RESEARCH ARTICLE

Study on ABCG2 Gene Polymorphism in Jaffarabadi and Surti Buffaloes by PCR-RFLP

Santul Patel, Balkrishna Brahmkshtri, Umed Ramani*, Guarav Pandya, Nikhil Dangar, Savita Devkatte, Vishnu Kharadi

Abstract

In the present study, considering the role of ABCG2 gene in milk production and composition traits, Jaffarabadi and Surti buffaloes were screened for its polymorphism. PCR- RFLP technique was used to detect the polymorphism in 292 bp (partial intron 13 and exon 14) and 240 bp (partial exon 14) fragments of ABCG2 gene using HhaI and PstI restriction enzymes, respectively. The absence of polymorphism in ABCG2 gene in the study for partial intron 13 and exon 14 fragment of 292 bp as well as partial exon 14 fragment of 240 bp indicated fixation of alleles at both the loci in Jaffarabadi and Surti buffalo breeds.

Keywords: ABCG2 gene, Jaffarabadi and Surti buffaloes, Milk production and composition trait, PCR-RFLP, Polymorphism.

Ind J Vet Sci and Biotech (2022): 10.21887/ijvsbt.18.3.6

Introduction

Water buffaloes (*Bubalus bubalis*) were domesticated in the Indian subcontinent about 5000 years ago. Buffaloes have a major role in the rural livelihood in most of milk producing states of India and contribute to food security of India (Thangaraju, 2019). Gujarat has four recognized breeds of buffalo, *viz.,* Jaffarabadi, Mehsani, Surti and Banni in which Jaffarabadi is one of the heaviest breed, whereas Surti is lightest one. The milk production and composition, being quantitative traits, are regulated by number of genes. Genetic improvements in these important economic traits have been conducted using conventional breeding and selection of buffaloes. Molecular genetic analysis of these traits leads to better understanding of genes governing these traits. ABCG2 gene belongs to the ATP binding cassette family of trans-membrane drug transporters which is expressed in a variety of tissues including mammary gland epithelial cells where it is strongly expressed during lactation in mice, cows and humans. It is considered an important protein that can transport a wide range of molecules including riboflavin and many lipophilic substrates into milk. The level of its expression significantly increases during lactation and reported to affect milk yield and milk composition (Farke *et al*., 2008). The present study was undertaken to study the polymorphism of ABCG2 gene using polymerase chain reaction for restricted fragment length polymorphism (PCR-RFLP) in Jaffarabadi and Surti buffaloes.

Materials and Methods

This research work was conducted on 50 Surti buffaloes maintained at Livestock Research Station under Navsari Agricultural University, Navsari and 50 Jaffarabadi buffaloes maintained at Patel Dairy Farm, Near Village Moti Pethan, Taluka: Jalalpore, Distt: Navsari. About 5-7 mL of the blood was collected aseptically from each animal from the vena jugularis in a sterile vacutainer containing 0.5 M EDTA (pH 8.0) solution as an anticoagulant. After collection of blood, the vacutainers were immediately transported to the laboratory in an icebox. The genomic DNA from whole blood samples was extracted by phenol-chloroform method as described by John *et al.* (1991) with some necessary modifications. Purity and concentration of genomic DNA were estimated in a Nanodrop 2000 c spectrophotometer by observing OD ratio 260/280 . The quality of genomic DNA was checked by running the samples in 0.8% agarose by submarine gel electrophoresis. Polymerase Chain Reaction was performed to amplify ABCG2 292 bp (partial intron 13 and exon 14) and 240 bp (partial exon 14) fragments, respectively, using gene specific primers (Table 1) in Jaffarabadi and Surti buffaloes. PCR was carried out in a final reaction volume of 25 µL (Table 2).

Amplification of the 292 bp region was carried out using 35 cycles each of denaturation at 94°C, annealing at 58°C and

Department of Animal Biotechnology, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

Corresponding Author: Umed Ramani, Department of Animal Biotechnology, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India, e-mail: umedramani@yahoo.co.in

How to cite this article: Patel, S., Brahmkshtri, B., Ramani, U., Pandya, G., Dangar, N., Devkatte, S., Kharadi, V. (2022). Study on ABCG2 Gene Polymorphism in Jaffarabadi and Surti Buffaloes by PCR-RFLP. Ind J Vet Sci and Biotech. 18(3), 26-29.

