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Polymorphism of Keratin – associated protein (*KAP*) 3.2 gene in Sandyno and Nilagiri breeds of sheep

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ABSTRACT

Polymorphic variants of keratin-associated protein (*KAP*) 3.2 gene in Sandyno and Nilagiri breeds of sheep were investigated. Genomic DNA was isolated from blood samples of 125 Sandyno, Nilagiri and Dorset x Nilagiri breeds and 76 numbers of wool samples were collected and processed. A 393 bp segment was amplified by PCR using ovine specific primers for *KAP* 3.2 gene. The genotyping was done by using PCR-SSCP. *KAP* 3.2 gene locus revealed 3 genotypes, viz. *AA, AB* and *BB* with a frequency of 0.84, 0.16 and 0; 0.86, 0.12 and 0.02 in Sandyno and Nilagiri breeds respectively with allele frequencies of A(0.92) and B(0.08) in both the breeds revealing monomorphic nature of this in this population. The result showed that the population was in Hardy-Weinberg equilibrium for *KAP* 3.2 with no significant difference. *KAP* 3.2 gene was found to have high degree of homozygosity (0.8824) in Nilagiri sheep. The effective number of alleles (Ne) for *KAP* 3.2 was 1.1716 and 1.1690 respectively in Sandyno and Nilagiri breeds of sheep. The PIC values for *KAP* 3.2 was 0.1356 and 0.1341 in Sandyno and Nilagiri breeds of sheep respectively. FIS values for *KAP* 3.2 was positive (0.1862) in Nilagiri breed and it was negative (– 0.0864) in Sandyno breed. The result revealed that presence of few alleles at the *KAP* 3.2 loci in Sandyno and Nilagiri breeds of sheep indicates monomorphic situation.

Keywords: Keratin Associated Protein (*KAP*) 3.2 , PCR-SSCP, Polymorphism, Sheep, Wool traits

INTRODUCTION

The Indian Wool Industry is the 7th largest in the world and it accounts for about 1.8 per cent of total world production of wool with annual production in the range of 45-48 million kg. Out of the total production of raw wool about 10 % was apparel grade, 70 % carpet grade and 20 % coarse grade. Wool yield per sheep in India is about 800-1000 g/year. The annual growth of wool production is marginal and wool production has remained static for last 10 years.

Keratin Associated Protein was one of the major genes that influence the economically important traits in wool sheep hence gene mapping studies of keratin proteins have identified some chromosomal regions associated with variation in wool quality and production traits. The keratin intermediate-filament proteins (*KRT*s) and keratin-associated protein (*KAP*s) are the major proteins that make up about 90 per cent of the wool fiber (McLaren *et al*., 1997). The *KRT*s form the skeletal structure of the wool fibre and are embedded in a matrix of *KAP*s. These proteins are connected through disulphide cross-linkages, which are important for the stability and the mechanical properties of wool (Feughelman, 1996; Schweizer *et al*., 2006). The *KAP* genes are small, between 0.6 and 1.5 kb in size and are intron less (Powell, 1996). The matrix *KAP*s are divided into 3 groups based on their amino acid compositions: the high-sulphur proteins (16–30% cysteine content) *KAP*1.n, *KAP*2.n, *KAP*3.n, ultra-high-sulphur proteins (30% cysteine content), *KAP*4.n, *KAP*5.n, *KAP*10.n and high- glycine-tyrosine proteins i.e., *KAP*6.n,

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*KAP*7.n, *KAP*8.n (Plowman, 2003; Rogers *et al.,* 2006, Barba *et al*., 2009). Among all the classes of Keratin Associated Protein gene, *KAP* 3.2 is found to be polymorphic having impact on wool characteristics and was reported by various researchers. The Nilagiri sheep is a dual utility (fine wool and meat), native to the Nilagiri hills of Tamil Nadu whose population was reported as 8000 by Ganesakale and Rathnasabapathy (1973). At present, this breed is endangered with 587 numbers existing, of which about 50 percent is maintained at Sheep Breeding Research Station, Sandynallah. The breed has been used along with Merino, in the development of another synthetic wool breed named Sandyno, which has better wool quality. So, Sandyno and Nilagiri breeds of sheep should be improved for fine wool production through Marker Assisted Selection. Considering above facts, the study was undertaken to investigate polymorphism of *KAP* 3.2 in Sandyno and Nilagiri breeds of sheep.

