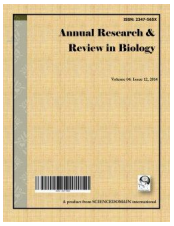

Annual Research & Review in Biology

22(5): 1-8, 2018; Article no.ARRB.39001
ISSN: 2347-565X, NLM ID: 101632869

Genetic Structure and Nucleotide Sequences of Three Productivity Trait Genes, in Egyptian Buffalo

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Authors' contributions

This work was carried out in collaboration between all authors. Author OEO designed the study, performed the data analysis, wrote the protocol and wrote the first draft of the manuscript. Author AAE performed the practical work and followed the publication process. Authors AEED and NAAEM managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/39001
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Jin Seop Bak, Republic of Korea.
(2) Khalifa Muhammad Aljameel, Usmanu Danfodiyo University, Nigeria.
(3) Zafer Bulut, Selcuk University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/22833

Original Research Article

Received 26th October 2017
Accepted 19th January 2018
Published 24th January 2018

ABSTRACT

One of the main sources of meat and milk in Egypt is buffalo, of river type. The marker assisted selection-depending on the promised genetic markers is considered to be the effective way for improvement of buffalo productivity. This work aimed to study the genetic structure and nucleotide sequences of three productivity genes namely; *LGB*, *PIT-1*, and *POUF-1* in Egyptian buffalo. This study is performed by using genomic DNA, which was extracted from 100 female buffaloes. The DNA extracts were subjected to PCR by using some specific primers of the tested genes. The PCR products were digested with dedicated restriction enzymes like; *Hae*III for *LGB* and *Hinf*I for both *PIT-1* and *POUF-1* genes. Depending on the appearance of restriction sites in the amplified fragments; GG^CC for *LGB*/*Hae*III and G^AATC for both *PIT-1*/*Hinf*I and *POUF-1*/*Hinf*I, digestion results also showed accordingly with the appearance like three, two and four digested fragments for *LGB*, *PIT-1* and *POUF-1*, respectively. As a result, the three tested productivity genes are found to be monomorphic in Egyptian buffalo with BB genotype. The nucleotide sequences of this

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genotype were submitted to GenBank under the accession numbers like JQ308794 (*LGB*), JQ885688 (*PIT-1*) and JQ885689 (*POUF-1*) respectively. As a conclusion, it can be said that irrespective of monomorphic genetic structure present in assessed DNA sequences of the Egyptian buffaloes, which ensures the production of good quality meat and milk, but still crossbreeds are highly needed for the betterment of the productivity.

Keywords: LGB; PIT-1; POUF-1; PCR-RFLP; Buffalo.

1. INTRODUCTION

One of the most important livestock in Egypt is buffalo; its meat and milk are the principal components of Egyptian's meal. Improvement in the buffalo productivity can be assured by the marker-assisted selection process which can be done depending on the genetic markers, which are responsible to generate supreme productivity. Among the important productivity trait genes, *LGB*, *PIT-1*, and *POUF-1* are promising markers, which promote the increased productivity.

β-lactoglobulin (*β-LG*) is the major whey protein of ruminant species and is also present in the milk of many, but not all mammalian species [1]. *β-LG* is located on chromosome 11 in the cow [2] which is similar to chromosome 12 in Buffalo [3]. Among the many alleles for the *β-LG* gene, A and B allele are the most frequent ones [4]. The contradictory reports of the association between the genetic structure of *β-LG* and milk production and composition were found [5].

Pituitary-specific transcription factor (*PIT-1*) is a protein consisting of 291 amino acids [6]. This protein is associated with the development of mammalian pituitary gland and the gene expression of hormones, which are secreting by it [7]. *PIT-1* gene was sequenced and mapped to the centromeric region of bovine chromosome 1 between TGLA57 and RM95 [8,9]. Bovine chromosome 1 is the equivalent q arm of buffalo chromosome 1 [3].

One of the promising genetic markers for the production traits in livestock is the growth hormone factor 1 (*POU1F1*) gene. The products of this gene have vital roles in the excretion, transcription, and regulation of many genes, including growth hormone, prolactin, and other pituitary genes [8,10,11].

