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SHORT REPORT

Single strand conformation polymorphism (SSCP) in 3' region of growth hormone gene in five breeds of Indian buffalo*

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Polymorphism in the 3' region of growth hormone (GH) gene in buffaloes of five Indian breeds was investigated using polymerase chain reaction (PCR) and single strand conformation polymorphism (SSCP). Screened were 100 Indian buffalo dams of Murrah, Nagpuri, Mehsana, Nili-Rawi and Surti breeds (20 of each). Based on the published nucleotide sequence information on bovine GH gene (GenBank M57764) oligonucleotide primer was designed to amplify a 280 bp orthologous sequence covering the 2473 to 2752 nucleotide (nt) sequence region. The PCR conditions were optimized to yield specific amplification of desired fragment length. The PCR product was denatured and subjected to polyacrylamide gel electrophoresis to detect SSCP of which six highly reproducible patterns were found. However, genotype frequencies varied in buffaloes of the breeds concerned, and in some of them some SSCP patterns were not identified at all.

KEY WORDS: buffaloes / DNA polymorphism / growth hormone / SSCP

Currently, the primary thrust of research in animaI genetics is the identification of genes (so-called major genes), which affect the expression of quantitative traits

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markedly. One of the potential major genes is that of growth hormone gene (*GH*). There is extensive literature on the possible relationship(s) between genetic polymorphism of *GH* and production and reproduction traits in cattle, but perusal of literature has indicated paucity of information on buffalo *GH*. Keeping in view that 54% of milk in India is yielded by buffaloes (The Hindu Agriculture Survey of India 2004) and the important role played by GH in growth and production of animals, this report has been prepared concerning the identification of DNA polymorphism in the Indian buffalo GH gene (*GH*). *GH* in the buffalo is located to chromosome 3p [Iannuzzi *et al*. 1999] and has been sequenced by Tiwari and Garg in 1998 (unpublished). The gene is approximately 1800 bp with five exons (I through V) and four introns (A through D). Unanian *et al*. [1994] reported a polymorphic site in the 3' region of the bovine *GH*, probably at position 2637. At this position, genotypes can be identified by PCR-RFLP because of the presence (or lack) of a *Taq*I restriction site. Rocha *et al*. [1992] reported an association between calf birth weight as a maternal trait and genomic DNA *Taq*I restriction endonuclease allele that hybridized to a growth hormone cDNA probe.

The objective of this study was to find a sequence variation in 3' region of *GH* in five Indian buffalo breeds based on single strand conformation polymorphism (SSCP).

Materials and methods

Included were 100 Indian buffalo (*Bubalus bubalis*) dams of Murrah, Nagpuri, Mehsana, Nili-Rawi and Surti breed, 20 of each breed. Approximately 15 ml blood was withdrawn from each animal and genomic DNA was isolated from leukocytes. Based on the published nucleotide sequence information on bovine *GH* [Gordon *et al*. 1983, Lagziel *et al*. 1996], a pair of oligonucleotide primers was designed, as follows:

GH 1: 3'- GTGGGGTGGGGCAGGACAGC-5' and

GH 2: 3'- TGGTTTGGTGGGCTGATGAG-5'.

With these primers a 280 bp of *GH*, from nt 2473 to nt 2752 was amplified by PCR. PCR was performed in reaction volume of 25 µl containing 100 ng of genomic DNA, 1 µl of each primer (50 pmoles), 1.5 µl $MgCl_2$ (25 mM) and 2.5 µl 10 × PCR buffer, 0.5 µl of dNTP (10 mM each), one unit *Taq* polymerase (SIGMA) and H_2O up to 25 µl. The first cycle was at 94°C for 4 min, followed by 35 cycles at 94°C for 45 s, 56°C for 30 s and 72°C for 20 s. The 10 min extension step at 72°C was added after the 35th cycle.

The single-strand conformation polymorphism (SSCP) analysis was carried out with the mini-electrophoresis apparatus (TARSONS). The 12% polyacrylamide gel was prepared with a 1 × TBE buffer and electrophoresis was run at 7 volts/cm for 12 hrs at room temperature. Ten µl of PCR product were mixed with 10 µl of denaturation buffer (formamide, 0.25% bromophenol blue, 0.25% xylene cyanole, 0.5 M EDTA), denatured for 5 min, rapidly chilled on ice and then loaded onto the gel. The gels were stained with the silver staining method.

