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"Study of DNA Polymorphism at β-Lactoglobulin Locus in Jamunapari and BarbariGoats"

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Abstract: *The present study was carried out of 44 animals (28, Barbari and 16 Jamunapari goats). The blood samples were collected aseptically. There were two primers (LGC1, LGC2) and two restriction enzyme (Sacll and Racl) used for study of polymorphism. Here two variant AA and AB were obtained in both populations at first locus at β-LG gene. Genotypic frequencies were AA = 0.142 AB = 0.857 in Barbari goats were observed. Genotypic frequencies were AA= 0.687 AB= 0.156 in Jamunapari goats were observed. Here, two variant AA and BB were obtained at second locus ofβ-LG gene. Genotypic frequencies of AA = 0.687 BB = 0.315 in Jamunapari goats were obtained. There were non-significantresults after chi-square test between observed and expected genotype in both populations at locus of β-LG gene.*

Keywords: *DNA, Polymorphism, Genotype, PCR, Lactoglobulin*

I. Introduction

India is agricultural country and Indian economy mostly based on agriculture. Livestock play a vital role in agriculture. Goats have important role in Indian economy and life of poor people. Among the livestock goats, first ruminant, which have been domesticated for meat purpose the time of domestication was well before 100bc and place of domestication are to be slopes of Zagros Mountain on the boarder of present day Iraq and Iran (Lush, 1957). Goats rearing have been recommended as best choice for the rural people in developing countries because of their wider adaptability, low investment, high fertility, and fecundity, high feed conversion efficiency and low risk involved.

The present worldwide distribution of goats shows that the number of milch type are more in temperate zone and dual types are located in sub tropical Asia and African countries. Indian rank first its genetic resources and numerical superiority in world. There are 20 breed goats in India. Jamunapari and Beetal are considered to be important milch breeds of goat in India. There are three important milch breeds found in northern region Jamunapari, Barbari and Beetel. Among them Jamunapari goat is dual purpose breed belonging to dry northern region (Jamuna and Chambal rivers, Chakarnagar, Etawah). However, Barbari breed of goat belonging northern central region of India best suited dual purposes, best for stall feeding.

Because of their contribution genetics study at cytogenetic as well as molecular level for increase the production. The recent past methods which utilize, polymorphic DNA loci, have been developed for selection of superior animals. Such developments in polymorphism at DNA sequence level and use them as markers, for evaluation of genetic basis for observed phenotypic variability.

The molecular techniques allow detecting variation or polymorphisms exists among individuals in population for specific of DNA. When a locus has allelic variants at frequencies too high to be polymorphic and the population exhibit polymorphism for that locus. Extensive polymorphism is present in genes structure mostly with co-dominant expression.

β-lacto globulin is major whey protein in milk of ruminant. It also found in milk of other Mammals as well, but absent from milk of rodents, lagomorphs or humans (Hamlinget al., 1992).

The goat β- LG gene has been assigned by in situ hybridization to chromosome 11q23 and also located GC rich region of genome. Monitoring of genetic variation at DNA level became possible with development of recombination DNA technology. Restriction enzymes and southern blot hybridization (Southern, 1975) were used to identify single-base pair changes in genomic DNA that result gain or loss of a restriction site. These nucleotide variants were called restriction fragment length polymorphisms (RFLPs).

The genetics polymorphisms at the DNA sequence level are used as markers. These markers revealing variations at DNA level referred to as molecular markers.

The progress in development of molecular markers suggests their potential use for genetic improvement in livestock species. In the recent year several markers, linked loci, claimed to be having major effects on quantitative traits. In farm animal species have been identified (Georges et al, 1988). The marker based breeding methods require a large number of markers evenly distributed in genome. However,

identification of complex traits responsible for economic traits in livestock species in far from completion and efforts are going on to evolve new DNA marker.

II. Materials and methods

The present study was carried out on 44 animals, out of 44 animals 28 animals belonging to Barbari and 16 animals belonging to Jamunapari breed of goat.All unrelated Barbari breed of goat selected from Waidaha village of sultanpur District. All Jamunapari breed of goat selected from Livestock Unit NDRI Karnal. The Blood samples were collected aseptically in vaccutainer tubes from juglar vein. The molecular work analysis was carried out at livestock Genomic Analysis Laboratory DCB Division, NDRI, Karnal (Haryana).

III. DNA Isolation

Genomic DNA was isolated from blood samples following phenol-chloroform extraction method (Sambrook etal. 1989). After isolation DNA pellet was dissolved in water and kept in water bath at 60℃ for 2hrs. After that DAN was cooled and stored at – 20 ℃ for use.

