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ANALYSIS OF POLYMORPHISM AT GROWTH-HORMONE LOCI IN GIR AND KANKREJ, INDIAN CATTLE

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ABSTRACT
This study test the distribution pattern of allelic variants at the GH-*Alu*I locus in gir and kankrej Indian native cattle breeds (*Bos indicus*). A 428 bp fragment of GH gene was amplified and digested with *Alu*I restriction enzymes. Two types of alleles L and V as well as two types of genotypes LL and LV for GH gene were observed. The frequency of L allele in gir and kankrej cattle was 1.00 and 0.994 respectively. Although two genotypes LL and LV were observed in kankrej cattle, none of the animal exhibited VV genotype either in gir or kankrej cattle. The One kankrej cattle has heterozygous genotype (LV). Because of very high frequency to almost fixed nature of L allele in gir and kankrej Indian cattle breeds, GH-*Alu*I polymorphism may not be a suitable candidate for animal selection purpose. However, the results could be used for association studies between this locus and milk production traits especially in kankrej cattle.

KEY WORDS: GH gene, Polymorphism, *Bos indicus*, PCR-RFLP

INTRODUCTION

Though India has 37 recognized cattle breeds, only Saihiwal, Red sindhi and Gir are dairy type milch breed. Gir cow is one of the prestigious zebu breed originating in Gujarat. Gir cattle is a milk purpose breed with a good milk production and kankrej cattle is a dual purpous breed with a draught and good milk yield belong to the *Bos indicus* type of cattle.

In India, genetic improvements in dairy cattle and buffalo carried out by traditional approach of progeny testing, pedigree selection and open nucleus breeding system. Progeny testing involves evaluation of bulls on the basis of their daughter's performance where as performance of parents and grandparents of the bull helpful in selecting the pedigree bull which is mainly depends on correct parentage records and its verification. Recently, technological development accelerates the detection of genes for desirable traits such as milk quantity and quality, growth, reproduction and disease resistance. This will helpful in marker assisted breeding and for deciding genetic merits in the dairy animals. Marker Assisted Selection is a method for the determination of such locus of quantitative traits (Lien *et al*., 1993). There are a number of genes discovered that affect milk production. They are GH (growth hormone), GHR (growth hormone receptor) (Iraz *et al*., 2012), LEP (leptin) genes (Rezaeia *et al*., 2015).

The total length of bGH gene is approximately 1800 bp in size. It contains 5 exons and 4 introns. The bGH is located on the 19th chromosome in q26-qter band region (Hediger *et al*., 1990). In livestock industries, higher concentrations of growth hormone are economic importance because they are associated with faster growth, if cattle stores less fat then in the dairy industry, more efficient milk quality and quantity production in dairy cattle (Mcmahon *et al*., 2001). Growth hormone has been known for many physiological activities, which include the regulation of growth, ageing, lactation, mammary gland development, gluconeogenesis and the activation of lipolysis (Burton *et al*., 1994).

Various studies have shown a possible direct relationship between allelic variants of bGH gene and milk productive traits in different cattle breed (Dybus *et al*., 2002 and Nina *et al*., 2012). Most of the studies describing bGH-*Alu*I polymorphism were in fundamentals focused on *Bos taurus* cattle populations, whereas limited systematic effort has been undertaken in the past to generate a comprehensive profile of bGH-*Alu*I polymorphism in Indian zebu cattle especially in Gujarat. *Bos indicus* populations possess different traits from those taurine breeds.

The polymorphic site in GH gene for *Alu*I restriction endonuclease, localized in the exon 5 and it is characterized by the substitution of cytosine for guanine at position 2,141 caused an amino acid change from leucine to valine at residue 127 (Lucy *et al*., 1993). The present study was with a view of the dominant contribution of gir and kankrej cattle breeds in Indian milk production to detect *Alu*I GH gene polymorphism in gir and kankrej cattle and estimate genotype and allele frequencies of this gene.

MATERIALS & METHODS

The genomic DNA for present study was extracted from blood samples collected from 177 Gir and 81 Kankrej cattle. They are randomly selected from the herds, maintained at Livestock Research Station College of Veterinary Science and Animal Husbandry cattle farm (AAU) in Anand, kankrej cattle breeding farm Thara and

Mandvi of Gujarat Livestock Development Board (GLDB) as well as private cattel farm of Anand Gujarat. 22 Gir cattle and 3 kankrej cattle semen straw were reccived from Amul Research and Development Association (ARDA), Ode farm and Sabarmati Ashram Gaushala (SAG), Bidaj farm. DNA was isolated by standard organic extraction method (Sambrook *et al*., 1989 and Miller *et al*., 1988).

