PCR-RFLP in IGFBP-3 Gene and its Association with Economic Traits in Muzaffarnagari Sheep

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Abstract—Using two resource populations, differing for body weight at 12 months of age (Resource population I) and for total greasy fleece yield (Resource population II), PCR-RFLP studies were carried out for Insulin like Growth Factor Binding Protein (IGFBP3) in Muzaffarnagari sheep. Significant differences (P < 0.01) between high and low 12 month body weight groups (43.22 ± 1.46 kg and 22.81 ± 0.99 kg, respectively); and between high and low greasy fleece yield groups (1676.18 ± 55.81 g and 625.24 ± 44.13 g, respectively) showed the effectiveness of resource populations. Monomorphic PCR-RFLP pattern of 654 bp fragment of IGFBP-3 (comprising of partial exon 2, complete intron 2 and exon 3, partial intron 3) with HaeIII restriction enzyme was observed between high and low 12 month body weight groups as well as between high and low greasy fleece yield groups. Similar trend was observed for PCR-RFLP of 333 bp fragment (comprising of intron 1, exon 2 and intron 2). Hence, no association could be established between Hae III PCR-RFLP pattern of 654 bp as well as 333 bp fragment of IGFBP-3 gene with either of the production traits, i.e. body weight or greasy fleece yield.

Keywords—Greasy fleece yield, IGFBP-3, monomorphic pattern, and resource populations.

I. INTRODUCTION

INSULIN-like growth factor binding proteins (IGFBPs) belong to a family of at least six homologous proteins that bind insulin-like growth factors (IGFs) and modulate many of their biological actions. IGFBP-3 is used as a marker for different body functions such as growth, metabolism, reproduction, in controlling body weight, immunity and energy balance etc. Due to the key role of IGFBP-3 in growth and development of animals, the IGFBP-3 gene is considered as a candidate gene for its use as a marker for growth and production traits. Hence the polymorphism in IGFBP3 has been studied in different live stocks such as pig, cattle, buffalo and goat for its association with economic traits [1], [2], [3]. Muzaffarnagari sheep is one of the tallest and heaviest mutton breeds of India. The inherent potential for growth and wool production of this breed has not been exploited due to inadequate information about genetic basis and the breeding strategies. Considering the importance of Muzaffarnagari sheep in production traits, the present research work was conducted to detect polymorphism in IGFBP-3 gene and to find out its association with body weight and wool production.

II. MATERIALS AND METHODS

Muzaffarnagari sheep flock maintained at Central Institute for Research on Goat for genetic improvement through selective breeding using quantitative genetic tools were used. Two resource populations were developed. For 1st resource population, a total of 10 individuals (5 males and 5 females) showing least and 10 individual showing maximum 12 month body weight were selected. Similarly 2nd resource population comprised of 10 individuals yielding least total greasy fleece yield and 10 individuals yielding maximum total greasy fleece yield. Approximately 10 ml of blood sample of each individual animal was collected from the jugular vein into a sterile 10 ml vacutainer tube containing 0.5 ml of 2.7% EDTA solution as an anticoagulant. Genomic DNA was isolated as per the standard phenol-chloroform extraction protocol [4] with some minor modifications. Two set of primers were used. While set 1 primers, adopted from [5] i.e. Forward (P3: 5′-CCA AGC GTG AGA CAG AAT AC-3′) and reverse (P4: 5′-AGG AGG GAT AGG AGC AAG AT-3′) was expected to amplify a 654 bp IGFBP3 gene fragment comprising of partial exon 2, complete intron 2, complete exon 3 and partial intron 3; second set of primers, adopted from i.e. Forward (TAT CAA TGA CCG TCA AGT CTG AT-3′) and reverse (GTG ATC TCT GGA TAC CCA GGC) was used to amplify a 333 bp fragment consisting of partial intron 1, complete exon 2 and partial intron 2. For amplification, 25 μl of PCR reaction was prepared by adding 10 pM of each primer, 200 μM of each dNTPs, 1.5 mM MgCl2, 1× PCR assay buffer, 50-75 ng DNA template and 1.5 Unit Taq DNA polymerase. The amplification programme used was comprised of initial denaturation of 1 min at 94 °C followed by 40 cycles of denaturation at 94 °C , annealing at 56 °C and extension at 72 °C each of 1 min each and lastly the final extension of 5 min at 72 °C. The PCR products were purified by using Promega kit. An aliquot of 20 μl of PCR product was digested overnight at 37 °C with 0.5 units of HaeIII restriction enzyme.
enzyme, as per manufacturer instructions. The restriction enzyme digested PCR products were electrophoresed in 4% agarose gel containing ethidium bromide as staining agent in 1× TAE buffer for 8 hours at 50 Volts. The digested products were visualized and documented under gel documentation system. The data generated by electrophoresis of Hae III digested product of each sample was used for estimating the frequency of different restriction fragment patterns. For this, gene and genotypic frequencies of alleles of IGFBP 3 gene were estimated by standard procedure [7].

