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Growth hormone gene polymorphism in Jaisalmeri and Sindhi camels

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ABSTRACT

The present study was aimed to assess the genetic polymorphism of growth hormone (GH) gene in Jaisalmeri and Sindhi camels using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique. Genomic DNA were isolated from venous blood samples collected from 93 Indian camels (Jaisalmeri-38 and Sindhi-55 camels) and 613-base pair fragment of GH gene was amplified from each genomic DNA sample by polymerase chain reaction. The PCR-RFLP was done using restriction enzyme MspI which revealed three genotypes, viz. CC, CT and TT in both the breeds. Our result indicated that both the breeds were polymorphic in nature. The corresponding genotype frequency in Jaisalmeri and Sindhi breeds were 0.474 and 0.345 for the CC, 0.395 and 0.564 for CT, 0.131 and 0.091 for TT genotypes and the corresponding C allele frequency was 0.671 and 0.627 and T allele frequency was 0.329 and 0.373. The CT genotype was found predominant in the Sindhi breed, whereas CC genotype was predominant in Jaisalmeri breed. The frequency of C allele was higher than the T allele in both of the breeds. These camel populations were in Hardy-Weinberg Equilibrium indicating that the populations meet the HWE assumptions and genetic variations were conserved. It could be concluded that Jaisalmeri and Sindhi camels having different genetic variants of GH gene might be investigated for production a DNA marker for growth and production traits in Indian camels.

Keywords: Camel, Gene, Growth hormone, Jaisalmeri, PCR-RFLP, Polymorphism, Sindhi

Camels are integral part of rural livelihoods in arid and semi-arid regions of India having multifaceted utility providing draught power, transport, milk and wool. The population of the camel in Rajasthan, and in India as a whole is experiencing a steep decline. The total camel population in the country was 2.5 Lakhs in 2019, which is 37.1% lower as compared to previous census (19th Livestock Census 2012). In this era of climate change, camels can perform better due to their adaptability to heat stress and drought like conditions unlike other livestock species. Genetic variability in indigenous breed is a major concern considering the necessity of preserving what may be a precious and irreplaceable richness, regarding new productive demands (Bastos *et al*. 2001). Researchers have earlier described the phenotypic characteristics of different camel breeds (Rathore *et al.* 1986). Candidate genes affecting growth and production traits may be studied in Indian camels for identifying genetic and phenotypic variants affecting the gene functions in order to increase their production potential.

Improvement of camel productivity and detection of biodiversity in different camel breeds has necessitated the genotyping of the productivity trait genes in these breeds (Abdel-Aziem *et al.* 2015). The growth hormone (GH) gene can act as a candidate gene for marker assisted selective (MAS) breeding in indigenous camels after possible association with the growth and production traits. Several studies have reported about the GH gene polymorphism and its influence on growth rate and milk production traits in different livestock species (Krenkova *et al.* 1999, Unanian *et al.* 2000, Hua *et al.* 2009, Kumari *et al.* 2014). Only few studies have been carried out to identify the GH gene variants in camel breeds found in Asian and African countries (Shah, 2006, Ishag *et al.* 2010, Afifi *et al.* 2014, Abdel-Aziem *et al.* 2015, Shawki *et al.* 2015, EL-Kholy *et al.* 2016). However, no such information is available for Indian camels.

Role of growth hormone (GH) gene as a candidate gene in controlling growth of farm animals has been widely studied. Growth hormone axis governs the important functions related with regulation of metabolism, lactation and reproduction in farm animals. Growth hormone gene codes a 22 KDa single chain polypeptide protein primarily produced and secreted by the somatotrophs of the anterior pituitary gland in circadian and pulsatile manner (Dybus *et al.* 2002). Camel growth hormone gene is of size 1.9 kb, and found similar with other mammalian species consisting of 5 exons and 4 introns (Maniou *et al*. 2003).

