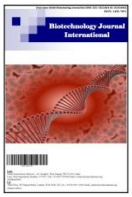

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A Study on Keratin-Associated Protein (*KAP*) 3.2 Gene and Its Polymorphism in Sandyno Breed of Sheep

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Authors' contributions

This work was carried out in collaboration among all authors. Author RB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RS, MJ and NM managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The current study investigates the polymorphic patterns of keratin-associated protein (*KAP*) 3.2 gene in Sandyno breed of sheep. Genomic DNA was isolated from blood samples of 51 numbers of Sandyno breed. Ovine specific primer associated PCR amplification of *KAP* 3.2 gene revealed product at 393 bp and genotyped by PCR-SSCP (Single Strand Conformation Polymorphism) method and visualized under silver staining technique. *KAP* 3.2 gene locus revealed 3 genotypes, viz. *AA, AB* and *BB* with a frequency of 0.84, 0.16 and 0 in Sandyno breed with allele frequencies of *A*(0.92) and *B*(0.08). Regarding population genetic indices, the effective number of alleles (N_e) for *KAP 3.2* in Sandyno breed of sheep was found to be 1.1716. The PIC values was 0.1356 and F_{IS} values was negative (– 0.0864) in this breed. The result revealed that the selected population of Sandyno breed of sheep was in Hardy-Weinberg equilibrium without any significant deviation from the population mean and was monomorphic for *KAP* 3.2 gene.

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Keywords: Keratin Associated Protein (KAP) 3.2; Sandyno; PCR-SSCP; silver stain; monomorphic.

1. INTRODUCTION

Keratin Associated Protein (*KAP*) 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 [1].

The *KAP* genes are small, between 0.6 and 1.5 kb in size and are intron less [2]. 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, *KAP*7.n, *KAP*8.n Barba et al. [3] Plowman, [4]; Rogers et al. [5], Schweizer et al. [6].

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 which is a dual utility (fine wool and meat), native to the Nilagiri hills of Tamil Nadu breed has been used along with Merino, in the development of another synthetic wool breed named Sandyno, which has better wool quality and it has been improved for fine wool production through Marker Assisted Selection [7]. Considering above facts, the study was undertaken to investigate polymorphism of *KAP* 3.2 in Sandyno breed of sheep.

2. MATERIALS AND METHODS

A total of 51 blood samples of Sandyno breed of sheep 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 [8] with slight modifications by using saturated Phenol: Chloroform: Isoamyl alcohol mixture. Good quality DNA samples with clear bands were selected for further study (Fig. 1).

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. [9]. PCR reactions were carried out with 20 µl of reaction mixture comprising 0.5 µl (5 picomoles) of each forward and reverse primers, 10 µl of 2 x PCR master mixes (1.5 mM $MgCl_2$, Taq DNA polymerase, 100 µM dNTPs) and 8.5 µl of nuclease free water was aliquoted in each PCR tube contaiing one µl template DNA. The thermal protocol consists of an initial denaturation step at 94°C for 4 min, followed by 35 cycles of denaturation (94°C, 30 sec), annealing (56°C, 45 sec) and DNA extension (72°C, 30 sec) and a final extension step at 72°C for 10 min. To PCR products was confirmed by 2 per cent (w/v) agarose gel electrophoresis. 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 (Single Strand Conformation Polymorphism) through 8% Polyacrylamide gel electrophoresis (acrylamide: bisacrylamide (29:1) 13.3 ml; 5 x TBE buffer 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. [10] with certain modifications to visualize the banding patterns (Fig. 1).

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 [11]. The polymorphism information content (PIC) was calculated by PIC calculator.

3. RESULTS

The quantity and quality of DNA was assessed by Biophotometer and the mean yields of DNA isolated from Sandyno breed of sheep was 319.98 ± 53.33 µg/ml. The PCR amplification yielded product at 393 bp (Fig. 2) as expected for *KAP* 3.2 gene. PCR amplicons were subjected to SSCP analysis to detect the polymorphic patterns of *KAP* 3.2 gene. PCR-SSCP analysis of *KAP* 3.2 gene (Fig. 3) revealed *AA*, *AB* and *BB* genotypes 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. The *A* and *B* allele frequencies were 0.92 and 0.08 respectively in Sandyno breed 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. The heterozygosity value (0.1591) in Sandyno breed was almost similar to the expected heterozygosity (0.1481) for *KAP 3.2* gene (Table 2). The effective number of alleles (N_e) was 1.1716 and the PIC values for *KAP* 3.2 gene was 0.1356 in Sandyno sheep (Table 2). The F_{IS} values were negative (– 0.0864) in the selected population for *KAP* 3.2 gene (Table 2).

