International Journal of Genetics
ISSN: 0975-2862 & E-ISSN: 0975-9158, Volume 12, Issue 4, 2020, pp.-725-727.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000226

Research Article

MOLECULAR SCREENING OF *FECB* GENE MUTATION THROUGH PCR RFLP METHOD IN DIFFERENT SHEEP BREEDS OF TAMIL NADU

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Received: March 16, 2020; Revised: April 07, 2020; Accepted: April 08, 2020; Published: April 30, 2020

Abstract: The indigenous sheep breeds Tamil Nadu were screened for the presence of fecundity gene: *FecB* as part of *FecB* gene introgression program. A total of 864 genomic DNA samples of Coimbatore, Mecheri, Niligiri, Trichy black and synthetic Sandyno sheep breeds were used to screen the *FecB* gene mutation through PCR RFLP method. The screening results showed that considerable percentage of *FecB* mutation present in both Nilagiri and Sandyno synthetic sheep breeds. In Nilagiri sheep the frequency of wild, *FecB* heterozygote (B+) and homozygote (BB) genotype were 54.71, 35.87 and 9.41 percent respectively. In Sandyno sheep the frequency of wild, *FecB* heterozygote (B+) and homozygote (BB) genotype were 88.14, 11.86 and 0.00 percent respectively. Whereas in Coimbatore, Mecheri and Tiruchy Black sheep *FecB* gene was absent in all samples screened and all animals showed only the uncut 140 bp band wild type (++) genotype.

Keywords: *FecB, Fecundity gene, PCR RFLP, Niligiri, Sandyno*

Citation: R. Saravanan, *et al*., (2020) Molecular Screening of *FecB* Gene Mutation through PCR RFLP Method in Different Sheep Breeds of Tamil Nadu. International Journal of Genetics, ISSN: 0975-2862 & E-ISSN: 0975-9158, Volume 12, Issue 4, pp.- 725-727.

Academic Editor / Reviewer: Dr M D Hivre

Introduction

Southern India in general and Tamil Nadu in particular are considered best suitable area for sheep production because of the semi-arid, dry climate and moderate rainfall. Tamil Nadu is having eight well-known breeds of sheep especially for meat production including one fine wool breed of Nilagiri Sheep breed which is in dwindling conditions. Sheep rearing is an economically important activity for the landless farmers and laborers for their livelihood. Most of sheep breeds present in Tamil Nadu are giving birth to singles and very rarely twins because of their genetic makeup and inadequate availability of feed. Sheep growers also prefer the singles, because of difficulty in external feed supplement and taboo on growing of twins. Nowadays, few progressive farmers prefer twins and the trend is increasing. Hence, the present study was carried out to screen for the presence of fecundity (*FecB*) gene among the native sheep breeds *viz*., Coimbatore, Mecheri, Niligiri, Trichy black and Sandyno of Tamil Nadu and also to screen the Indian Garole sheep crosses as a part of *FecB* introgression program. The *FecB* (Booroola) is a mutant gene known for its fecundity and is responsible for increasing the ovulation rate and litter size in sheep [1, 2]. Mutational effect of *FecB* results in higher ovulation rate in sheep and the allele variations increases the twining among the sheep approximately 1.6 times higher than the normal [3].

