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MOLECULAR CHARACTERISATION OF GROWTH HORMONE (GH) GENE IN INDIAN DROMEDARY AND BACTRIAN CAMEL

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ABSTRACT

Molecular characterisation of 613 bp long growth hormone (GH) gene fragment spanning partial exon-1, intron-1, exon-2 and partial intron-2 was done in Indian (one humped) dromedary and Indian (double humped) bactrian camel through PCR amplification, sequencing and bioinformatics analysis. The sequence variations within and between single and double humped Indian camels were observed. In the Indian single humped camels of Mewari, Kachchhi and Bikaneri breed at position 264 C>T variation was seen. At this particular locus animals with single and double peaks in the sequence chromatograms were observed. Accordingly, two allele (C, T) and three genotype (CC, CT, and TT) were identified in the Indian dromedary camels. In Indian Bactrian camel at position 264 only C allele and CC genotype was identified. In double humped camel at position 242, A>G and at position 469 G>A transition variation was observed compared to single humped camel. The different sequences obtained were submitted to NCBI and Gen Bank Accession No. MT478653 for C allele Indian Dromedary, MT478654 for T allele Indian Dromedary and MT478655 for Indian Bactrian camel were obtained. Similarity ranging from 98% to 100 % was observed with available GH Sequences of camel at GenBank. Camel sequence was found to have close similarity with other Camelidae family members like *Lama pacos* (97.07%) and *Lama glama* (96.58%). The evolutionary relationship between sequences showed close relationship between dromedary and bactrian camel followed by vicugna and llama. The domesticated species like cattle, buffalo, sheep, goat, yak and mithun were distantly related to camel. The present study showed close similarity between GH gene sequence pattern of Indian one and double humped camel except transition variation at two positions in bactrian camel.

Key words: Bactrian, camel, dromedary, growth hormone (GH) gene, sequence

The efforts to improve the productivity of camels can be accelerated by supplementing the conventional genetic improvement programmes by molecular genetic techniques. Camel presents a unique case where success of conventional breeding methods and constrained by the biological and economical limitations on the applying desired selection intensity, as a result of which, response is affected, so is the selection process. However, with the advancement in molecular genetics technology, the identification of molecular markers and their subsequent utilisation in the breeding programme has become possible. To maximise the benefits of the camel production potential, improved understanding of the genetics underlying their unique biology is needed. Till date, there are relatively few published studies in the area of camel genetics and genomics. The candidate gene strategy involves study of genes that are supposed to be responsible for a considerable amount of the genetic variation for the traits of interest based on their known physiological function (Moioli *et al,* 2007). In farm animals, promising candidate genes for many traits are located in the growth hormone axis. The growth hormone is a polypeptide hormone with diverse biological activities. It is necessary to select genotypes with high growth and meat quality for more contribution of camels to the agricultural economy (Ramadan and Inoue-Murayama, 2017). Growth hormone (GH) is an anabolic hormone which plays an important role in postnatal longitudinal growth, tissue growth, lactation, reproduction as well as protein, lipid and carbohydrate metabolism (Dybus, 2002; Daverio *et al,* 2012). Among the several candidate genes, growth hormone (GH) gene structure and its role in farm animals like cattle, buffalo, sheep and goat production is widely studied. But only few reports on growth hormone gene in camels are available (Maniou *et al,* 2001; Shah, 2006; Ishag *et al,* 2010; Afifi *et al,* 2014; Abdel Aziem *et al,* 2015' Shawki *et al,* 2015;

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El-Kholy *et al,* 2016). The camel growth hormone (GH) gene extends over about 1900 bp, and like other mammalian GH genes; it splits into 5 exons and 4 introns (Maniou *et al,* 2001). The molecular structure of GH gene in Indian dromedary and bactrian camels are not available. Hence, the present study was undertaken to characterise the growth hormone (GH) gene in Indian dromedary and bactrian camel.

