



Absence of overdominance phenotype of Callipyge gene in Indian sheep

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In the world, the best documented mutation for muscle development in sheep is callipyge (*CLPG*), which causes a postnatal muscle hypertrophy that is restricted at the pelvic limbs and loin with little or no effect on anterior skeletal muscles (Cockett *et al.* 1996). Callipyge phenotype in sheep is also known as 'beautiful buttocks'. This phenotype develops only in paternal heterozygous animals (*CLPG*) mutant allele inherited from father and wild type allele inherited from mother. The maternal heterozygous and homozygous genotype pattern does not express the callipyge phenotype in animals. This type of non-Mendelian inheritance pattern is known as 'Polar Overdominance' (Cockett *et al.* 1996 and Vuocolo *et al.* 2007). The callipyge gene was mapped on ovine chromosome number 18 in the telomeric region within a cluster of imprinted genes (Tellam *et al.* 2012). India has 42 recognised sheep breed (NBAGR 2016) which are found in the different geographical region of the country. The present study was undertaken with the objective to study the genetic polymorphism of callipyge gene in Indian sheep breeds and fat tailed dumba sheep by PCR-RFLP method.

Collection of blood sample and DNA isolation: A study was designed to identify the genetic polymorphism of callipyge gene in sheep breeds of India. About 4 ml of blood samples were collected randomly from 239 adult sheep comprising 15 different breeds of Avikalin (10), Bharat Merino (10), Chokla (10), Decanni (20), Dumba (14), Garole (16), Jaisalmeri (16), Kendrapada (8), Magra (15), Malpura (10), Mandya (44), Marwari (15), Nellore (10), Nali (12), Patanwadi (17) and Fat tailed dumba sheep (12) by jugular vein puncture using disposable needle containing ACD (Citric acid, Sodium citrate and D-glucose) as anticoagulant. DNA was extracted from white blood cells using standard phenol-chloroform extraction method. DNA

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sample were dissolved 0.1× TE buffer (pH 8.0). The quality of the genomic DNA was checked by 0.8% agarose gel electrophoresis.

PCR amplification and RFLP analysis: PCR amplification was carried out in total volume of 20 µl containing 100–150 ng genomic DNA as template, 1 unit Taq DNA Polymerase, 1.5 mM MgCl₂, 1× PCR 10×buffer, 200 µM dNTPs, 10 picomoles of primers and final volume was adjusted with distilled water. *CLPG* locus was amplified by forward 5'-TGA AAA CGT GAA CCC AGA AGC-3' and reverse 5'-GTC CTA AAT AGG TCC TCT CG-3' primers mentioned by Freking *et al.* (2002). The amplification included a touch-down PCR method as mentioned by Freking *et al.* (2002). The amplification was checked at 2% agarose gel electrophoresis at 90V for 1 h. The amplified PCR product of *CLPG* was digested with the restriction enzyme in 13 µl reaction volume at 37°C for 16 h containing 2 units of restriction enzyme *FaqI* (*BsmFI*), 1 × Tango buffer with BSA and 0.3 µl of 50 × SAM solutions. The restriction enzyme digested products were resolved on 2% agarose gel electrophoresis and genotype patterns were visualized by ethidium bromide staining under UV illuminator.

In this study, genetic polymorphism of Callipyge gene was studied for the 15 Indian sheep breeds and fat tailed dumba sheep. Callipyge (426 bp fragment) gene was amplified (Fig. 1) from sheep genomic DNA. The *FaqI* (*BsmFI*) digestion of the PCR amplicon produced two type of digestion patterns for mutant allele G of callipyge having 395 bp and 31 bp fragment and wild type allele A having 278 bp, 117 bp and 31 bp fragments. In present study, only homozygous genotype AA and allele A was observed in all the population of sheep studied (Fig. 2). The genotype GG and GA were not detected in studied Indian sheep breeds. Therefore, *CLPG* locus was found to be monomorphic in all (239) samples of the sheep. The result of the present study was in agreement with the other studied sheep breeds like Lacaune, Tsigai, Improved Valachian, East Friesian and crossbreed Tsigai and Lacaune breeds of Slovakia (Gabor *et al.* 2009); Iranian Afshari and Lori sheep (Quanbari *et al.* 2007 and Nanekarani *et al.* 2014); Najdi and Harri (Alakilli 2015) sheep breeds of Saudi Arabia; Karakachan sheep breed of Bulgaria (Dimitrova *et al.* 2016). The result of present study was not in agreement with callipyge gene mutation (allele G) in Dorset, Rambouillet and Hampshire