Source of support: Nil

Conflict of interest: None.

Submitted: 21/10/2021 **Accepted:** 10/02/2022 **Published:** 10/07/2022

© The Author(s). 2022 Open Access This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Table 1: The primer pairs, expected product size and restriction enzyme of the ABCG2 gene

Gene	*Region of gene*	*Primer Sequence*	*Reference*	*Product size*	*Restriction enzyme*
ABCG2	Partial intron 13 and exon 14	F-5'AACAGCCTCAGCTCCAGAGAGATAT3' R-5'CGGTGACAGATAAGGAGAACATACT3'	Ghombavani *et al.* (2016)	292 bp	PstI
	Partial exon 14	F-5'GTATTCACGAGACTGTCAGGG3' R-5'GGCTTTATTCTGGCTGTTTCC3'	El-Nahas *et al.* (2018)	240 bp	HhaI

Table 2: Components of each reaction used for PCR

Components	*Volume*	*Final concentration*
Final master mix		
2X Master Mix(Takara)	12.5 µL	1X
Deionised water	07.5 µL	………
Forward Primer	01.0 µL	10 pmole
Reverse Primer	01.0 µL	10 pmole
Genomic DNA (30 ng/µL)	03.0 µL	90 ng
Total	25.0 µL	

Table 3: Restriction enzymes and their restriction sites

Restriction Enzymes	*Restriction site*
PstI	5'...C T G C A G…3' 3'...G A C G T C…5'
HhaI	5'...G C G C…3' 3'...C G C G…5'

extension at 72°C each for 45 second. Initial denaturation was carried out at 95°C for 5 minutes, while the final extension was performed at 72°C for 10 minutes. To amplify the 240 bp another fragment, similar PCR protocol was used, except annealing temperature of 56°C.

The PCR amplification of ABCG2 292 bp and 240 bp fragments were confirmed by running 5 µL of PCR products along with 50 bp DNA ladder on 2% agarose gel at constant voltage 80 V for 90 min in 0.5X TBE buffer. The amplified products were visualized under UV light and photographed by gel documentation system.

The 292 bp and 240 bp fragment PCR products were digested with PstI (RE 0.25 µL, 10X buffer 1.5 µL, PCR product 5.0 µL, autoclaved water 8.25 µL) and HhaI (RE 0.6 µL, 10X buffer 2.0 µL, PCR product 10.0 µL, autoclaved water 7.4 µL) restriction enzymes, respectively (Table 3). These reaction samples were incubated at 37°C for 60 min and inactivated by adding 1 µL 0.5 M EDTA solution and at 80°C for 10 min, respectively.

The digested 292 bp and 240 bp fragment PCR products were run on 2% agarose gel at constant voltage 80 V for 90 min in 0.5X TBE buffer. The digested products were visualized under UV light and photographed by gel documentation system. Genotyping was performed according to the band patterns of digested PCR products.

Results and Discussion

In present study, screening of the Jaffarabadi and Surti buffaloes for polymorphism of partial intron 13 and exon 14 fragment as well as partial exon 14 fragment of ABCG2 gene was done from blood of each buffalo under study and DNA was extracted. All extracted DNA samples were of good quality showing single compact band on agarose and OD ratio of 260/280 was between 1.8-2.0. In the present study, PCR product size of ABCG2 gene was found to be 292 (Fig. 1 & 2) and 240 (Fig. 3 & 4) bp for two different regions of the gene under study which corroborates with the reports of Ghombavani *et al.* (2016) and El-Nahas *et al.* (2018), respectively.