Results and discussion

The studies of genetic markers applied to animal breeding and production is focused mainly on analyses of mutations located within economically important structural genes and searching association between them and quantitative traits *loci* (QTLs). The *GH* is a candidate gene for quantitative traits in cattle.

We used the PCR-SSCP method to identify sequence variation in 3' region of *GH* in Indian buffalo. Searching for SSCP is a powerfu1 method for screening of DNA. SSCP is based on sequence-specific differential migration of single-strand DNA through a non-denaturing gel matrix. Under appropriate conditions, single-stranded nucleic acids form unique secondary structures depending on their sequence. A single nucleotide substitution can alter the secondary structure and result in differences in electrophoretic mobility of the nucleic acid. Multiple fragments can be analysed in a lane of an electrophoresis gel, making this method efficient for discovering new mutations [Orita *et al*. 1989].

First we obtained a specific PCR product of desirable size of 280 bp. Then the DNA amplification product was subjected to SSCP analysis to identify the DNA sequence variation. The variation was clearly shown by the number of bands and their position in the gel.

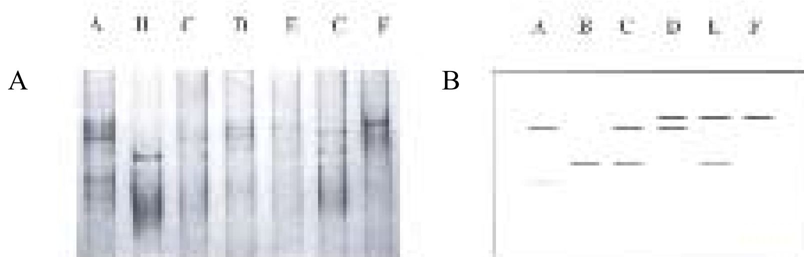

Fig. 1. SSCP patterns of 3' region of buffalo growth hormone gene. A – Polyacrylamide gel electrophoresis of the 280 bp GH gene fragment amplified from DNA of 100 animals. B – Schematic representation of individual SSCP patterns.

Table 1. Frequency of six SSCP patterns (genotypes) in 3' region of GH gene in different Indian buffalo (*Bubalus bubalis*) breeds

Breed (%)	Genotype					
	A	B	C	D	E	F
Murrah	35	15	-	10	40	-
Mehsana	15	10	30	20	5	20
Nagpuri	35	30	15	10	-	10
Surti	15	5	10	35	15	10
Nili-Rawi	35	15	-	25	-	25

Within the buffalo population analysed, six SSCP patterns were observed (Fig. 1). The individual SSCP patterns were highly reproducible, but frequency of genotypes varied in different breeds (Tab. 1).

This is the first study that shows the polymorphic nature of the 3' region of buffalo *GH*. DNA sequencing is needed to find nucleotide sequence variation in different SSCPs patterns. The difference in genotype frequency of SSCP patterns and absence of some SSCP patterns among the buffalo breeds may be due to stochastic factors such as genetic drift and founder group effect. The results of our study may be used to search for associations between different SSCP patterns and buffalo performance traits.

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SSCP w rejonie 3' genu hormonu wzrostu pięciu ras bawołu azjatyckiego (*Bubalus bubalis*)

S t r e s z c z e n i e

Na materiale złożonym ze 100 samic bawołu azjatyckiego (*Bubalus bubalis*) należących do pięciu ras (po 20 zwierząt każdej rasy) badano polimorfizm PCR-SSCP w rejonie 3' genu hormonu wzrostu (*GH*). Na podstawie informacji o sekwencji nukleotydowej genu *GH* (GenBank M57764) opracowano startery przeznaczone do amplifikacji ortologicznej sekwencji, obejmującej rejon położony między nukleotydem 2473 a 2752. Zoptymalizowano warunki PCR i uzyskano fragment DNA o założonej długości 280 nt. Produkt PCR zdenaturowano i poddano elektroforezie w żelu poliakryloamidowym. Wykryto sześć wysoce powtarzalnych wzorów SSCP. Frekwencje poszczególnych genotypów (wzorów SSCP) były różne i zależały od rasy zwierząt; niektóre polimorficzne układy SSCP nie występowały w ogóle u pewnych ras.