MATERIALS AND METHODS

A total of 125 blood samples (51 numbers of Sandyno, 54 numbers of Nilagiri and 20 numbers of Dorset x Nilagiri crossbred sheep) and 76 numbers of wool samples (35 numbers of Sandyno and 41 numbers of Nilagiri) were collected from the Sheep Breeding Research Station (SBRS), Sandynallah, the Nilgiris. Genomic DNA was isolated from whole blood using a modified method of Montgomery and Sise (1990) with slight modifications by using saturated Phenol: Chloroform: Isoamyl alcohol mixture. Good quality DNA samples with clear bands were selected for further study. Primers of *KAP* 3.2 F (5'-CCAAGACTTCTCTCATCAACC-3') and *KAP* 3.2 R (5'-GCATTAAGACTTGAGCAGCTC-3') were used for the amplification of the *KAP 3.2* gene as described by Mahajan *et al*. (2015). PCR reactions were carried out with nineteen µl of reaction mixture comprising 0.5 µl (5 picomoles) of each forward and reverse primers, 7.5 µl of 2 x PCR master mixes (1.5 mM $MgCl_2$, Taq DNA polymerase, 100 µM dNTPs) and 10.5 µl of nuclease free water was aliquoted in each PCR tube and one µl template DNA was added to each tube to make the final volume with thermal protocol of an initial denaturation step at 94º C for 4 min, followed by 35 cycles of denaturation (94º C, 30 sec), annealing(56º C, 45 sec) and DNA extension (72º C, 30 sec) and a final extension step at 72º C for 10 min. To confirm the targeted PCR amplification, five µl of each of PCR amplicons were electrophoresed through 2 per cent (w/v) agarose gel containing 0.5 µg/ml ethidium bromide in 1x TAE buffer. The sizes and quantities of PCR products were verified by comparison with 100 bp DNA ladder.

To explore genetic polymorphism in *KAP* 3.2 gene, amplified PCR products were subjected for SSCP through 8 % Polyacrylamide gel electrophoresis (acrylamide: bisacrylamide (29:1) 13.3 ml; 5 x TBE 10 ml; Ammonium persulfate (10 %) 250 µl; TEMED 100 µl; Triple distilled water 26.35 ml and total volume of 50 ml). After the run was completed, silver staining was carried out according to Bassam *et al.* (1991) with certain modifications to visualize the banding patterns.

The allele and genotype frequencies were calculated and Hardy-Weinberg equilibrium was tested by comparing expected and observed genotype frequencies using a Chi-square (χ^2)-test along with population genetic indexes such as gene homozygosity (Ho), gene heterozygosity (He), effective allele numbers (Ne), fixation index (Fis) and Shannon's Information index (I) were executed in POPGENE 32 version 1.32 software (Yeh *et al*., 1999).The polymorphism information content (PIC) was calculated by PIC calculator.

RESULTS

The quantity and quality of DNA was assessed by Biophotometer and the mean yields of DNA isolated from Sandyno and Nilagiri breeds of sheep were 319.98 ± 53.33 and 483.38 ± 80.56 µg/ml respectively. The ratio of optical density at 260/280 of DNA for the above genetic groups ranged from 0.95 to 1.95 and from 1.28 to 1.99 respectively. The PCR amplification yielded product at 393 bp (Figure-1) as expected for *KAP* 3.2 gene.

PCR amplicons were subjected to SSCP analysis to detect the polymorphic patterns of *KAP* 3.2 gene in three different genetic groups of sheep. PCR-SSCP analysis of *KAP* 3.2 gene (Figure-2) revealed *AA*, *AB* and *BB* genotypes in the two breeds with predominance of *AA* genotype. The genotype frequencies of *AA, AB* and *BB* were in the order of 0.84, 0.16 and 0.0 in Sandyno breed and 0.86, 0.12 and 0.02 in Nilagiri breed respectively. The *A* and *B* allele frequencies were 0.92 and 0.08 respectively in both Sandyno and Nilagiri breeds of sheep (Table-1). The present populations were consistent with Hardy-Weinberg equilibrium and had no significant difference (P>0.05) in *KAP* 3.2 gene for both Sandyno and Nilagiri breeds.

The heterozygosity value (0.1591) in Sandyno breed was almost similar to the expected heterozygosity (0.1481) for *KAP 3.2* gene (Table-2).