Towards the genetic screening of some important productivity trait genes in Egyptian buffalo, PCR-RFLP, and sequencing techniques were used to identify the genetic structure and DNA sequences of three genes; *LGB*, *PIT-1,* and *POUF-1* which are related to the production traits in Buffalo.

2. MATERIALS AND METHODS

2.1 Ethics Statement

The blood samples used in this study were collected by veterinarians during routine blood sampling on commercial farm animals (for medical care or follow up). These animals were not linked to any experimental design, and the blood sampling was not performed specifically for this study. All the samples and data processed in this study were obtained with the breeders and breeding organizations' consent.

2.2 Genomic DNA Extraction

Genomic DNA was extracted from the whole blood of 100 female buffaloes according to the method described by Miller et al. [12] with minor modifications. Briefly, blood samples were mixed with cold 2x sucrose triton and centrifuged at 5000 rpm for 15 min at 4°C. The nuclear pellet was suspended in lysis buffer, sodium dodecyl sulfate and proteinase K and incubated overnight in a shaking water bath at 37°C. Nucleic acids were extracted with saturated NaCl solution. The DNA was picked up and washed with 70% ethanol. The DNA was dissolved in 1x TE buffer. DNA concentration was determined, using NanoDrop 1000 Thermo Scientific spectrophotometer and then diluted to the working concentration of 50 ng/µl.

2.3 Polymerase Chain Reaction (PCR)

A PCR cocktail consists of 1.0 µM upper and lower primers specific for tested genes (Table 1), 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin (w/v), 0.1% Triton X-100 and 1.25 units of *Taq* polymerase. The cocktail is aliquot into PCR tubes with 100 ng of buffalo DNA. The reaction is cycled for 1 min. at 94°C, 2 min at an optimized annealing temperature that is determined for each primer (Table 1) and 2 min. at 72°C for 30 cycles. The

Table 1. The sequences and information of primers used in this study

Gene	Primer sequence 5′ -------- 3′	PCR conditions (30 cycles)	PCR product size	Restriction enzyme used	References
LGB	TGT GCT GGA CAC CGA CTA CAA AAA G GCT CCC GGT ATG TGA CCA CTC TCT	94°C 1 min 60°C 2 min 72°C 2 min	248-bp	*Hae*III	Heidari et al. (2009) [18]
PIT-1	GAG CCT ACA TGA GAC AAG CAT C AAA TGT ACA ATG TGC CTT CTG A	94°C 1 min 60°C 2 min 72°C 2 min	614-bp	*Hinf*I	Javanmard et al. (2005) [31]
POUF-1	CAA TGA GAA AGT TGG TGC TCT GCA TTC GAG ATG CTC	94°C 1 min 54°C 2 min 72°C 2 min	1301-bp	*Hinf*1	Curi et al. (2006) [36]

PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide.

2.4 Restriction Fragment Length Polymorphism (RFLP)

The PCR products for the three tested genes were digested with a specific restriction enzyme for each gene (Table 1). 10 µl of PCR product was digested with 1 µl of Fast Digest restriction enzymes specific for each tested gene for 15 min at the optimum temperature for maximum activity of each restriction enzyme. The restriction fragments were subjected to electrophoresis on 2% agarose/ethidium bromide gel in 1x TBE buffer (0.09 M Tris-boric acid and 0.002 M EDTA). Gels were visualized under UV light and documented in FX Molecular Imager apparatus (BIO-RAD).

2.5 Sequence Analysis

The PCR products of each tested gene were purified and sequenced by Macrogen Incorporation (Seoul, Korea). Results of endonuclease restriction were carried out using FastPCR. The nucleotide sequences of the three tested genes were submitted to GenBank (NCBI, BankIt).

