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Association between Microsatellite Markers and Milk Production Traits in Egyptian Buffaloes

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ABSTRACT

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The objectives were to evaluate polymorphism in ten microsatellite markers and to demonstrate their association with milk production traits in the Egyptian buffaloes, based on the cross-species transferability of microsatellites from cattle to buffalo. A total of 17 439 daily milk records from the first five lactations were subjected to analyses, in which records from 5 to 290 days in milk were only included. The analysis revealed that eight out of the ten bovine markers analyzed were polymorphic. The means of the number of alleles, effective number of alleles, and fixation index within markers were 4.125, 2.479, and 0.062, respectively. The means of the observed and expected heterozygosity were 0.491 and 0.527 per marker, respectively. The eight polymorphic microsatellites (*BM1706, BMS711, BM143, BM415, BM6438, ETH131, BM1443, ETH2*) showed significant ($P < 0.001$) associations with average daily milk yield deviation. Protein percentage was significantly associated with microsatellites *BM6438* ($P < 0.01$) and *ETH131* ($P < 0.001$). Only marker *BM415* had a significant ($P < 0.05$) influence on protein yield. None of the analyzed markers revealed significant effect on fat yield and percentage. The results obtained support future application of the polymorphic microsatellites for detailed studies of the Egyptian buffalo genome.

Keywords: relationship; microsatellites; milk yield; milk composition; bubaline genome

Most of the economically important traits in animal production are quantitative in nature and complex in etiology, such as milk yield and composition. These traits are controlled by many genomic regions and affected by the surrounding environment as well as the interaction between the genotype and the environmental factors. The identification of such genomic regions, termed as quantitative trait loci (QTL), is very important to assign genes underlying a trait variation. Mapping these loci can be achieved through analyzing their linkage with known molecular markers, such as microsatellites. Because microsatellites are highly polymorphic markers distributed through the entire genome, they are useful for identification and mapping QTL associated with variation in traits of economic importance. Such QTL enhance our understanding of genetics and physiology of those traits while providing practical benefits to the dairy industry by increasing the rate of genetic improvement using genomic information (Iheshiulor et al. 2016).

Nowadays, dense genetic linkage maps are available for bovine species, with polymorphic microsatellite markers being their fundamental components.

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Microsatellites have contributed to detecting the QTL influencing milk production in dairy cattle (Heyen et al. 1999; Nadesalingam et al. 2001) and also in buffalo (Michelizzi et al. 2011; Mekkawy et al. 2012; Venturini et al. 2014) using mainly cattle-derived markers due to the scarcity of specific microsatellites originating from buffalo genome and genetic similarity between the two bovid species. More details on QTL mapped in different farm animal species can be retrieved from the Animal QTLdb database (http://www.animalgenome.org/cgi-bin/QTLdb; Hu et al. 2016).

Comparative genomic studies have shown that microsatellite primer pair sequences are often conserved across cattle and buffalo genomes and can be used for the development of markers and linkage maps in closely related species (Navani et al. 2002; Wu et al. 2013).

To the best of our knowledge, molecular markers specific for buffaloes (*Bubalus bubalis*) are still limited, compared to other livestock species. The aims of the present study were to evaluate the genetic variation in ten cattle-derived DNA microsatellites and their relationship with milk production traits in the Egyptian buffaloes.

MATERIAL AND METHODS

Population and phenotypic data. Data on 102 multiparous Egyptian buffaloes kept in a herd belonging to the Agricultural Experiment Station, Faculty of Agriculture, Cairo University, located in the province of Giza, Egypt were collected between 2010 and 2015. A total number of 32 087 daily milk yield records were initially at disposal. To ensure homogeneity of the dataset and to guarantee quality of the data to be analyzed, a number of filtration steps were performed. Raw data were thoroughly edited and validated and animals with irrecoverable errors, such as unknown birth dates and missing calving dates, were excluded. Also production records for lactating buffaloes less than 5 days and over 290 days in milk (DIM) were discarded. Data from the first five lactations were only involved. After data editing, the remaining 17 439 daily milk yield records were submitted to subsequent analysis (Table 1).

