RESEARCH ARTICLE

Genetic Polymorphism of GHR, LEP and MSTN Genes in Surti Goats from Organized Farm

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Abstract

Genetic studies pertaining to genes related to growth parameters can give idea about growth, meat as well as milk production performance of the animals in advance. Surti is considered as a dual purpose goat breed mainly of South Gujarat region. The aim of the present study was to know the genetic polymorphism in growth related genes, *viz.,* growth hormone receptor (*GHR*), leptin (*LEP*) and myostatin (*MSTN*) through PCR-RFLP technique. The blood samples were collected from 62 Surti goats and DNA was extracted. Amplification of the genes was carried out using suitable primers. The PCR products of *GHR*, *LEP* and *MSTN* were digested with *AvaII*, *HinfI* and *HaeIII* enzymes, respectively. PCR products were found to be monomorphic on digestion.

Keywords: Genetic polymorphism, Growth related genes, Surti goats, PCR-RFLP.

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Introduction

Goat husbandry plays a pivotal role in the livelihood of landless and marginal farmers in India. Goat contributes significantly, after poultry and buffalo, in the total meat production of the country by about 13.5 % (Anonymous, 2019). Although the contribution of goat in the total milk production of India is only about 4 % (Anonymous, 2019), but because of certain physical characteristics and medicinal values, the goat milk remains in specific demand. Surti is a small sized goat breed of South Gujarat region. The breed is mainly reared for both milk and meat purposes. Body weight, heart girth, body length parameters at early stage of life are good indicators of growth of the animals, and receive high priority in breeding programmes for milk as well as meat purpose. Marker Assisted Selection can be a useful approach for early selection of goat with high genetic merit. Studies pertaining to genetic variability in growth parameters controlling genes can help in identification of marker genes for growth traits. Growth hormone (*GH*), Growth hormone receptor (*GHR*), Leptin (*LEP*) and Myostatin (*MSTN*) are some of the identified genes known to affect growth parameters in various livestock species including goats (Supakorn, 2009). Among these, growth hormone (GH) is the most studied gene for polymorphism in India as well as abroad (Marques *et al.,* 2003; Gupta *et al*., 2009; Hua *et al.,* 2009; Kumar *et al.,* 2011; Bayan *et al.,* 2018). However, the information on the other genes studied in present study is scanty.

Various studies have been reported to find the polymorphism in growth parameters controlling genes in goats across the world (Singh *et al.,* 2009; Alakilli *et al.,* 2012; An *et al.,* 2011[a,b]; Bayan *et al.,* 2018). However, the genetic polymorphism is still required to be studied more in Surti goats. Hence the present study was planned to assess the genetic variability in growth related genes, *viz.,* GHR, LEP and MSTN genes in Surti goats using PCR-RFLP technique.

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Materials and Methods

Blood samples were collected in EDTA vacutainers from jugular vein of 62 Surti goats (males and females) maintained at Livestock Research Station, NAU, Navsari. Phenol-chloroform method was used for DNA extraction as described by John *et al.* (1991). Quality and quantity of the extracted DNA was checked by 0.8 % agarose gel electrophoresis and Nanodrop 2000c spectrophotometer, respectively. PCR was performed using gene specific custom synthesized (Europhins) primers (either previously reported or designed using PRIMER-3 tool) as given in Table 1. PCR was set in the Veriti thermal cycler (Thermo Fisher) using EmeraldAmp® GT PCR Master mix (Takara Clontech) as per the composition and protocol given in Table 2 and 3, respectively. The PCR amplification was checked on 2% agarose gel electrophoresis.

The master mix composition for PCR-RFLP along with the restriction enzyme (Manufacturer) is given in Table 4. Digestion and inactivation protocol was carried out in PCR thermal cycler as per the manufacturer's instructions. PCR products digested with the restriction enzymes were run on 2 % agarose gel electrophoresis to check the polymorphism.

Results and Discussion

The good quality DNA samples with spectrophotometric 260/280 ratio between 1.8 – 2.0 and revealing single band on electrophoresis were diluted to 30 ng/µl concentration followed by PCR amplification.

