Original Research

Polymorphism of Exon 2-3 of Growth Hormone Gene in Surti and Mehsani Goats by PCR-RFLP

Jyotishree Bayan[1*], Vishnu Kharadi[2], Umed Ramani[3], Mamta Janmeda[1], Pawar Vallabh[1] and Balkrishna Brahmkshtri[1]

College of Veterinary Science, Navsari Agricultural University, Navsari, Gujarat, INDIA

[1]Department of Animal Genetics and Breeding
[2] Livestock Research Station
[3] Department of Animal Biotechnology

***Corresponding author:** jyotishreebayan89@gmail.com

Rec. Date:	May 14, 2018 11:38
Accept Date:	Jul 01, 2018 05:01
DOI	10.5455/ijlr.20180514113801

Abstract

The investigation was planned to study GH gene exon 2-3 polymorphism using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) in Surti and Mehsani goats. GH gene exon 2-3 region was found to be polymorphic on restriction digestion with Hae III which revealed two genotypes AA and AB with genotypic frequencies of 0.24 and 0.76 in Surti goats and 0.20 and 0.80 in Mehsani goats respectively. Both the population of Surti and Mehsani goats were not found to be in genetic equilibrium for GH locus exon 2-3 indicating selection pressure for growth. Polymorphism in exon 2-3 region of GH gene in both the goat breeds can be utilized in formulating breeding strategies based on marker assisted selection in both the goat breeds.

Key words: Goat, Growth Hormone Gene, Polymorphism, PCR-RFLP

How to cite: Bayan, J., Kharadi, V., Ramani, U., Janmeda, M., Vallabh, P., & Brahmkshtri, B. (2018). Polymorphism of Exon 2-3 of Growth Hormone Gene in Surti and Mehsani Goats by PCR-RFLP. International Journal of Livestock Research, 8(11), 64-72. doi: 10.5455/ijlr.20180514113801

Introduction

Goat has distinct social, economical, managerial and biological advantages over other livestock species and often termed as the "Poor man's cow. Out of the 28 defined goat breeds in India (NBAGR), five breeds namely, Surti, Mehsani, Kutchi, Zalawadi and Gohilwadi are native to Gujarat. The Surti goat breed derives its name from the name of the place called Surat in Gujarat where it is found in most pure form and is widely distributed in parts of South Gujarat. It is a small to medium sized goat with compact body and is used for milk and meat purpose and is highly prolific having 50% twinning rate. The Mehsani breed of goat derives its name from the place of its origin called Mehsana in Gujarat where it is found in its most pure

form and is widely distributed in parts of north Gujarat in varying intensity. The breed is large in size and kept for meat and milk purpose. In the livestock industry, growth traits are always of primary concern during breeding, for its determinant economic values (Zhang, 2008). Growth is considered as a main economic trait in the goat industry. The growth traits are complex traits involving multiple genes, loci and interactions (Hua *et al*., 2009). Animals with high milk yield reveal superior Growth Hormone (GH) average levels than animals with lower production, namely during peak lactation (Reinecke *et al*., 1993).

Development in DNA technologies have made it possible to uncover a large number of genetic polymorphisms at the DNA sequence level and to use them as markers for evaluation of the genetic basis for observed phenotypic variability which makes a vital part in animal breeding (Athe *et al*., 2018) .With the development of molecular biology and biotechnology, researchers are able to achieve more accurate selection goals through marker assisted selection (MAS). Therefore, in establishing a MAS system, finding and verifying genetic markers related to growth and milk traits are essential (Allan *et al*., 2007). GH gene, with its functional and positional potential, has been widely used as a marker in several livestock species including the cattle (Sorensen *et al*., 2002) sheep (Marques *et al*., 2006) and goat (Malveiro *et al*., 2001). GH gene may be used as a candidate gene for studying its polymorphism and association in relation to growth due to its vital role of GH in animal growth and development, (Supakorn, 2009).

