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Identification of polymorphism of *FecX* gene in indigenous sheep of Meghalaya

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ABSTRACT

Fecundity gene such as *FecX*, govern fertility and litter size in sheep. Selection of animals with the help of molecular markers through identification of polymorphism of fecundity genes is an impactful method of genetic improvement. The present study was carried out to identify the polymorphism of *FecX* gene in indigenous sheep of Meghalaya. Blood samples were collected from 50 randomly selected ewes from 2 districts of Meghalaya namely, East Khasi Hills and West Khasi Hills. Genomic DNA was extracted from the samples and PCR amplification of *FecX* gene with specific primer set was done. PCR product size of 141 bp was obtained. Further, digestion of the PCR product with restriction enzyme *HinfI* through PCR-RFLP technique was conducted to identify the variants in *FecX* gene. Upon digestion, 2 types of fragment pattern identified, arbitrarily designated as AA and AB genotype, where AA genotype yielded one fragment (141 bp) and AB genotype yielded 2 fragments (100 and 41 bp). Presence of polymorphism can be an important indicator for selection with the help of molecular markers.

Keywords: Fecundity, *FecX*, Indigenous sheep, PCR-RFLP, Polymorphism

In sheep, major economic traits, which affect the annual meat production from a herd is litter size, as higher number of lamb born give an opportunity to produce more meat at the same time frame (Roy *et al.* 2011). Genetic variation associated with ovulation rate in sheep has been widely documented and the evidences show substantial variation (Bindon *et al.* 1996). This variability suggests that selection for improvement of litter size is possible. Studies indicated that the reproductive processes like ovulation rate, litter size, fertility, normal follicular growth etc. can be genetically regulated by a set of different genes, collectively named as fecundity (Fec) genes (Hanrahan *et al.* 2004, Davis 2005). One of the fecundity genes *FecX* gene, also known as BMP15 gene, was described first in Romney sheep and named as Inverdale gene or *FecXI* (Lassoued *et al*. 2017). BMP-15 locus is X-linked gene and its expression occurs in the oocytes from the primary stage through ovulation (Otsuka *et al*. 2001). The role of *FecX* in early follicle development is species-specific and related to the differences between mono- and polyovulatory species (Silva *et al.* 2004 and Moore *et al*. 2005). *FecX* gene has a crucial role in granulosa cell proliferation and differentiation during ovarian follicular development (Galloway *et al.* 2000 and Crawford *et al*. 2011). It stimulates granulosa cell mitosis and suppresses FSH receptor's expression (Moore *et al.* 2005).

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Indigenous sheep of Meghalaya is a unique germplasm which is yet to be explored. The native sheep of Meghalaya are medium in size and mainly reared for meat purpose. An attempt to investigate the presence of fecundity genes in the indigenous sheep population of Meghalaya is required for better understanding and improvement of reproduction. Further, identification of polymorphism of fecundity genes can be utilized for marker assisted selection for increased litter/reproductive performances. Hence, the present study was carried out to identify presence of *FecX* gene and its polymorphism in indigenous sheep of Meghalaya.

MATERIALS AND METHODS

The investigation was carried out on the indigenous sheep of Meghalaya. Blood samples were collected from different areas in the breeding tract of the indigenous sheep, viz. Nongrum, Thynroit and Mawjrong villages of East Khasi Hills district and Mawthungkper, Marang and Jaidoh villages of West Khasi Hills district of Meghalaya. Fifty randomly chosen ewes were utilized to study polymorphism of *FecX* or BMP15 gene. Approximately 5 ml blood samples were collected. Genomic DNA was isolated using phenol chloroform extraction procedure (Sambrook and Russell 2001) with slight modifications. The purity of the genomic DNA was assessed by UV spectrophotometer (Nanodrop Spectrophotometer, Model- UV/VIS 916) by checking the optical density (OD) value at 260 and 280 nm, which indicated the amount of DNA and the amount of protein in a given sample, respectively. The samples having OD ratio