The PCR-RFLP technique is one among the several methods used for genotyping of specific polymorphic loci. This method involves the amplification of a DNA segment

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using PCR followed by digestion with respective restriction enzymes and analysis of the digested fragments by gel electrophoresis. Among several genetic markers available, RFLP is advantageous in being a fast, simple and accurate molecular tool for the profiling and identification of population (Marty *et al*. 2012). Sindhi is a unique camel population found in the areas of Jaisalmer and Barmer which is closely situated to border of Pakistan. Their population is clearly separated from other registered breeds of Indian camel revealing its separate gene pool (Sharma *et al.* 2020). The breeding tract of Jaisalmeri camels encompasses the Jaisalmer, Barmer and parts of Jodhpur districts in Rajasthan (Mehta *et al.* 2006). The camels of Jaisalmeri and Sindhi breed are adapted to the climatic conditions of the Thar desert where the temperature goes very high in summer and very low in winter. Considering the important contribution of these two breeds to desert agro-ecosystem, our study aimed to investigate the genetic polymorphism at GH locus in Jaisalmeri and Sindhi camels using PCR-RFLP method.

MATERIALS AND METHODS

Experimental animals and samples preparation: Blood samples of 55 Sindhi camels were collected from camel keepers of Barmer and Jaisalmer districts of Rajasthan and 38 Jaisalmeri camel blood samples from ICAR-NRCC, camel farm. DNA was extracted from white blood cells using standard phenol/chloroform/isoamyl alcohol extraction protocol (Sambrook *et al.* 1989). PCR amplification of 613-bp fragment of GH gene were done using the forward primers sequences (5' GTCCTGTGGACAGCTCAC3') and reverse primers sequences (5' TGTCCTCCTCACTGCTTTA3') as reported by Abdel-Aziem *et al.* (2015). Custom DNA oligonucleotide primers in tubes were synthesized in 0.01 mM concentration by Eurofins genomics. The PCR reaction was carried out in 25µl of total volume, containing ready to use GO taq PCR master mix –12.5 µl (Promega), 1 µl of each primer with concentration of 10 pM, 1 µl of 80–100 ng camel genomic DNA and nuclease free water to make total volume up to 25 µl. Amplification was performed in Mastercycler® Gradient (Eppendorf AG, Hamburg, Germany) programmed for initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 57°C for 45 sec, extension at 72°C for 45 sec, and final extension at 72°C for 10 min. PCR products were checked for amplification by electrophoresis on 1.5% agarose gel (Sigma-aldrich, Life Science), in parallel with 100 bp DNA marker (Thermo scientific).

Investigating genetic polymorphs: Amplified PCR fragment was digested using restriction endonuclease MspI. Each PCR products were digested separately in a total volume of 30 µl with 8 µl PCR product, 1.5µl restriction enzyme MspI, 2 µl of 10× buffer and 18.5 µl nuclease free water (Promega). The tube containing the reaction mixture was incubated at 37º C for overnight. The digested products were resolved by electrophoresis on 2.5% agarose gel at a constant voltage of 80 V for 90 to 120 min in 0.5× TBE buffer. The digested products were run in parallel with a 50-bp DNA marker (Thermo Scientific). After electrophoresis, the genotype patterns were visualized in UV transilluminator after staining with ethidium bromide. The genotypes were denoted based on the size and number of DNA bands resolved during electrophoresis of digested PCR products and the genotypic and allelic frequencies were calculated for each camel breed.

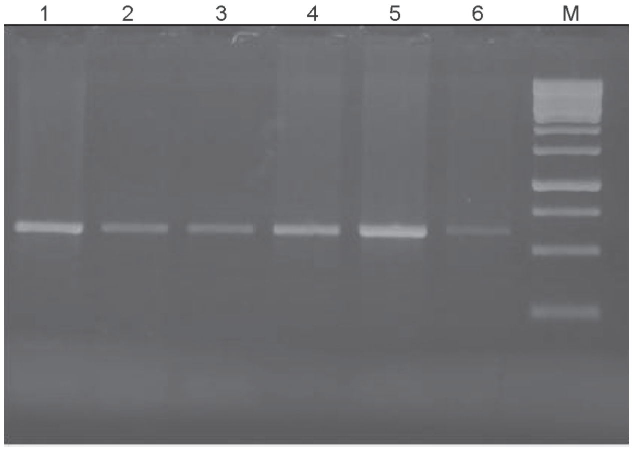

Fig. 1. PCR amplification of GH gene in camels resolved on 2.5% agarose gel. M, 1kb marker; lanes 1–6, 613 bp PCR product.

Statistical analysis: The genotype and allele frequencies were estimated by standard procedure (Falconer and Mackey, 1998). Chi-square (χ^2) test was carried out to estimate whether the distribution of the genotype frequencies was in the Hardy Weinberg equilibrium or not.