Materials and Methods

Blood samples were collected from 5 camels each from Bikaneri, Mewari, Kachchhi breeds maintained at ICAR-National Research Centre on Camel Farm at Bikaner and 5 double humped camels from farmer's herd at Nubra valley, Ladakh in 10 ml vacutainer tubes containing EDTA. The DNA was extracted from blood cells using standard phenol-chloroform extraction protocol (Sambrook *et al,* 1989). PCR amplification of 613 bp GH gene fragment was done utilising primers reported by Abdel Aziem *et al* (2015). The amplified GH gene fragment covered partial exon-1, intron-1, exon-2 and partial intron-2 Primers were synthesised from Eurofins genomics. The PCR reaction was carried out in 25µl of total volume, containing ready to use Go taq Green master mix-12.5 µl (Promega, USA), 1µl of each primer with concentration of 10 pM, 1µl of 80-100 ng camel genomic DNA and nuclease free water (Promega, USA) to make total volume up to 25µl. 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 s, annealing at 57°C for 45 s, extension at 72°C for 45 s, and final extension at 72°C for 10 min. PCR products were checked for amplification by electrophoresis on 2.0% agarose gel (Himedia), in parallel with 100 bp DNA marker (Thermoscientific). After purification of the amplified fragment bidirectional sequencing using forward and reverse primers was done using Sanger Dideoxy Chain termination method (Eurofins Genomics). The forward and reverse sequences obtained for each animal were edited using Codon Code Aligner software (USA) and different sequences patterns were generated. The pair wise and multiple alignment of the different sequence pattern was done to analyse the differences and relationship between Indian camels GH gene sequences. The pairwise and multiple alignment of identified Indian camel GH gene sequences were done with other reported dromedary and bactrian camels as well as other related and domesticated species GH sequences available at National Centre for Biotechnology Information (NCBI) database using BLAST software program (http://www. ncbi.nlm.nih.gov/) to study the sequence variation and relationship. The estimation of evolutionary relationship between different species and sequences obtained were inferred by neighbour joining method using Molecular Evolutionary Genetics Analysis software (MEGA 7.0).

Results and Discussion

The annealing temperature of 57^{o} C was found optimal for amplification of the target GH gene fragment. A single clear band was observed when the PCR products were checked for amplification by electrophoresis on 2.0 % agarose gel in parallel with 100 bp DNA marker (Fig 1). After bidirectional sequencing of PCR products and its editing using Codon Code aligner software, 613 base pair GH gene fragment's genetic variant were identified (Table 1). The different sequence pattern thus obtained were submitted to NCBI with GenBank Accession No. MT478653 for Indian dromedary (C allele), MT 478654 for Indian dromedary (T allele) and MT478655 for Indian bactrian. The sequence variations within and between single and double humped camels are depicted in table 1. In the 613 bp long GH gene fragment sequence, the nucleotides were conserved at all positions except 3 position in Indian dromedary and bactrian camel. Sequence variation was seen at position 264 C>T in Indian one humped camels of Mewari, Kachchhi and Bikaneri breed. At this particular locus, the dromedary camels with one and two peaks in the sequence chromatograms were observed. Accordingly, 2 allele (C, T) and 3 genotype (CC, CT, and TT) were identified in the Indian dromedary camels (Fig 2). In the Indian bactrian camel at position 264, only C allele and CC genotype were identified (Fig 2). In Indian double humped camel, transition variation was present at position 242, A>G and 469 G>A as compared to single humped camel. All the observed variation were in the intronic region. The exon sequences were similar in double hump and single hump camels and similar amino acids were coded. The sequences

Table 1. Sequence variation between Indian dromedary and Bactrian camel GH gene.

Name	Accession No.	Nucleotide position		
		242	264	469
Indian Dromedary C allele	MT478653	A	C	G
Indian Dromedary T allele	MT478654	A	T	G
Indian Bactrian	MT478655	G	C	A

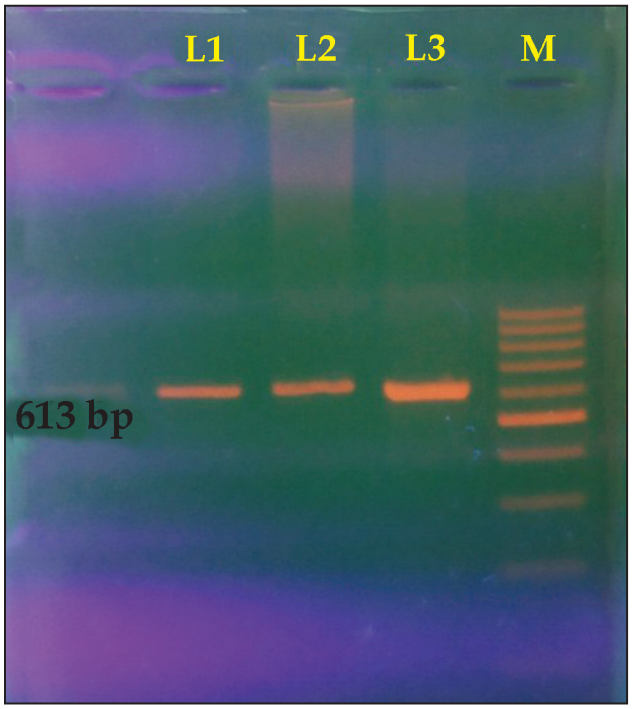

Fig 1. PCR amplification of Growth Hormone (GH) gene resolved on 2.0% agarose gel M marker 100 bp DNA ladder, L1, L2, L3 GH gene product.

obtained was similar to finding of Shah (2006); Ishag *et al* (2010); Abdel Aziem *et al* (2015); Shawki *et al* (2015) in different Asian and African camel breeds. The sequence identity matrix of 3 identified

Table 2. Sequence Identity (above diagonal) and Genetic distance (below diagonal) between Indian dromedary and Bactrian camel GH gene.