GH is an anabolic hormone synthesized and secreted by the somatotroph cells of the anterior lobe of the pituitary in a circadian and pulsatile manner (Ayuk and Sheppard, 2006). Various studies carried out in ruminants confirm a role of GH in regulation of mammary growth (Akers, 2006) and show the effects of GH on growth in several tissues, including bone, muscle and adipose tissue (Hua *et al*., 2009). GH affects a wide variety of physiological processes such as lactation, reproduction, growth and metabolism (Ayuk and Sheppard, 2006; Akers, 2006). GH is associated with both animal's growth (Hua *et al*., 2009) and milk yield traits (Malveiro *et al*., 2001; Marques *et al*., 2003) in many livestock animals. GH gene is physically located on goat chromosome 19q22 (Pinton *et al*., 2000). GH is a peptide and encoded by 1,800 base pairs consisting of five exons and four intervening introns (Missohou *et al*., 2006; Wickramaratne *et al*., 2010). This gene produces the GH from the anterior pituitary. This hormone is necessary for postnatal growth and metabolism in vertebrates (Ayuk and Sheppard, 2006).

GH gene polymorphism has been reported in some of the Indian goat and sheep breeds by various researchers including Malabari goats (Chitra and Aravindrakshan, 2004), Black Bengal goats (Gupta *et al*., 2007; Shankar *et al*., 2014), Jhakrana goats (Gupta *et al*., 2009), Sangamneri goats (Wickramaratne *et al*., 2010), Osmanabadi goats (Wickramaratne *et al*., 2010), Sirohi goats (Kumar *et al*., 2001; Singh *et al*., 2015), Barbari goats (Singh *et al*., 2015), Atapaddy Black, Malabari and Malabari crossbreds (Radhika *et al*., 2016), Chokla, Magra, Malpura, Nellore, Patanwadi, Sonadi, Garole, Bharat Merino, Avikalin sheep (Kumari *et al*., 2014) and Vembur sheep (Sheevagan *et al*., 2015).

 DOI 10.5455/ijlr.20180514113801

The present investigation was undertaken to study the genetic polymorphism in the region of exon 2-3 in GH gene in Surti and Mehsani goats.

Materials and Methods

Blood Sampling

Blood samples were collected from 50 Surti goats maintained at Livestock Research Station under Navsari Agricultural University, Navsari, Gujarat and 50 Mehsani goats maintained at Livestock Research Station under Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Dantiwada, Gujarat. About 5-7 ml of the blood was collected from the jugular vein of each animal into sterile 10 ml Ethylene diamine tetra-acetic acid (EDTA) coated vacutainers and stored at -4 ºC till further processing.

Genomic DNA Extraction

The genomic DNA from whole blood samples of 50 Surti and 50 Mehsani goats were extracted by Qiagen DNeasy Blood Kit. Purity and concentration of genomic DNA were estimated by Nanodrop 2000c spectrophotometric reading at OD_{260} and OD_{280}. The quality of genomic DNA was checked by in 0.8 % agarose gel electrophoresis at a constant voltage of 80V for 60 minutes.

Polymerase Chain Reaction (PCR)

Oligo primers (Table 1) specific to caprine GH gene locus as specified by Hua *et al*., 2009 were custom synthesized at Eurofins Genomics India Pvt. Ltd. Bengaluru and utilized to amplify the desired fragments in the present study.