Materials and Methods

Blood samples were collected from five indigenous sheep breeds *viz*., Nilagiri, Coimbatore, Mecheri, Tiruchy Black and Sandyno of Tamil Nadu and the animals were selected randomly based on history of twining [Table-1] as the part of *FecB* gene introgression program [Fig-1]. A total of 864 blood samples were collected and the genomic DNA was extracted [4]. Forced PCR and RFLP method was used to screen the 140 bp of BMPR-1B region using F- 5' TCGCTATGGGGAAGT TTGGATG 3' and R- 5' CAAGATGTTTTCATGCCTCATCAACACGGTC 3' primers from Bioserve [5]. The reverse primer consciously introduced by a point mutation would create a restriction site in mutated strand, wild type ewes lacking the sites. About 100 ng of template DNA was put in 25µl reaction volume from Amplicon Taq DNA 2x Master Mix in PCR with the following amplification conditions; initial denaturation at 94°C for 1 min, followed by 94°C for 15s, 60°C for 30s, 72°C for 30s for 35 cycle and final extension at 72°C for 5 min. The products were tested in 3% agarose gel and then 10 µl PCR products was digested with AvaII enzyme from Thermo Scientific with recognition sequence (G/GACC) mixture which was prepared according to the pamphlet at 37°C for 4 h and loaded in 3.0 percent agarose gel with 5 µl DNA molecular size marker (50 base pair ladder ready-to-use) from Bio-Rad. To confirm the nucleotide variation in the PCR RFLP pattern, amplified *FecB* 140 bp gene PCR products was carried out. The 140 bp fragments was sequenced to ascertain the presence of *FecB* mutation. The sequencing was performed at twice in SciGenom, Cochin and the resulted sequences were aligned in DNASTAR LaserGene 7.1.0 MegAlign software by ClustalW method to find out nucleotide variation.

Table-1 *The FecB mutation in Bone Morphogenetic Protein Receptor-1B gene (BMPR-1B) through forced PCR-RFLP technique*

Breeds	No. of sample collected	*FecB* Genotype*		
		++	B+	BB
Nilagiri sheep	223	122	80	21
Sandyno sheep	178	157	21	0
Mecheri sheep	193	193	0	0
Tiruchy Black sheep	125	125	0	0
Coimbatore sheep	105	105	0	0
Garole (Male)	7	0	0	7
NARI Composite	8	0	0	8
F1 Garole X Nilagiri	27	0	19	6

** ++: Homozygous wild type; B+: Heterozygous for FecB; BB: Homozygous for FecB gene*

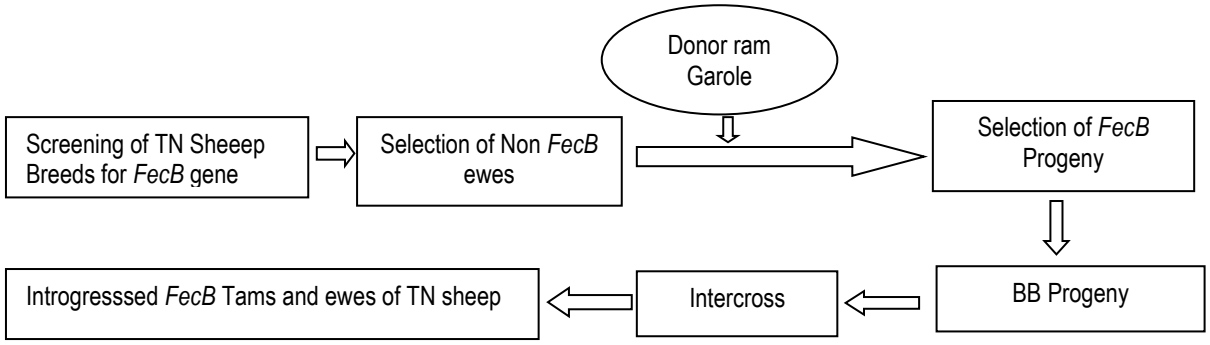

Fig-1 *Flow diagram of FecB gene introgression program of Tamil Nadu Sheep Breeds*

Results

The frequency distribution of *FecB* mutation in five local Nilagiri, Coimbatore, Mecheri, Tiruchy Black and Sandyno sheep breeds are given in [Table-1]. The resultant 140-base pair (bp) PCR product digested with AvaII restriction enzyme produced three different genotypic patterns *viz*., BB homozygote with 110 bp band, B+ heterozygote showed 140 and 110bp bands and the wild ++ homozygote revealed uncut 140 bp band [Fig-2].