3. RESULTS AND DISCUSSION

3.1 Beta-lactoglobulin (*β-LG*) Gene

The primers used in this study flanked a 248-bp fragment from exon 4 of buffalo *β-LG* gene (Fig. 1). To identify its genetic structure (polymorphism), the PCR amplified fragments were digested with a *Hae*III endonuclease. The digestion results showed the appearance of three fragments at 133, 74 and 41-bp in all tested animals (Fig. 2) resulted from the presence of two restriction sites (GG^CC) at positions 74^75 and 207^208 in the amplified PCR products (Fig. 3). This result declared that all studied buffaloes are genotypes as BB, which is characterized by these three digested fragments. The nucleotide sequence of this genotype was submitted to GenBank under the accession number: JQ308794.

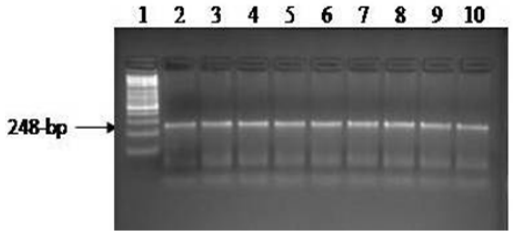

Fig. 1. Agarose gel showed the PCR products of the *β-LG* gene in Egyptian buffalo
Lane 1: 100-bp ladder marker
Lanes 2-10: 248-bp PCR products amplified from Egyptian buffalo DNA

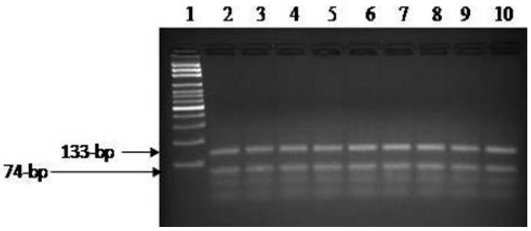

Fig. 2. The Electrophoretic pattern of PCR amplified *β-LG* fragments after digestion with a *Hae*III restriction enzyme
Lane 1: 100-bp ladder marker.
Lanes 2-10: Homozygous BB genotype characterized by three restricted fragments at 133, 74 and 41-bp (not showed in the figure)

TGTGCTGGACACCGACTACAAAAAGTACCTGCTTTTCTGCATGGAGAACAGTGCTGAGCCCGAGCAAAGCCTGG^
CCTGCCAGTGCCTGGGTGGGTGCCAACCCTGGCTGCCCAGGGAGACCAGCTGTGTGGTCCTCGCTGCAACGGGGC
GGCGGGGGGACGATGGGAGCAGGGAGCTTGATTCCCAGGAGGAGGAGGGATGGGGGGG^CCCCGAGTCCCGCCAG
GAGAGAGTGGTCACATACCGGGAGC

Fig. 3. The nucleotide sequences of PCR amplified fragment from buffalo *β-LG* gene
GG^CC is the restriction site at positions 74^75 and 207^208

Many reports showed that BB genotype *of β-LG* is associated with higher fat and increased cheese yields [13]. An association of *β-LG* BB with higher protein yield has also been reported [14]. On the contrary, the association between the *β-LG* AA genotype and best milk trait was identified [15,16,17]. On the other hands, the association between AB genotype of *β-LG* gene and good milk components, including lactose and protein was declared by Tsiaras et al. [5].

Heidari et al. [18] studied polymorphism of *β-LG* gene in 101 superior cows belonging to the Holstein herd, producing more than 150 kg milk/day, together with four offspring. Genotypes were distributed according to the Hardy-Weinberg equilibrium, and the results indicated that the *β-LG* genotypes significantly affected (P<0.01) milk yield where genotype AB is the best one.

Ren et al. [19] digested the PCR products of the *β-LG* gene with *Hae*III endonuclease in Chinese Murrah, Nili-Ravi and hybrid buffalo. They reported the appearance of one allele (B) in all studied buffaloes. These observations are similar to the findings of Meignanalakshmi and Nainar [20] for β-lactoglobulin, but different from the result of Vohra et al. [21] where they reported the presence of two alleles; A and B in Murrah and Bhadawari breeds.