Samples and DNA extraction. The buffalo resource population for the present study consisted of 30 randomly selected lactating buffaloes. Blood samples were collected from jugular vein into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant, and sent in dried ice to the Molecular Biology Laboratory of the Genetic Engineering Research Center (GERC), Faculty of Agriculture, Cairo University, Egypt for DNA extraction. Genomic DNA was isolated from whole blood according to the manufacturer's instructions (Fermentas® kits, cat. No. k0512 (Fermentas Life Sciences/Thermo Fisher Scientific, USA) and was stored at –20°C till further analyses. Milk samples were collected during the morning milking from each animal to perform chemical analyses of milk constituents. The analyses were carried out using a Bentley 150 Infrared Milk Analyzer (Bentley Instruments, Inc., USA).

Microsatellites. For genotyping assays, ten microsatellite markers located on bovine autosomes were selected using already published cattle linkage maps as a guide (Ihara et al. 2004). Some of the selected markers have been reported to be associated with milk production traits in preceding studies (Table 2).

Polymerase chain reaction (PCR). The polymerase chain reaction was carried out in a Biometra thermal cycler (Biometra GmbH, Germany) using the primers listed in Table 2. The PCR reaction mix included the following: 10 ng/µl DNA; 0.5 U of Red Hot *Taq* DNA polymerase and 10X *Taq* polymerase buffer (ABgene Limited, UK/Thermo Fisher Scientific); 10 mM dNTPs; 50 mM $MgCl_2$; 10 uM of each forward and reverse primer. The PCR protocol started with 95°C for 5 min, followed by denaturation (35 cycles at 94°C for 1 min), annealing at 56°C for 1 min, and polymerization (extension) at 72°C for 2 min, followed by a final extension step at 72°C for 7 min. After thermocycling, the PCR products were checked electrophoretically on 2% (w/v) agarose gel in 1X TBE buffer, for the presence or absence of amplified DNA.

Table 1. Descriptive statistics for daily milk yield after quality check

Lactation	Records *n*	Mean ± SD (kg)	Min	Max
1	1 937	5.866 + 2.727	0.2	14
2	3 762	7.174 ± 2.838	0.2	15.4
3	4 272	7.373 ± 3.214	0.2	17.6
4	4 458	7.654 ± 3.713	0.2	20
5	3 010	6.318 ± 3.194	0.2	18
1–5	17 439	7.052 ± 3.278	0.2	20

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Table 2. Characteristics of cattle microsatellite markers analyzed in the present study (Ihara et al. 2004)

Marker	Chromosomes n^1	Alleles n^2	Primer sequence 5'–3'	T_m (°C)
BMS711	1 (1)	9	F: AGCTTCTTATGGCAACACCTG R: TGAAATCGCAGAGTTGTACATG	58
BM1443	23 (2)	11	F: AATAAAGAGACATGGTCACCGG R: TCGAGGTGTGGGAGGAAG	56
BM1706	16 (5)	10	F: ACAGGACGGTTTCTCCTTATG R: CTTGCAGTTTCCCATACAAGG	56
BM6438	1 (1)	6	F: TTGAGCACAGACACAGACTGG R: ACTGAATGCCTCCTTTGTGC	56
BM143	6 (7)	12	F: ACCTGGGAAGCCTCCATATC R: CTGCAGGCAGATTCTTTATCG	58
BM415	6 (7)	15	F: GCTACAGCCCTTCTGGTTTG R: GAGCTAATCACCAACAGCAAG	54
ETH131	21 (20)	19	F: GTGGACTATAGACCATAAGGTC R: GCTGTGATGGTCTACGAATGA	48
ETH2	5 (4)	6	F: CCCACAGGTGCTGGCATGGCC R: CCATGGGATTTGCCCTGCTAGCT	56
BM1258	23 (2)	9	F: GTATGTATTTTTCCCACCCTGC R: GAGTCAGACATGACTGAGCCTG	58
BM1905	23 (2)	11	F: GTCCATGGGTTCACAAAGAG R: ACGCCTGCTGATGCTGTAG	58

F = forward, R = reverse, T_m = annealing temperature

[1]chromosomal number in cattle (values in parentheses are the corresponding chromosome in buffalo as reported by Cribiu et al. 2001), [2]number of alleles in cattle

Individual genotyping. The amplified PCR products were separated using polyacrylamide vertical gel electrophoresis under denaturing conditions. To measure the actual allele sizes, specific size marker was run on a special well, and then bands were determined through extrapolation with known-sized bands in marker lanes.