Table-1. Genotype and allele frequencies of *KAP* 3.2 gene in Sandyno and Nilagiri breeds of sheep

Breed / Group	Total number of animals (n)	Observed Genotypic frequency			Allele frequency		Expected Genotype frequency			χ^2 value	P value
		AA	*AB*	*BB*	*A*	*B*	*AA*	*AB*	*BB*		
Sandyno	44	0.84 (37)	0.16 (7)	0	0.92	0.08	0.85 (37.24)	0.15 (6.51)	0.00 (0.24)	0.28 ^{NS}	0.60
Nilagiri	51	0.86 (44)	0.12 (6)	0.02 (1)	0.92	0.08	0.85 (43.28)	0.15 (7.44)	0.00 (0.28)	2.18 ^{NS}	0.14

Table-2. Heterozygosity statistics and genetic diversity at *KAP 3.2* gene in Sandyno and Nilagiri breeds of sheep

Breed	Gene	Observed homozygo-sity	Observed heterozygo-sity	Expected homozygo-sity	Expected heterozygosity	*Ne*	PIC	F_{IS}
Sandyno	*KAP 3.2*	0.8409	0.1591	0.8519	0.1481	1.1716	0.1356	-0.0864
Nilagiri	*KAP 3.2*	0.8824	0.1176	0.8540	0.1460	1.1690	0.1341	0.1862

Ne = Effective number of alleles; PIC = Polymorphic information content; F_{IS} = Fixation index

DISCUSSION

The PCR amplification yielded product at 393 bp (Figure 1) as expected for *KAP* 3.2 gene. Similarly, Mahajan *et al*. (2015) and Wang *et al.* (2010) also obtained products at 393 bp whereas McLaren *et al.* (1997) observed product at 424 bp.

PCR amplicons were subjected to SSCP analysis to detect the polymorphic patterns of *KAP* 3.2 gene in three different genetic groups of sheep. PCR-SSCP analysis of *KAP* 3.2 gene (Figure-2) revealed *AA*, *AB* and *BB* genotypes in the two breeds with predominance of *AA* genotype. The genotype frequencies of *AA, AB* and *BB* were in the order of 0.84, 0.16 and 0.0 in Sandyno breed and 0.86, 0.12 and 0.02 in Nilagiri breed respectively. The *A* and *B* allele frequencies were 0.92 and 0.08 respectively in both Sandyno and Nilagiri breeds of sheep (Table-1). Wang *et al*. (2011) observed similar type of polymorphism in *KAP* 3.2 gene with three genotypes (*AA, AB* and *BB*) in Tibetan sheep. Similarly, Itenge-Mweza, (2012) in Merino sheep and Mahajan *et al.* (2015) in Rambouillet sheep observed three genotypes by PCR-SSCP analysis. Contrary to the present findings, Mahajan *et al.* (2015) in Rambouillet sheep observed the genotypic frequency for *KAP* 3.2 gene as 0.46 , 0.40 and 0.14 for *AA, AB* and *BB* genotypes respectively. Whereas, the gene frequencies were 0.66 and 0.34 for *A* and *B* alleles, respectively in Rambouillet sheep. The present populations were consistent with Hardy-Weinberg equilibrium and had no significant difference (P > 0.05) in *KAP* 3.2 gene for both Sandyno and Nilagiri breeds.

The heterozygosity value (0.1591) in Sandyno breed was almost similar to the expected heterozygosity (0.1481) for *KAP 3.2* gene (Table-2). In Nilagiri breed of sheep, the heterozygosity value (0.1176) was less than the expected heterozygosity (0.1460) suggesting a high degree of homozygosity (0.8824). However, Mahajan *et al*. (2015) reported expected heterozygosity (He) value of 0.45 in Rambouillet sheep and Wang *et al.* (2010) for Tibetan sheep (0.50) and Wang *et al.* (2011) for Tibetan (0.50), Oula (0.47) and Qiaoke (0.29) sheep.

The effective number of alleles (N_e) for *KAP 3.2* gene was 1.1716 and 1.1690 in Sandyno and Nilagiri breeds of sheep respectively (Table-2). Mahajan *et al*. (2015) observed almost similar value of 1.81 in Rambouillet sheep. The results obtained in this study were not in agreement with those reported by Wang *et al.* (2010) for Tibetan sheep (2.00) and Wang *et al.* (2011) in Tibetan (2.00), Oula (1.87) and Qiaoke (1.40) sheep.

The PIC values for *KAP* 3.2 gene was 0.1356 and 0.1341 in Sandyno and Nilagiri sheep respectively (Table 2). However, Mahajan *et al*. (2015) estimated polymorphic information content (PIC) values with medium polymorphism as 0.35 in Rambouillet sheep. The result is deviated from the findings of Wang *et al.* (2010) for Tibetan sheep (0.38) and Wang *et al.*

(2011) in Tibetan (0.38), Oula (0.36) and Qiaoke (0.24) sheep.