3.2 Pituitary-specific Transcription Factor 1 (*PIT-1*) Gene

A 614-bp fragment covering apart from intron 5 (206-bp), 210-bp from exon 6 and a part of intron 6 (198-bp) of buffalo *PIT-1* gene was amplified using PCR (Fig. 4).

These PCR products (614-bp) were digested with *Hinf*I endonuclease, and the result showed the appearance of two fragments at 369, 245-bp in all tested animals (Fig. 5) resulted from the restriction site (G^AATC) at positions 369^370 in the amplified PCR products (Fig. 6). This result declared that all buffaloes under study are genotyped as BB, which is characterized by these two digested fragments. The nucleotide sequence of this genotype was submitted to GenBank under the accession number: JQ885688.

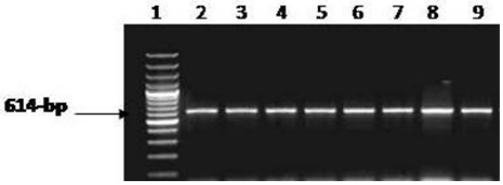

Fig. 4. Agarose gel showed the PCR products of *PIT-1* gene in Egyptian buffalo
Lane 1: 100-bp ladder marker
Lanes 2-9: 614-bp PCR products amplified from Egyptian buffalo DNA

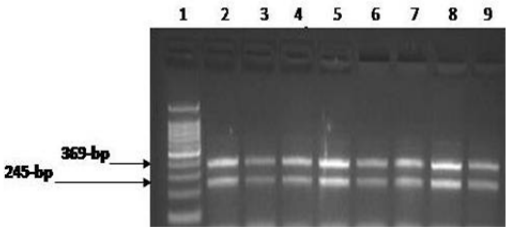

Fig. 5. The Electrophoretic pattern of PCR amplified *PIT-1* fragments after digestion with *Hinf*I restriction enzyme
Lane 1: 100-bp ladder marker
Lanes 2-9: Homozygous BB genotypes showed two digested fragments at 369- and 245-bp

The *PIT-1* locus has potential as a marker for genetic variation in milk production traits. The frequencies of different *PIT-1* alleles and genotypes, as well as their associations with the bovine milk traits parameters, were reported by many investigators [22,23,24,25,26]. The positive association between AA genotype of *PIT-1* gene and milk yield was reported in Baja California Holstein cattle [27]. This finding confirmed the results of Renaville et al. [22] about the significant effect of this genotype (P<0.05) on milk yield. Viorica et al. [28] used PCR-RFLP technique *PIT-1/Hinf*I in Romanian

GAGCCTACCTGAGACAAGCATCTAAATGTTCAAAAAAACTTCACATTTATTATTGTTGAAGAACTTTGAAGGTGT
TTTCAGCATCTTTAGGTTTCCTTTTTACGTTAATGTTAATACTAATATTTAGGAAATGTAACCTACCTTGATTTT
GATGGGCCTAAACCATCATCTCCCTTCTTCTTTCCTGCCAACTCCCCACCTCCCAGTATTGCTGCTAAAGACGCC
CTGGAGAGACACTTTGGAGAACAGAATAAGCCTTCCTCTCAGGAGATCCTGCGGATGGCTGAAGAACTAAACCTG
GAGAAAGAAGTGGTGAGGGTTTGGTTTTGTAACCGAAGGCAGAGAGAAAAACGGGTGAAGACAAGCCTG^AATCA
GAGTTTATTTACTATTTCTAAGGAGCATCTCGAATGCAGATAGGCTCTCCTATTGTGTAATAGCGAGTTTTTCTA
CTTTTCATTCCTTTCTCTTCTCCAGCCAAAATAGAAATTAGTTATTTGGTTAGCTTCAAAAAATCACATCAGTAA
TTTTTGCAGAAGTGTTTCTTTTCTACTTTAAAAATAAATACAATTTAAATTATGTTGATGAATTATTCTCAGAAG
GCACATGGTAACATT

Fig. 6. The nucleotide sequences of PCR amplified fragment from buffalo *PIT-1*gene
G^ANTC is the restriction site at position 369^370

Simmental cattle to declare that the A allele was superior over allele B for milk yield as well as protein and fat percentage. These results confirmed the previous findings reported by Wieckowski et al. [29], Vlaic et al. [30] and Zhao et al. [25].

