Short communications

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Genetic polymorphisms in growth hormone gene in Patanwadi, Marwari and Dumba breeds of sheep

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India ranks third in the world in sheep population. It has 62.5 million sheep (FAO, 2011). India is endowed with wide diversity of sheep genetic resources, which forms the backbone of its rural livelihood security. Sheep contributes about 238.8 million kg meat/year. Sheep are reared for carpet wool as the major produce. However, with the increase in requirement for food and nutrition, meat has replaced wool. It is highly important to increase carcass weight with a strategy to produce more quality meat/animal. Genomic structure of growth hormone (GH) gene has been studied in different animal species including human (Fiddies *et al*. 1979), bovine (Woychick *et al*. 1982), pig (Vize and Wells 1987), sheep (Bryne *et al*. 1987) and goat (Koika *et al*. 1989). The growth hormone plays an important role in many physiological actions. It involved in the processes of sexual diûerentiation and pubertal maturation and it participates in gonadal steroidogenesis, gametogenesis and ovulation. It also has additional roles in pregnancy and lactation. The objective of any sheep breeding programme is to determine the gene sets that positively affect production traits and to improve phenotypic structures by means of transferring these gene sets to the next generations. The strategy based on the selection of animals with an endogenous GH secretion resulting in a superior quantitative trait is desirable. This productive advantage could be the result of a more active GH variant or of a more favourable upstream regulation directing its synthesis. The present investigation was undertaken with the objective to study the polymorphism of growth hormone gene by PCR-RFLP in Patanwadi, Marwari and Dumba sheep.

Collection of blood samples and DNA isolation: A study was designed to characterize the growth hormone gene in Patanwadi, Marwari and Dumba sheep breeds of Gujarat. About 8 ml of blood sample were collected from 150 adult sheep (50 animals of each of Patanwadi, Marwari and Dumba) by jugular vein puncture using disposable needle containing EDTA as anticoagulant. The genomic DNA was isolated from blood by phenol-chloroform extraction method (Sambrook and Russel 2001). The quality of DNA was checked by 0.8% agarose gel electrophoresis.

PCR amplification: Primers and protocol for amplification were followed from Hua *et al*. (2009). Total two primers used for the polymorphism study of GH gene were as follows:

GH1 F: 5' CTCTGCCTGCCCTGGACT3',
GH1R: 5' GGAGAAGCAGAAGGCAACC 3'
GH2 F: 5' TCAGCAGAGTCTTCACCAAC3'
GH2R: 5' CAACAACGCCATCCTCAC 3'

The amplification included a initial denaturation at 94°C for 5 min, 35 cycle of denaturation at 95°C for 30 sec; annealing at 65–52°C for 30 sec; extension at 72°C for 45 sec followed by final extension at 72°C for 7 min. The amplicons were checked by 2% agarose gel electrophoresis at 90V for 1 h. The PCR amplified product of GH Exon 2 and 3 (A781G) and Exon 4 (A1575G) locus was cleaved with the HaeIII restriction enzyme in 12µl reaction volume at 37°C for 16 h. The RE digested products were electrophoresed in 2% (G1) and 3% (G2) agarose gel, and the genotyping patterns were visualized by ethidium bromide staining.

DNA quality was checked on 0.8% agarose gel electrophoresis visualized with ethidium bromide under UV illuminator (Fig.1).

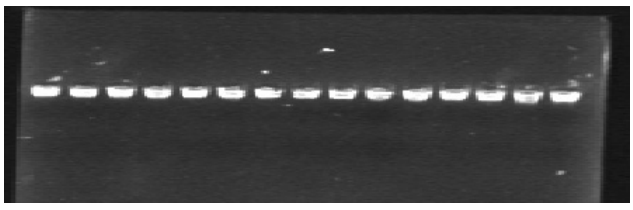

Fig. 1. DNA quality checked on 0.8% agarose gel

PCR amplification for growth hormone gene

Analysis of genotypic frequencies and allele frequencies of growth hormone gene: Genotypic frequencies of growth hormone gene locus A781G in Marwari breed of sheep were 0.08 and 0.92 for AA and AB, respectively; while, the allelic frequencies were 0.54 and 0.46 for A and B, respectively. The Chi square test for Hardy Weinberg equilibrium indicated significant differences (P>0.01) among the gene and genotypic frequencies with respect to GH gene (χ^2=35.44).

