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Identification of polymorphism in ABCG2 gene in Rathi cattle

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Abstract
In present study, Rathi cattle were selected to analyze *ABCG2* genes for polymorphism. Total 160 lactating animals of Rathi cattle from livestock research station (LRS), Nohar and Bikaner were selected. Blood samples were collected and DNA isolated by kit method. For Exon 7-partial intronic region, Exon 14 and Intron 15 region of *ABCG2* gene primers were constructed based on available sequences of *ABCG2* gene in the NCBI GenBank database. Fragments of 393, 207 and 323-bp of exon 7-partial intronic region, exon 14 and intron 15 region of *ABCG2* gene, respectively were amplified by a polymerase chain reaction in a final reaction volume of 25µl. The PCR-RFLP and PCR-SSCP method was used according to region of *ABCG2* gene. In exon 7-partial intronic region and exon 14 of *ABCG2* gene shows monomorphism. In intron 15 region of *ABCG2* gene two SSCP patterns were observed and it shows polymorphic nature of this region. In intron 15 region of *ABCG2* gene observed two alleles and two genotypes with frequency 0.85, 0.15, 0.70 and 0.30, respectively, were observed. In present study, two regions of *ABCG2* gene observed monomorphic nature and one region, intron 15 region of *ABCG2* gene revealed polymorphic nature.

Keywords: Rathi cattle, *ABCG2* gene, polymorphism and PCR

Introduction
In the world, India holds the first rank in milk production with 22 percent of global production [1]. Total milk production in-country is 187.75 million tonnes which are increased by 6.5% than the previous year. India has great diversity in livestock population and possesses 50 registered, well-recognized breeds of cattle [2]. Native milch cattle breed Rathi has breeding tract in Hanumangarh, Loonkaransar tehsil of Bikaner, Shriganganagar districts of the state [3]. Rathi cattle contribute 0.83 percentage to the total population of indigenous cattle in India[4].Rathi cattle breed is known for its hardiness to withstand the harsh agro-climatic conditions in the arid and semi-arid zone of Rajasthan. Even in dry conditions, Rathi animals have good potential for milk production. Rathi cattle produce good lactation milk yield in the semi-arid region of Rajasthan [5]. Conservation and improvement can be possible by the use of molecular tools in selecting animals for breeding. Milk production-related gene i.e. *ABCG*2 is located on bovine chromosome 6. This is responsible for genetic variation in milk production traits, milk fat and its composition of bovine milk [6, 7] affect absorption and distribution, altering the effectiveness and toxicity of drugs in animals and human [8, 9]. During lactation, in the mammary gland, the *ABCG2* efflux transporter has induced the secretion of different compounds into milk. *ABCG2* gene mapping and characterization of genes controlling important milk performance, production, health and quality traits have become an important field of research in livestock [10, 11]. Therefore, the excellent candidate genes, *ABCG2* gene having a crucial role in milk production and milk composition were included in the present investigation.

Materials and Methods
An overall 160 Rathi animals were selected from Livestock Research Station Nohar (LRS), Hanumangarh and Bikaner (80 animals from each farm). Only milking cows with a minimum of 120 days of lactation were included in the study. After approval of ethical committee, Blood samples were collected aseptically from jugular vein puncture into the anticoagulant EDTA containing vacutainers tube and were transported to Molecular Genetics Laboratory in an

icebox. Genomic DNA from the whole blood sample was extracted through the spin column method as per standard method [12]. Three primers according to the Exon 7, Exon 14 and Intron 15 region of *ABCG2* gene were constructed based on available sequences of *ABCG2* gene in the NCBI GenBank database. The sequences of primers, the accession number of the reference sequence and expected fragment length of the different selected regions are represented in Table 1.

Table 1: Primer sequences and expected fragment sizes of PCR products of selected genomic regions

Selected Region	Primer Sequences	Gen Bank Accession No.	Expected Fragment Length	References
ABCG2 Exon 7 and partial Intronic region	Forward 5'-TAAAGGCAGGAGTAATAAAG-3'	NC007304.4	393	[13]
	Reverse 5'-TAA CAC CAA ACT AAC CGA AG-3'			
ABCG2 Exon 14	Forward 5' CACGAGACTGTCAGGGACTT 3'	AJ871176	207	[14]
	Reverse 5' GGACAATGAACCAGCACGGT 3'			
ABCG2 Intron 15	Forward 5'-TGTATCAGAGCCCCAAATCC-3'	AJ871176	323	This study
	Reverse 5'-AGCCACTGTGACACTGAACG-3'			

PCR reaction mixture (25µl) used for amplification of genomic DNA of different region of *ABCG2* gene used in present study are presented in Table 2.