RESULTS AND DISCUSSION

Growth hormone gene fragment consisting of 613 bp size spanning partial exon-1 (36 bp), intron-1 (243 bp), exon-2 (161 bp), and partial intron-2 (173 bp), were successfully amplified in 93 camels of two breeds (Fig. 1). The restriction digestion of the amplified 613 base GH gene fragment using restriction endonuclease MspI detected restriction site at position C264T in the intron-1 region of the GH gene. Depending upon the presence and absence of restriction site (C^CGG) at position 264^265, three genotypes were identified by PCR-RFLP. On visualising the digested PCR product under UV-transilluminator, CC genotype was identified as two digested fragment of 349 and 264 bp, TT genotype as one undigested fragment of 613 bp and CT genotype as three fragments of 613, 349 and 264 bp (Fig. 2). Our result was in the line of the earlier reports of a SNP in the non-coding region (Intron-1) at same position in different camel breeds (Ishag *et al.* 2010, Afifi *et al.* 2014, Abdel-Aziem *et al.* 2015). Daverio *et al.* (2012) studied the organization of the GH gene sequence, its promoter region and investigated SNPs in the llama (Lama glama) depicting that introns were less conserved. Some introns also regulate gene expression (Yang *et al*. 2013). Moreover, introns play a pivotal role in mRNA export, transcription coupling, splicing, etc. (Maniatis *et al*. 2002).

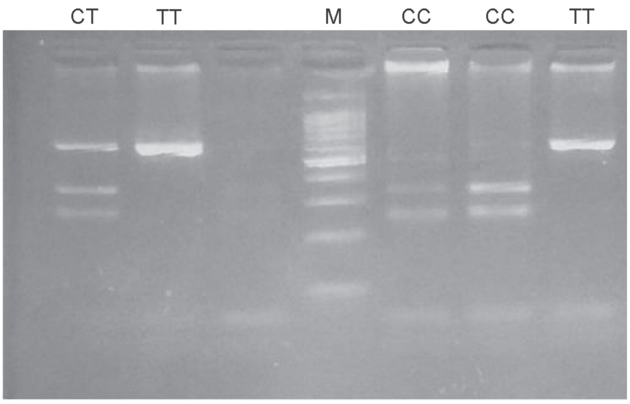

Fig. 2. PCR-RFLP of GH gene resolved on 3% agarose gel electrophoresis. Lane 1 represents CT (3 band); lane 2, 7 represents TT (single band) and Lane 5, 6 represents CC genotype (2 band). Lane M, 100 bp ladder.

Table 1. Genotype and allele frequency of the GH gene fragment for MspI site in Jaisalmeri and Sindhi camels

Breed	Genotype frequency				Allele frequency		
	N	CC	CT	TT	C	T	χ^2
Jaisalmeri	38	0.474 (18)	0.395 (15)	0.131 (5)	0.671	0.329	0.426
Sindhi	55	0.345 (19)	0.564 (31)	0.091 (5)	0.627	0.373	2.319
Mean	93	0.41	0.479	0.111	0.649	0.351	

The polymorphisms as observed in intronic region of the growth hormone gene may affect the expression of the gene. Intronic regions of GH gene also showed high level of polymorphism in pig breeds (Ashok *et al*. 2014).

The allele and genotype frequencies for GH gene in Jaisalmeri and Sindhi camels are presented in Table 1. The corresponding mean allelic frequencies detected in Jaisalmeri and Sindhi Camels were 0.649 (C) and 0.351 (T) and the genotype frequencies were 0.410 (CC), 0.479 (CT) and 0.111 (TT). In Jaisalmeri breed, the allele frequency was 0.329 for T and 0.671 for C, and genotypes frequency was 0.474 (CC), 0.395 (CT), and 0.131 (TT). In Sindhi camel, allele frequency was 0.373 (T) and 0.627 (C), and genotype frequency was 0.345 (CC), 0.564 (CT), and 0.091 (TT). The study revealed that the heterozygote genotype CT was more predominant in the Sindhi camel population, whereas CC genotype was predominant in Jaisalmeri camel population. In both of the breeds, TT genotype was least abundant. The allele frequency of C allele was found higher in both the breeds. Both the populations were in Hardy-Weinberg equilibrium as the observed frequencies and expected frequencies conform to Hardy-Weinberg expectations.