Statistical analysis. The GenAlEx 6.5 software package (Peakall and Smouse 2012) was used to calculate the allele frequencies, deviation from Hardy-Weinberg proportion, effective number of alleles, observed and expected heterozygosity, and fixation index at each locus.

Since a few individuals have been genotyped, initial adjustments for the environmental factors have been performed on the whole dataset based on all available contemporary animals using the PROC MIXED in the SAS software (Version 9.4, 2013). In this model, the first order autoregressive covariance structure for repeated statement was used:

$$Y_{ijklmno} = \mu + MF_i + AC_j + YM_k + b_{l1}\,(DIM) + b_{l2}\,(\exp(-0.05 \times DIM)) + lac_m + p_n + \varepsilon_{ijklmno} \quad (1)$$

where:

$Y_{ijklmno}$ = daily milk records
μ = overall mean of observations
MF_i = fixed effect of each day milking frequency
AC_j = fixed effect of age at calving
YM_k = fixed combined effect of year (Y) and month (M) of calving
b_{l1}, b_{l2} = two regression coefficients associated with fixed lactation function
DIM = days in milk (Wilmink 1987)
lac_m = random effect of lactation number
p_n = random permanent environmental effect
$\varepsilon_{ijklmno}$ = residual error

Afterwards, the average of the adjusted daily milk yield deviations for the genotyped animals was used as a response variable for the association analysis in a mixed model as follows:

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

where:

Y_{ijk} = average daily milk yield deviation (ADMYD)
G_i = fixed effect of an animal's genotype for each marker
$sire_j$ = random effect of a sire
ε_{ijk} = residual error

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For milk composition (fat and protein percentages and contents), only the model (2) was used. *Post hoc*, effects among genotype classes for each marker in each trait were tested for significance using a Tukey-Kramer test as implemented in the SAS (Version 9.4, 2013).

RESULTS AND DISCUSSION

Details of the microsatellites analyzed in the present study are given in Table 3. Means of the observed number of alleles, effective number of alleles, observed heterozygosity, expected heterozygosity, and fixation index within markers were 4.125, 2.479, 0.491, 0.527, and 0.062, respectively.

Among the ten bovine-derived microsatellite markers initially tested in buffalo samples, two loci showed technical problems during the genotyping process and therefore were removed from the subsequent analysis. Marker *BM1258* was poorly amplified, while microsatellite *BM1905* resulted monomorphic. This finding indicates the conservation of DNA sequences flanking microsatellites within the Bovidae family.

The possibility to amplify buffalo DNA based on cattle markers has been confirmed in several studies (Mekkawy et al. 2012; Wu et al. 2013; Venturini et al. 2014). In this regard, a set of 61 polymorphic microsatellite markers for riverine buffaloes were identified using cattle-derived primers (Navani et al. 2002). The authors concluded that the extensive conservation of bovine microsatellites in the buffalo genome means that cattle primer pairs can be a very cost effective and time saving resource for analysis of bubaline genome, including the construction of a linkage map, QTL detection, parentage verification, assessment of genetic diversity and gene flow.

A total of 33 alleles were detected across the investigated loci. Markers *BMS711* and *BM1443* had the greatest observed number of alleles (Na) per locus (6), while *BM1706*, *BM415,* and *ETH131* revealed the lowest (2). In an earlier study carried out by El-Kholy et al. (2007) in the Egyptian buffaloes, 12 alleles were observed for each of the *ETH2* and *BM1706* markers. Not surprisingly, in our study the two markers showed 5 and 2 alleles, respectively. The differences in the number of observed alleles between the two studies seem to be logical, since our samples were collected from one herd compared to six herds in the study of El-Kholy et al. (2007). The success to amplify *ETH2* and *BM1706* in these studies may recommend their future use to provide quick entry points to QTL detection in buffaloes without the need to develop specific-buffalo markers and thus save time, cost, and laboratory effort. In cattle, Ihara et al. (2004) reported that microsatellites *ETH2* and *BM1706* exhibited 6 and 10 alleles. Alternatively, on average 22 alleles per locus were found in the Egyptian buffalo by Mekkawy et al. (2012). The authors identified 17 and 19 alleles for markers *BM415* and *BM143* analyzed in 436 and 373 animals, compared to 2 and 5 alleles in the present study, respectively. Sikka and Sethi (2008) stated that genomic DNA of Murrah buffalo was amplified by PCR using specific primers for *ETH131*. However, the extent of polymorphism demonstrated in buffaloes was lesser than that previously reported in cattle (6 vs 10 alleles, respectively). The limited sample size in our study may be a cause for the lower Na in comparison to other studies carried out in Egyptian, Iranian, and Indian buffalo populations.