The quality and purity of DNA were checked and quantization was done by UV-spectrophotometery by taking ratio O.D. at 260 and 280 nm. DNA samples with an OD_{260} :OD_{280} ratio of 1.8 to 2.0. DNA concentration was estimatedusing the formula Con. Of DNA(µg/ml) = OD_{260}× dilution factor × 50. The DNA concentration was determined approx. 30 ng/µl with HPCLwater.

PCR amplification of β-lacto globulin gene

A 426 bp and 120 bpfragment of β-lacto globulin gene spanning over part of exon 7 and entron 7 was amplified two primers with pair of forward (5'-CGGGAGCCTTGGCCCTCTGG-3') reverse(5'CCTTTGTCGAGTTTGGGTGT-3')andforward(5'-CAACTCAAGGTCCCTCTCCA-3') reverse (5'CTTCAGCTCCTCCTCCACGTACA-3'). PCR mix containing PCR buffer 100µl, $MgCl_2$ 4µl, dNTPs 100µl, forward primer 74µl, reverse primer 54µl.For PCR each tube contained 44µl PCR mix, 2µl DNA sample and 4µl of tag polymerase.

PCR reaction was carried out in programmed thermal cycler machine. The cycling condition for primer LGC1 DNA amplification were 35 cycles of denaturation at 95℃ for at 30sec. annealing at 65^0C for 1 min. extension at 72℃ for 90 sec followed by final extension at 72℃ for 5 min. The cycling condition for primer LGC2 DAN amplification were 31 cycles of denaturation at 94℃ at 30 sec. annealing 60℃ and extension at 72℃ for 90 sec followed by final extension at 72℃ for 5 min. Amplified product were checked at 1.5% agarose in gel electrophoresis.

Restriction digestion

The 426 bp and 120 bpamplicon was treated with Racl enzyme to identify RFPLs of β-lacto globulin gene.14 µl PCR product was digested with 0.30 µl Racl enzyme and digested buffer at 37℃ for 3 hrs. The digestion reaction was stopped by adding 0.5m EDTA(pH 8.0). The 120 bpamplicon was treated with Scall enzyme to identify RFPLs of β -lacto globulin gene.14 µl PCR product was digested with 0.30 µl Scall enzyme and digested buffer at 37℃ for 3 hrs. The digestion reaction was stopped by adding 0.5m EDTA (pH 8.0).

