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Determination of MSTN Gene Polymorphism in the Iraqi Awassi Sheep

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ABSTRACT: The present study aimed to investigate the polymorphism of MSTN gene in Awassi sheep. PCR-RFLP method was used to detect of MSTN gene polymorphism. Two genotypes (MM and mm) were detected in Awassi sheep. The allele frequency of M and m were 0.07 and 0.93, respectively, while genotype frequency of mm and MM were 0.85 and 0.15 respectively. The chi-square χ^2 test showed an agreement to Hardy-Weinberg equilibrium (P>0.05).

Keywords: Awassi, MSTN, polymorphism, PCR-RFLP, SNP.

1. Introduction

Sheep are the most suitable agricultural animals that adapted for grazing in dry and harsh environmental conditions. Iraqi sheep belong to the fat-tailed Asian sheep and include three breeds; Karadi, Arabi and Awassi. Awassi constitutes about 60% of native sheep breeds in Iraq and characterized by good meat quality (Al Qasimi et al. 2019). MSTN which is known as growth and differentiation factor 8 (GDF8), plays to inhibit muscle growth by preventing the formation of muscle fibers (Grobet et al. 1997; Kambadur et al. 1997; McPherron and Lee 1997). The ovine MSTN gene is located at chromosome 2(BTA2) and consists of 3 exons and 2 introns (Jeanplong et al. 2001; Bellinge et al. 2005; O'Rourke 2010). It was found that the mutations that occur in MSTN gene are associated with the double-muscled phenomenon in various mammalian species (Casas et al. 1998; Schuelke et al. 2004; Clop et al. 2006; Mosher et al. 2007). The aim of this study is to determine MSTN gene polymorphism in Iraqi Awassi sheep.

2. Material and method

A total of 80 (male) from Iraqi Awassi sheep were used in this study. Disodium EDTA containing tubes were used to prevent coagulation of blood during collection of samples. Then, blood samples storage was carried out at - 20°C until DNA extraction procedures. Blood samples were taken from the Tail Vein of animals. Genomic DNA was extracted from whole blood by using the phenol chloroform methods. 337 bp length of MSTN (Exon 3) region was amplified with forward (5'CCGGAGAGACTTTGGGCTTGA-3') and reverse (5'TCATGAGCACCCACAGCGGTC-3') primers reported by Smith et al. (1997). The PCR was done in a reaction volume of 10 µL according with some modifications. The reaction consists of 5µL of 2X Dream Taq Green PCR Master Mix (Thermo Scientific), 0.30µL primer each primer forward and reverse (10 pmol) and 3.4µL ddH_2O which finally added to 1 µL genomic DNA. The cycling protocol followed with initial denaturation at 94°C for 1 min followed by 33 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 60 sec, extension at 72°C for 2 min with a final extension at 72°C for 2 min. The PCR product of each sample (5 µL) and 1000 bp DNA marker was loaded in 2% agarose gel in 0.5X Tris-Borate-EDTA (TBE) buffer and ethidium bromide was used for staining gel. The electrophoresis was carried out for 50 min at 100 V. The electrophoresis gel was examined on an UV trans-illuminator and bands were visualized and photographed. The PCR products of MSTN gene were digested by *HaeIII* fast digest (Thermo Scientific) at 37. The reaction volume was 15 µL consisted of 5 µL PCR product, 8.5 µL ddH_2O, 1 µL 10X buffer and 0.5 µL restriction enzyme. The polymorphism of the cleaved fragments recognition was carried out by %2 agarose gel electrophoresis then the digested PCR products was obviously envisioned under UV light and scored in a gel documentation system.

3. Result and discussion

337 bp of PCR product was amplified. The PCR product was digested with *HaeIII* restriction enzyme. Two genotypes (MM, and mm) were observed. MM genotype was 337 bp, whereas mm genotype was 131 and 123 bp (figure 1). χ^2 test showed agreement to Hardy-Weinberg equilibrium (p>0.05). The allele frequency of M and m were 0.07 and 0.93, respectively, while genotype frequency of mm and MM were 0.85 and 0.15 respectively. Soufy et al. (2009) observed three genotypes in Sanjabi Sheep with genotype frequency 0.02(MM), 0.01(Mm) and 0.97(mm). Sahu et al. (2017) reported two genotypes (MM and Mm) in Madras Red and Mecheri sheep breeds at G5622C locus in exon 3 of MSTN/MspI polymorphism with genotype frequency as 0.41(MM) and 0.58(Mm); 0.48(MM) and 0.51(Mm) respectively. Whereas two genotypes (Mm and mm) have also been reported in Kordi, Kalehkoohi, Farahani, Mehraban and Teleorman sheep breeds (Ebrahimi et al. 2014; Jamshidi et al. 2014; Shariatzadeh et al. 2014; Akbari et al. 2015; Lazar et al. 2016). Most studies confirm that the MSTN/*HaeIII* at exon 3 is monomorphic in the different sheep breed due to the small sample size, environmental effect, geographical position, and mating strategies.

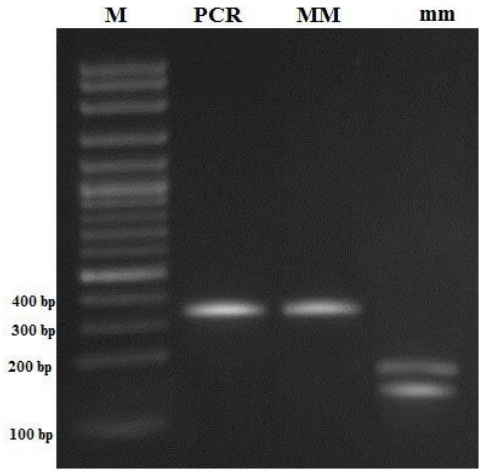

Figure 1. PCR-RFLP analysis of the MSTN/*HaeIII* polymorphism, 337 bp PCR fragment; 337 bp for MM genotype; 131 bp and 123 bp for mm genotype

4. Conclusion

MSTN gene shows polymorphism in Iraqi Awassi sheep so they can be considered important genetic markers and used as markers in genetic improvement programs for improving the growth traits.

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