Table 1: Sequence of growth hormone (GH) gene primers, PCR product size and amplified region (Hua *et al.*, 2009)

Primers	Primer sequence	Product size	Amplified region
GH 1 F	CTC TGC CTG CCC TGG ACT	422 bp	Exons 2 and 3
GH 1 R	GGA GAA GCA GAA GGC AAC C		

GH= Growth Hormone, F= Forward, R= Reverse

Touch-down PCR was performed to amplify exon 2-3 region of GH gene in Surti and Mehsani goats. PCR was carried out in a final reaction volume of 20 µl consisting of 10 µl of 2X master mix (Puregene), 3 µl genomic DNA (60 ng), 0.8 µl (8 pmole) of each forward and reverse primer and 5.4 µl of nuclease free water. In touch-down PCR, 11 cycles of denaturation at 95^0C for 30 seconds, annealing temperature at 65^0C (-1^0C per cycle) for 30 seconds and extension at 72^0C for 30 seconds followed by constant annealing temperature of 54^0C for 30 seconds for the remaining 24 cycles with same denaturation and extension step was performed. Initial denaturation was carried out at 94^0C for 5 minutes, while the final extension was performed at 72^0C for 10 minutes.

DOI 10.5455/ijlr.20180514113801

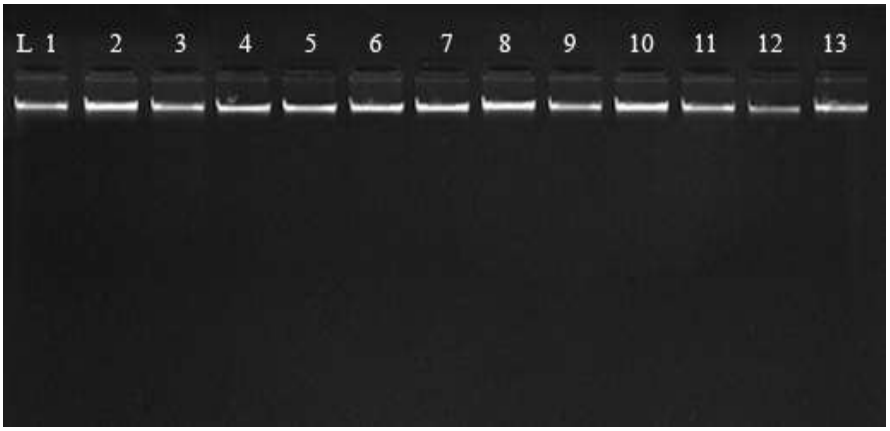
The PCR products were analysed by electrophoresis on 2% agarose gel and stained with ethidium bromide for 60 minutes at 80V. 100bp DNA ladder was used as a molecular size marker. The bands were visualized under UV light and documented by gel documentation system (Alpha Imager HP).

Restriction Fragment Length Polymorphism (RFLP)

Restriction digestion of the amplified PCR products were carried out in a total volume of 30 µl having 10 µl PCR product, 3 µl 10X buffer, 16.5 µl nuclease free water and 0.5 µl restriction enzyme, *Hae III* (NEB). The restriction mixtures were incubated at 37^0C for 60 minutes and enzyme inactivation was done at 80^0C for 20 minutes. The digested PCR products were run on 2.0 percent agarose gel at a constant voltage 80 V for 90 to 120 min in 0.5X TBE buffer. 50bp DNA ladder was used as a molecular size marker. The restriction patterns were visualized under UV light and photographed by gel documentation system. Upon electrophoresis, the segments resolved in the gel and the genotypes were recorded after observing the PCR-RFLP patterns, and the genotypic and allelic frequencies were calculated for Surti and Mehsani goat populations under study. Chi-square test was carried out to evaluate allelic and genotypic frequency differences across the investigated goat breeds.

Results and Discussion

On amplification of exon 2-3 region of GH gene, amplified PCR product of 422 bp size was observed in both the breeds (Plates 1&2).