Table 2: PCR reaction mixture used for amplification of genomic DNA of different region of *ABCG2* gene

S. No.	Content	Volume		
		Exon 7	Exon 14	Intron 15
1.	5X PCR buffer	5µl	5µl	5µl
2.	1.5mM MgCl2	3µl	3µl	2.5µl
3.	10 Mm dNTP's mix	1µl	1µl	1µl
4.	forward primer (70pmol/µl, 80pmol/µl, 70pmol/µl)	1µl	0.75µl	0.75µl
5.	reverse primer 70 pmol/µl (70pmol/µl, 80pmol/µl, 70pmol/µl)	1µl	0.75µl	0.75µl
6.	Genomic DNA 25 ng/µl	4µl	4.5µl	5µl
7.	Taq DNA polymerase 5U/µl	0.2µl	0.3µl	0.3µl
8.	DNAase free water	10.8µl	9.7µl	9.7µl

Amplification was carried out in PCR thermocycler with a program illustrated in Table 3. After the amplification, the PCR products were stored at -20°c for further analysis.

Table 3: PCR programming for amplification of regions of *ABCG2* gene

Steps / parameters	Initial denaturation	Cycle Denaturation	Annealing			Synthesis	Final extension	Hold
			Exon 7	Exon 14	Intron 15			
Temperature	95 ºC	95 ºC	54 ºC	59	57	72 ºC	72 ºC	4 ºC
Time	4min.	45 sec	45 sec			1min	10min	5 min
No. of Cycle	1 cycle	35 cycles					1cycle	1 cycle

The quality and size of the PCR amplicons for different studied locus were assessed on 1.5% agarose gel containing ethidium bromide (1% solution) by electrophoresis method. The genetic variation in the selected genomic regions of *ABCG2* gene was identified through two different approaches RFLP and SSCP methods.

The digestion of the amplified fragment of *ABCG2* exon-14 region was carried out through *Hinf* I restriction enzyme. Restriction digestion of the amplified products of exon 14 of the *ABCG2* gene was carried out separately in a 30 µl reaction mixture containing 10X buffer (2µl), amplified product (10µl), *Hinf* I 10 units (1µl) and nuclease-free water (17 µl). Uniform mixing of the reaction mixture was assured through the spinning of samples for a few seconds before incubation at 37ºC for 6 hr in a water bath. The separation of the restricted products was carried out on 8% polyacrylamide gel at 120 V to obtain a clear picture of the digested samples. The polyacrylamide gel after 2/3 migration of the digested sample, was stained in a gel tray containing 1% ethidium bromide solution. The digested bands were visualized under UV light and documented by a gel documentation system.

Aliquots of 5µL PCR products were mixed with 5µL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene cyanole and 0.025% bromophenol blue), denatured for 10 min at 95 °C followed by a rapid chill on ice for 10 min. Denatured PCR products were subjected to 8% polyacrylamide gel electrophoresis in Tris-Borate-EDTA buffer and constant voltage (120 V) for 15 h at a constant temperature of 4 °C, and then gels were stained with 1% ethidium bromide solution and visualized with under UV light and documented by gel documentation system. Individual genotypes were defined according to band patterns. The frequencies of different electrophoretic patterns were recorded under each group.

The genetic structure of the studied population at two locations (LRS, Nohar and Bikaner) for gene and genotypic frequencies, observed heterozygosity (Ho), expected heterozygosity (HE) and expected unbiased heterozygosity (HE unbiased), an effective number of alleles and Nei's genetic distances were analyzed through POPGENE program (version 3.1) [15].

Results and Discussion

Variation in regions of *ABCG2* gene

Single SSCP bands pattern ('AA') was observed for the 393 fragment of exon-7-partial intronic region of the *ABCG2* gene in Rathi cattle (Fig 1). Thus monomorphism was observed in all studied animals for this region. Three genotypes for exon 7 of the *ABCG2* gene were observed that dissimilarity in Chinese Holstein cows with present study [16].