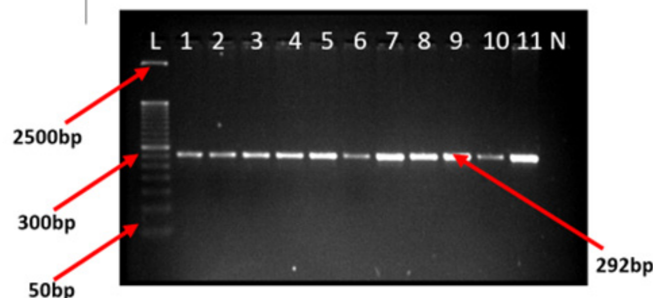

Fig.1: PCR products of partial intron 13 and exon 14 ABCG2 292 bp fragments of Jaffarabadi buffaloes.
Lane: L– 50 bp Ladder, 1 to 11-292 bp PCR products, N– Negative control

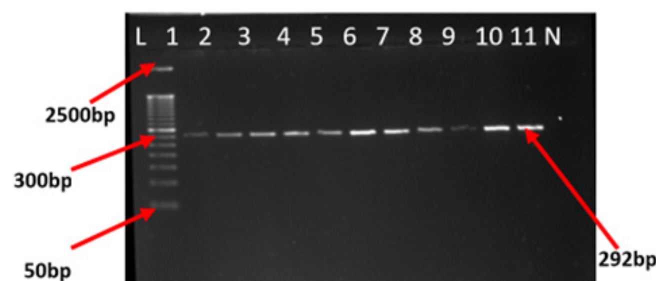

Fig. 2: PCR products of partial intron 13 and exon 14 ABCG2 292 bp fragments of Surti buffaloes.
Lane: L– 50 bp Ladder, 1 to 11-292 bp PCR products, N–Negative control

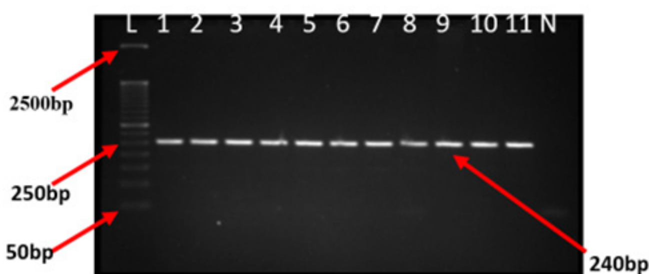

Fig.3: PCR products of partial exon 14 ABCG2 240 bp fragments of Jaffarabadi buffaloes.
Lane: L– 50 bp Ladder, 1 to 11-240 bp PCR product, N– Negative control.

Many polymorphic studies on the bovine ABCG2 have been reported in Indian and exotic cattle (Ghombavani *et al.*, 2016; Sharma *et al.*, 2016; El-Nahas *et al.*, 2018), however, the

reports of polymorphic studies in Indian buffaloes are very few (Tantia *et al.,* 2006).

The 292 bp (partial intron 13 and exon 14) amplicons in Jaffarabadi and Surti buffaloes were digested with restriction enzymes PstI. PCR-RFLP pattern revealed one genotype (AA) and one allele (A) for the locus (Fig. 5 & 6).

In the present study the gene frequency (A) and genotypic frequency (AA) was observed 1.0 in both the Jaffarabadi and Surti buffaloes (Table 4). The findings of this study for digestion of 292 bp ABCG2 gene fragment with PstI was not in agreement with the results of Ghombavani *et al.* (2016) who reported two RFLP bands of 292 bp and 268 bp. They reported two genotypes: AA, AC with their genotype frequencies of 0.94; 0.06, and allelic frequencies for A and C as 0.97; 0.03 respectively, in Holstein dairy cows. Similar findings were reported by Sharma *et al*. (2016) in Sahiwal and Hariana cattle breed and Tantia *et al.* (2006) in Indian cattle and buffaloes. Contrarily, Ates *et al.* (2014) found allelic frequencies of A and C alleles of 0.63; 0.37 in SAR and 0.64; 0.36 in EAR breed of Turkey cattle, whereas Kowalehiska-Luczak *et al.* (2009) found allelic frequencies of 0.80 and 0.20, respectively, in Jersey cows.