(269 nm/280 nm) 1.7 to 1.9 were used for the experiment. The concentration of genomic DNA was estimated spectrophotometrically taking OD value at 260 nm. Genomic DNA quality was checked to ensure the presence of intact DNA without any shearing. Horizontal submarine agarose gel electrophoresis was performed to check the quality of DNA. PCR was carried out in a final reaction volume of 50 µl. Each reaction volume contained following components: 2 µl DNA template; 1 µl each of forward and reverse primers; 26 µl Master mix and 20 µl nuclease free water. The primer set as suggested by Hanrahan *et al.* (2004) was used to amplify *FecX* gene. The forward and reverse primers were: Forward: 5′CAC TGT CTT CTT GTT ACT GTA TTT CAA TGA GAC3′ and Reverse: 5′GAT GCA ATA CTG CCT GCT TG-3′. Cycling condition for PCR amplification of *FecX* gene were; initial denaturation at 94°C for 5 min; followed by 35 cycles of denaturation at 94°C for 45 sec; annealing at 63°C for 40 sec and extension at 72°C for 45 sec. PCR amplification was confirmed by running 5 µl of PCR product mixed with 2 µl of 6 × gel loading dye on 1.5% agarose gel (depending on the expected size of the amplified product) at a constant voltage of 85 V for 60 min in 1× TAE buffer. Ethidium bromide was added @ 0.5 µl/100 ml gel solution in the gel itself. The amplified product was visualized as a single compact fluorescent band of expected size under UV light and documented by gel documentation system (Gel Logic 100, Kodak). The restriction enzyme used for *FecX* was *Hinf1* (Restriction site: 5′G↓ANTC3′3′CTNA↑G5′). The enzyme digested products were loaded @ 10 µl on 2.5% agarose gel along with 20 bp and 50 bp ladders. Electrophoresis was carried out at 110 V for 75 min and the bands were visualized, and documented using gel documentation system. The bands were analyzed by comparing with the ladder. Further, the enzyme-digested products were analyzed for confirmation by polyacrylamide gel electrophoresis (PAGE). Gene and genotypic frequencies were calculated as given by Falconer and Mackay (1996). Chi-square (χ^2) test was performed to test if the population was in Hardy-Weinberg Equilibrium. Samples were outsourced for sequencing through Molbiogen. The analysis of molecular data was carried out by Bioedit software.

RESULTS AND DISCUSSION

In the present study, the size of the PCR product for *FecX* gene was 141 bp (Fig. 1). Amplification of *FecX* gene in all the samples of indigenous sheep of Meghalaya resulted in a product size of 141 bp. This was in agreement with the results reported by Barzegari *et al.* (2010) in Moghani and Ghezel Sheep in Iran, and Kumar *et al.* (2015) in Garole sheep.

The PCR-RFLP technique was used to identify the variants in *FecX* gene based on the variations produced by digestion of a 141 bp amplified product with restriction enzyme *HinfI*. The amplified 141 bp fragment of *FecX* gene upon *HinfI* digestion yielded 2 types of fragment pattern, arbitrarily designated as AA and AB genotype, where AA

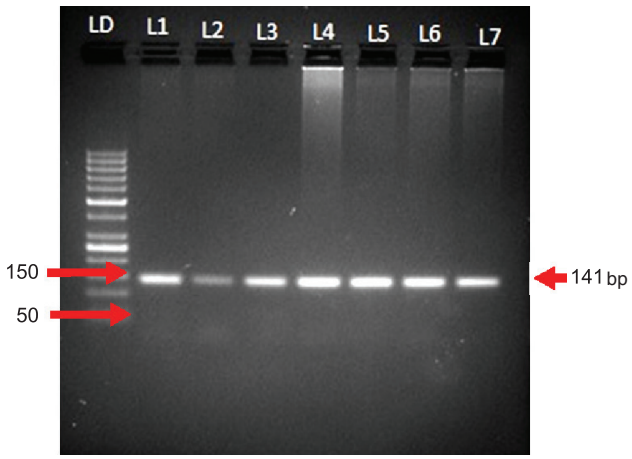

Fig. 1. L1-L7, PCR amplicon (141 bp) of *FecX* gene; LD, 50 bp Ladder.

genotype yielded 1 fragment (141 bp) and AB genotype yielded 2 fragments (100 and 41 bp) as shown in Fig. 2. The enzyme-digested products were analyzed for confirmation by polyacrylamide gel electrophoresis (PAGE).

