MOLECULAR GENOTYPING OF GROWTH HORMONE AND GROWTH HORMONE RECEPTOR IN SURTI BUFFALO

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ABSTRACT

The study was conducted to find out polymorphism of different growth hormone (bGH) and growth hormone receptor loci (bGHR) by using PCR-RFLP technique and association of different polymorphic bGH and bGHR loci with milk production in Surti buffalo. 72 blood samples of Surti buffalo were collected from LRS, Navsari Agricultural University, Gujarat. The DNA samples were subjected to PCR amplification using bGH and bGHR specific primers GH1, GH2, GH3 and GHR1. The PCR products of GH1 (427 bp), GH2 (891 bp), GH3 (441bp) and GHR1 (approx 640 bp) loci were digested with *Alu 1*, *Msp 1, Hae III* and *Mae II* restriction enzymes respectively. Only AA, CC, FF and RR genotypes were found in Surti buffalo. All the bGH and bGHR loci studied were monomorphic indicating monomorphism at these loci may be a species characteristic of Surti buffalo.

KEYWORDS: Buffalo, Growth hormone, Growth hormone receptor, PCR-RFLP

INTRODUCTION

Growth hormone (GH) is a polypeptide hormone secreted by somatotroph of the anterior pituitary. Biologically it helps in body growth through rapid cell division and skeletal growth. It also influences metabolism, mammogenesis, galactopoiesis, lypolysis etc (Bauman and McCutchean, 1986). Growth hormone receptor (GHR), a single-pass trans membrane protein of the cytokine receptor superfamily and is required to regulate the action of growth hormone. Allelic variation in the structural or regulatory sequences of the GH and GHR genes would be of interest because of possible direct or indirect effects on milk production and growth performance.

Considering the limited studies that had been carried out in buffaloes using molecular genetic techniques, the present study was undertaken in buffaloes to find out polymorphism at different bGH and bGHR loci i.e. GH1 (Growth Hormone1), GH2 (Growth Hormone 2), GH3 (Growth Hormone 3) and GHR1 (Growth Hormone Receptor) by using PCR-RFLP technique and their association with milk production.

MATERIALS AND METHODS

Experimental materials for the present study comprised of 72 blood samples of Surti buffalo maintained at Livestock Research Station, Navsari Agriculture University, Gujarat.

DNA extraction:

The DNA was extracted by phenol- chloroform method as per method described by John *et al.* (1991). DNA was kept for incubation at 55^{0C} for 45 min to enhance the dissolution and then stored at 4^{0C}.

Polymerase chain reaction:

Bovine GH gene specific primers (**GH1 F**: 5'-CCG TGT CTA TGA GAA GC-3' and **GH1 R**: 5'-GTT CTT GAG CAG CGC GT-3'; Lucy, 1991), (**GH2 F**: 5'- ATC CAC ACC CCC TCC ACA CAG T-3' and **GH2 R**: 5'-CAT TTT CCA CCC TCC CCT ACA G-3'; Zhang *et al.*, 1993), (**GH3 F**: 5'-ACG

CGC TGC TCA AGA AC-3’ **GH3 R**: 5’-GGC TGG AAC TAA GAA CC-3’; Unanian *et al.*, 1994) and bovine GHR gene specific primer (**GHRI F**: 5’-GCG TAG CTA CTC AAC TCA TCA AAC TGC CCA TAC-3’ and **GHR1 R**: 5’-AGC CAA CCC TGT GCC ATT CAA-3’, Ge *et al.*, 2000) were custom synthesized at Sigma, India and were used to amplify different fragments.

PCR was carried out in a final reaction volume of 25 ml. Each reaction volume contained 12.5µl of MBI Fermentas 2X PCR Master Mix used at 1X concentration (Composition: Taq DNA polymerase (recombinant) 0.05 unit/µl, 4mM $MgCl_2$, 0.4mM dNTPs, 1.0 µl of primers (10 pmole each), 3.0 µl template DNA (90ng) and 7.5 µl deionised water. The reaction mixture was subjected to 32 cycles of denaturation at 94^{0C}, annealing at appropriate temperature (GH1 & GH3 loci-60^0C, GH2 loci -64^0C and GHR1 loci-50^{0C}) and extension at 72^{0C}. Initial denaturation was carried out at 94^{0C} for 5 minutes, while the final extension was performed at 72^{0C} for 10 minutes.