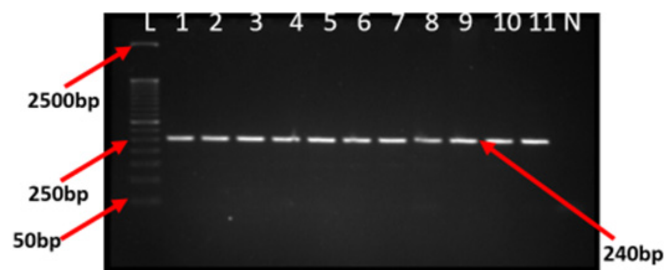

Fig. 4: PCR products of partial exon 14 ABCG2 240 bp fragments of Surti buffaloes.
Lane: L– 50 bp Ladder, 1 to 11-240 bp PCR product, N– Negative control

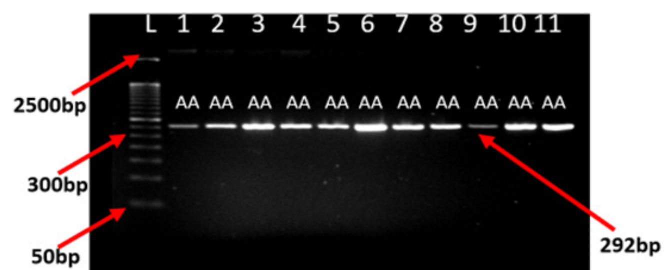

Fig. 5: Restriction patterns of partial intron 13 and exon 14 ABCG2 292 bp fragments of Jaffarabadi buffaloes on digestion with PstI.
Lane : L– 50 bp Ladder, 1 to 11-292 bp PCR products.

The 240 bp (partial exon 14) amplicons in Jaffarabadi and Surti buffaloes were digested with restriction enzymes HhaI. PCR-RFLP pattern revealed one genotype (AA) and one allele (A) for the locus (Fig.7 & 8) with gene frequency of genotype AA and allele A as 1.00 in both Jaffarabadi and in Surti buffaloes (Table 4). These findings for its restriction digestion with HhaI were not in agreement with El-Nahas *et al.* (2018) who reported two RFLP bands of 240 bp and 128 bp with genotype AA and AB in Egyption native (Baladi) cattle and Hybrid cattle. However, in Holstein Friesian cattle they found

Fig. 6: Restriction patterns of partial intron 13 and exon 14ABCG2 292 bp fragments of Surti buffaloes on digestion with PstI
Lane : L– 50 bp Ladder, 1 to 11-292 bp PCR products.

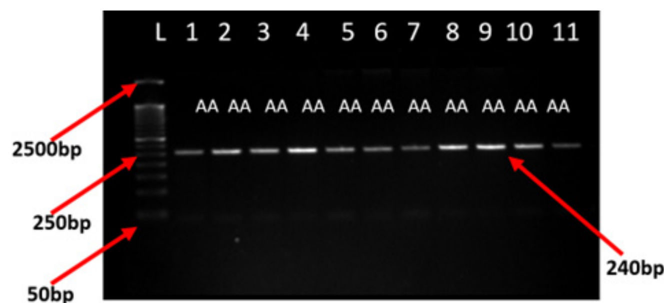

Fig. 7: Restriction patterns of partial exon 14 ABCG2 240 bp fragments of Jaffarabadi buffaloes on digestion with HhaI.
Lane : L– 50 bp Ladder, 1 to 11-240 bp RFLP product.

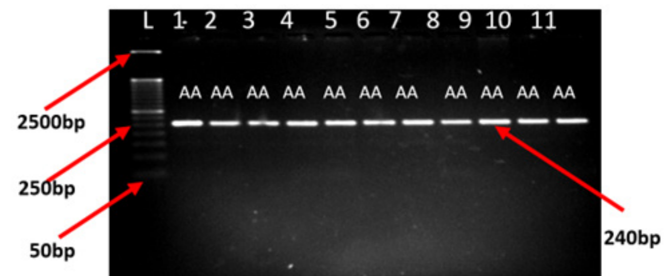

Fig. 8: Restriction patterns of partial exon 14 ABCG2 240 bp fragments of Surti buffaloes on digestion with HhaI.
Lane : L– 50 bp Ladder, 1 to 11-240 bp RFLP product.