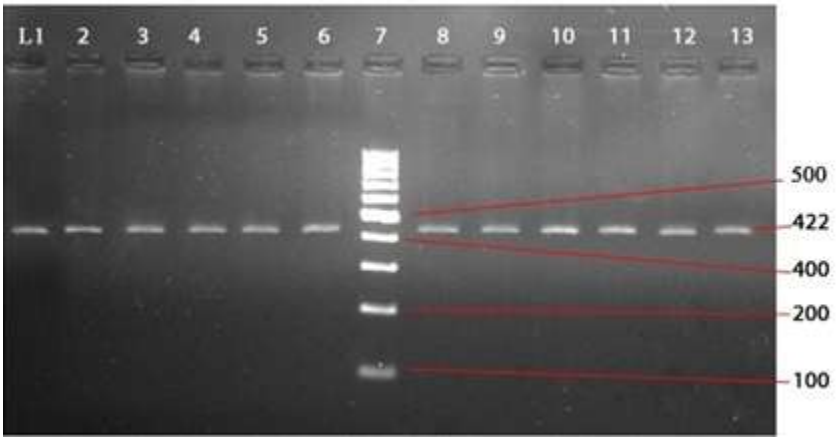
Lane: L1 to L6: Genomic DNA in Surti goat, L7-L13: Genomic DNA in Mehsani goat

Plate 1: Genomic DNA in Surti and Mehsani goats

The PCR product of similar size has also been reported in various breeds of goats and sheep like Boer bucks (Hua *et al*., 2009), Boer and Matou dams (Zhang *et al*., 2011), Barki, Zaribi, Ardi and Masri goats (Alakilli *et al*., 2012), Savanna and Kalahari goats (Amie *et al*., 2012), Sirohi and Barbari goats (Singh *et al*., 2015), Egyptian sheep and goats (Othman *et al*., 2015), Malabari and Atapaddy Black goats (Radhika *et al*., 2016),

 DOI 10.5455/ijlr.20180514113801

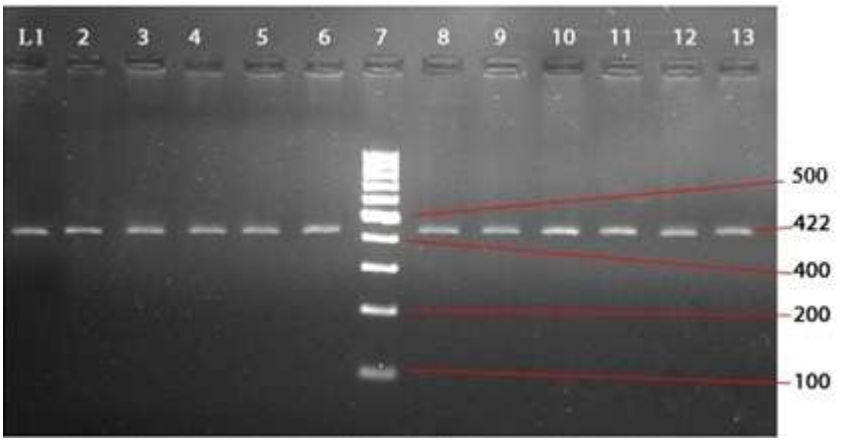
Chokla, Magra, Malpura, Nellore, Patanwadi, Sonadi, Garole, Bharat Merino, Avikalin sheep breeds (Kumari *et al*., 2014) and in Vembur sheep (Sheevagan *et al*, 2015) by amplification of this region of the GH gene.



Lane: L7: 100 bp DNA ladder, L1-L6 and L8-L13: 422 bp PCR products.

Plate 2: PCR products of exon 2-3 (422 bp) of GH gene in Surti goat

In present study on restriction digestion of exon 2-3 region of GH gene using *Hae III*, two types of genotypes AA (366bp and 56bp) and AB (422bp, 366bp and 56bp) (Plates 3&4) were found in both Surti and Mehsani goats respectively, whereas the genotype BB (422bp) was absent in both the breeds of goats.

