Mitochondrial Cytochrome b gene polymorphism of Zom sheep breed in Karacadağ region of Turkey by PCR-RFLP method

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Abstract
The present study was conducted to identify Zom sheep breed (Ovis aries) by using mitochondrial cytochrome-b (CYTB) gene. The aim of this study was genetic identification as CYTB gene by using PCR-RFLP analysis. For this study, the DNA was isolated from the whole blood through commercial DNA kit. The DNA samples were amplified by PCR using CYTB primers. CYTB gene partial region was amplified of sheep. The amplified product was digested with restriction enzymes *Hae*III. The DNA fragments were viewed on 2.0 % agarose gel. Different bands were observed as compared with 100 bp DNA ladder. PCR product was is 1124 bp. Restriction endonucleases were cutted 110, 138, 159, and 717 bp. Zom sheep breed did not show polymorphisms for *Hae*III estriction sites of mitochondrial CYTB gene.

Keywords: Cytochrome b gene, *Hae*III, PCR-RFLP, Zom sheep

Introduction

Domestic sheep (*Ovis aries*) belong to the *Bovidae* family within the *artiodactyla* class. The *Bovidae* family, which dates back to the myocene period (about 20 million years ago), has shown a rapid spread until today (Gatesy et al., 1992). Sheep (*Ovis aries*) is domesticated livestock in the world since 11.000 years ago (Galal, 2005). sheep is an important source of meat, milk, and wool. Sheep (*Ovis aries*) is the most cultivated farm animal in different parts of in the world in 2018, the Food and Agriculture Organization (FAO) reported around 1,209 billion heads sheep were raised of in world (http://faostat.fao.org/). Turkey is one of the world's most important sheep farming countries and there are about 33.7 million head in 2018 (www.tuik.gov.tr).

Native sheep breeds in Turkey, as fat-tailed and thin-tailed sheep breeds are discussed in the two groups. Essentially, Turkish sheep breeds are genarally fatty tailed breeds. Among the fat-tailed sheep breeds, Akkaraman (47%), Morkaraman (20%), Dağlıç (16%) and İvesi (1.6%), respectively. Our thin-tailed domestic sheep breeds are Kivircik (6.3%), Karayaka (3%), Sakız sheep (Özcan, 1997). Turkish sheep breeds are fat-tailed breeds such as Zom sheep breed.

The domestic sheep genome (2n = 54) includes 26 pairs of autosomal chromosomes, 2 pairs of sex chromosomes and mitochondrial genome. In addition to the DNA (nDNA) found in the nucleus of eukaryotic cells, a small amount of cytoplasmic DNA is also encountered. These cytoplasmic DNAs are in mitochondria (mtDNA) and have double helix and circular structure (Hiendleder et al., 1998a). mtDNA was used as molecular markers in molecular studies such as determining phylogenetic relationships by using genetic similarities or differences of populations (Meadows et al.2007). Sheep mitochondrial genome; protein-coding 13 regions (cytochrome c oxidase complex I, II and III subunits, ATPase complex 6 and 8 subunits, NADH dehydrogenesis 1, 2, 3, 4L, 4, 5 and 6 and cytochrome b), 2 ribosomal RNA regions (12S rRNA , 16S rRNA), control region (D-loop) and 22 tRNA regions (Hiendleder et al., 1998). Sheep mtDNA is 16640 bp.

Cytochrome b gene (CYTB) is one of the genes that is coded by mtDNA. Cytochrome B proteins are produced in the immune system, brain, liver, and other tissues throughout the body. The Cytochrome b gene family member expressed in the liver is important in the production of haemoglobin. (Gacon et al., 1980). Cyto-chrome B is believed to play a major role in iron

metabolism in tissues of duodenum (Latunde-Dada et al., 2002). The sheep genetic resources conservation of sheep breeds by means of the identification of genetic variability. Mitochondrial Cytochrome b gene is an important tool. CYTB has been widely used to study genetic variability of small ruminant animals (Loehr et al., 2006; Joshi et al., 2004).

Hiendleder et al. (1998b) investigated the phylogenetic relationship between domestic sheep (*O.aries*) and wild sheep by using the PCR-RFLP method in the mtDNA. According to RFLP data, the average gene sequence difference in domestic sheep haplotypes was 0.492%, 0.091% in mouflon haplotypes, 0.865% between two argali subspecies, and between urial, argali and mouflon sheep and domestic sheep as 2.724, 2.115 and 0.465, respectively. Here, genetic diversity and parcinom analyzes have been reported to support that the domestic sheep are of two different lineages, mostly European lineage including European domestic sheep and one being Asian lineage. The Asian lineage includes some European and central Asian domesticated sheep breeds.