The effective number of alleles (Ne) obtained (2.479) is markedly lower than that of 3.78 reported by Shokrollahi et al. (2009) in Iranian river buffaloes. Large differences were observed between Na and Ne especially for highly polymorphic markers in the present study, such as *BMS711*, *BM143*, *BM6438*, *BM1443*, and *ETH2*. These results indicate the probability of fixation of specific alleles in the population analyzed. Distinguishing the different allele substitution effects is essential for

Table 3. Summary statistics for microsatellite loci analyzed

Marker	*n*	Na	Ne	Ho	He	F
BM1706	21	2	1.893	0.667	0.472	–0.413
BMS711	29	6	3.579	0.793	0.721	–0.101
BM143	29	5	3.298	0.690	0.697	0.010
BM415	21	2	1.508	0.238	0.337	0.293
BM6438	29	5	3.162	0.483	0.684	0.294
ETH131	21	2	1.100	0.095	0.091	–0.050
BM1443	29	6	2.170	0.310	0.539	0.424
ETH2	29	5	3.121	0.655	0.680	0.036
Mean	–	4.125	2.479	0.491	0.527	0.062

n = sample size, Na = observed number of alleles, Ne = effective number of alleles, Ho = observed heterozygosity, He = expected heterozygosity, F = fixation index

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the future decision-making process in the genetic improvement of the Egyptian buffaloes.

Observed heterozygosity (Ho) ranged from 0.095 to 0.793 for markers *ETH131* and *BMS711*. Similarly, the same two markers revealed the lowest and the highest estimates for expected heterozygosity (He), Na and Ne, respectively (Table 3). In general, estimates of He were higher than their Ho counterparts for all markers, except *BM1706*, *BMS711*, and *ETH131*. Comparable results in river buffaloes were found by Navani et al. (2002) in India, El-Kholy et al. (2007) and Mekkawy et al. (2012) in Egypt, and Shokrollahi et al. (2009) in Iran.

With the exception of three loci (*BM1706*, *BMS711,* and *ETH131*), all estimates of fixation index (F) were positive (Table 3), indicating the presence of a level of inbreeding within the herd. Because sample size largely affects Na, all the measures of genetic diversity computed in the current study were generally of low to intermediate magnitude. However, these findings may present a good opportunity for genetic improvement of indigenous Egyptian buffaloes by means of within-breed selection. Chi-square tests for Hardy-Weinberg equilibrium (HWE) were highly significant ($P < 0.001$) for all markers, except *BM1706*, *BM415*, and *ETH131*. Although significant deviations from HWE have been observed for some loci, none of them was excluded from further calculations. Departure from HWE in buffaloes has been documented (El-Kholy et al. 2007; Shokrollahi et al. 2009).

Results of the association analysis between the microsatellites and the average daily milk yield deviation (ADMYD) are summarized in Table 4. All markers involved in the present study revealed highly significant ($P < 0.001$) effects on ADMYD. Markers *BM1706* and *ETH2* had the lowest and the highest *F*-value, respectively. It should be mentioned that marker *BM1706* possessed the lowest Na estimate (2). Evidently the larger the *F*-test statistic value, the stronger the marker–trait association.