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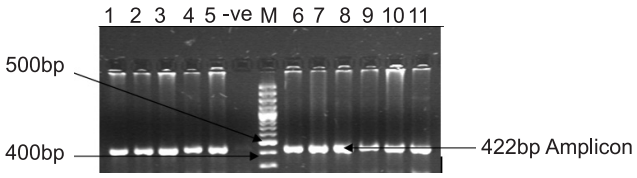

Fig. 2a. Representative genotyping of GH2 gene

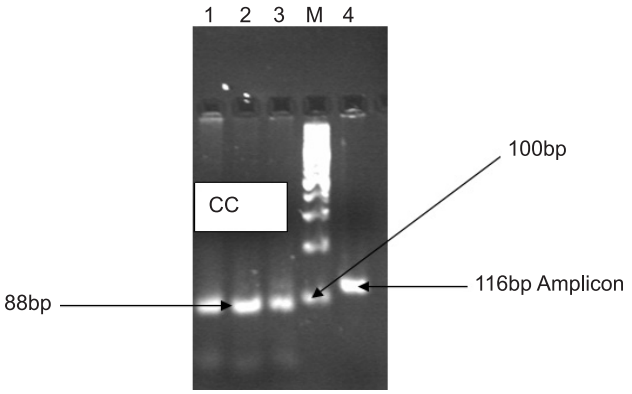

Fig. 2b. Amplification of growth hormone (GH1) fragment

Genotypic frequencies of growth hormone (GH) gene locus A781G in Patanwadi breed of sheep were 0.00 and 1.00 for AA and AB, respectively; while, the allelic frequencies were 0.50 and 0.50 for A and B, respectively. The Chi square test for Hardy Weinberg equilibrium indicated significant differences ($P<0.01$) among the gene and genotypic frequencies with resepect to GH gene (=49.00). Genotypic frequencies of growth hormone gene locus A781G in Dumba breed of sheep were 0.04 and 0.96 for AA and AB, respectively; while, the allelic frequencies were 0.52 and 0.48 for A and B, respectively. The Chi square test for Hardy Weinberg equilibrium indicated significant differences ($P<0.01$) among the gene and genotypic frequencies with resepect to GH gene (=41.68). Hence it was inferred that Marwari, patanwadi and dumba breed populations were not in Hardy Weinberg equilibrium with respect to locus A781G. Genotypic frequencies in Marwari, Patanwadi and Dumba indicated that heterozygotes were favoured for better growth and higher birth weight. Patanwadi population showed 100% heterozygotes, with AB genotypes, could be due to sampling error or breeding homozygotes male and female, resulting in all the heterozygotes in the sample. All breeds exhibited clear predominance of the A allele, with the highest frequency in samples from Marwari breed (0.54) and Dumba breed (0.50).

Growth hormone gene fragment was amplified using gene specific primers for both the locus (A781G and A1575G). PCR products of 422 bp (GHR1/GHF1) and 116 bp (GHR2/GHF2) were resolved on 2% agarose gels (Fig. 2a, b). These amplified products were further used for the RFLP analysis.

A781G locus, 144 sheep were heterozygous (AB) and 6 sheep were homozygous (AA) but no homozygous (BB) individuals were found. At A1575G locus, all sheep were homozygous (CC) but no homozygous (DD) as well as heterozygous (CD) individuals were found.

Allelic frequency was 0.52 for A allele and 0.48 for B allele and the overall genotypic frequencies for AA, AB, BB and CC were 0.06, 0.94, 0 and 1.00 respectively. Genotypic frequencies for GH gene locus A1575G in all the 3 population indicated the fixation at this locus (CC) its frequency being 1.00 in Marwari, Patanwadi and Dumba population. In accordance with the present finding, Hua *et al.* (2009) also observed in Boer goat bucks similar results wherein they observed at A781G locus 25 bucks were heterozygous while no homozygous (BB) individuals were found. In this study they also observed that CD genotype animal measured smaller than CC genotype in height at weaning. It can be assumed that selection for higher growth favoured for CC genotype and this could be the reason as to why CC genotype was fixed in all the 3 populations in the present study.

The molecular genetic analysis of GH gene revealed that the restriction fragments generated for A781G locus were of 366 and 56 bp for AA genotype and 422, 366 and 56 bp for AB genotypes, respectively. Greater frequencies of allele A and genotypes AB and AA showed that selection for higher growth favoured AA homozygotes and AB heterozygotes. The restriction fragments generated for GH gene A1575G locus were if 88 and 28 bp for CC genotype. Individuals with AB heterozygous genotype exhibited higher birth weight. Complete absence of BB genotype could be due to the fact that selection did not favour BB genotype due to the negative influence of B allele on growth in sheep. The genotypic and allelic frequencies of growth hormone gene showed that all 3 breeds were significantly away from Hardy Weinberg equilibrium.

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