	Indian Dromedary C allele	C allele Dromedary T allele	Indian Bactrian
Indian Dromedary C allele	1	0.998	0.996
Indian Dromedary T allele	0.0016	1	0.995
Indian Bactrian	0.0033	0.0049	1

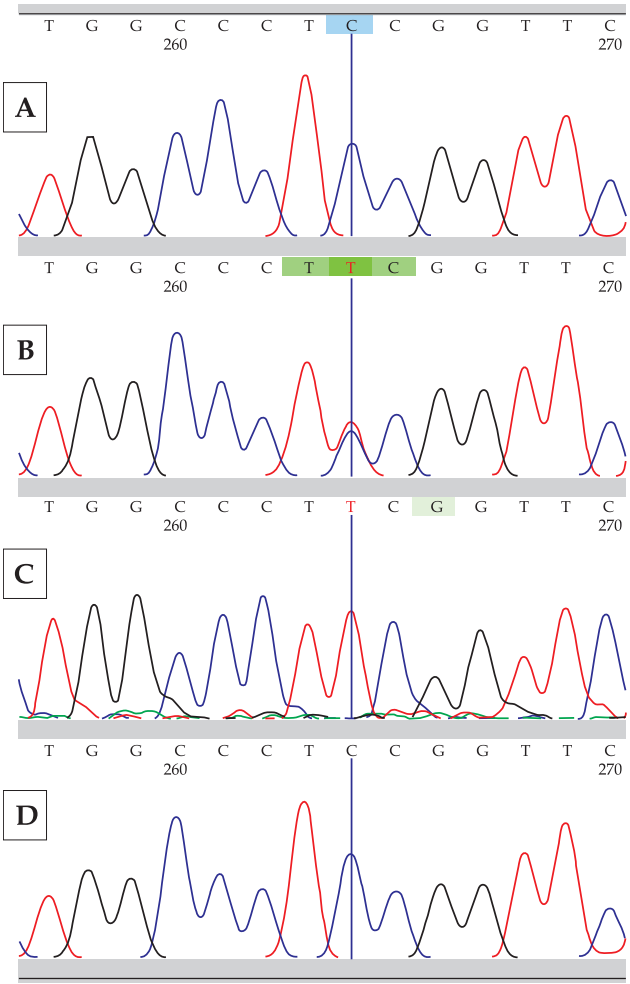

Fig 2. Sequence variation at position 264 in GH gene sequence, A depicts CC, B depicts CT, C depicts TT genotype of Indian dromedary camel and D depicts CC genotype in Indian bactrian camel.

sequences showed more than 99 percent identity and the average genetic distance between 3 sequences were 0.0032 between Indian dromedary GH alleles and bactrian camel GH genes (Table 2). The sequence variation and percent similarity determined on the basis of pairwise nucleotide BLAST of Indian

Table 3. Variation in the camel GH gene sequences relative to Indian dromedary GH sequence (Accession No. MT478653).

Accession No	Total sequence differences	Position and type of variation	Insertion	Deletion	% Identity
AJ575419.1	0	-	-	-	100
J X891650.1	0	-	-	-	100
KP1435181.1	1	480 (G/T)	-	-	99.84
JX891651.1	2	242 (A/G), 469 (G/A)	-	-	99.67
KP143517.1	1	264 (C/T)	-	-	99.67
MK986663.1	3	264 (C/T), 490 (A/G), 491 C/T	-	-	99.42
KR902744.1	7	62 (C/T), 63 (T/A), 225 (G/A), 513 (G/A)	516 (GA)	508 (A)	98.66
KR902745.1	7	12 (A/G), 264 (C/T), 492 (C/T), 510 (T/G), 514 (A/G)	454(A)	508 (A)	98.65