The PCR-RFLP analysis of the 207-bp exon 14 of *ABCG2* gene PCR product with Hinf I enzyme resulted in three restriction fragments of 207, 151 and 56 bp for the AC fragment (Fig. 2). The present study revealed the presence of single genotypic SSCP pattern. Similar results were reported in Modicana cow [18], in German dairy cow [18] and indigenous cattle of Turley [19].

The PCR-SSCP analysis of the 323-bp product of the *ABCG2* gene in the Rathi breed revealed the presence of two unique patterns reflecting their respective SSCP genotypic pattern (Fig. 3). The SSCP pattern resolved for animals of Rathi cattle was considered as AA to AB.

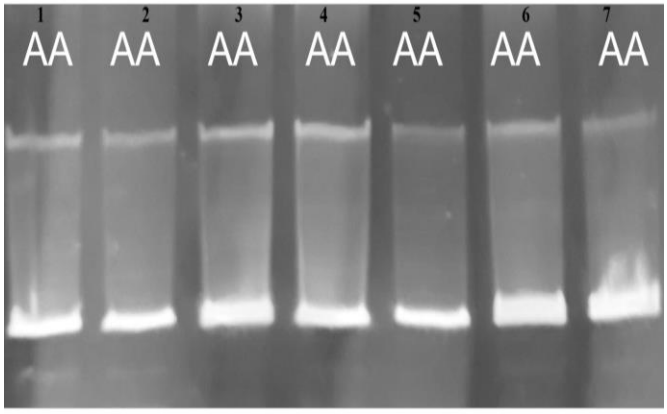

Fig 1: PCR-SSCP patterns of exon 7 of *ABCG2* gene in Rathi cattle Lane 1-7: AA genotype

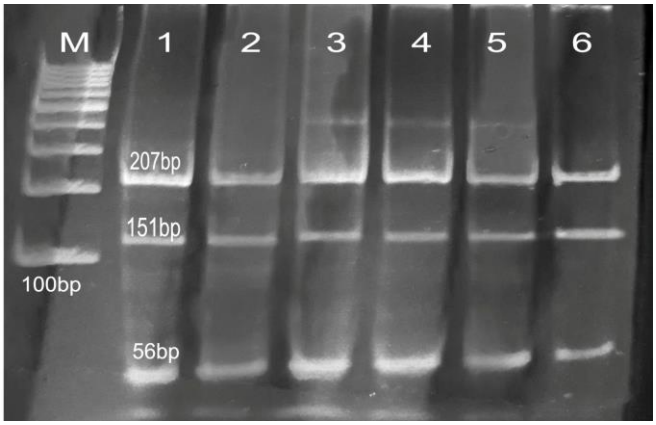

Fig 2: PCR-RFLP PATTERNS OF exon 14 of *ABCG2* gene in Rathi cattle Lane M: Marker (100 bp) Lane 1-6: AB genotype (207bp, 151 bp, 56 bp)

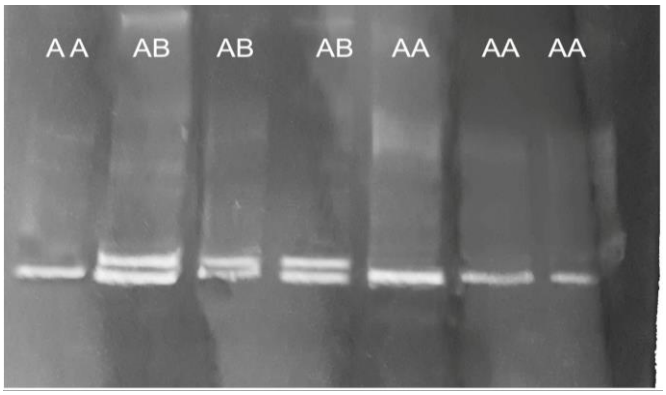

Fig 3: PCR-SSCP patterns of intron 15 of *ABCG2* gene in Rathi cattle Lane 1-7: AB genotype

Gene and genotypic frequency of regions of *ABCG2* gene

In our study, all animals at both locations have the same single SSCP pattern in exon-7 and exon 14 of *ABCG2* gene and monomorphism observed in these regions. As per available literature, in Chinese Holstein cows identified three genotypes for exon 7 of the *ABCG2* gene [17]. As per available literature, a study show dissimilarities with the present work, in two population SAR and EAR cattle [19], in Chinese Holstein cattle [18, 20] identified polymorphism and found A and B allele and three genotypes AA, AB and BB for exon-7 of *ABCG2*.

