17.841	0.271	NS
	2	15	17.847	0.271	NS

DF: degrees of freedom, ChiSq: Chi square test, Prob: probability, *: P<0.05, **: P<0.01, ***: P<0.001, NS: not significant

The results of the association analyses of marker loci and milk production traits are illustrated in Table 5. A cumulative analysis of the two herds was applied. Evidences in favor of five significant QTL for only three out of the five studied milk production traits can be observed. Such QTL were linked to four different markers. Microsatellite BM143 showed significant (P<0.05) effect on average daily milk yield deviation and protein percent. Also, the second trait was linked significantly (P<0.05) to locus CSSM047. Fat yield (kg) was associated with microsatellites BM1443 (P<0.05) and CSSM061 (P<0.01). On the other hand, marker ETH3 presented proofs approximate to the significance level of P<0.05 on two traits (fat percent and protein yield). All other markers had no significant linkage to the traits studied.

Considering average daily milk yield deviation (ADMYD), a significant (P<0.05) association between locus BM143 and the trait in question was detected. This finding coincides well with the present author's previous work (Rushdi *et al*., 2017). In this study based only on animals belonging to herd 1 (H1), a set of eight microsatellites revealed significant effects on ADMYD. Among them, curiously, five markers were in common with the present study (BMS711, BM143, BM6438, BM1443, ETH2). However, in the current study no other markers showed significant association with ADMYD, rather than BM143. The reason for absence of significant association may be due to lack of linkage disequilibrium between marker and QTL, especially for animals belonging to H2, which affects accuracy and power of QTL mapping.

Table 5. Association of microsatellites with milk production traits

Marker	Trait	F-value	P-value	Significance
BMS711	ADMYD	0.58	0.566	NS
	Fat %	0.18	0.835	NS
	Protein %	1.25	0.315	NS
	Fat kg	0.79	0.469	NS
	Protein kg	1.14	0.345	NS
BM143	ADMYD	3.54	0.048	*
	Fat %	1.35	0.290	NS
	Protein %	5.05	0.022	*
	Fat kg	2.35	0.126	NS
	Protein kg	2.16	0.148	NS
BM6438	ADMYD	0.49	0.619	NS
	Fat %	0.43	0.658	NS
	Protein %	2.54	0.116	NS
	Fat kg	1.59	0.234	NS
	Protein kg	1.85	0.189	NS
BM1443	ADMYD	0.47	0.629	NS
	Fat %	0.10	0.904	NS
	Protein %	2.34	0.132	NS
	Fat kg	3.93	0.040	*
	Protein kg	2.03	0.164	NS
ETH2	ADMYD	1.52	0.241	NS
	Fat %	0.32	0.729	NS
	Protein %	2.59	0.108	NS
	Fat kg	0.66	0.532	NS
	Protein kg	0.37	0.697	NS
CSSM061	ADMYD	0.75	0.702	NS
	Fat %	0.93	0.575	NS
	Protein %	1.40	0.586	NS
	Fat kg	8.31	0.008	**
	Protein kg	3.33	0.129	NS
ETH225	ADMYD	0.61	0.802	NS
	Fat %	2.25	0.229	NS
	Protein %	0.41	0.914	NS
	Fat kg	0.47	0.863	NS
	Protein kg	0.47	0.828	NS
ETH3	ADMYD	0.76	0.685	NS
	Fat %	5.02	0.059	NS
	Protein %	0.26	0.967	NS
	Fat kg	1.87	0.193	NS
	Protein kg	4.53	0.061	NS
BMC1013	ADMYD	1.84	0.160	NS
	Fat %	0.57	0.783	NS
	Protein %	0.68	0.716	NS
	Fat kg	0.78	0.648	NS
	Protein kg	0.45	0.872	NS
CSSM047	ADMYD	2.20	0.102	NS
	Fat %	1.08	0.497	NS
	Protein %	3.75	0.030	*
	Fat kg	0.62	0.769	NS
	Protein kg	1.42	0.303	NS