The breed wise differences observed in genotype and allele frequencies was in accordance with the earlier reports in camels (Table 2). The frequency of CC genotype was higher in Jasialmeri and Sindhi camel as compared to majority of the previous reports (Table 2) except for reports in Bishari camel (Ishag *et al.* 2010) and Maghrabi Camel (Abdel-Aziem *et al.* 2015). The frequency of heterozygote genotypes CT observed in our study was comparable to many of the earlier reported breeds (Table 2). However, Ishag *et al.* (2010) reported lower CT frequency in Kennani and Lawhee camel. Abdel-Aziem *et al.* (2015) also reported same findings in Somali camel. Abdel-Aziem *et al.* (2015) reported higher heterozygote frequency in Sudany camel as compared to this study. In contrast to previous reports, frequency of TT genotype in our study was lower (Table 2) except for reports in Maghrabi camel (Abdel-Aziem *et al.* 2015) in which absence of TT genotype was reported. Higher frequency of C allele in both Jaisalmeri and Sindhi breed was in agreement with earlier reports in which higher frequency for C allele has been reported (Table 2) except Bishari breed (Ishag *et al.* 2010) and Maghrabi and Sudany camel (Abdel-Aziem *et al.* 2015).

It could be concluded that the growth hormone (GH) gene is polymorphic in Jaisalmeri and Sindhi camel breeds and their populations is being maintained in Hardy-Weinberg Equilibrium. This study may provide basic

Table 2. Reported frequency distribution for MspI RE site polymorphism of GH gene fragment

Breed	N	Country	CC	CT	TT	T	C	Reference
Saheli	200	Saudi Arabia	0.29	0.50	0.21	0.46	0.54	Afifi *et al.* 2014
Majaheem	200	Saudi Arabia	0.28	0.50	0.22	0.47	0.53	
Homor	200	Saudi Arabia	0.28	0.50	0.22	0.47	0.53	
Waddah	200	Saudi Arabia	0.32	0.49	0.19	0.44	0.56	
Kenani	31	Sudan	0.19	0.26	0.55	0.32	0.68	Ishag *et al.* 2010
Rashaidi	30	Sudan	0.10	0.47	0.43	0.33	0.67	
Lahwee	30	Sudan	0.17	0.27	0.57	0.30	0.70	
Anafi	30	Sudan	0.10	0.37	0.33	0.48	0.52	
Bishari	30	Sudan	0.37	0.40	0.23	0.57	0.43	
Kabbashi	30	Sudan	0.13	0.40	0.47	0.33	0.67	
Fallahy	15	Egypt	0.10	0.40	0.40	0.40	0.60	Abdel-Aziem *et al.* 2015
Maghrabi	15	Egypt	0.50	0.50	0.00	0.75	0.25	
Mowaled	15	Egypt	0.10	0.40	0.50	0.30	0.70	
Somali	15	Egypt	0.09	0.18	0.73	0.18	0.82	
Sudany	15	Egypt	0.23	0.62	0.15	0.54	0.46	

information on existence of genetic variation in GH gene of camels. The different genotypes observed may be associated with growth and production traits in camels. Further investigations could be carried out to define the relationship between the GH gene variants and traits related to growth and development in camels. To note, our study may be first attempt to identify the genetic variants of growth hormone gene in Indian camel.

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REFERENCES

19th Livestock Census. All India Report. 2012. Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India, Krishi Bhavan, New Delhi.

Abdel-Aziem S H, Abdel-Kader H A M, Alam S S and Othman O E. 2015. Detection of MspI polymorphism and the single nucleotide polymorphism (SNP) of GH gene in camel breeds reared in Egypt. *African Journal of Biotechnology* **14**: 752–57.

Afifi M, Metwali E M and Brooks P H 2014. Association between growth hormone single nucleotide polymorphism and body weight in four saudi camel (*Camelus dromedarius*) breeds. *Pakistan Veterinary Journal* **34**(4): 494–98.

Ashok S K, Kuwabara, Y, Abe S and Morita E. 2014. Polymorphism in growth hormone gene sequence from microminipig (MMP) with direct Sequencing PCR. *International Journal of Scientific and Technology Research* **3**: 83–92.

Bastos E, Cravador A, Azevedo J and Guedes-Pinto H. 2001. Single strand conformation polymorphism (SSCP) detection in six genes in the Portuguese indigenous sheep breed "Churra da Terra Quente". *Biotechnology, Agronomy, Society and Environment* **5**(1): 7–15.

