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Molecular Genetic Analysis of Three Turkish Sheep Breeds by RAPD-PCR Method

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ABSTRACT

In this study determined genetic polymorphisms within and among in three Turkish sheep breeds namely Sakız, Karayaka and Bafra by the RAPD technique. These breeds were screened by 20 primers having arbitrary sequences. DNA samples were extracted from blood. The samples were amplified by polimerase chain reaction (PCR). Amplified products were resolved on 1.5% agarose gel and stained with ethidium bromide (EtBr). The RAPD data from 6 primers were evaluated. Genetic distances were calculated which ranged from 0.040 to 0.470 between the breeds. The highest heterozigocity was observed in Bafra breed. In addition, all individuals were tested in Bafra containing 1250 bp allel which was assumed as a distinguishable character. This result can be suggested as a specific DNA marker for Bafra breed.

Key Words: genetic diversity, random amplified polymorphic DNA, sheep.

INTRODUCTION

Sheep breeding is important in raising livestock all over the world. Turkey is not exception to this. Of the total sheep population 97 percent is composed by the local sheep breeds while 3 percent foreign ones, particularly the breed Merinos and ıts hybrids [1, 2]. Productivity features of the local breeds that noticably vary among them are considered low in general. Much efforts have been therefore given to increase the level of productivity in local breeds of Turkey. Breeding programmes for breed development have been undertaken numerously to achive for this aim. One of the most prominent studies were carried out on Bafra sheep breed that was obtained by the crossbreeding of Sakız and Karayaka sheep. Sakız sheep breed is distrubuted along the coastal towns of Çeşme, Urla and Seferihisar of Izmir province. Although the breed has a high milk yield and outstanding prolificacy, it is poorly adapted in other parts of Turkey. Its rams are sometimes crossed with other breeds such as Karayaka to improve productivity. The sheep breed is distributed along the eastern half of the Black Sea coast, especially in Ordu, Giresun, Samsun, Tokat and Sinop.

That is, the sheep breed is well adapted to rainy climate. Additionally, milk production of the Karayaka is one of the best among other local breeds in Turkey and also meat quality is considered good. Because of these features, for the first time, Sakız and Karayaka sheep breeds were crossed in the Karaköy state farm. New breed which was called Bafra has been produced in Gökhöyük state farm in Middle Black Sea Region. Moreover, productivity features of Bafra are better than Karayaka [3, 4, 5, 6].

The present study was made to determine genetic polymorphisms within and among the three Turkish sheep breeds. Samples of Karayaka and Sakız breeds were collected from two main sources which were state farm and communal farm. Our aim to use different livestocks for Sakız and Karayaka was to compare the individuals obtained from state and communal farms. We did not apply this for Bafra breed because it was produced only in a state farm and distrubuted from this farm to communal farms [4].

Historical, morphological and anthropological evidence is not satisfactory for classiffication of sheep breeds. Breed characterization requires knowledge of genetic variation that can be effectively measured within and among populations [7, 8]. Recent studies for conservation and improvoment of breeds have been based on molecular analysis methods. For this reason, different techniques are used [9, 10, 11]. For example, various tools used to identify DNA marker in livestock are based either on length polymorphism or Polymerase Chain Reaction (PCR). RAPD markers, which is one of the methods based on PCR were established by Williams et al. [11] and Welsh and McClelland et al. [12, 13]. This technique amplifies several discrete loci in the genome using a single primer of arbitrary sequence, usually 8-10 mer long. These markers have provided quickly and efficient detecting polymorphisms for genetic mapping and breed identification. [14, 15, 16, 17, 18]. In addition, this method has been used in taxonomic studies, forensic medicine, improvement of plants and animals, hereditary diagnosis of Fragile X Syndrome [11, 15, 19, 20, 21]. This method was chosen for our study because it has some advantages such as simplicity, not being very expensive, and also the method requires no need to know the genome sequences for the primer design.

MATERIAL AND METHODS

Blood Samples: The sampling was made randomly from the flock. However, attention is paid to pick up the individuals with an older look. The blood samples of Karayaka and Sakız breeds collected from two main sources; Agricultural Managements The Institute of Animal Research and from communal farms. But the samples of Bafra breed were obtained from only Govermental Animal Research Center. The number of animals examined is given in Table 2.1.