4. DISCUSSION

The PCR amplification yielded product at 393 bp (Fig. 2) as expected for *KAP* 3.2 gene. Similarly, Mahajan et al. [9] and Wang et al. [12] also obtained products at 393 bp whereas McLaren et al. [13] observed product at 424 bp.

PCR-SSCP analysis of *KAP* 3.2 gene (Fig. 3) revealed *AA*, *AB* and *BB* genotypes 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. The *A* and *B* allele frequencies were 0.92 and 0.08 respectively in Sandyno breed of sheep (Table 1). Wang et al. [14] observed similar type of polymorphism in *KAP* 3.2 gene with three genotypes (*AA, AB* and *BB*) in Tibetan sheep. Similarly, Itenge-Mweza, [15] in Merino sheep and Mahajan et al. [9] in Rambouillet sheep observed three genotypes by PCR-SSCP analysis. Contrary to the present findings, Mahajan et al. [9] 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 of *A* and *B* alleles were 0.66 and 0.34 respectively in Rambouillet sheep.

The heterozygosity value (0.1591) in Sandyno breed was almost similar to the expected heterozygosity (0.1481) for *KAP 3.2* gene (Table 2). However, Mahajan et al. [9] reported expected heterozygosity (He) value of 0.45 in Rambouillet sheep and Wang et al. [12] for Tibetan sheep (0.50) and Wang et al. [14] 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 in Sandyno breed of sheep (Table 2). Mahajan et al. [9] 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. [12] for Tibetan sheep (2.00) and Wang et al. [14] in Tibetan (2.00), Oula (1.87) and Qiaoke (1.40) sheep.