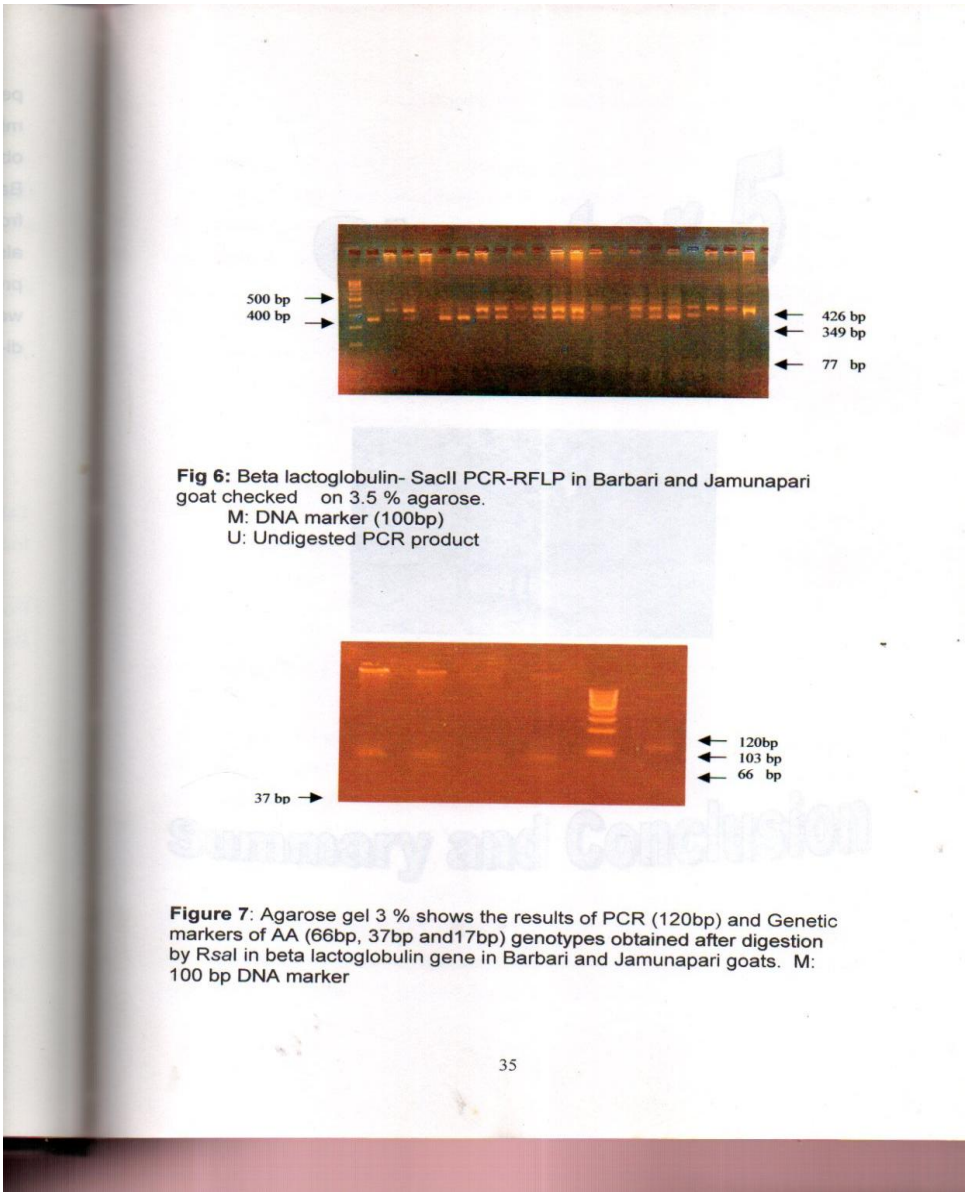

Fig 6: Beta lactoglobulin- SacII PCR-RFLP in Barbari and Jamunapari goat checked on 3.5 % agarose.
M: DNA marker (100bp)
U: Undigested PCR product

Figure 7: Agarose gel 3 % shows the results of PCR (120bp) and Genetic markers of AA (66bp, 37bp and17bp) genotypes obtained after digestion by *Rsa*I in beta lactoglobulin gene in Barbari and Jamunapari goats. M: 100 bp DNA marker

IV. Statistical analysis

Gene and genotype frequency was estimated as per described by Falconer (1998). Data was tested by x^2-test.

V. Result and Discussion

Genotyping at β-LG first locus at DNA level revealed the presence of two alleles. The spanning from exon 7 to 3' flanking region of the goat β-LG gene was amplified from genomic DNA sample belonging to 2 genetic group.PCR products was digested with Scall enzyme and subsequently analyzed for mutation in the fragment. Two variants AA and AB were observed in the both population. Two different group (349bp and 78bp) and undigested were obtained. PCR-RFPL with Sacll enzyme recognized the polymorphic site, which was produced by a single nucleotide substitution in position +4601(Pena et al.2000).

Proportion of AA was high 68.7% in Jamunapari breed and AB proportion was high 85.7% in Barbari breed. Proportion of AA in Barbari breed was 14.28% and proportion of AB in Jamunapari breed was 31.2%. There were non- significant results after x^2-test between observed and expected genotype. Means population was in HW equilibrium. Result on second locus of β-LG gene PCR product was observed 120bp PCR product digested with Racl enzyme subsequently analyzed for mutation in fragment. AA (66bp, 37bp and 17bp) and BB (103bp and 17 bp) genotypes obtained after digestion by Racl. The result after used primer LGC2 and enzyme Racl out of 28 sample of Barbari and 16 Jamunapari samples homozygous AA genotype was most frequent while heterozygous AB genotype was absent in Barbari and Jamunapari goats. AA genotype in Barbaribeed was

71.4% and in Jamunapari breed was 68.75%. BB genotype in Barbari breed was 24.8% and in Jamunapari breed was 31.5%. there were non-significant results after x^2-test between observed and expected genotype. Population was in HW equilibrium.

VI. Conclusion

The result show that at both loci of β-LG gene DNA polymorphism exists. This polymorphism shows that β-LG gene can be used in making early selection. The molecular markers can be used to increase intensity of selection at younger age, thus reducing expenditure on unproductive stage of life. Also these primers can be used for making marker-assisted selection for profitable goat farming enters prices.

More information is available on β-LG gene in cattle however less work has been done in case of goat. Thus this information will be helpful for rearing the goat as dairy animal to fulfill the demand much needed.

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