The F_{IS} values were negative (– 0.0864) in Sandyno breed and were positive (0.1862) in Nilagiri breed for *KAP* 3.2 gene (Table 2). However, Mahajan *et al*. (2015) observed Fixation index (F_{IS}) value of 0.11 in Rambouillet sheep. Deviation from the reported studies at *KAP* 3.2 gene may be due to breed differences and selective breeding practices. However, presence of few alleles at the *KAP* 3.2 loci in Sandyno and Nilagiri breeds of sheep indicates monomorphic situation.

CONCLUSION

PCR-SCCP analysis revealed that *KAP* 3.2 gene was monomorphic in Sandyno and Nilagiri breeds of sheep. Three PCR-SSCP patterns were observed in both Sandyno and Nilagiri breeds of sheep in *KAP* 3.2 loci and the highest *KAP* 3.2 genotypic frequency was observed for *AA* genotype (0.86) in Nilagiri breed of sheep. The allele frequency of *A* allele was almost nearing to fixation in both Sandyno and Nilagiri sheep (0.92).

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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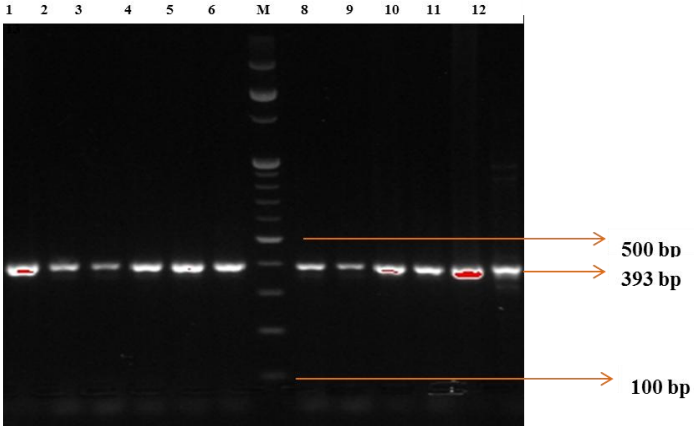
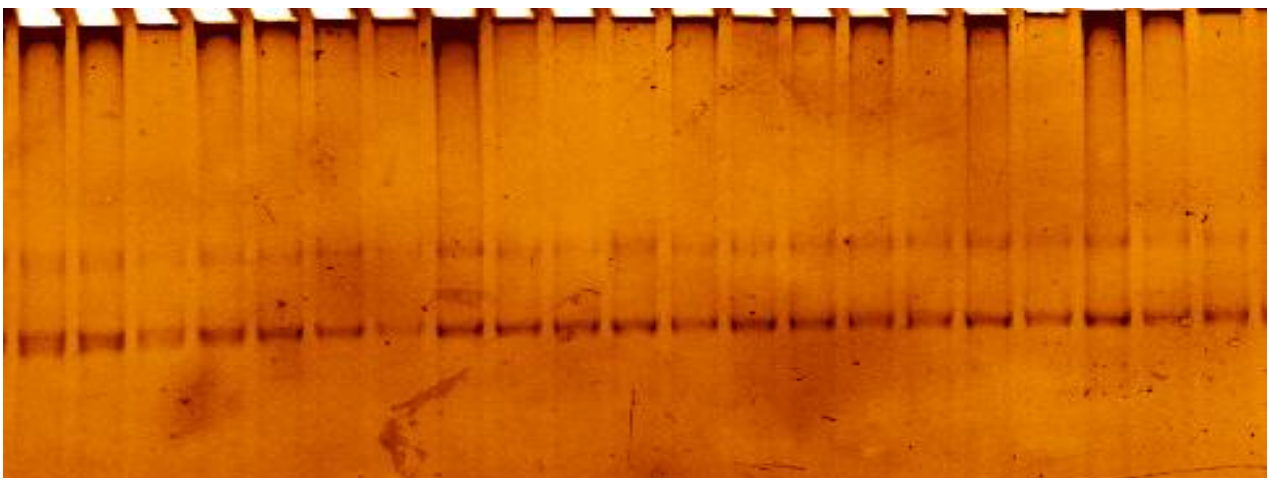
Figure-1. PCR amplified product of KAP 3.2 gene of Sandyno and Nilagiri breeds of sheep (2 % agarose gel electrophoresis)



KAP 3.2 gene {Lane: 1 to 6 and 8 to 13 samples; Lane M: Marker (100 bp)}

Figure-2. SSCP patterns of PCR products of *KAP* 3.2 gene in Sandyno and Nilagiri breeds of sheep (8 % PAGE electrophoresis)



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