In White Fulani and Muturu cattle breeds [21], in Sahiwal and Hariana cattle breed [22], in Dutch Holstein–Friesian [23], also observed similar results with our present study and reported monomorphism for exon-14 of *ABCG2*. Some workers show dissimilarities with present work, in SAR and EAR cattle [19].

The group-wise gene and genotypic frequency observed for SSCP genotypic patterns of intron 15 of *ABCG2* gene are represented in Table 4.

Table 4: Gene and genotypic frequencies of intron 15 of *ABCG2* gene detected through SSCP analysis

Group	N	Genotypic Pattern		Gene frequency	
		AA	AB	A	B
1	80	0.55 (44)	0.45 (36)	0.775	0.225
2	80	0.85 (68)	0.15 (12)	0.925	0.075
Overall	160	0.7 (112)	0.3 (48)	0.85	0.15

Note: group 1=LRS, Nohar; group 2 =LRS, Bikaner, Number in parenthesis are number of observations

The overall studied population of Rathi cattle revealed non-significant deviation from Hardy-Weinberg equilibrium (p 0.05) that indicates that animals similar in their genotypic distribution for gene frequency. However, in group 2 Chi square and G square test value observed for the *ABCG2* intron 15 gene showed significant deviation from Hardy-Weinberg equilibrium (p 0.05) that showed that animals differ in their genotypic distribution concerning gene frequency. The results indicate the presence of sufficient genetic variation at the intron 15 locus though in the form of heterozygote combination (Table 5).

Table 5: Within-population heterozygosity estimates, PIC and FIS values of Rathi variant of intron 15 of *ABCG2* gene

Group	Sample size (N)*	Observed Heterozygosity (H_o)	Expected Heterozygosity (H_E)	Nei's unbiased Heterozygosity (H_e)	PIC	Fixation index (F_{IS})
1	80	0.1500	0.1396	0.1387	0.2437	-0.0811
2	80	0.4500	0.3509	0.3487	0.2437	-0.2903
Overall	160	0.3000	0.2558	0.2550	0.2437	-0.1765

The Shannon index for LRS, Nohar cattle was observed lowest among all studied animals with a value of 0.2664 (Table 5). Shannon index indicate the heterozygosity in LRS, Bikaner cattle, higher index value was recorded.

Table 6: Observed and effective number of alleles; and Shannon information index for variants of intron 15 of *ABCG2* gene

Group	Sample size of alleles	Observed number of alleles	Effective numbers of alleles	Shannon's Information Index
1	160	2.0000	1.1611	0.2664
2	160	2.0000	1.5355	0.5332
Overall	320	2.0000	1.3423	0.4227

Conclusion

The present study concluded polymorphic nature of intron 15 region of *ABCG2* gene and monomorphic nature of exon 7-partial intronic region, exon 14 of *ABCG2* gene in Rathi cattle of studied cattle.