ADMYD: average daily milk yield deviation, FY: fat yield, PY: protein yield, FP: fat percentage, PP: protein percentage, *: P<0.05, **: P<0.01, NS: not significant

Microsatellite BM143 has been mapped to bovine chromosome 6 (*Bos taurus* 6, BTA6). Many studies have agreed that BTA6 harbors several QTL underlying milk yield and composition (Georges *et al*., 1995; Nadesalingam *et al*., 2001; Ron *et al*., 2001; Olsen *et al*., 2005; Hu *et al*., 2010). In Egyptian buffalo, Mekkawy *et al*. (2012) found that locus BM143 was significantly linked to a QTL affecting milk yield. Hu *et al*. (2010) stated that the mapping resolution obtained by linkage analysis is generally weak due to few crossing over incidences in pedigrees and poor marker density. As the confidence intervals for the majority of localized QTL could span

approximately 30 cM, positional cloning of the underlying genes seems inaccurate due to the large distance between the candidate genes and QTL.

As mentioned earlier, only marker BM143 illustrated significant effect on ADMYD. On the other hand, some of the markers employed in the present study (CSSM047, BMC1013, ETH3) resulted highly polymorphic when used to study genetic biodiversity and relationship of buffalo populations (Moioli *et al*., 2001; Tantia *et al*., 2006). Subsequently, they may be successfully applied in further studies on QTL identification and parentage verification in bubaline species. Association between microsatellite markers and milk yield in different buffalo and cattle breeds has been reported in several studies (Georges *et al*., 1995; Olsen *et al*., 2005; Sikka and Sethi, 2008; Zabolewicz *et al*., 2011; Rushdi *et al*., 2017). Recently, many studies identified SNPs linked to milk production traits in livestock (Michelizzi *et al*., 2011; Meredith *et al*., 2012; Iamartino *et al*., 2017).

Four loci demonstrated strong effects on the chemical composition of milk. Microsatellite BM143 was significantly ($P<0.05$) associated with protein percent (PP). The segregation of many QTL for milk production traits in the middle of BTA6 close to locus BM143 has been reported (Nadesalingam *et al*., 2001; Ron *et al*., 2001; Hu *et al*., 2010). Olsen *et al*. (2005) fine mapped a QTL for PP to a 7.5 cM interval near to BM143 in Norwegian dairy cattle. Later, genetic orthology between BTA6 and buffalo chromosome 7 (*Bubalus bubalis* 7, BBU7) has been confirmed (Goldammer *et al*., 2007; Venturini *et al*., 2014). Another QTL with significant ($P<0.05$) influence on PP was linked to locus CSSM047. The marker has been localized to BTA8. Homology between BTA8 and BBU3 has been reported by Cribiu *et al*. (2001). To the best of our knowledge, microsatellite CSSM047 has never been used for QTL detection studies in buffalo, but yes for assessment of genetic variability among various buffalo breeds, including the Egyptian buffalo (Moioli *et al*., 2001; Tantia *et al*., 2006). The observed number of alleles for this marker ranged from 9 to 12. Interestingly, the highest number of alleles was recorded in the Egyptian buffalo populations. Only 6 alleles have been detected in the current study. This finding would encourage the use of locus CSSM047 to further study buffalo genome.

In a previous work undertaken in the same herd "H1" (Rushdi *et al*., 2017), two QTL primarily influencing PP were identified through their linkage with markers BM6438 ($P<0.01$) and ETH131 ($P<0.001$). Significant association between BM6438 and PP has been found in buffalo (Sikka and Sethi, 2008) and in cattle (Zabolewicz *et al*., 2011). Curiously, locus BM6438 was also analyzed in the current study; however, this microsatellite presented P-value estimate far away from the minimal significance level for all the studied phenotypes. On the contrary, locus BM143 did not demonstrate any significant effect on PP in the study of Rushdi *et al*. (2017), compared to the present study. As mentioned before; however, both studies agreed in detecting significant association of locus BM143 and ADMYD. Additional investigation is required to confirm the relationship between BM143 and lactation traits in Egyptian buffalo.