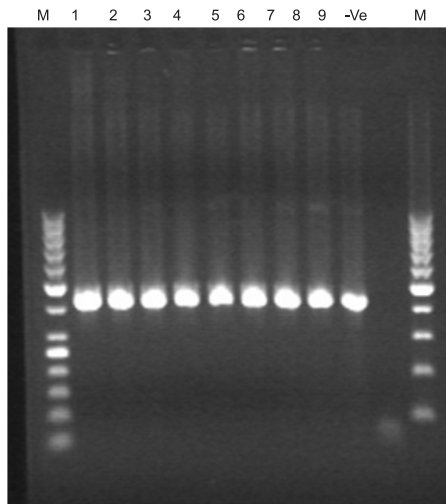


Fig. 1. Amplification of callipyge gene 226 bp fragment by PCR in 2% agarose gel. Lane M, 50 bp and 100 bp DNA ladder; Lane 1 to 9 are the 426 bp amplicon of callipyge gene and -ve is the negative control without genomic DNA.

sheep breeds (Jackson *et al.* 1997). PCR product of AA genotype from Malpura and Avikalin sheep were sequenced by both forward and reverse primers. Nucleotide sequences (426 bp) were submitted to GeneBank with accession no. JN227864 and JN227865 for Malpura and Avikalin, respectively. The BLAST comparison of nucleotide sequence of allele A revealed high similarity (99%) with *Ovis aries*, 97 to 98% with *Capra hircus* followed by 95% with *Bos taurus*. This is the first report on the genetic polymorphism of the callipyge (*CLPG*) gene in Indian sheep breeds.

It was difficult to identify the 31 bp fragment on 2% agarose gel, since it could not be resolved; however, the 278 bp and 117 bp fragments could distinguish the genotypes clearly, therefore, 31 bp was omitted.

SUMMARY

PCR amplification of callipyge gene yielded an amplified product of 426 bp in Indian sheep breeds. RFLP study of callipyge gene with *FaqI* (*BsmFI*) restriction enzyme revealed presence of wild type allele A with only one genotype (AA) and absence of the mutant allele G which is responsible for callipyge phenotype and enhanced mutton quality. The results revealed that there is absence of polymorphism at this locus and callipyge mutation is not found in all the studied Indian sheep breeds and fat tailed dumba sheep.

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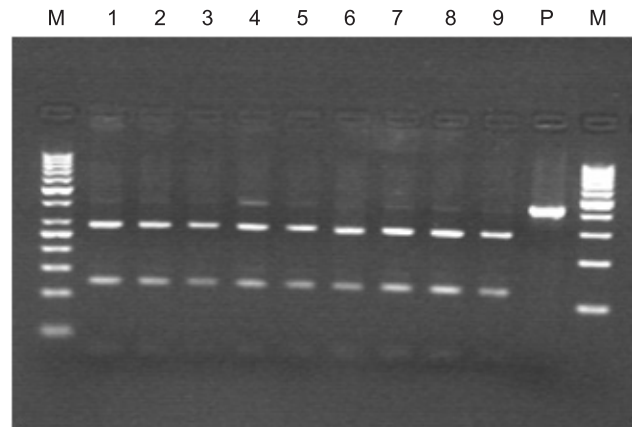


Fig. 2. PCR-RFLP analysis of callipyge gene in sheep. DNA electrophoresis patterns of amplicon after digestion with *FaqI* (*BsmFI*) restriction endonuclease enzyme. Lane M, 50 bp and 100 bp DNA ladder, Lane 1 to 9 are the AA genotype (278 bp, 117 bp and 31 bp). P is the undigested PCR amplicon.

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