GHR gene

The amplification of exon 3 and 4 region GHR gene using the custom designed specific primers revealed the expected product size of 344 bp (Figure 1). The PCR products remained undigested on digestion with AvaII enzyme revealing monomorphic band pattern of AA genotypes with frequency 1.0 (Table 5). Complete absence of corresponding allele B may be due to fixation of allele A. These findings were in agreement

Table 1: Primer details for the genes under study and corresponding restriction enzymes

Gene	*Primers (5′ – 3′)*	*Region*	*Product size (bp)*	*Ref.*
GHR	F: CATTCCCACCACTGCATGAC R: ACAGCAGCTCTGAAGCTATGG	Exon 3 and 4	344	Newly designed
LEP	F: TGCAGTCTGTCTCCTCCAAA R: CGATAATTGGATCACATTTCTG	Exon 2	152	Singh *et al.* (2009)
MSTN	F: AACGTTTGGCTTGGCGTTAC R: GGAGCCTTGGGCAAAAGTTG	Promoter and 5′ UTR	337	Newly designed

Table 2: PCR master mix composition for the genes under study

	Total reaction volume 20 µl		
Components	*GHR*	*Leptin*	*MSTN*
Master mix (2x)	10.0	10.0	10.0
FP (10 pmole /µl)	0.7	0.8	0.4
RP (10 pmole /µl)	0.7	0.8	0.4
gDNA (30 ng /µl)	2.5	2.5	2.5
MiliQ water	6.1	5.9	6.7

Table 3: PCR protocol for the genes under study

	Genes under study		
Steps	*GHR*	*Leptin*	*MSTN*
1. Initial denaturation	94 °c – 10 min	94 °c – 10 min	94 °c – 10 min
2. Denaturation*	94 °c – 45 sec	94 °c – 1 min	94 °c – 1 min
3. Annealing*	54 °c – 45 sec	58 °c – 1 min	54 °c – 1 min
4. Extension*	72 °c – 1 min	72 °c – 1 min	72 °c – 1 min
5. Final extension	72 °c – 10 min	72 °c – 10 min	72 °c – 10 min

* Total 35 cycles for step 2, 3 and 4

Table 4: PCR-RFLP master mix composition for the genes under study

	Total reaction volume 20 µl		
Components	*GHR (AvaII)*	*Leptin (HinfI)*	*MSTN (HaeIII)*
Enzyme buffer (10x)	2.0	2.0	2.0
Enzyme (10U/µl)	0.4	0.5	0.4
PCR product	10.0	10.0	10.0
MiliQ water	7.6	7.5	7.6

* Names in the parentheses indicate the restriction enzymes used for the digestion

Table 5: Genotypes and gene frequencies for the genes under study in Surti goats

Gene (Total number of animals)	*Genotypes*	*Observed genotypes*	*Observed genotypic frequencies*	*Observed gene frequency (A)*	*Observed gene frequency (B)*
Growth Hormone Receptor (*GHR*) (58)	AA	58	1.0	1.0	0.0
Leptin (*Leptin*) (62)	AA	62	1.0	1.0	0.0
Myostatin (*MSTN*)(55)	BB	55	1.0	0.0	1.0

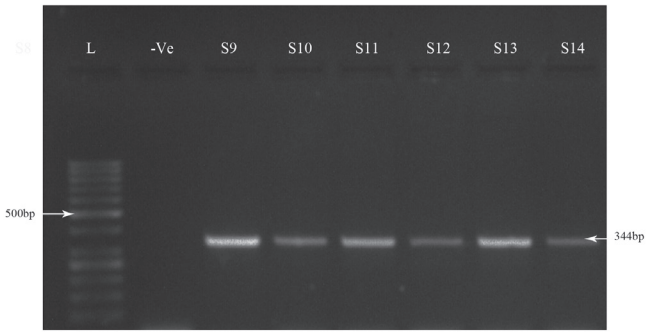

Figure 1: PCR Product of Growth Hormone Recepter (GHR) gene of Surti Goats.
Lane: L: DNA Ladder, -Ve: Negative Control, S9 to S14- PCR products of GHR of Surti Goats.

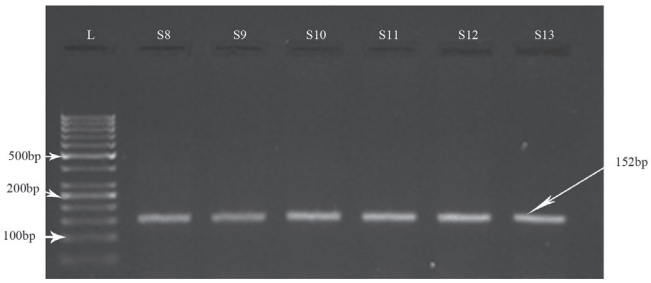

Figure 2: PCR Product of Leptin gene of Surti Goats.
Lane: L: DNA Ladder, S8 to S13- PCR- products of Leptin of Surti Goats.