No significant evidence for QTL affecting fat percent (FP) was detected. The highest F-value for FP (5.02) was recorded for locus ETH3. Typically, the higher the F-test statistic value, the stronger the microsatellite-trait relationship. However, from the standard deviations it is clear that the influence was not significant (0.059). It should be indicated that marker ETH3 had moderate to high estimates for all measures of genetic diversity calculated in the present study. The result obtained may be due to limitation of phenotypic data on milk composition to only one measure per each individual, as well as the small set of individuals and markers genotyped. In dairy cattle, evidence for the segregation of a single QTL for FP located near to BM143 on BTA6 has been presented (Ron *et al*., 2001; Olsen *et al*., 2005; Hu *et al*., 2010). Marker BM143 was included in the current study; however, no proof for significant QTL underlying FP was observed.

Clear evidences in favor of two QTL primarily underlying fat yield (FY) were obtained. Markers BM1443 ($P<0.05$) and CSSM061 ($P<0.01$) were associated significantly with the genetic variation in FY. Locus CSSM061 proved to be rich in the observed number of alleles (FAO, 2011). Microsatellite BM1443 has been mapped to BTA23 (Ihara *et al*., 2004). Ron *et al*. (2001) found a significant association of marker BM143 and all phenotypes of milk production studied in Israeli Holsteins, expect for FY which was non-significant. On the other hand, Zabolewicz *et al*. (2011) reported significant QTL-linked microsatellite BM6438 and FY trait in Polish Holstein Friesian (HF) cattle. However, no statistically significant differences were observed among the possible genotypes for FY. Based on haplotypes of all 14 microsatellite markers studied in Chinese Holstein cattle, Hu *et al*. (2010) confirmed the segregation of highly significant QTL affecting FY located in the adjacent marker bracket MNB208-BMS5010, near to marker BM143. The ratio of QTL genetic variance to total variance for FY was estimated to be 10.3%. Sequencing this interval has shown that approximately 18 genes potentially associated with lactation traits are involved (https://www.ncbi.nlm.nih. gov/genome/ gdv). Sequence identity between bovine and bubaline species has been evaluated to be between 78-95%, suggesting that cattle marker primers are able to detect homologous loci in buffalo (Sikka and Sethi, 2008; Michelizzi *et al*., 2011; Venturini *et al*., 2014).

In regard to protein yield (PY), no significant association between the set of analyzed loci and PY was identified. However, a marginally significant effect on PY was observed through locus ETH3 ($P<0.061$). Microsatellite ETH3 has been mapped to BBU3, while its corresponding position in cattle is on BTA19. This marker has been used to study genetic biodiversity among different buffalo breeds (Moioli *et al*., 2001). The number of observed alleles for this marker ranged between 4 and 9. Nevertheless, there is no published data on the association between microsatellite ETH3 and milk production traits in buffalo. On the contrary, our previous linkage analyses in buffalo (Rushdi *et al*., 2017) found a QTL affecting PY associated with marker BM415. This marker has been localized to BTA6 and BBU7. Orthologous sequences between BTA6 and BBU7 have been identified (Cribiu *et al*., 2001; Venturini *et al*., 2014).

Taking in account the high similarity between bovine and bubaline genomes as confirmed in several studies (Cribiu *et al*., 2001; Venturini *et al*., 2014), the shortage in genetic markers and linkage maps specific for buffalo can be compensated through using bovine markers to study buffalo genome. Navani *et al.* (2002) suggested that cattle microsatellites linked to QTL may also be useful in studying causes of genetic variation in buffalo; however, synteny groups and gene order may be varied between the two members of Bovidae family.