Molecular techniques are used in genomic studies for genetic polymorphism analysis. Genetic markers have been used in agriculture with PCR techniques. These are; restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), , microsatellites (SSR), single nucleotide polymorphisms (SNP), and direct sequencing.

The objective of this research study was to identify polymorphisms for CYTB gene region of Zom sheep by PCR-RFLP technique.

Material and Methods

Three (3) mL blood sample was collected from 36 unrelated sheep of into ethylenediamine tetra-acetic acid (EDTA) tubes and transported to laboratory in thermos. DNA was isolated from whole blood using DNA isolate kit (Gene JET Whole Blood Genomic DNA Purification Mini Kit # K0781, Thermo). DNA were indentified using 1 % agarose gel stained with ethidium bromide. . DNA samples were to stored at -86 °C. Primers were designed from partial region of cytochrome b gene using software Primer3 (http://frodo.wi.mit.edu/) (Steve and Skaletsky, 2000) from complete mitochondrial genome of Ovis aries (NC_001941) available on NCBI (http:// www. ncbi. nlm. nih.gov) for PCR amplification of Mitochondrial Cytochrome b gene. Based on used to primers for CYTB gene F (5'- CAACATCCGAAAAACCCACC - 3') and R (5'- GCATCATCGAAAACAACCTCC - 3') (Kiraz, 2009), it was amplificated the region of 1124 bp of CYTB gene.

Boeco thermocycler was used for PCR reactions of DNA samples. PCR reactions were prepared in a volume of 20 µL for each samples as follows; 2.0 µL 10X PCR buffer, 1.0 µL $MgCl_2$ (25mM), 1.0 µL dNTP mix (10 mM), 1.0+1.0 µL F/R primers (20 pmol), 0.5 µL *DNA Taq polymerase* (5U/ µL), 1.0 µL template DNA and 11.5 µL ddH_2O. PCR conditions: 94 °C for 5 minutes as initial denaturation, 94 °C for 60 seconds of denaturation, 60 °C for 60 seconds for annealing, 72 °C for 60 seconds for extension and 72 °C for 5 minutes for final extension. PCR products were indentified using 1% agarose gel.

PCR-RFLP prosedure: the amplified PCR product of CYTB gene region were cutted by *Hae*III restriction enzyme. Digestion reaction: in 15 µl reaction volume containing 3.0 µl 10X reaction buffer, 1 µl restriction enzyme (10U/µl), 6 µl ddH_2O and 5 µl PCR product. The digestion reaction was mixed by incubated at least for 6 h. The digested products were run in a 2% agarose gel stained with ethidium bromide . Ladder was ran as 100 bp DNA (Fermentas). The digested product was visualized by gel UV system (Daihan). Then, samples of sheep were sequenced from both directions to confirm the results obtained with PCR-RFLP technique.

The restriction fragment length polymorphisms (RFLP) band profiles set were scored for their presence (1) or absence (0) and prepared data files. Calculations were done at POPGEN32Ver. 1.33 package (Yeh et al. 1999).

Sheep *Cyt b* gene: 1140 bp, PCR product: 1124 bp (NC_001941), HaeIII cut site GG↓CC

ATGATCAACATCCGAAAAACCCACCCACTAATAAAAATTGTAAACAACGCATTCATTG
ATCTCCCAGCTCCATCAAATATTTCATCATGATGAAACTTTGGCTCTCTCCTAGGCATT
TGCTTAATTTTACAGATTCTAACA**GG↓CC**TATTCCTAGCAATACACTATACACCTGACA
CAACAACAGCATTCTCCTCTGTAACCCACATTTGCCGAGACGTAAACTATGGCTGAATT
ATCCGATATATACACGCAAACGGGGCATCAATATTTTTTATCTGCCTATTTATGCATGT
AGGACGA**GG↓CC**TATACTATGGATCATATACCTTCCTAGAAACATGAAACATCGGAGT
AATCCTCCTATTTGCGACAATAGCCACAGCATTCATAGGCTATGTTTTACCATGAGGAC
AAATATCATTCTGAGGAGCAACAGTTATTACCAACCTCCTTTCAGCAATTCCATATATT
GGCACAAACCTAGTCGAATGAATCTGGGGAGGATTCTCAGTAGACAAAGCTACCCTCA
CCCGATTTTTCGCCTTTCACTTTATTTTCCCATTCATCATCGCAGCCCTCGCCATAGTTC
ACCTACTCTTCCTCCACGAAACAGGATCCAACAACCCCACAGGAATTCCATCGGACAC
AGATAAAATTCCCTTCCACCCTTATTACACCATTAAAGACATCCTAGGTGCTATCCTAC
TAATCCTCATCCTCATGCTACTAGTACTATTCACGCCTGACTTACTCGGAGACCCAGAC
AACTACACCCCAGCAAACCCACTTAACACTCCCCCTCACATCAAACCTGAATGATACT
TCCTATTTGCGTACGCAATCTTACGATCAATCCCTAATAAACTAGGAGGAGTCCTCGCC
CTAATCCTCTCAATCCTAGTCCTAGTAATTATACCCCTCCTCCATACATCAAAGCAACG
GAGCATAATATTCCGACCAATCAGTCAATGTATATTCTGAATCCTAGTAGCCGACCTAT
TAACACTCACATGAATTGGA**GG↓CC**AGCCAGTTGAACACCCCTACATCATTATTGGAC
AACTAGCATCTATTATATATTTCCTTATCATTCTAGTCATAATACCAGTAGCTAGCATC
ATCGAAAACAACCTCCTAAAATGAAGA