The quality of DNA was checked on 0.8% agarose and quantity by nanodrop spectrophotometer (Thermo Scientific, USA) at A 260/A 280 nm. The samples having OD ratio between 1.75-1.9 were considered good and used for polymerase chain reaction (Eppendrof mastercycler, Germany). Genotyping analyses were performed by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. The 428 bp fragment of exon 5 in bovine GH gene was amplified by PCR using forward and reverse primers. F 5′-CGG ACC GTG TCT ATG AGA AGC TGA AG-3′, R5′- GTT CTT GAG CAG CGC GTC GTC A -3' (Balogh *et al*., 2009). The PCR mix contained 1X PCR buffer, 0.4 mM dNTPs, 1 Unit of *Taq* DNA Polymerase (Fermantas), 100 ng genomic DNA, 10 pM each of sense and antisense primer (Sigma) and sterilized distilled water to make a final volume of 25 µl.

The PCR reaction contains the following steps: Predenaturation for 5 minute at 94ºC followed by 32 cycles of 94ºC for 30 seconds, 60ºC for 1minute, 72ºC for 1 minute and final extension for 10 minutes at 72ºC. The PCR performance was verified by electrophoresis of 283 amplified products in 1.5% agarose gel for 70 min and stained with ethidium bromide, photographed under UV transilluminator (Labnet). The amplified PCR product was digested by using *Alu*I restriction enzyme and 1X reaction buffer (NEB) at 37ºC for overnight. The digested product was loaded and visualized on 4% agarose gel after staining with ethidium bromide and photographed under UV transilluminator. The genotype and allele frequencies were estimated by direct counting.

RESULTS & DISCUSSION

A 428 bp fragment of exon 5 in GH gene was amplified from all the samples and presented in (Figure.1). The GH allelic variation by PCR-RFLP showed two different alleles via *Alu*I restriction enzymes. Three kinds of band patterns corresponding to genotypes LL (homozygous leucine, 265, 96, 51 and 16 bp), LV (265, 147, 96, 51 and 16 bp) and VV (homozygous valine, 265, 147 and 16 bp) were observed for the GH gene (Figure. 2). The result of the present study showed that the GH *Alu*I loci allele L was more frequent than the V allele (0.9982 vs. 0.0018). Most of cows were homozygous for leucine allele 282 where as only 1 cow has heterozygous allele. Gir cattle was found to be monomorphic with only LL genotypes, The LV genotypes were found only in Kankrej cattle, its frequency was very much less (0.02).

The frequencies of L alleles of GH gene (1.00) obtained in this study for gir cattles were similar to the frequencie obtained earlier for the Nelore, Gyr and Guzera cattle (Kemenes *et al*., 1999). Jakaria *et al*., reported that the L allele frequency of GH *Alu*I loci was higher in *Bos indicus* cattle than *Bos taurus* origin cattle. The similar result was found by Ozdemir *et al*.,2011, in his study reported higher frequency of L allele (0.976 and 0.905) of GH gene for native East Anatolian red and brown swiss cattle which was almost equal to presently observed (0.998). It can be seen that gir and kankrej cattle frequency of the V allele was low (0.0 to 0.0060), suggesting that there may be a selection force acting against the V allele or favoring the L allele in these populations.

TABLE 1: Gene and genotypic frequencies of GH *Alu*I loci in gir and kankrej cattle

	Genotypes			Allele frequency	
Breed	LL	LV	VV	L	V
Gir (199)	199	00	00	1.0	00
Kankrej (84)	83	01	00	0.9940	0.0060
Total (283)	282	01	00	0.9982	0.0018