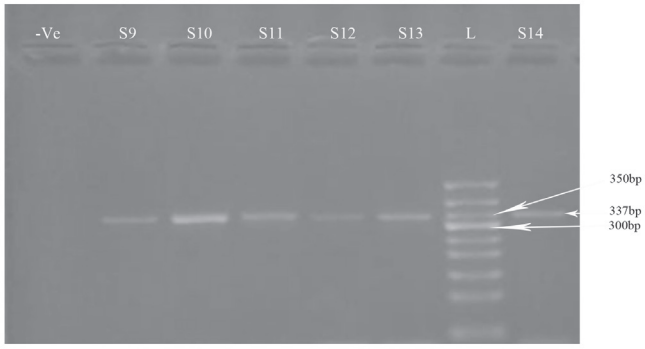

Figure 3: PCR Product of MSTN gene of Surti Goats.
Lane: L: DNA Ladder, -Ve: Negative control, S9 to S14- PCR products of *MSTN* of Surti Goats.

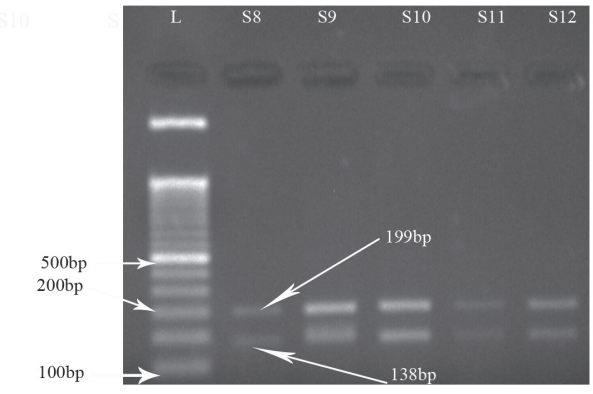

Figure 4: HaeIII enzyme digested MSTN PCR products of Surti goats.
Lane: L: DNA Ladder, S8 to S12- PCR-RELP products of *MSTN* of Surti Goats.

with the earlier reports for Beetal and Changthangi goat breeds (Sharma *et al.,* 2014). However, the studies carried out in other Indian goat breeds revealed 1 SNP per 126 bp sequenced of GHR gene (Sharma *et al.,* 2014). Polymorphism with microsatellite markers in the 5′ non-coding region has also been reported for two Polish goat breeds (Maj *et al.,* 2007). Lack of genetic variability in the present population for GHR gene may be due to the selection practiced over the years on the farm. The reports with polymorphism in GHR gene may be due to the more dense markers chosen or larger population involved in the studies.

LEP gene

The PCR products on amplification of exon 2 region of LEP gene using previously reported primers revealed 152 bp PCR products (Figure 2). The restriction digestion analysis using HinfI enzyme revealed the undigested PCR products with single band of 152 bp. Only one allele A with frequency of 1.0 was found in the population under study (Table 5). Amplification of the same region in Jamunapari and Barbari goats by Singh *et al.* (2009) also revealed 152 bp PCR products. However, in contradiction to the present findings, sequencing and restriction analysis of the same region revealed five major haplotypes in Jamunapari and Barbari goat breeds (Singh *et al.,* 2009). Seven SNPs had also been reported on sequencing of the entire gene from seven Indian goat breeds (Maitra *et al.,* 2014). Monomorphic pattern of the genotypes in the present study may be the result of fixation of the gene due to selection.

MSTN gene

The amplification of promoter and 5′ UTR region of *MSTN* by the newly designed primers revealed the expected product of 337 bp (Figure 3). On digestion with HaeIII restriction enzyme, the PCR product revealed one site at 199 bp resulting BB genotype with two bands of 199 and 138 bps in all the samples (Figure 4) (Table 5). PCR based SSCP and DNA sequencing techniques in Boer and its crossbreds revealed polymorphism for exon 1 and exon 3 regions of the gene (An *et al.,* 2011[a]). Similarly, polymorphism had also been reported in 5′UTR and exon 1 regions of MSTN gene in Anhui and Boer goats by PCR-SSCP and sequencing (Zhang *et al.,* 2013). The lack of polymorphism under present study might be due to selection operating at organized farm. The gene would have been fixed on account of the selection practiced on the farm over the years.

In conclusion, the present study did not reveal any polymorphism for GHR, LEP and MSTN genes in the Surti goats maintained at Livestock Research Station, Navsari Agricultural University, Navsari. The studies related to polymorphism in all the genes known to control growth parameters and its association with growth parameters should be carried out in a wider population to use them as marker for early selection.

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