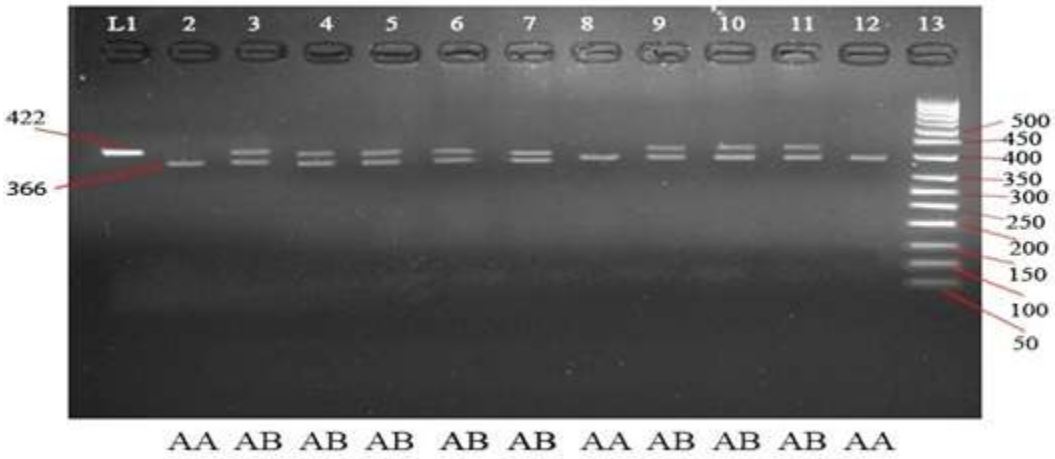


Lane: L6: 100 bp DNA ladder, L1-L5 and L7-L13: 422 bp PCR products.

Plate 3: PCR products of exon 2-3 (422 bp) of GH gene in Mehsani goat

The genotypic frequencies of AA and AB were observed as 0.24 and 0.76 in Surti goats and 0.20 and 0.80 in Mehsani goats, respectively. The frequency of allele A was observed as 0.62 and 0.60, whereas, the frequency of allele B was observed as 0.38 and 0.40 in Surti and Mehsani goats respectively (Table 2).

DOI 10.5455/ijlr.20180514113801



Lane: L1: undigested PCR product, L2-L12: RFLP products of Surti goat, L13: 50 bp DNA ladder.

Plate 4: Restriction enzyme (*HaeIII*) digestion of GH gene exon 2-3 (422 bp) PCR products in Surti goat

Table 2: Genotypic and allelic frequencies for GH1 locus in Surti and Mehsani goats with *HaeIII* and Chi-square test

Goats	Locus	Genotype	Observed number of genotype	Genotypic frequency	Allele frequency		Chi square test-P value
					A	B	
Surti	GH1 422	AA	12	0.24	0.62	0.38	p<0.01
		AB	38	0.76			
		BB	0	0			

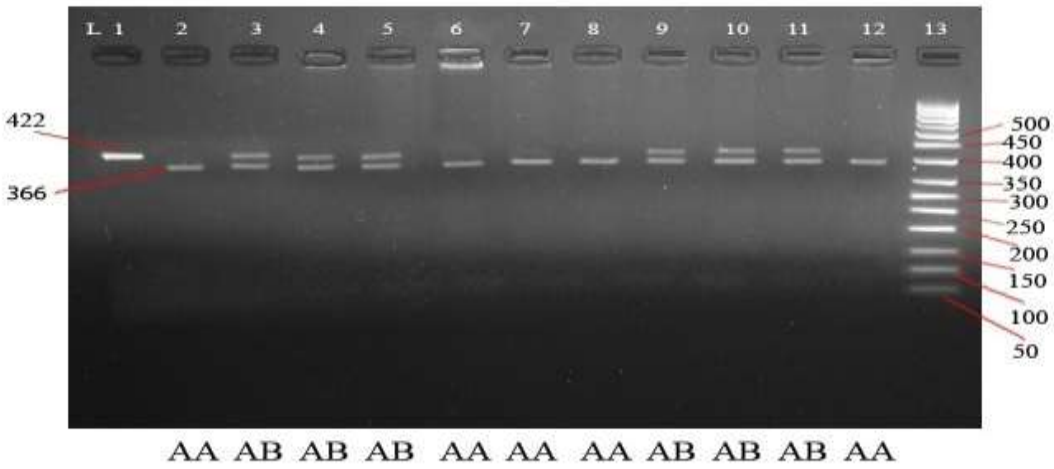
The frequencies of genotype AB and A allele were higher in both the goat breeds. The chi-square test shows significant ($p \leq 0.01$) departure of the genotypes from the expected frequencies with regards to GH exon 2-3 *Hae III* patterns for both Surti and Mehsani breeds of goats. This shows that population was not under Hardy-Weinberg equilibrium indicating that the animals were under indirect selection for the GH gene on both the organized farms.