Javanmard et al. [31] showed the presence of one restriction site of *Hinf*I in *Pit-1* exon 6 PCR product (600-bp) in cattle and buffalo. The digested PCR products by *Hinf*I endonuclease exhibited three genotypes AA (600-bp), AB (600-, 357- and 243-bp) and BB (357- and 243-bp). Sequence analysis revealed a variation (G to A) at the polymorphic *Hinf*I site. The genotype frequencies ranged from 0 to 0.842 (AA), from 0 to 0.769 (AB) and from 0 to 0.375 (BB). For Iranian buffalo, Javanmard et al. [31] reported the frequency of different genotypes for *Pit-1* gene after digestion of PCR products with *Hinf*I endonuclease; AA (0.567), AB (0.400) and BB (0.033) whereas *Pit-1/Hinf*I technique in our study declared that all buffaloes genotyped as BB where the digestion of 614-bp PCR products yielded two fragments at 369- and 245-bp.

3.3 Growth Hormone Factor 1 (*POUF-1*)

The primers used in this study flanked a 1301-bp fragment covering 54-bp from exon 5, 1038-bp from intron 5 and 209-bp from exon 6 of buffalo *POUF-1* gene (Fig. 7). The PCR products were treated with *Hinf*I endonuclease, and the digestion results declared the appearance of four fragments at 617, 379, 260 and 45-bp in all tested animals (Fig. 8) resulted from the presence of three restriction sites (GG^CC) at positions 260^261, 877^878 and 1256^1257 in PCR amplified products (Fig. 9). The result showed that all buffaloes under study are genotyped as BB, which is characterized by four digested fragments. The nucleotide sequence of this genotype was submitted to GenBank under the accession number: JQ885689.

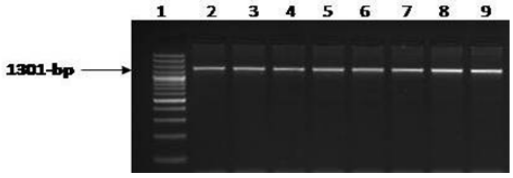

Fig. 7. Agarose gel showed the PCR products of *POUF-1* gene in Egyptian buffalo
Lane 1: 100-bp ladder marker
Lanes 2-9: 1301-bp PCR products amplified from Egyptian buffalo DNA

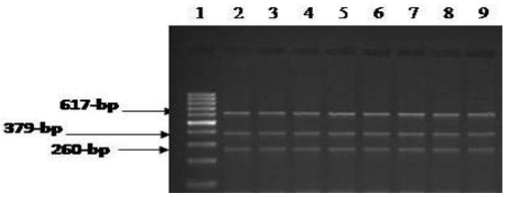

Fig. 8. The Electrophoretic pattern of PCR amplified *POUF-1* fragments after digestion with *Hinf*I restriction enzyme
Lane 1: 100-bp ladder marker
Lanes 2-9: Homozygous BB genotypes showed four digested fragments at 617-, 379-, 260- and 45-bp (not showed in the figure)

Moody et al. [32] identified the polymorphism in *POU1F1* gene and reported the presence of alleles A and B. This polymorphism (A→G) was present in bovine *POU1F1* exon 6 [33]. The frequencies reported in the literature for the A allele of *POU1F1*/*Hinf*I in different cattle breeds ranged from 0.25 in Piedmontese [34], 0.53 in Belgian Blue [22], 0.45 in Angus [32] and 0.86 in Canchim cattle breeds [35]. Furthermore, Curi et al. [36] estimated the allele and genotype frequencies of *POU1F1*/*Hinf*I in four cattle breeds. The allele frequencies of A allele ranged from 0.641 to 0.897 with a mean 0.731 whereas the allele B frequencies ranged from 0.103 to 0.359 with a mean 0.269.