In the population studied, the groups of sheep exhibiting polymorphism of *FecX* gene could be distinguished as AA and AB. Notably, there was a greater number of AB heretozygotes in comparison to AA homozygotes. The frequencies of A and B alleles were calculated as 0.55 and 0.45, and those of AA and AB genotypes as 0.10 and 0.90 respectively. Barakat *et al.* (2017) in Barki, Ossimi and Rahmani sheep breeds of Egypt; Getmantseva *et al.* (2019) in Volgograd breed of Russia and Mohamed *et al.* (2020) in Watish Sudanese desert sheep obtained two types of fragments of *FecX* gene upon digestion which corroborated with the present findings. On the contrary, 3 genotypes were found by Davis *et al.* (2006) in Hu and Han sheep breed of China; Zhang *et al.* (2011) in small tailed Han sheep; Wang

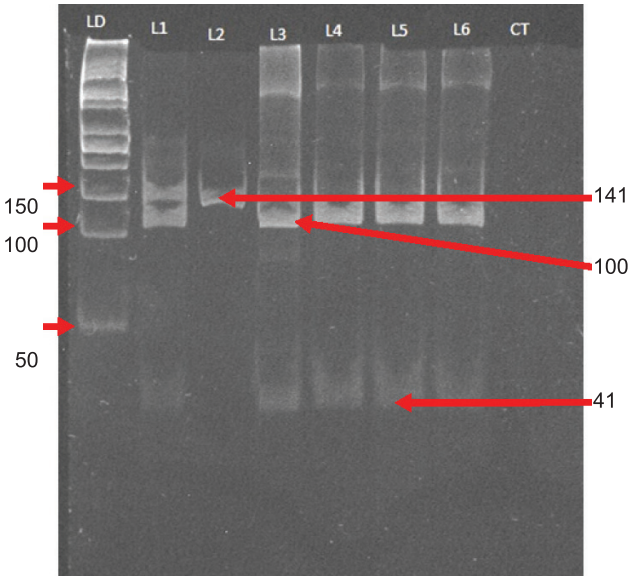

Fig. 2. L1-L6, PCR-RFLP of *FecX* gene using *HinfI* (LD, 50 bp Ladder; CT, Non template control).

et al. (2015) in Small Tailed Han sheep and Hu sheep; Dash *et al.* (2017) in Indian Kendrapada sheep and Praveena *et al.* (2017) in Deccani and Nellore sheep.

Sequencing of PCR amplicons: Sequencing was done using both forward and reverse direction for a few samples representing AA and AB genotype in *FecX* gene by automated DNA sequencer (Applied Biosystem, USA) as shown in Figs 3–6.

The sequence obtained was analysed by BLAST and submitted to NCBI. Based on scores obtained by BLAST, other nucleotide sequences of *FecX* gene of *Ovis aries* were obtained from NCBI for comparative analysis. One *HinfI* restriction site was detected at 100th position of the 141 bp fregment of *FecX* gene (Fig. 7). This is in similarity with Volgograd breed of Russia (Getmantseva *et al*. 2019) and

CCGCCTAAAAAGAGAAGGGTCTTTTTCTGTAACTCTTTCAGGCCTTTAGGGAGA GGTTTGGTCTTCTGAACACTCTGAGATCTCATTGAAATAGAGTAACAAGAAGAC AGTGAG

Fig. 3. *FecX* gene sequence of AA genotype.