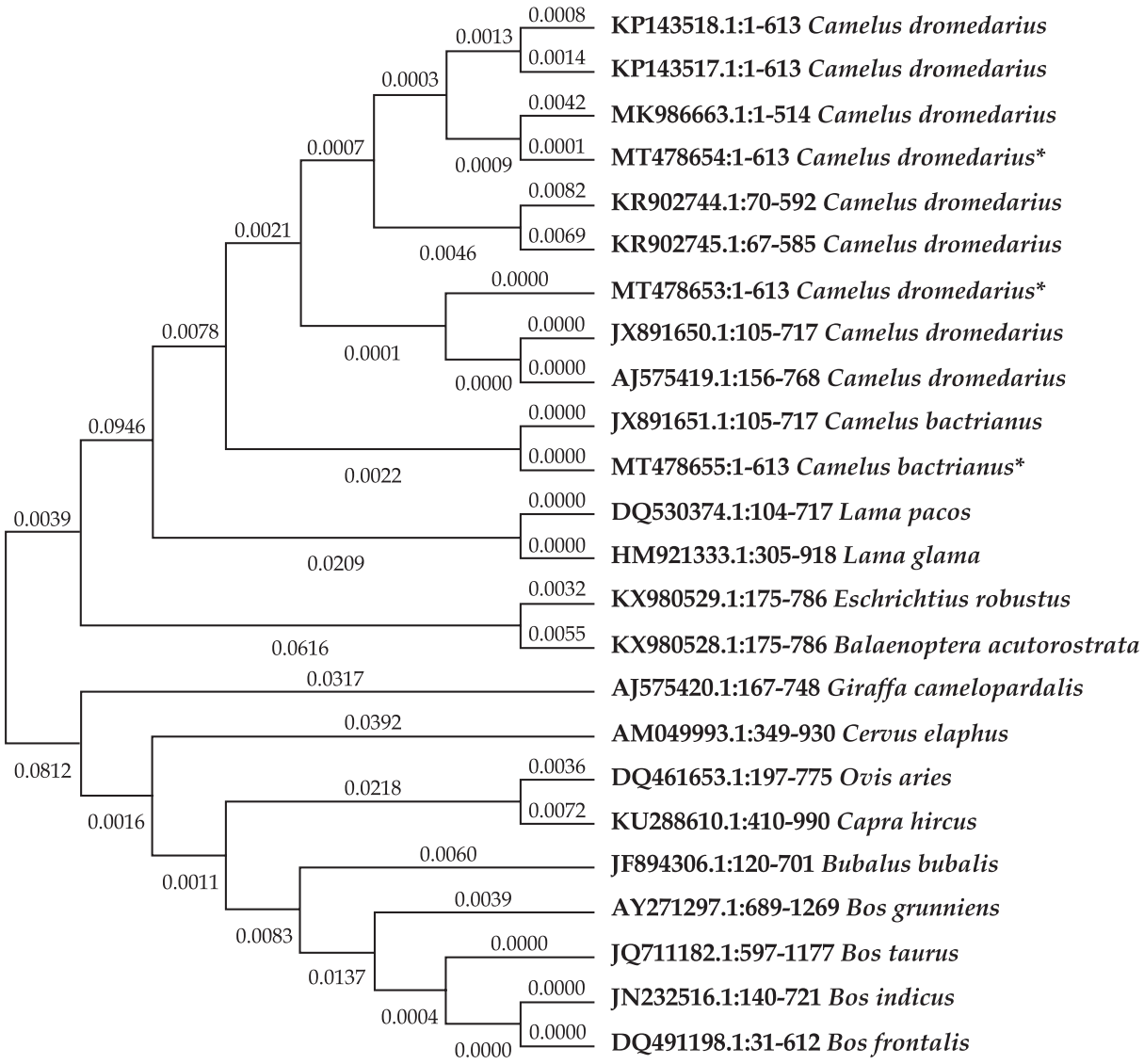

Fig 3. Phylogenetic tree between GH gene sequences from different species. Sequences with * asterisk sign show sequence under study, at extreme right of Fig Sequence name is denoted as accession number, sequence range and zoological name of species.

dromedary GH gene (C allele) with published camel GH gene sequences at GenBank repository is given in table 3. Similarity ranging from 98% to 100 % was obtained with available GenBank camel GH sequences. The GH gene (C allele) sequences also differed at few position to other camel GH Gen bank sequences. The differences were due to transition, transversion, insertion and deletion (Table 3). Camel sequences has close similarity with other camelids family members *Lama pacos* (97.07%) and *Lama glama* (96.58%). With other species the sequence identity varied from 83.65% (*Eschrichtius robustus*) to 78.63% (*Capra hircus*). The evolutionary relationship between GH genes of different species was inferred using Neighbour joining method by analysing 24 nucleotide sequences including three consensus sequences generated in the present study. The sum of the branch length of optimal phylogeny tree (Fig 2) was 0.4507. The evolutionary relationship between sequences showed close relationship between dromedary and Bactrian camel species followed by vicugna and llama. The domesticated species like cattle, buffalo, sheep, goat, yak and mithun were distantly related to camel. Thus, present study showed close similarity between GH gene sequence pattern of Indian Single and double humped camel. Further Indian camels share GH gene structure similar to its Asian and African counterparts. The variation observed at different locus need to be investigated in larger population for gene and genotype frequency and possible association of GH genotypes with performance traits.

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