The present RFLP pattern for exon 2-3 region of GH gene of our study with two genotypes AA and AB is in agreement with the findings of Hua *et al.* (2009) in Boer bucks, Zhang *et al*. (2011) in Matou and Boer breeds of goat, Amie *et al*. (2012) in Savanna and Kalahari goats, Kumari *et al*. (2014) in 324 sheep which included 9 different sheep breeds of India. The present RFLP pattern for exon 2-3 of GH gene of our study is also comparable to the findings of Othman *et al*. (2015) who also reported two genotypes, GG (366 and 56 bp) designated as AA in our study and AG (422, 366 and 56 bp) designated as AB in our study in major Egyptian goats and sheep. The PCR-RFLP studies of Sheevagan *et al*. (2015) using *Hae III* also revealed polymorphism at exon 2-3 region of GH gene in Vembur sheep with two genotypes AA and AB.

However, the studies of GH gene exon 2-3 region by Alakilli *et al*. (2012) in four Egyptian and Saudi goat breeds (Barki, Zaribi, Ardi and Masri) revealed three genotypes AA (422 bp) designated as BB in our study,

DOI 10.5455/ijlr.20180514113801

AB (422, 366 and 56) and BB (366 and 56 bp) designated as AA in our study. The PCR-RFLP studies of exon 2-3 region of the GH gene in Sirohi and Barbari goats by Singh *et al*. 2015) using *Hae III* showed the presence of two genotypes AB and BB. Radhika *et al*. (2016) reported polymorphism of exon 2-3 region of GH gene in two native goat breeds of Kerala namely, Malabari and Attappady Black, along with Malabari crossbreds with three genotypes AA, AB and BB.



Lane: L1: undigested PCR product, L2-L12: RFLP products of Mehsani goat, L13: 50 bp DNA ladder.

Plate 5: Restriction enzyme (*HaeIII*) digestion of GH gene exon 2-3 (422 bp) PCR products in Mehsani goat.

Conclusion

GH gene exon 2-3 region amplified with caprine specific primers was found to be polymorphic on restriction digestion with *Hae III* which revealed two genotypes AA and AB with genotypic frequencies of 0.24 and 0.76 in Surti goats and 0.20 and 0.80 in Mehsani goats respectively. The chi-square test shows significant *($p \leq 0.01$)* departure of the genotypes from the expected frequencies with regards to GH exon 2-3 *Hae III* patterns for both Surti and Mehsani breeds of goats. This shows that population was not under Hardy-Weinberg equilibrium indicating that the animals were under indirect selection for the GH gene on both the organized farms. Polymorphism in exon 2-3 region of GH gene on digestion with *Hae III* in both Surti and Mehsani breeds of goats using PCR-RFLP indicates that the molecular method used is an appropriate tool for detecting genetic polymorphism and can be utilized in formulating breeding strategies based on marker assisted selection in both the goat breeds.

Acknowledgment

We acknowledge the Dean, College of Veterinary Science and Animal Husbandry, NAU, Navsari, for providing necessary financial support and laboratory facilities to carry out the research work. We also thank

DOI 10.5455/ijlr.20180514113801

Research Scientist, Livestock Research Station, Navsari and Livestock Research Station, Dantiwada, Gujarat, respectively, for providing necessary help in sample collection for this study.

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DOI 10.5455/ijlr.20180514113801