CCGCCTAAAAAGAGAAGGGTCTTTTTCTGTAACTCTTTCAGGCCTTTAGGGAGA GGTTTGGTCTTCTGAACACTCTGAGTCTCATTGAAATACAGTAACAAGAAGACA GTGAG

Fig. 4. *Fec X* gene sequence of AB genotype.

CCTAGAAAGAGAAGGGTCTTTTTCTGTAAACTCTTTCAGGCCTTTAGGGAGAGG TTTGGTCTTCTGAACACTCTGAGTCTCATTGAAATACAGTAACAAGAAGACAGT GAGT

Fig. 5. *FecX* gene sequence of allele A for AB genotype.

CCGCCTAAAAAGAGAAGGGTCTTTTTCTGTAACTCTTTCAGGCCTTTAGGGAGA GGTTTGGTCTTCTGAACACTCTGAGTCTCATTGAAATACAGTAACAAGAAGACA GTGAG

Fig. 6. *FecX* gene sequence of allele B for AB genotype.

Watish Sudanese desert sheep (Mohamed *et al.* 2020).

The frequencies of A and B alleles of *FecX* gene were 0.55 and 0.45, those of AA and AB genotypes were 0.10 and 0.90 respectively (Table 1).

The present findings revealed a higher frequency of AB genotype followed by AA genotype. This was in compliance with the observations reported by Zhang *et al.* (2011) in small tailed Han ewes and Barakat *et al.* (2017) in Barki, Ossimi and Rahmani sheep breeds of Egypt. However, in contradiction to present observation, a higher frequency of homozygotes than heterozygous were reported by Getmantseva *et al*. (2019) in Volgograd breed of Russia; Ghoreishi *et al.* (2019) in case of Markhoz goats; and Mohamed *et al.* (2020) in Watish Sudanese desert sheep. The chi-square (χ^2) test revealed that the calculated value for *FecX* gene was more than the tabulated value at 1% level of significance with 1 degree of freedom. Hence the population under study revealed deviation in the genotype distribution from the Hardy-Weinberg Equilibrium for *FecX* gene in Indigenous sheep of Meghalaya.

The presence of polymorphism in fecundity genes opens interesting prospects for future selective breeding programme, especially based on marker assisted selection for reproductive improvement of this germplasm. Deviation

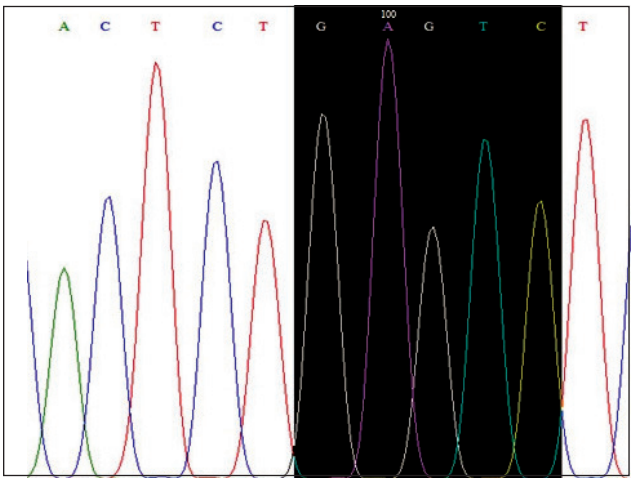

Fig. 7. *HinfI* restriction site of *FecX* gene.

Table 1. Distribution of genotype and allele frequencies of *FecX* gene along with χ^2 value

Genotype	Observed number	Expected number	Genotype frequency	Allele	Allele frequency
AA	5	15.125	0.1	A	0.55
AB	45	24.750	0.9	B	0.45
BB	0	10.125	0.0		

χ^2 =33.4711**.χ^2, chi-square value **(P<0.01).

of the studied population from Hardy Weinberg Equilibrium in respect to *FecX* gene might be due to the fact that the population of indigenous sheep of Meghalaya is relatively small and one of the conditions for Hardy Weinberg equilibrium might have been disturbed.

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