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PCR-SSCP ANALYSIS OF EXON 2 OF MYOSTATIN (*MSTN*) GENE IN MARWARI GOATS

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Marwari goat is an important meat breed of Rajasthan and distributed in Barmer, Bikaner, Jaisalmer, Jalore, Jodhpur, Nagour, and Pali districts. The breed is sturdy, well adapted to the arid environment and has a higher tolerance to salt than any other species of the region (Rohilla and Patel, 2003). Myostatin (*MSTN*) gene, also known as growth and differentiation factor 8 (GDF8) gene is a member of transforming growth factor-β super family that plays an important role in the regulation of muscle growth and meat quality (Zhang et al., 2013). *MSTN* gene has 3 exons and 2 introns that encode a glycoprotein which is expressed widely in skeletal muscle (Bellinge et al., 2005). Changes in the gene structure and expression of myostatin may regulate the expression of target genes, thereby changing the composition of muscle fibres and causing variation in muscle weight (Chen, 2008). A dramatic muscularity and a "double-muscling" phenomenon has been observed in Nellore cattle due to mutation in *MSTN* gene that inactivates its expression or produce a non-functional protein (Grisolia et al., 2009). A number of studies in pigs (Fan et al., 2010) and sheep (Boman et al., 2009) have detected the role of mutations in *MSTN* genes in muscular development. However, similar investigations about the properties of the *MSTN* gene in goat are limited (Liu et al., 2006).

Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) technique is simple and sensible and has emerged as a powerful tool for single nucleotide polymorphism (SNP) detection and genotyping (Orita et al., 1989). The unique characteristic of Marwari goat requires its molecular characterization, genetic differentiation and relationships with other breeds. Therefore the present study was undertaken to investigate the genetic variation in exon 2 of the myostatin gene in Marwari goats using PCR-SSCP technique.

MATERIALS AND METHODS

All essential procedures of sample collection were performed strictly as specified by the Institutional Ethics Committee with minimal stress to the animals. Blood samples in EDTA were collected from 120 unrelated Marwari goats maintained in the field units (Deshnoke, Kalyansar, Raisar and Daiya villages, District Bikaner, Rajasthan) of All India Coordinated Research Project on Goat Improvement. Genomic DNA was extracted from blood samples using Blood Genomic DNA purification kit (HiMedia Pvt. Ltd.). The quality and quantity of extracted DNA were checked on 1.0% agarose gel and nano-spectrophotometer, respectively. The exon 2 fragment of the *MSTN* gene was amplified from genomic DNA using a set of

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primers (Forward 5'- AAAAACCCAAATGTTGCTTCT TTA-3' and Reverse 5' - CAGTCCTTCTTCTCCTGGT TCTGG -3') designed from the caprine *MSTN* gene sequence (Gene bank accession no. DQ167575). The PCR reactions were performed in a final volume of 25 µL (4 µL DNA template, 0.5 µL each primer, 2.0 µL dNTPs, 0.5 µL *Taq* DNA polymerase (Promega, Madison, USA), 5 µL 5X reaction buffer, 1.5 µl $MgCl_2$ and 11µL ddH_2O). A total of 40 cycles of PCR amplification was carried out at 94°C for 45 sec, annealing at 55°C for 45 sec and 60 sec at 72°C and final extension of 10 min at 72°C. The quality of amplified product was checked on 1.5 % agarose gel (Plate 1).

SSCP was carried out to detect polymorphism in the exon 2 of *MSTN* gene. Aliquots of 5 µL of PCR products were mixed with 5 µL denaturing 2X gel loading dye and heated for 8 min at 95°C and chilled in ice immediately for 7 min. The denatured PCR products were subjected to 8% polyacrylamide gel electrophoresis (PAGE) at 120 V and 8 watt till marker dye reached bottom. The gels were stained with ethidium bromide and the different patterns were analyzed under UV light and documented by UVP gel-doc system.