Restriction Fragment Length Polymorphism (RFLP) and Agarose gel electrophoresis:

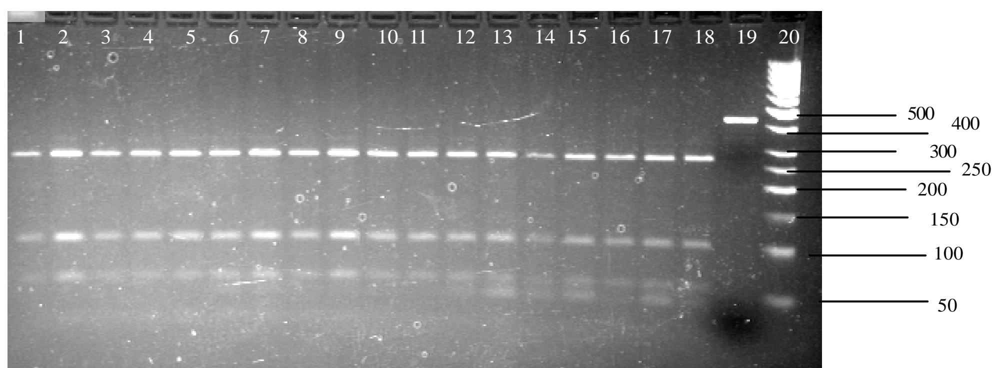
10 µl of PCR products were digested with *Alu I*, *Msp I, Hae III* and *Mae II* restriction enzymes, respectively by incubating them at 37^0C for 2 hours (Mini Cycler) except for *Mae II* which was incubated at 65^{0C} for 2 hours and electrophoresed on 2.5% agarose gel for 60-90 min (80 V) to reveal the restriction pattern. Single stained GelStar loading dye containing stock GelStar and Dimethylsulfoxide at the ratio of 1: 99 was used to load the digested PCR samples. 50bp DNA Ladder was used as a molecular size marker. The bands were visualized under UV light and documented by gel documentation system (Syngene, Gene Genius Bio Imaging).

RESULTS AND DISCUSSION

PCR amplification generated segments of 427 bp, 891 bp, 441 bp and approx 640 bp for GH1, GH2, GH3 and GHR1 loci respectively which is homologous to the cattle GH gene of similar length.

As per Zhang *et al.* (1992) bovine GH gene RFLP for *Alu I* restriction enzyme, present within exon fifth results in three genotypes: AA (274, 96, 50 and 16 bp fragments), BB (274,146 and 16 bp) and AB (274, 146, 96, 50 and 16 bp) based on presence and absence of restriction site. In the present study, only AA genotype was found in all the animals (Fig.1.)

Fig.1. GH1 gene 427 bp PCR fragment in Surti buffalo digested by *Alu I*



Lanes: 1-18 Surti (all AA genotype)

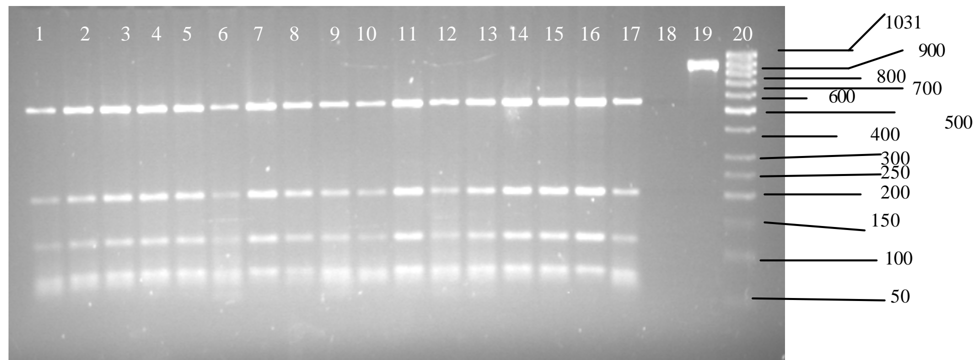
Lanes: 19- Positive Control (undigested PCR product)

Lanes: 20- 50 bp DNA Ladder

As per Zhang *et al.* (1993) bovine GH gene RFLP for *Msp I* restriction enzyme, present within intron third were designated as ‘C’ and ‘D’ alleles, recorded three genotypes as CC (526, 193, 109 and

63bp fragments) DD (635, 193 and 63 bp) and CD (635, 526, 193 109 and 63bp) for presence and absence of restriction site. In the present study, only one type of genotype i.e. CC is found in all the animals (Fig.2.)

Fig.2. GH2 gene 891 bp PCR fragment in Surti buffalo digested by *Msp I*



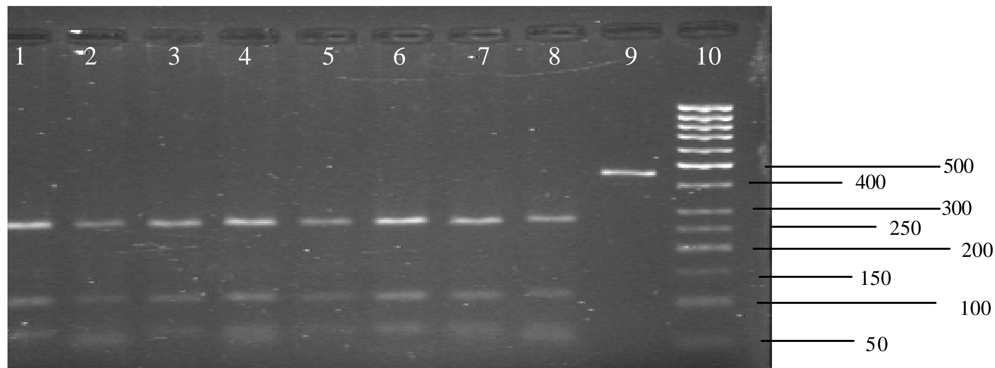
Lanes: 1-17 Surti (CC genotype)