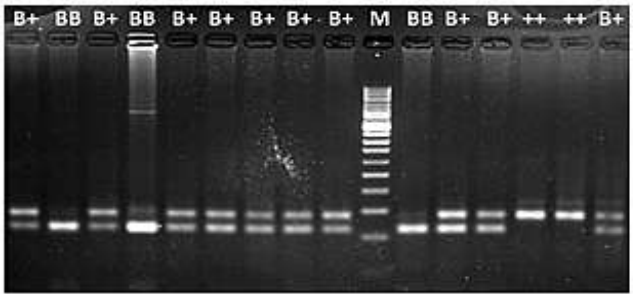

Fig-2 The forced PCR-RFLP showing different banding patterns of *FecB* genotype in 3 percent agarose gel electrophoresis. ++: Homozygous wild type;
B+: Heterozygous for *FecB*; BB: Homozygous for *FecB* gene. M-Molecular Marker

In Nilagiri sheep, the frequency of wild, *FecB* heterozygote (B+) and homozygote (BB) genotype were 54.71, 35.87 and 9.41 percent respectively, where as in Sandyno sheep, the frequencies were 88.14, 11.86 and 0.00 percent respectively. In Coimbatore, Mecheri and Tiruchy Black sheep *FecB* gene was absent in all samples screened and all animals showed only the uncut 140 bp band *i.e*., wild type (++) genotype [Fig-3]. Further, the *FecB* genotypic was confirmed in the introgressed F1 population of Nilagiri X Garole and Mecheri X Garole crosses. The Nilagiri showed higher the *FecB* gene frequency when compared to all other Tamil Nadu sheep breeds.

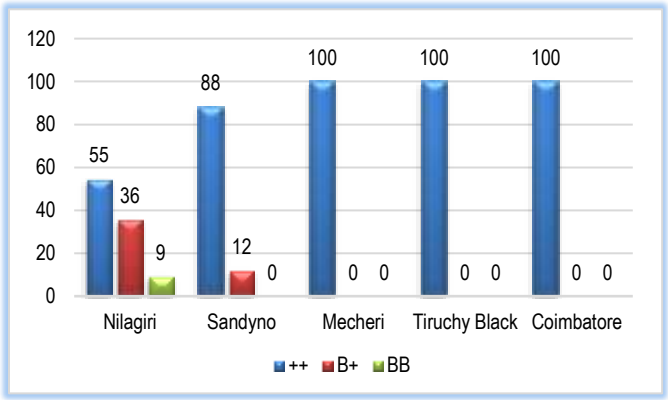

Fig-3 Presence of *FecB* gene (percent) in Nilagiri, Sandyno, Mecheri, Tiruchy Black and Coimbatore sheep breeds of Tamil Nadu

The nucleotide sequencing of PCR products of *FecB* region (140 bp) was confirmed by the nucleotide at 110 bp levels from Adenine (A) to Guanine (G) and the observed sequence pattern hosted in the DNA Data Bank of Japan (DDBJ) with GenBnak Accession No. LC152969.1, LC152970.1 and LC152971.1 [Fig-4]. The nucleotide variation confirmed that the presence of wild genotype (Adenine) in Coimbatore, Mecheri and Tiruchy Black sheep breed population and the presence of both wild (Adenine) and mutant (Guanine) genotypic pattern in Sandyno and Nilagiri population.