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\text{Total No. of individuals of a particular genotype} = \frac{\text{Total No. of individuals of all genotypes}}{(2D + H)}
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\text{Gene Frequency} = \frac{2N}{2N}
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Where, D is number of homozygotes of particular gene, H is number of heterozygotes having that gene; and N is total no. of individuals

III. RESULTS & DISCUSSION

For 1st resource population, average 12th month body weight was 22.81 ± 0.99 kg in low body weight group and 43.22 ± 1.46 kg in high body weight group. Similarly, average G FY was 625.24 ± 44.13 g in low G FY group and 1676.18 ± 55.81 g in high G FY group of second resource population. Significant differences (P < 0.01) between high and low group showed the marked divergence of the resource populations.

Both the sets of primers i.e. Set I and Set II amplified the 654 bp and 333 bp fragments, respectively in all the individuals of both the resource populations. Typical restriction enzyme profile of 654 bp band with Hae III restriction enzyme includes eight restriction fragments of 56 bp, 7 bp, 201 bp, 19 bp, 16 bp, 201 bp, 67 bp and 87 bp. On resolution of Hae III digested PCR products at 1.2 % agarose gel, only 4 of the total 8 restriction fragments were visualized as two fragments of 201 bp appeared as single band and other three restriction fragments of 7 bp, 16 bp and 19 bp were not visible due to their very small sizes. Hae III PCR RFLP of 654 bp fragment in resource population I as well as resource population II yielded monomorphic restriction enzyme profile (Fig.1). Only AA genotypes showing the restriction enzyme profile of 201 bp, 87 bp, 67 bp and 56 bp were observed.

Similarly, typical restriction enzyme profile of 333 bp with Hae III restriction enzymes including 4 restriction fragments of 100 bp, 205 bp, 8 bp and 20 bp sizes were observed. On resolution of Hae III digested PCR products at 1.2 % agarose gel, only 2 of the total 4 restriction fragments were visualized as two fragments 8 bp and 20 bp were not visible due to their very small sizes. Hae III PCR RFLP of 333 bp fragment in resource population I as well as resource population II yielded monomorphic restriction enzyme profile (Fig.2). Only AA genotype showing the restriction enzyme profile of 205 bp and 100 bp were observed. Earlier workers have also reported monomorphic restriction enzyme profile of 654 bp in four different breeds of sheep including Muzafaramnagari with Hae III [8] as well as with MspI, TaqI and Hae III [9]. Similar to the above findings recently, [10] reported similar restriction pattern of IGFBP-3 gene in four Egyptian local sheep breeds namely Rahmani, Ossimi, Awassi and Barki in his PCR-RFLP studies. Similarly, [10] and [2] reported monomorphic restriction pattern of IGFBP-3 gene with Hae III in bovines. We have used more number of samples as well as also expanded the region of IGFBP3 gene, but still could not detect any polymorphism in this region, which suggested that the IGFBP3 region, more particularly exon 2 and exon 3 have very high sequence homology within sheep, especially Muzaffarnagari sheep. However, the 333 bp fragment of IGFBP3 gene was not explored for PCR-RFLP in sheep. We have attempted for Hae III PCR-RFLP of the 333 bp fragment, but could not detect polymorphism in either of the resource populations; hence no association could be established between PCR-RFLP pattern of this fragment with Hae III and body weight as well as GFY. [11] although detected polymorphism in IGFBP-3 gene but could not find any significant association of IGFBP-3 genotype with milk traits in goat (P > 0.05).

In conclusion, the Hae III RFLP studies showed single restriction pattern of IGFBP-3 gene or presence of only one genotype in both the resource populations under study indicating fixation of gene in Muzaffarnagari sheep population maintained at C.I.R.G. However, the length of amplified product of IGFBP-3 gene differed with that of earlier reports of cattle and buffalo. Hence, no association could be established between polymorphic pattern of IGFBP-3 gene and production traits.

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Fig. 1 *Haeill* PCR-RFLP of 654 bp IGFBP3 fragment in resource population I. Lane 1-10: Low body weight group; Lane 11-20: High body weight group. Lane M: Trackit 100 bp ladder, Invitrogen)

Fig. 2 *Haeill* PCR-RFLP of 333 bp IGFBP3 fragment. Lane 11-20: Low body weight group; Lane 11-20: High body weight group. Lane M: Trackit 100 bp ladder, Invitrogen)