RESULTS AND DISCUSSION

The exon 2 of *MSTN* gene showed two types of conformation patterns on PAGE gel (Plate 2). One of the patterns revealed an extra band in 18 samples (out of 120 samples) which could be suggestive of possible mutation in the exon 2 of myostatin gene in Marwari goat. The pattern that showed only two bands was considered as AA genotype, whereas another pattern that showed an extra band was designated as AB genotype (Zhang et al., 2013). The frequencies of AA and AB genotypes at exon 2 locus of *MSTN* gene were 0.85 and 0.15, respectively. The gene frequencies of A and B alleles in the population were estimated as 0.925 and 0.075, respectively.

Plate 1. PCR amplification of myostatin (Exon 2) gene in Marwari goats (Lane M: DNA ladder; lane 1-6: MSTN (exon 2) gene, 375 bp in Marwari goat; lane 7: Negative control

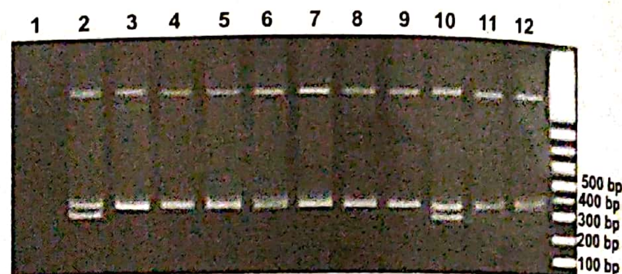

Plate 2. PAGE electrophoresis and SSCP pattern of PCR amplified product of myostatin (Exon 2) gene in Marwari goats (Lane 1: Negative control; Lane 2 and 10: AB genotype; Lane 3-9, 11-12: AA genotype; lane 13: DNA ladder

Polymorphism in *MSTN* gene was found to be significant in different goat breeds. It was reported that the TTTTA deletion phenomenon of the *MSTN* gene had emerged in different species and might be unique for goats (Zhang et al., 2013). Li et al. (2008) found significant effects of a 5 bp deletion on early body weight and sizes of goats. The present study on the genetic diversity analysis of Marwari goats for *MSTN* exon 2 gene through PCR-SSCP technique revealed low genetic diversity. The study revealed highest frequency of pattern AA followed by very low frequency of pattern AB. The results observed were in agreement with similar studies conducted in Boer goats by Zhang et al. (2013). Soufy et al. (2009) observed monomorphic pattern in exon 3 of myostatin gene in Sanjabi sheep. Nada et al. (2013) detected a monomorphic pattern in myostatin gene in five Egyptian and Saudi sheep breeds using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). However, Dehnavi et al. (2012) reported high polymorphism in exon 3 of the myostatin gene in Zel sheep on RFLP-PCR. The

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inconsistency in the results may be due to differences in species and breeds, population structure, sample size, environmental factors, mating strategies, geographical position and frequency distribution of genetic variation. The present study provides the base information for further investigation of *MSTN* gene in Marwari goats and other native breeds. However, further studies should be conducted with large number of samples before they are used in goat breeding and genetics.

SUMMARY

A study was conducted with the objective to detect genetic polymorphism in exon 2 of myostatin (*MSTN*) gene in Marwari goats by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) technique. Genomic DNA was isolated from blood of 120 randomly selected Marwari goats maintained in field units of All India Coordinated Research Project on Goat Improvement. The exon 2 of *MSTN* gene was amplified using specific primer designed from caprine *MSTN* gene sequence (Gene bank accession no. DQ167575). SSCP was carried out on 8% non-denaturing polyacrylamide gel to resolve the different genotypic pattern. Exon 2 of *MSTN* gene revealed two types of conformation patterns. The SSCP pattern that showed only two bands was designated as AA genotype, whereas the other pattern with an extra band was considered as AB genotype. The frequencies of AA and AB genotypes were 0.85 and 0.15, respectively. The dimorphic pattern observed for exon 2 of *MSTN* gene could be utilized to identify and characterize Marwari goats.

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