Multiple chromosomal regions harbouring the genes underlying economically important traits have been mapped in dairy cattle through the linkage analysis using microsatellites (Georges et al. 1995; Heyen et al. 1999; Nadesalingam et al. 2001; Zabolewicz et al. 2011). Similarly, numerous single-nucleotide polymorphisms (SNPs) were re-

Table 4. Association between microsatellite markers and milk production traits

Marker	Trait	*F*-value	*P*-value
BM1706	ADMYD	142.84	0.0001***
	FP	0.02	0.8894
	PP	0.46	0.5200
	FY	0.00	0.9541
	PY	0.01	0.9356
BMS711	ADMYD	2527.41	0.0001***
	FP	1.17	0.3406
	PP	0.08	0.9203
	FY	0.25	0.7808
	PY	1.21	0.3289
BM143	ADMYD	1411.74	0.0001***
	FP	0.86	0.4892
	PP	0.05	0.9832
	FY	0.31	0.8144
	PY	0.75	0.5425
BM415	ADMYD	259.96	0.0001***
	FP	0.49	0.4993
	PP	0.00	0.9544
	FY	0.23	0.6426
	PY	8.62	0.0125*
BM6438	ADMYD	2747.16	0.0001***
	FP	2.45	0.1081
	PP	8.21	0.0042**
	FY	0.41	0.7973
	PY	1.25	0.3446
ETH131	ADMYD	196.34	0.0001***
	FP	2.61	0.1350
	PP	31.38	0.0003***
	FY	0.59	0.4573
	PY	1.30	0.2767
BM1443	ADMYD	3060.12	0.0001***
	FP	0.53	0.7186
	PP	1.27	0.3407
	FY	0.11	0.9765
	PY	0.58	0.6864
ETH2	ADMYD	3627.58	0.0001***
	FP	0.73	0.5543
	PP	0.17	0.9140
	FY	0.16	0.9235
	PY	0.75	0.5436

ADMYD = average daily milk yield deviation, FP = fat percentage, PP = protein percentage, FY = fat yield, PY = protein yield

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

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lated to milk traits in cattle (Meredith et al. 2012). If it turns out that synteny and gene order over these regions are conserved between cattle and buffalo genomes, it is fair to expect that bovine microsatellites linked with some of those QTL may also be useful in defining genetic variability in buffaloes (Navani et al. 2002).

The bovine-based markers analyzed in this study were mapped to different cattle chromosomes (1, 5, 6, 16, 21, and 23) as reported in earlier studies. Genome homology between cattle (*Bos taurus*, BTA) and buffalo (*Bubalus bubalis*, BBU) chromosomes was confirmed (Cribiu et al. 2001; Michelizzi et al. 2011). Venturini et al. (2014) found 2 significant SNPs placed on BTA15 and BTA20, which are homologous to BBU16 and BBU19, respectively. Results indicate that some buffalo chromosomes like BBU16 need more detailed studies to confirm the presence of QTL affecting milk yield and also to estimate their positions.

In India, cluster analysis based on sharing specific DNA lengths indicated about 91% DNA similarities between low and high yielding Murrah buffalo heifers (Sikka and Sethi 2008). This depicts the co-segregation of close genotypes in the same cluster consistent with the variation observed in milk production. The authors concluded the possible association of microsatellite *ETH131* with milk yield (MY) and subsequently the ability to incorporate it in early selection plans for MY. Controversially, this marker showed the lowest observed number of alleles in the current study, in parallel with *BM1706* and *BM415*. Mekkawy et al. (2012) reported that microsatellites *BM143*, *BM1329*, and *ILSTS097* are significantly associated with MY, and may be considered as potential candidates for QTL detection in Egyptian buffalo populations. In general, heritability estimates of microsatellites associated with MY were close to zero during the whole lactation period. The authors mentioned that those estimates could be underestimated because of the expected high level of linkage equilibrium within the studied population based on unrelated animals. Weller et al. (1990) reported that when pedigree information is available, linkage disequilibrium can be exploited within families using daughter or grand-daughter designs for increasing accuracy and power of QTL mapping.

Recently, Wu et al. (2013) have observed 8 SNPs in buffaloes that were significantly associated with MY. Also, Venturini et al. (2014) identified 1429 SNPs for milk yield at 305 days (MY305), 798 SNPs for fat yield at 305 days (FY305), 1448 SNPs for protein yield at 305 days (PY305), 860 SNPs for fat percentage (FP), and 714 SNPs for protein percentage (PP) at a significance level of 5%. Adjustment for Bonferroni multiple testing led to insignificant results for all traits except MY, where only two SNPs placed on BTA15 and BTA20 were significant. These chromosomes are homologous to BBU16 and BBU19, respectively. However, the two previous studies did not coincide in the SNPs associated with MY.