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References

1. Anonymous. Dairy production and products: production, Food and agriculture organization of United Nations 2019. (http://www.fao.org/dairy-production-products/production/en/).
2. NBAGR National Bureau of Animal Genetic Resources, Karnal, Haryana 2020.
3. Anonymous Annual Report, Department of Animal Husbandry, Government of Rajasthan 2018. (http://animalhusbandry.rajasthan.gov.in/)
4. Breed Survey. Ministry of Agriculture and Farmers Welfare, Department of Animal Husbandry, Dairying and Fisheries, Government of India. Krishi Bhawan, New Delhi 2013.
5. Dhaka CS, Kachwaha RN, Bais B. Performance of Rathi Cattle under organized farm management conditions. Research and review: journal of veterinary science 2015;2(1):8-10.
6. Bouwman AC, Bovenhuis H, Visker MH, van Arendonk JA. Genome-wide association of milk fatty acids in Dutch dairy cattle. BMC genetics 2011;12(1):43.
7. Leonard S, Khatib H, Schutzkus V, Chang YM, Maltecca C. Effects of the osteopontin gene variants on milk production traits in dairy cattle. Journal of Dairy Science 2005;88(11):4083-4086.
8. Sarkadi B, Özvegy-Laczka C, Német K, Váradi A. ABCG2–a transporter for all seasons. FEBS letters 2004;567(1):116-120.
9. Jonker JW, Merino G, Musters S, van Herwaarden AE, Wagenaar E, Mesman E *et al.* The breast cancer resistance protein BCRP (ABCG2) concentrates drugs and carcinogenic xenotoxins into the milk. Nature Medicine 2005;11:127-129.
10. Weikard R, Widmann P, Buitkamp J, Emmerling R, Kuehn C. Revisiting the quantitative trait loci for milk production traits on BTA6. Animal genetics 2012;43(3):318-323.
11. Olsen HG, Lien S, Gautier M, Nilsen H, Roseth A, Berg PR *et al.* T. THE: Mapping of a milk production quantitative trait locus to a 420-kb region on bovine chromosome 6. Genetics 2005;169:275-283.
12. Sambrook J, Russell DW. *Molecular cloning:* A laboratory manual. 3rd Edition. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press 2001
13. Alim MA, Xie Y, Fan Y, Wu X, Sun D, Zhang Y *et al.* Genetic effects of *ABCG2* polymorphism on milk production traits in the Chinese Holstein cattle. Journal of Applied Animal Research 2013;41(3):333-338.
14. Kowalewska-Łuczak INGA, Kulig H, Kmieć MAREK. Amplification created restriction sites for genotyping SNPs in the bovine ABCG2 and its association with milk production traits (Brief Report). Archives Animal Breeding 2009;52(6):647-649.
15. Yeh FC. Population genetic analysis of co-dominant and dominant marker and quantitative traits. Belgian journal of Botony 1997;130:129-157.
16. Li C, Sun D, Zhang S, Yang S, Alim MA, Zhang Q *et al.* Genetic effects of FASN, PPARGC1A, ABCG2 and IGF1 revealing the association with milk fatty acids in a Chinese Holstein cattle population based on a post genome-wide association study. BMC genetics. 2016;17(1):110.
17. Valenti B, Criscione A, Moltisanti V, Bordonaro S, De Angelis A, Marletta D *et al.* Genetic polymorphisms at candidate genes affecting fat content and fatty acid composition in Modicana cows: Effects on milk production traits in different feeding systems. *Animal*. 2019;13(6):1332-1340.
18. Mömke S, Brade W, Distl O. Co-segregation of quantitative trait loci (QTL) for milk production traits and length of productive life with QTL for left-sided displacement of the abomasum in German Holstein dairy cows. Livestock science. 2011;140(1-3):149-154.
19. Ateş A, Hoştürk GT, Akiş I, Gürsel FE, Yardibi H, Öztabak K. Genotype and allele frequencies of polymorphisms in ABCG2, PPARGC1A and OLR1 genes in indigenous cattle breeds in Turkey. Acta veterinaria. 2014;64(1):73-80.
20. Yue W, Fang X, Zhang C, Pang Y, Xu H, Gu C *et al.* Two novel SNPs of the ABCG2 gene and its associations with milk traits in Chinese Holsteins. Molecular biology reports 2011;38(5):2927-2932.
21. Ahmed RO, Bello SF, Adeyemi MO, Bolaji UFO. Genetic Polymorphism of ABCG2 and DGAT1 Genes in White Fulani and Muturu cattle breeds. *International* Journal of Agriculture, Environment and Bioresearch 2020;5(2):118-127.
22. Sharma A, Tiwari M, Singh SP, Sharma D, Kumar S, Sharma A *et al.* Study of ABCG2 gene polymorphism in Sahiwal and Hariana Cattle by PstI/PCR-RFLP assay. Journal of Animal Research 2016;6(3):475-477.
23. Schennink A, Bovenhuis H, Léon-Kloosterziel KM, Van Arendonk JA, Visker MH. Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. Animal genetics 2009;40(6):909-916.