Table 2.1: Distribution of samples according to the regions, sexuality and the number of breeds**.**

Breed name	Male number	Female numbe r	Obtained regions
Bafra	20	20	Amasya Gökhöyük Agricultural Managements The Institute of Animal Research
Sakız	10	10	Bandırma-Marmara The Institute of Animal Research
Sakız -local	20	20	A communal farm in İzmir-Çeşme
Karayaka	10	10	Amasya Gökhöyük Agricultural Managements The Institute of Animal Research
Karayaka -local	20	20	A communal farm in Giresun

Genomic DNA Isolation: Genomic DNA was isolated from 200 µl of blood in the tubes with EDTA according to Fermentas Genomic DNA Purification KIT®. The manufacturer's instructions was followed in DNA extraction. Then DNA was disolved approximately 100 µl in sterile deionized water by gentle vortexing. DNA concentration was measured with spectrophotometer and DNA stock was diluted in order to prepare of 5 ng/µl stock solution.

Primer d esign: Primers were selected from Operon Tech. Alameda CA (OP). These primers were considered containing 60–70% G+C and have no self-complimentarity. The list of used in the study 10 base long RAPD primers and their sequences are given in Table 2.

Table 2.2: The list of 10 base long RAPD primer used in this study.

Number of the primers	The sequences of the primers (5'---3')	G-C%
1. OPA-02*	TGCCGAGCTG	70
2. OPA-18	AGGTGACCGT	60
3. OPC-08	TGGACCGGTG	70
4. OPD-15	CATCCGTGCT	60
5. OPE-02	GGTGCGGGAA	70
6. OPF-05*	CCGAATTCCC	60
7. OPF-06	GGGAATTCGG	60
8. OPG-02	GGCACTGAGG	70
9. OPK-20	GTGTCGCGAG	70
10. OPA-20*	GTTGCGATCC	60
11. OPB-08	GTCCACACGG	70
12. OPB-13	TTCCCCCGCT	70
13. OPB-20	GGACCCTTAC	60
14. OPC-05*	GATGACCGCC	70
15. OPC-07	GTCCCGACGA	70
16. OPC-12	TGTCATCCCC	60
17. OPC-13	AAGCCTCGTC	60
18. OPD-02*	GGACCCAACC	70
19. OPD-09	CTCTGGAGAC	60
20. OPE-04*	GTGACATGCC	60

* Primers which were shown reliable polymorphic bands.

PCR react ion con ditions: Each PCR tubes were performed in a 25 µl total volume containing 0.6 U Taq DNA polymerase, 0.10 mM of each dNTP, 3mM $MgCl_2$, 0.4 µM of primer, 20-40 ng of DNA. Amplifications were performed on Hybaid thermal cycler programmed for 40 cycles 1min at 49^0C, 1min at 35^0C, 2 min at 72^0C. An initial denaturation time was 2 min at 94 ^{0}C and final extension time was 10 min at 72 ^{0}C. Amplification products were resolved by electrophoresis at 5 V/cm for 2.5 hr in 1.7% agarose gel with 1X TBE buffer. The products were detected by staining with ethidium bromide and the gels were photographed under UV light [22].

The Evaluation of Data: Polymorphisms were scored according to presence (1) or absence (0) of DNA fragments. RAPD data were used for estimating polymorphic loci, Nei's genetic distance (D), genetic diversity (h), gene frequency, number of observed alleles (Na), number of effective alleles (Ne) among the breeds using computer programe POPGENE (Version 1.31 Microsoft WINDOW-based Freeware for Population Genetic Analysis). Estimated pairwise distances in Phylip software were used to construct a neighbor joining tree [23, 24, 25].

RESULTS AND DISCUSSION

RAPD-PCR is a powerful molecular technique for detection of genetic variability in the different breeds or populations [17]. This technique was used to determine the genetic diversity in three Turkish sheep breeds. But this technique is highly sensitive for minor alterations in the PCR. For this reason reaction conditions were optimized to get clear and reproducible bands. In this study, 20 primers were used. Six of these primers were produced reproducible and scorable polymorphic fragments. The highest number of polmorphic bands was 15 with OPC-05 and the lowest polymorphic bands with OPA-20. The size of the polymorphic bands from approximately 200 bp to 1250 bp were recorded (Table 3).

Table 3.1. Occurrance of approximate size of allelles (bp) in three sheep breeds by OPA02, OPA20, OPC05, OPD02, OPE04, OPF05.

Primers	Approximate base pairs (bp)
OPA02	225-550
OPA20	350-650
OPC05	300-1250
OPD02	325-800
OPE04	300-650
OPF05	150-1200

The highest percentage of the polymorphic locus was observed in Bafra (53.73%) and the lowest in Karayaka (22.39%). A specific allel (1250 bp) was found for Bafra breed with OPC-05 primer. But this band was not observed in the other breeds. Also 1200 bp allel was obtained with OPF-05 for some individuals in the same breed so this primer assumed as polymorphic (Figure 1.a). The OPF05 primer was not detected as polymorphic in Sakız and Karayaka sheep breeds. (Figure 1. b, 1.c)