Lanes: 19- Positive Control (undigested PCR product)

Lanes: 20- 50 bp DNA Ladder

As per Unanian *et al.* (1994) bovine GH gene RFLP for *Hae III* restriction enzyme, present within 3' flanking region were designated as 'E' and 'F' alleles, showing three genotypic patterns: EE (268bp, 102bp, and 71bp fragments), FF (268bp, 102bp, and 50bp) and EF (268bp, 102bp, 71bp and 50 bp).In the present study, only genotype FF was found in all the animals (Fig.3.)

Fig.3. GH3 gene 441 bp PCR fragment in Surti buffalo digested by Hae III



Lanes: 1--8 Surti (FF genotype)

Lanes: 9- Positive control (undigested PCR product)

Lanes: 10- 50 bp DNA Ladder

Mitra *et al.* (1995) studied 57 Indian Sahiwal cattle, 53 Murrah, 19 Nili-Ravi, 11 Egyptian buffaloes for GH *Alu I and* GH *Msp I* polymorphism resulted in monomorphic pattern for all buffaloes in contrast to presence of polymorphism in cattle.

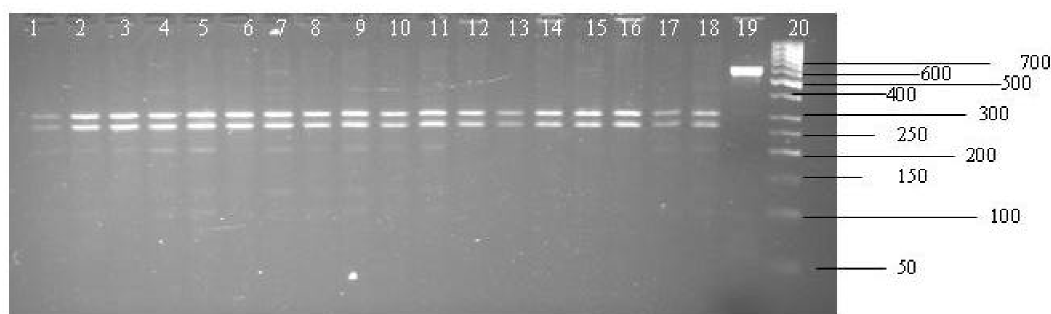
Biswas *et al.* (2003) carried out a study in Sahiwal, Holstein-Friesian, Jersey and crossbred cattle and Murrah, Bhadwari, Surti, Nagpuri and Surti buffaloes for *Alu I* polymorphism also showing

monomorphism in buffaloes and polymorphism in cattle.

Ge *et al.* (2000) have observed four SNP (single nucleotide polymorphisms) at the position of 76 (T↔C), 200 (G↔A), 229 (T↔C), and 257 (A↔G) bp from the 5' end of the growth hormone receptor fragment amplified with GHR1 primer sets.

Results obtained for growth hormone receptor gene in the present study are contrary to the earlier report of Ge *et al*. (2000) i.e. the C allele had been recognized using *Mae II* at positions 76 and 229 bp but are in accordance with the results of Pawar (2005) i.e. cattle GHR1 gene comprising of two fragments of around 280 and 300 bp with only one internal restriction site for *Mae II*. (Fig.4).

Fig.4. GHR1 gene 640 bp PCR fragment in Surti buffalo digested by *Mae II*



Lanes: 1-18 Surti (RR genotype)

Lanes: 19- Positive Control (undigested PCR product)

Lanes: 20- 50 bp DNA Ladder

Since all the loci studied were monomorphic in all the animals, indicating monomorphism at these loci may be a species characteristic of buffalo.

CONCLUSION

Only AA, CC, FF and RR genotypes were found in Surti buffalo at GH1, GH2, GH3 and GHR1 loci respectively. The allelic frequencies of A, C, F and R alleles were 1.00 with absence of B, D, E and S alleles respectively. Monomorphism at these loci may be a species characteristic of buffalo, probably due to absence of any mutation and high degree of sequence conservation. As buffalo GH and GHR gene loci studied at present are monomorphic, they can not be used as genetic markers for selection purpose.

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