Table 4: Genotypic and allelic frequency in ABCG2 gene in Jaffarabadi and Surti buffaloes

Buffaloes	*Region*	*Genotype*	*Observed number of genotype*	*Expected number of genotype*	*Genotype frequency*	*Allele frequency*
						A
Jaffarabadi (n=50)	292 bp (intron 13 and exon 14)	AA	50	50	1.00	1.00
	240 bp (Exon 14)	AA	50	50	1.00	1.00
Surti (n=50)	292 bp (intron 13 and exon 14)	AA	50	50	1.00	1.00
	240 bp (Exon 14)	AA	50	50	1.00	1.00

monomorphic band pattern with AA genotype only which supports results of our study. El-Nahas *et al.* (2018) found two genotypes AA and AB with their genotype frequencies of 0.70; 0.25 and allelic frequencies for A and B as 0.88; 0.12, respectively, in Baladi cows. In hybrid cows also they found two genotypes AA and AB with their genotype frequencies of 0.84; 0.16, and allelic frequencies for A and B as 0.92; 0.08, respectively. They reported significant association for lactose percent in which ABCG2-HhaI-AA genotype was found to be significantly associated with higher lactose percent and lower milk yield in Baladi cattle.

Conclusions

In the present study, we observed absence of polymorphism in ABCG2 gene for partial intron 13 and exon 14 fragment of 292 bp as well as partial exon 14 fragment of 240 bp alleles at both the loci in Jaffarabadi and Surti buffalo breeds. Both these buffalo breed populations were observed to be fixed for the regions of the ABCG2 gene which reveals substantial conservation of genome organization among the higher vertebrates.

Acknowledgement

Authors are thankful to the authority of the Veterinary College, Navsari for finance and research support, Head of LRS, Head of Animal Biotechnology as well as Ronakbhai Patel (Owner of Patel Dairy Farm) particularly for sample collection and cooperation extended for this work.

References

Ateş, A., Hoştürk, G.T., Akiş, I., Gürsel, F.E., Yardibi, H., & Öztabak, K. (2014). Genotype and allele frequencies of polymorphisms in ABCG2, PPARGC1A and OLR1 genes in indigenous cattle breeds in Turkey. *Acta Veterinaria*, *64*(1), 73-80.

El-Nahas, A.F., Basiony, W.M., El-Kassas, S., & Mahmoud, S. (2018). Variation in the genetic effects of ABCG2, growth hormone and growth hormone receptor gene polymorphisms on milk production traits in Egyptian native, Holstein and hybrid cattle populations. *Pakistan Veterinary Journal*, *38*(4), 371-376.

Farke, C., Meyer, H.H., Bruckmaier, R. M., & Albrecht, C. (2008). Differential expression of ABC transporters and their regulatory genes during lactation and dry period in bovine mammary tissue. *Journal of dairy research*, *75*(4), 406-414.

Ghombavani, M.S., Mahyari, S.A., Rostami, M., Baghenoei, S.G., & Edriss, M.A. (2016). Effect of polymorphisms in the ABCG2, LEPR and SCD1 genes on milk production traits in Holstein cows. *South African Journal of Animal Science, 46*(2), 196-203.

John, S.W., Weitzner, G., Rozen, R., & Seriver, C.R. (1991). A rapid procedure for extracting genomic DNA from leukocytes. *Nucleic acid research, 19*, 408-409.

Kowalewska-Łuczak, I.N.G.A., Kulig, H., & Kmieć, M.A.R.E.K. (2009). Amplification created restriction sites for genotyping SNPs in the bovine ABCG2 and its association with milk production traits (Brief Report). *Archives Animal Breeding*, *52*(6), 647-649.

Sharma, A., Tiwari, M., Singh, S.P., Sharma, D., Kumar, S., Sharma, A., & Verma, A.K. (2016). Study of ABCG2 Gene polymorphism in Sahiwal and Hariana Cattle by PstI/PCR-RFLP Assay. *Journal of Animal Research*, *6*(3), 1-4.

Tantia, M.S., Vijh, R.K., Mishra, B.P., Mishra, B., Kumar, S.B., & Sodhi, M. (2006). DGAT1 and ABCG2 polymorphism in Indian cattle (Bos indicus) and buffalo (*Bubalus bubalis*) breeds. *BMC Veterinary Research*, *2*(1), 1-5.

Thangaraju, P. (2019). Status of buffalo husbandry in India and its role in rural upliftment. In: Compendium of National Conference on *Enhancing Rural livelihood through improved buffalo productivity and health,* held at College of Veterinary Science and Animal Husbandry, NAU, Navsari, Gujarat, 17th-19th January, 2019, pp 19-30.