Daverio S, Di Rocco F and Vidal Rioja L. 2012. The Llama (Lama glama) growth hormone gene: sequence, organization and SNP identification. *Small Ruminant Research* **103**(2–3): 108–11.

Dybus A. 2002. Associations of growth hormone (GH) and prolactin (PRL) genes polymorphisms with milk production traits in polish black-and-white cattle. *Animal Science Papers and Reports* **20**: 203–12.

EL-Kholy A F, Zayed M A, Shehata M F, Salem M A I, El-Bahrawy K A, El-Halawany N and Hassanane M S. 2016. Association of single nucleotide polymorphisms for myogenic factor 5 and growth hormone genes with meat yield and quality traits in one humped camel (*Camelus dromedarius*). *Asian Journal of Animal and Veterinary Advances* **11**: 263–71.

Falconar D S and Mackay T F C. 1998. Introduction to Quantitative Genetics. 4th ed, Addison Wesley Longman Ltd, Essex, England, pp.1–2.

Hua G H, Chen S L, Yu J N, Cai K L, Wu C J, Li Q L, Zhang C Y, Liang A X, Han L, Geng L Y, Shen Z, Xu D Q, and Yang L G. 2009. Polymorphism of the growth hormone gene and its association with growth traits in Boer goat bucks. *Meat Science* **81**(2): 391–95.

Ishag I A, Reissmann M, Peters K J, Musa L M and Ahmed M K. 2010. Phenotypic and molecular characterization of six Sudanese camel breeds. *South African Journal of Animal Science* **40**(4): 319–26.

Krenkova L, Kuciel J and Urban T. 1999. Association of the RYR1, GH, LEP and TF genes with carcass and meat quality traits in pigs. *Czech Journal of Animal Science* **44**(11): 481–86.

Kumari R, Kumar R, Meena A S, Jyotsana B, Prince L L L and Kumar S. 2014. Genetic polymorphism of growth hormone gene in native sheep breeds of India. *Indian Journal of Small Ruminants* **20**: 15–18.

Maniatis T and Reed R. 2002. An extensive network of coupling among gene expression machines. *Nature* **416**: 499–506.

Maniou Z. 2003. Molecular evaluation of pituitary growth hormone (AJ575419). PhD Thesis, Biological Sciences, University of Sussex, Brighton, UK.

Marty E, Buchs J, Eugster-Meier E, Lacroix C and Meile L. 2012. Identification of staphylococci and dominant lactic acid bacteria in spontaneously fermented Swiss meat products using PCR–RFLP. *Food Microbiology* **29** (2): 157–66.

Mehta S C, Mishra B P and Sahani M S. 2006. Genetic differentiation of Indian camel (*Camelus Dromedarius*) breeds using random oligonucleotide primers. *Animal Genetic Resources Information* **39**: 77–88.

Moradian C, Mohamadi N, Sheshdeh S A R, Hajihosseinlo A and Ashrafi F. 2013. Effects of genetic polymorphism at the growth hormone gene on growth traits in Makooei sheep. *European Journal of Experimental Biology* **3**(3): 101–05.

Rathore G S. 1986. *Camels and their Management*. ICAR Publication, New Delhi, India

Sambrook J, Fritsch E R and Maniatis T. 1989. Molecular cloning: A laboratory manual. 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Shah M G. 2006. Differentiation of six Pakistani camel breed by phenotype and molecular genetics analysis. PhD thesis, University of Agriculture, Faisalabad, Pakistan.

Sharma R, Ahlawat S, Sharma H, Prakash V, Shilpa, Khatak S, Sawal R K and Tantia M S. 2020. Identification of a new Indian camel germplasm by microsatellite markers based genetic diversity and population structure of three camel populations. *Saudi Journal of Biological Sciences* **27**(7): 1699–1707.

Shawki I, Mourad M, Rashed M A and Ismail I M. 2015. Molecular characterization of camel growth hormone gene in Maghraby camel breed. *Animal Science Reporter* **9**: 50–55.

Unanian M M, Barreto C C, Freitas A R D and Cordeiro C M T. and Josahkian L A. 2000. Associations between growth hormone gene polymorphism and weight traits in Nellore bovines. *Revista. Brasiliera de Zootecnia* **29**: 1380–86.

Yang, Y F, Zhu, T, Niu, D K. 2013. Association of intron loss with high mutation rate in Arabidopsis: implications for genome size evolution. Genome *Biology and Evolution* **5**(4): 723–33.