Bovine SNPs have been developed to deeply study genetic variation in the dairy cattle during the last decade. Consequently, several QTL associated with different milk production traits in dairy cattle have been identified (Meredith *et al*., 2012). Using the Illumina BovineSNP50 BeadChip that features 54,001 informative SNPs uniformly distributed along the entire bovine genome, Michelizzi *et al*. (2011) reported that 41,870 of the 54,001 SNPs were fully scored on the ten buffalo animals studied. These findings indicate that the SNP sites, but not the SNP polymorphisms, are conserved between cattle and buffalo. Recently, a species-specific medium-density 90K SNP panel was created (Axiom® Buffalo Genotyping Array 90K) and seems to be qualified for deeply genomic analysis of river buffalo, investigating genetic biodiversity and performing genome-wide association studies for economically important traits in buffalo populations (Iamartino *et al*., 2017). More studies are required to characterize the SNP evolutionary process, thus incrementing understanding of genetic diversity within and between species, phylogenetics and molecular adaption to continuously changing environmental conditions.

CONCLUSION

A total of five significant QTL were detected in Egyptian buffalo using microsatellites. The identified QTL were associated with genetic variation in average daily milk yield deviation, protein percent and fat yield. The present study provides an initial step towards addressing the causal mechanisms controlling the milk production traits. No doubt, our understanding of buffalo genome will help to optimize production potential and efficiency which could assist in the sustainable development of water buffalo role in agriculture and food production in Egypt. This study confirms the potential use of bovine-derived microsatellites in detecting chromosomal regions underlying milk production traits in the Egyptian buffalo populations.

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التحليل الوراثى الجزيئى للواسمات الوراثية الدقيقة المرتبطة بمحصول اللبن وتركيبه فى الجاموس المصرى

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صفات إنتاج اللبن مهمة إقتصادياً لصناعة إنتاج اللبن. كان الهدف من الدراسة الحالية هو الكشف عن المواقع الوراثية للصفات الكمية (QTL) المسئولة عن كمية اللبن وتركيبه الكيميائى فى الجاموس المصرى. إشتملت الدراسة على عدد إجمالى قوامه 106 من إناث الجاموس المصرى الأصيلة، التى تم تحديد التركيب الوراثى لها بالنسبة إلى مجموعة مكونة من عشرة واسمات وراثية دقيقة (microsatellite). إستُخدم 33299 سجل لبن يومى فى التحليل، الذى إقتصر فقط على تلك السجلات الخاصة بمواسم الحليب الخمس الأولى، والتى تراوحت بها فترة الحليب من 5 إلى 290 يوم. تم بنجاح تكبير جميع الواسمات الوراثية الدقيقة التى تضمنتها الدراسة الحالية، مما يدل على درجة كبيرة من تعدد الصور الأليلية. تراوحت تقديرات التراكيب الوراثية الخليطة المُلاحظة من 0.5 لـ BM6438 إلى 0.950 لـ CSSM061. كما تباينت قيم التراكيب الوراثية الخليطة المُتوقعة بين 0.505 و 0.855 لنفس زوج الواسمات الوراثية الدقيقة، على الترتيب. أظهر الواسم الوراثى الدقيق BM143 تأثيراً معنوياً ($P<0.05$) على كل من متوسط إنحراف إنتاج اللبن اليومى (ADMYD) ونسبة البروتين. تأثرت أيضاً الصفة الآخيرة معنوياً ($P<0.05$) بالواسم الوراثى الدقيق CSSM047. تأثرت كمية دهن اللبن (كجم) معنوياً بإثنين من الواسمات الوراثية الدقيقة، BM1443 ($P<0.05$) و CSSM061 ($P<0.01$). أظهر الواسم ETH3 تأثيراً معنوياً حدياً على نسبة الدهن وكمية البروتين (كجم). قد تكون النتائج التى تم الحصول عليها مفيدة لزيادة دقة الإنتخاب ومعدل التحسين الوراثى فى الجاموس المصرى.