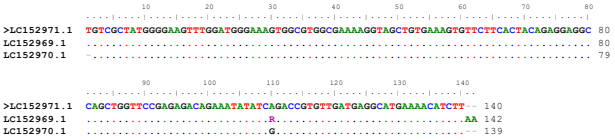

Fig-4 The nucleotide sequencing of *FecB* region in the Nilagiri Breed of Sheep

Discussion

The *FecB* genotyping was carried out in five native breeds of Tamil Nadu and the donar breed of *FecB* homozygote Garole and NARI Composite rams. The homozygote Garole rams for introgression purpose and he confirmed that *FecB* allele has been fixed in Garole population [6]. The results showed the absence of *FecB* mutation in Mecheri, Tiruchy Black and Coimbatore sheep. The similar result has been observed in sixty-seven animals of Zel sheep breed with non-carrier (++) 190 bp band (wild type) in the AvaII RFLP pattern [7]. The non-carrier *FecB* mutation reports were also observed in Deccani sheep breed [8, 9] and in contrary to various *FecB* genotypic frequencies were observed in Nilagiri and Sandyno sheep breeds. But the results are in agreement to the presence of *FecB* mutation in the Kendrapada sheep of Orissa [10]. Further, F1 generation of Mecheri X Garole crossbred individuals having 100 percent *FecB* carrier in their genotypes and results confirmed that only one allele of *FecB* received from the Garloe homozygote (BB) rams. Whereas in Nilagiri X Garole crossbred individuals' different genotypic frequencies of 0.76 (B+) and BB (0.24) was observed. This carrier heterozygote (B+) individual received one allele from Garloe ram but the homozygote individual received from both ewe and ram. This indicates that Nilagiri ewes used for breeding were also a heterozygote individual and it confirms that presence of *FecB* alleles in Nilagiri population.

Nucleotide sequence of cDNA of the *BMPR1B* and *GDF9* genes of 20 Black belly ewes for the presence of *FecB* mutations which is responsible for high prolificacy and they concluded that high prolificacy is a main characteristic of the hairy breeds [11]. Further, expression level of *BMPR1B* gene was significantly higher in uterus tissue of small Tail Han BB ewes than in Han B+ and Han ++ sheep [12]. The prolificacy in Nilagiri sheep was higher and mean number of lambs born per ewe over lifetime for heterozygous mutant (B+) groups was 5.73 ± 0.70 [13]. Presence of *FecB* allele in Sandynallah breed is due to this prolific gene has been introgressed from the Booroola Merino while it was crossing with Merino/ Rambouillet with Nilagiri breed of sheep. Booroola Merino has been crossed with several breeds in different countries to improve the reproduction rate at desirable levels of performance for along with other traits [14, 15]. When compared to both breeds, the Nilagiri sheep has highest *FecB* mutants and recorded incidence of a greater number of twins.

Culling of ewes those giving twins may be the reasons for the absence of homozygote *FecB* mutation in Sandyno sheep breed. When compared to all sheep breeds of Tamil Nadu these two breeds have higher incidence of twins which may due to the presence of *FecB* gene responsible for the higher ovulation rate. Thus, the presence of *FecB* mutation in the Nilagiri and Sandyno ewes will have additive effect on the ovulation rate and increased litter size [13].

Conclusion

The result of the study revealed that the *FecB* gene were identified only in Nilagiri and Sandyno sheep breeds whereas the *FecB* gene could not be detected in any animals tested belongs to Coimbatore, Mecheri and Tiruchy Black breeds of sheep. Forced PCR-RFLP technique allows rapid screening *FecB* gene mutation and this technique markedly reduce the sequencing cost involved in mutational screening of *FecB* genotype. The introduction of homozygous *FecB* (BB) mutant Nilagiri and Sandyno rams in to respective farmers flock and in farm would be a welcome innovation, resulting in increased profitability without increasing flock size in a local breed of sheep

Application of research: Forced PCR-RFLP technique cheaper method to screen *FecB* gene mutation in sheep breeds.

Research Category: Animal genetics

Abbreviations: PCR-RFLP: Polymerase Chain Reaction and Restriction Fragment Length Polymorphism

Acknowledgement / Funding: Authors are thankful to Principal Investigator, Central Sector Scheme on "Biotech Centre for Fecundity Genes" and Tamil Nadu Veterinary and Animal Sciences University, Chennai, 600051, Tamil Nadu, India.

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Research project name or number: Biotech Centre for Fecundity Genes

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Namakkal, 637002

Breed name: Sheep Breeds

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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