Result and Discussion

DNA was isolated from Zom sheep. The agarose gel image of isolated genomic DNA figure is given in Figure 1. PCR study was performed. Its were obtained with PCR products. In sheep, 1124 bp part region of CYTB gene were amplificated with PCR (Figure 2).

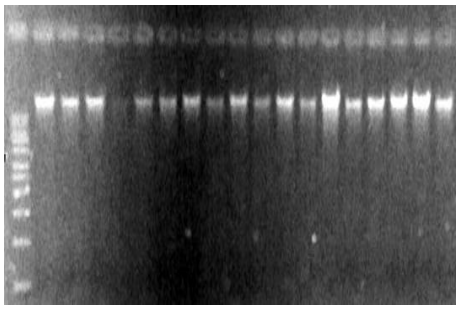

Figure 1. Genomic DNA isolated from sheep (ladder DNA 1kb)

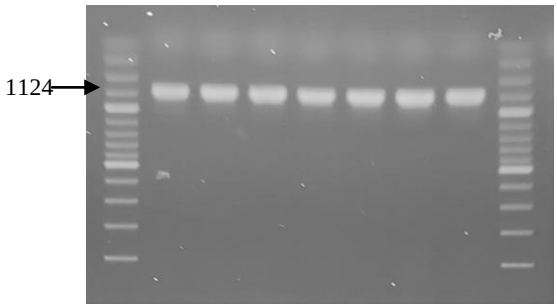

Figure 2. Gel photo of PCR yields of mitochondrial CYTB gene (ladder DNA 100 bp)

The results of this study indicated the presence of tree *Hae*III *c*leavage sites within the CYTB *g*ene region sequence. In this research, *Hae*III restriction enzyme showed tree cleavage sites (143, 303 and 1021 bp) within the CYTB gene region sequence. It is clear from *Hae*III RFLP pattern represented in Figure 3, that, there was no polymorphism Zom sheep breed in respect to CYTB gene. Digestion of the PCR product of CYTB gene with *Hae*III revealed only one type of restriction pattern including fragments of sizes 717, 159, 138 and 110 bp (Figure 3).

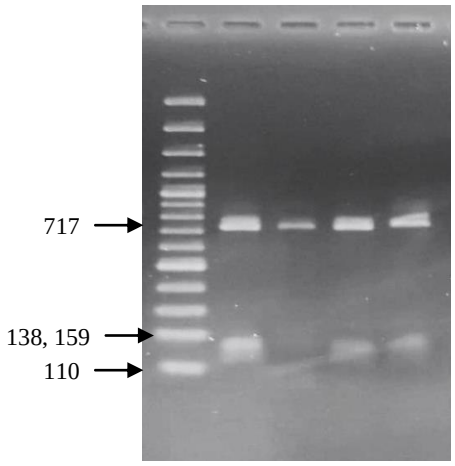

Figure 3. RFLP pattern of mitochondrial CYTB gene by *Hae*III (110, 138, 159, and 717 bp fragments)

In the phylogenetic tree established according to mtDNA domestic sheep, two as A and B. They reported that they came from maternal lineage. In later studies, strain A in sheep and B, as well as Chinese and Near Eastern A new maternal line C in sheep breeds (Guo et al., 2005; Pedrosa et al. al., 2005). However, Pereira et al.(2006), have reported C lineage is found at low frequency in Portuguese domestic sheep. Tapio et al. (2006) In North Caucasian, Karachai sheep this is stateda fourth maternal line separated from three lines, they detected the existence of (strain D). Finally, Meadows et al. (2007) detected, the presence of the noble lineage E in Turkey the first time.

In the present study, RFLP-PCR profile of the Cyt-b region in the varied results. The CYTB gene region using RFLP-PCR technique was suitable tool for analyzing genetic variability Zom sheep

breed. Further study is required to sequence the complete of CYTB gene of sheep breed to understand the genome of Turkish sheep breeds.

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