Considering milk composition, the results of the association of marker loci with milk constituents are given in Table 4. Despite the entire set of microsatellites analyzed showed a significant association with ADMYD, only three loci yielded significant evidence in favour of a segregating QTL for milk composition. These markers are *BM415*, *BM6438*, and *ETH131* located on BTA6, BTA1, and BTA21, respectively. The first marker has been mapped to BTA6, a chromosome that harbours many QTL underlying milk composition and yield (Georges et al. 1995). Homology between BTA6 and BBU7 has been confirmed by Cribiu et al. (2001). In the current study, it is amazing that the lowest estimate of Ne was recorded for the locus *ETH131*, followed by *BM415*. In general, the two markers had low estimates regarding all statistics for the marker loci analyzed. On the other hand, microsatellite *BM6438* exhibited moderate to high estimates of the population parameters computed.

No proof for QTL with a significant influence on fat percentage (FP) has been obtained. The reasons for this result are clear; nevertheless, the low variation in FP due to the limited number of genotyped individuals and analyzed markers seems to be the most probable explanation. In Holstein-Friesian cows in Ireland, Meredith et al. (2012) identified 370, 370, and 385 SNPs significantly ($P < 0.05$) associated with MY, FY, and PY, respectively. In buffaloes, Venturini et al. (2014) reported that among 15 745 SNPs analyzed, only 1562 revealed significant ($P < 0.01$) influence and 4742 showed significant ($P < 0.05$) effect on all the traits studied.

A clear evidence in favour of two QTL primarily affecting protein percentage (PP) has been found. The first QTL was linked to locus *BM6438* ($P < 0.01$). In cattle, this microsatellite is localized

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at 1.78 cM on BTA1 (AnimalQTLdb: 7127; http://www.animalgenome.org/cgi-bin/QTLdb/BT/index). Cribiu et al. (2001) demonstrated that BTA1 and BTA27 were a fusion of BBU1. Zabolewicz et al. (2011) found three alleles (*256*, *258*, and *268*) for *BM6438*, forming 6 genotypic groups in Polish Holstein-Friesian cattle. However, no statistically significant differences were found out among the potential genotypes for PP. According to our knowledge, the finding obtained in the present study for *BM6438* has not been previously reported. On the other hand, the second QTL underlying PP was in linkage disequilibrium with *ETH131* ($P < 0.001$). This marker, mapped to BTA21, proved to have a significant effect on MY in Murrah buffaloes as indicated by Sikka and Sethi (2008), suggesting its future use in buffalo breeding programs based on molecular markers. A one-to-one correspondence between BTA21 and BBU20 has been documented (Cribiu et al. 2001). Unfortunately, association analysis studies relating microsatellites to milk production traits in buffaloes are generally scarce.

No significant associations of segregating markers with fat yield (FY) have been detected. However, preceding studies reported significant QTL underlying FY in buffalo (Mekkawy et al. 2012; Venturini et al. 2014) and cattle (Zabolewicz et al. 2011; Meredith et al. 2012). The limited sample size could be a cause for this finding. Not surprisingly, neither FP nor FY presented evidence for linkage to a specific marker. As the two traits are positively correlated, the results obtained seem acceptable.

Just the microsatellite *BM415* demonstrated a significant ($P < 0.05$) influence on protein yield. This marker has been mapped to BTA6, the richest cattle chromosome in the number of QTLs underlying milk yield and composition (Georges et al. 1995; Nadesalingam et al. 2001; Venturini et al. 2014).

Although several studies have mentioned segregation of QTLs for milk production traits close to marker *BM143* placed at the middle of BTA6 (Georges et al. 1995; Nadesalingam et al. 2001), no significant effect of this microsatellite on milk composition was detected in the present study. This finding may be attributed to low linkage disequilibrium level, as reported by Iheshiulor et al. (2016).

Generally speaking, the poor number obtained for the significant associations of microsatellites with milk components is expectable given the small number of animals genotyped and limitation of data on milk composition to only one measure per each animal.

CONCLUSION

Eight out of the ten cattle microsatellites analyzed in the present study successfully amplified DNA from buffaloes. This result shows the transferability of microsatellite markers for studying buffalo genome. In addition, the analyzed markers show significant associations with milk production traits. The results obtained provide a basis for understanding the molecular mechanisms underlying genetic variation in milk production traits in Egyptian buffaloes.

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