CAATGAGAAAGTTGGTGCAAATGAAAGAAAAAGGAAACGGAGAACAACAATCAGGTATACTTTTGAGATATTAAG
AGTTAGTGGAGAAGAAAATGATATTTTACAAATGGAATGAACATTTGAGTATAATATAGTTTCAATATAACATAA
AAATGAATAGAGGCAATTGAGAAAATAGGTGAAAAAGCACAACATTCAATAAATTACTGCTGAGAAACAGCTGGA
AATTTAAAATTTGATGGAAAAATATGTATTGTTTG^ATTCAAGAACAGTTTTGCTCTGCAAGTTTTGGATAAAAC
AGAAGCTATACAATCACAGCTAAAAAGAATGACTGTTTCTACTGTGTCATAATGTGTTGATTTATGTTTAGACAT
AAATCTTGCTCCGGGAAAGACCCCATGGACTGTAGCCTACAGGTTCCTCTGTCCATGGGATTTTCCAGGCAAGAA
TAATGGAGTGGGTTGCCATTTCCTTCTCCAGGAGATCTTCCCGACCCAGGGATTGAACCCGGGTCTCCTACATTG
TAGGCAGATGCTTTACCATCTGAGCCACAAGGGAAGTCAGCTATCTATATTATTTCAAATTAACAAAACTGGTCA
CTAGTATTTTAGTTGCTTAAAGTTCAAAATGACTTCTAGCATTTCAAGCCAGATTGTTCATTTATCTTTTTGTAG
TTTCCGTGAGGCTCATGGAGGAATTGCTAAAATACAGGTTTTGTTTTGGTTGGTTAGTTGTACACTAAACATTTC
AATAACCTGAGTTCTGGGGGACATTTAGAAATGCATACAGAATTATTTTCTTCTCAGTAAGTCAGTGCCCTCTTG
TGGCAGAAAGTGGATAAACAATGTCGGGGTTCCCTCCTTAATTTCTTCCTGTG^ACTCTGGTAAAAGGAGCCTAC
ATGAGACGAGCATCTAAATGTTCAAAAAAACTTCACATTTATTATTGTTGAAAAGCTTTGAAGGTGTTTTCAGCA
TCTTTAGGTTTCCTTTTTACGTTAATGTTAGTACTAATATTTAGGAAATGTAACCTAACTTGATTTTGATGGGCC
TAAACCATCATCTCCCTTCTTTCCTGCCAACTCCCCACCTCCCAGTATTGCTGCTAAAGACGCCCTGGAGAGACA
CTTTGGAGAACAGAATAAGCCTTCCTCTCAGGAGATCCTGCGGATGGCTGAAGAACTAAACCTGGAGAAAGAAGT
GGTGAGGGTTTGGTTTTGTAACCGAAGGCAGAGAGAAAAACGGGTGAAGACAAGCCTG^AATCAGAGTTTATTTA
CTATTTCTAAGGAGCATCTCGAATGCAGA

Fig. 9. The nucleotide sequences of PCR amplified fragment from buffalo *POUF-1* gene
G^ANTC is the restriction site at positions 260^261, 877^878 and 1256^1257

In contrast to the low frequency of B allele for *POU1F1*/*Hinf*I reported in cattle by Curi et al. [36], all Egyptian buffalo animals investigated during this study are genotyped as BB where all tested buffalo DNA amplified fragments (1301-bp) were digested with *Hinf*I endonuclease and gave four digested fragments at 617-, 379-, 260- and 45-bp. This result indicated that Egyptian buffaloes are superior for meat and growth traits where Renaville et al. [22] and Carrijo et al. [35] reported that *POU1F1*/*Hinf*I BB animals were significantly superior over animals with the AB and AA genotypes in body weight, weaning and yearling weight traits in cattle.

4. CONCLUSION

Due to the monomorphic genetic structure are found within the three tested productivity trait genes, so it can be said that the Egyptian buffaloes are much potent to give enough amount of milk and meat. Though cross-breeding of these traits with other improved Italian species can generate some more advance traits with advance genomic sequences to come up with quality and quantity upgraded milk as well as meat.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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