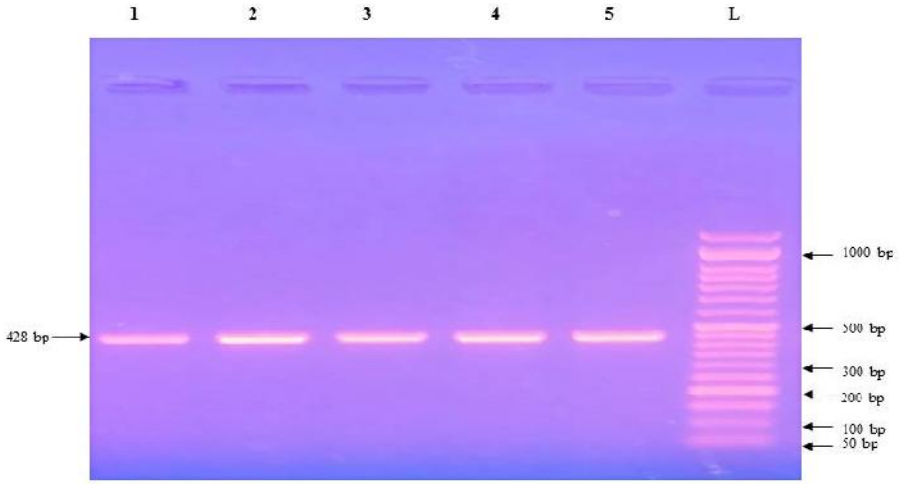

FIGURE 1: Electrophoretic pattern of PCR products (428 bp) generated by amplification of DNA using GH gene specific primer. Representative Five cattle samples PCR Product along with low range ladder (50 bp), Lane 1 to 5 PCR Products, Lane 6- Ladder.

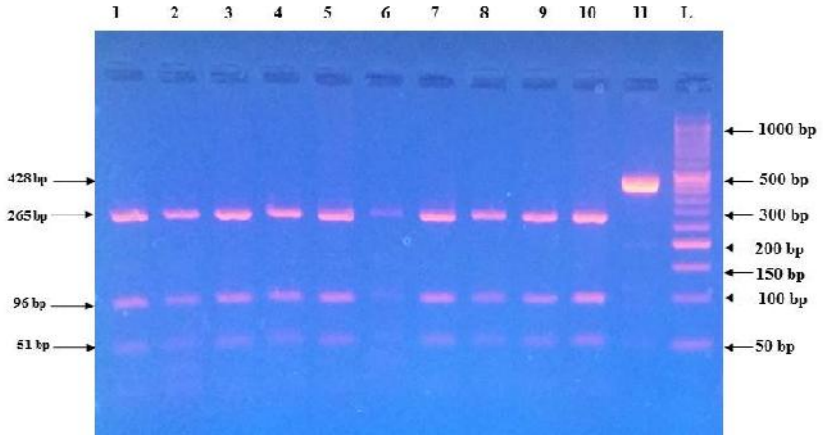

FIGURE 2: Electrophoretic pattern of *Alu*I digested PCR product of representative samples on 4 % agarose, Lane 1 to 10 Four fragment with 265 bp, 96 bp, 51 bp and 16 bp (LL genotype), Lane 11-PCR product, Lane 12 Low range DNA ladder (50 bp). The small 16 bp fragments were invisible in the gel.

It is obvious that the *Alu*I L allele frequencies in Indian zebu cattle (*Bos indicus*) are quite different from those reported for taurine breeds (*Bos taurus*) from Europe and America, where very low frequencies of the GH-*Alu*I L allele and high frequencies of the GH-*Alu*I V allele were detected. For example, GH-*Alu*I L allele frequencies 0.56 and 0.43 were reported in the South Anatolian and East Anatolian red cattle breed respectively (Yardibi *et al*., 2009), 0.3363 in Slovak spotted cattle (Nina *et al*., 2012), 0.47 and 0.44 in jersey cattle breed (Komisarek *et al*., 2011) and 0.31 in Holstein dairy cattle (Hadi *et al*., 2015).
On the basis of statistical analyses many scientist found that LL genotyped cattle produced milk significantly higher milk fat and protein percent. Dybus *et al*., 2002 found higher milk fat and protein yield in cows with LL genotype compared to LV individuals, the findings in Australian Holstein Friesian cows that animals with L/L and L/V genotypes had significantly greater than cows with V/V genotype (Shariflou *et al*., 2000). Sadeghi *et al* 2008 found that L allele was associated with higher milk protein yield. Biswas *et al*., 2003 reported an association in Holstein Friesian cattle the LV heterozygous had significantly higher birth weight than LL genotype (26.75 vs 22.00 kg).

CONCLUSION

Out of 283 gir and kankrej cattle 282 had LL genotype and only 1 kankrej cattle had LV genotype for locus GH-*Alu*I. The monomorphism of GH-*Alu*I is species specific, 199 samples from gir cattle were analysed and all found to be homozygous for the L allele. Therefore, the V allele does not exist in gir cattle (*Bos indicus*) or it is very rare. The Indian zebu (gir and kankrej) cattle have maintained the indicine characteristics at the growth hormone gene locus. The diversity of GH-*Alu*I gene in gir and kankrej cattle (*Bos indicus*) was very low. The low diversity in gir and kankrej can be caused by a limited number of bulls in the population, and the high inbreeding frequency.
In conclusion, it is noted that growth hormone gene is polymorphic in kankrej cattle and monomorphic in gir cattle. However, for better molecular study more populations of gir cattle from different locations of India are required to get accurate allele and genotype frequency

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