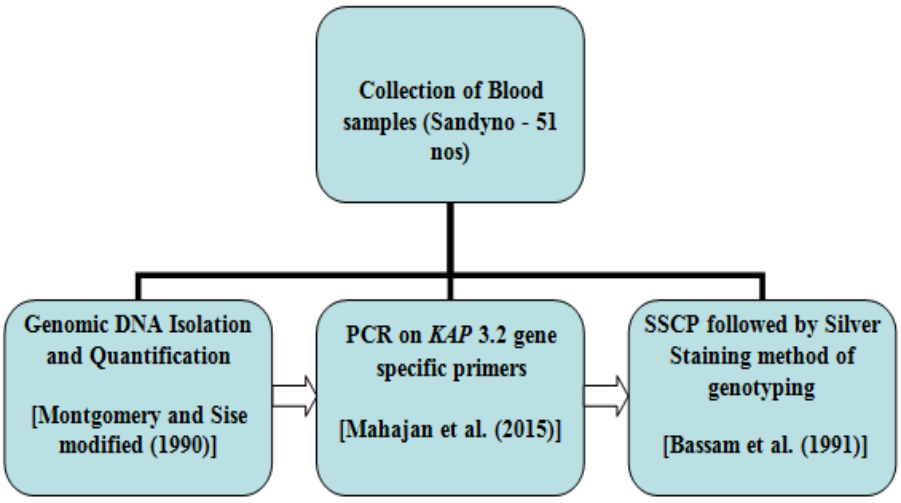


Fig. 1. Pictorial representation depicting the methodology of *KAP* 3.2 gene

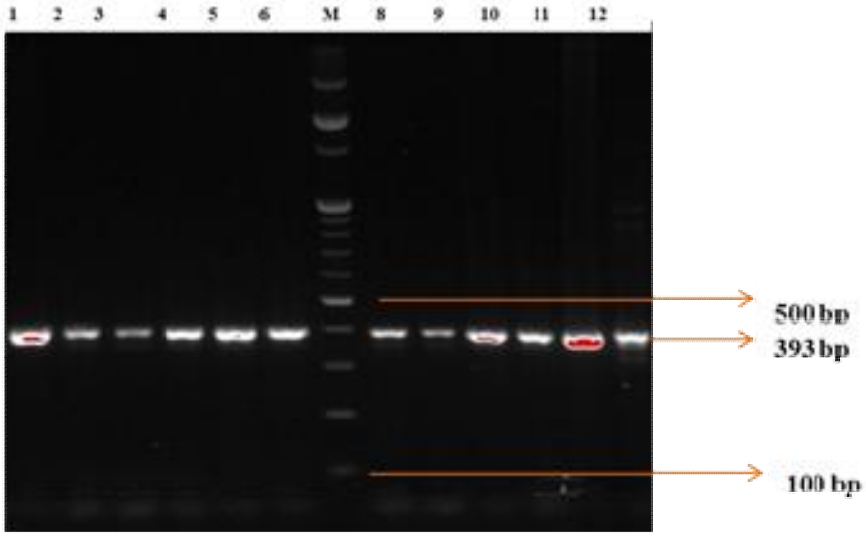
Table 1. Genotype and allele frequencies of *KAP* 3.2 gene in Sandyno breed of sheep

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	51	0.84 (44)	0.16 (7)	0	0.92	0.08	0.85 (37.24)	0.15 (6.51)	0.00 (0.24)	0.28^{NS}	$^{0.60}$

Table 2. Heterozygosity statistics and genetic diversity at KAP 3.2 gene in Sandyno breed of sheep

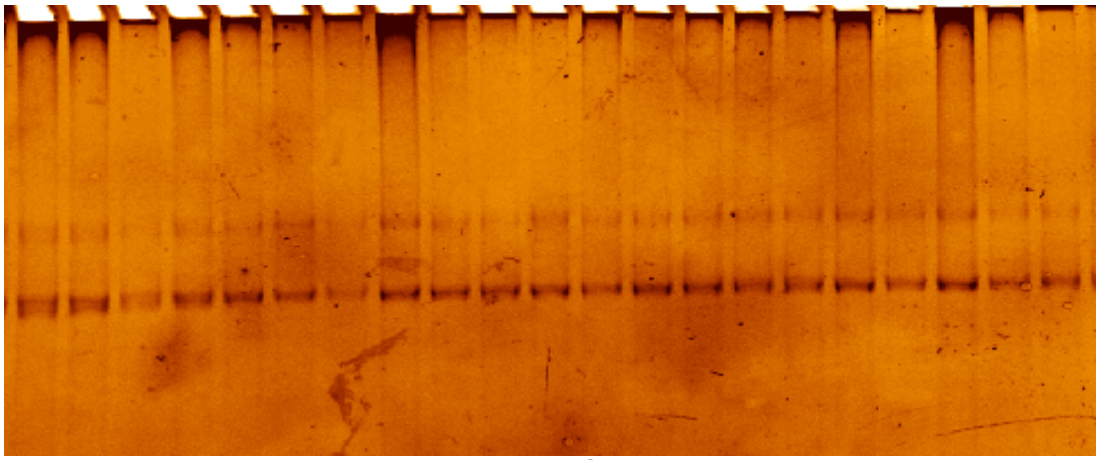
Breed	Gene	Observed homozygosity	Observed heterozygosity	Expected homozygosity	Expected heterozygosity	*Ne*	PIC	F_{IS}
Sandyno	*KAP 3.2*	0.8409	0.1591	0.8519	0.1481	1.1716	0.1356	-0.0864

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



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

Fig. 2. PCR amplified product of *KAP* 3.2 of Sandyno sheep (2 % agarose gel electrophoresis)



***KAP* 3.2 (393 bp PCR product)**

Fig. 3. SSCP patterns of PCR products of *KAP* 3.2 genes in Sandyno breed of sheep (8% PAGE electrophoresis)

The PIC values for *KAP* 3.2 gene was 0.1356 in Sandyno sheep (Table 2). However, Mahajan et al. [9] 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. [12] for Tibetan sheep (0.38) and Wang et al. [14] in Tibetan (0.38), Oula (0.36) and Qiaoke (0.24) sheep.

The F_{IS} values were negative (– 0.0864) in Sandyno breed for *KAP* 3.2 gene (Table 2). However, Mahajan et al. [9] 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 breed of sheep indicates monomorphic situation.

5. CONCLUSION

The selected population of Sandyno breed of sheep was analysed for *KAP* 3.2 gene and their polymorphism. PCR-SCCP analysis revealed the monomorphic pattern in *KAP* 3.2 loci with three genotypes. The population genetic indices were calculated and the resulted allele frequency was

almost nearing to fixation in Sandyno sheep (0.92).

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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