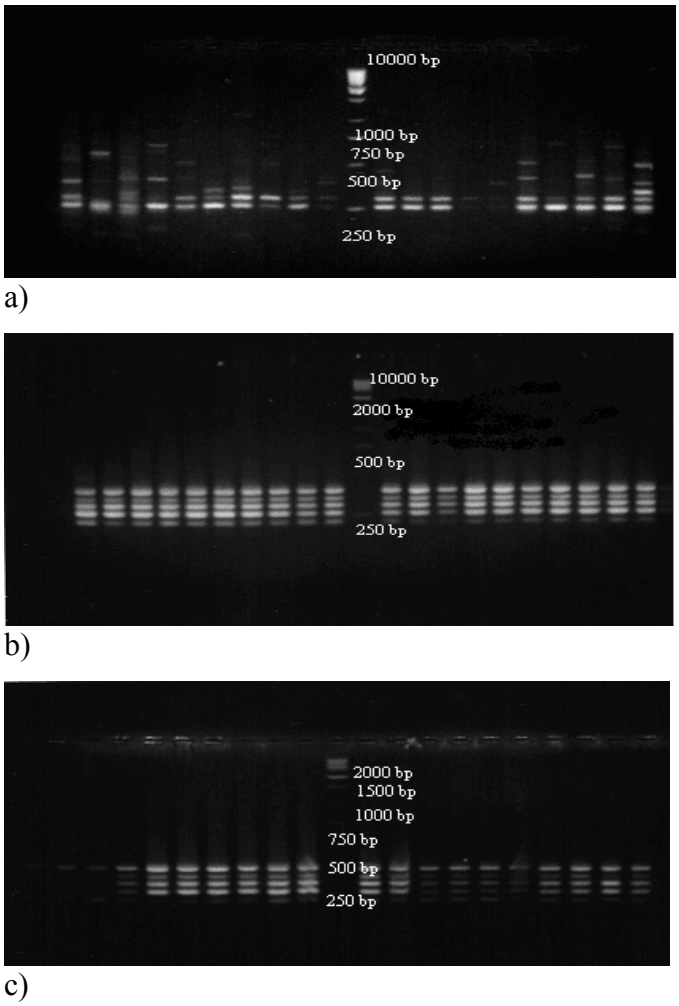

Figure 3.1. Agarose gel electrophoresis of the RAPD products were observed using primer OPF05. a) Lane 11: 1 kb marker; lanes 1-10: females and lanes12-20: males of Bafra sheep breed. b) Lane 11: 1 kb marker; lanes 1-10: females and lanes12-20: males of Sakız sheep breed. c) Lane 11: 1 kb marker; lanes 1-10: females and lanes12-20: males of Karayaka sheep breed.

Observed number of alleles and effective number of alleles were calculated for all the loci. The number of observed alleles (Na) within the breeds differed between 1.22-1.54. Number of effective alleles (Ne) was always lower than Na as it is expected and it was ranged from1.15 to 1.32. Nei's genetic diversity (h) was observed the highest in Bafra (1.19) and the lowest value was observed in Sakız-local (0.09). Summary of variation statistics for all the loci were given in the Table 4.

Table 3.2: Summary of genetic variation ststistics for all the loci in three Turkish sheep breeds.

Breed name	*Sample size*	*Na*[1] (n±SD)	*Ne*[2] (n±SD)	*h*[3] (n±SD)	*# p. lokus*[3]	*p. lokus%*[3]
Bafra	35	1.54± 0.50	1.32± 0.37	1.19± 0.20	36	53.73
Sakız	18	1.39±0.49	1.22±0.34	0.13±0.19	26	38.81
Karayaka	17	1.22±0.42	1.17±0.33	0.10±0.18	15	22.39
Sakız-local	31	1.24±0.43	1.15±0.31	0.09±0.17	16	23.88
Karayaka-local	33	1.40±0.50	1.24±0.36	0.14±0.20	27	40.30

1 Na= Observed number of alleles ± standard deviation (n±SD)

2 Ne= Effective number of alleles ± standard deviation (n±SD)

3 h= Nei's genetic diversity ± standard deviation (n±SD)

The highest level of gene frequency value (1.0000) was observed in allele 0 with the primers of OPE-04 and OPC-05. The lowest frequency value (0.0033) was observed in allele 1 using OPA-02 primer.

Unbiased measures of genetic identity (above diagonal) values ranged from 0.0432 to 0.4709 and genetic distances (below diagonal) values ranged from 0.6244 to 0.9577 (Table 6).

Table 3.3: Pair-wise mean genetic distance of the breeds.

Breeds	Bafra	Sakız	Karayaka	Sakız-local	Karayaka-local
Bafra	****	0.8072	0.8130	0.6244	0.6768
Sakız	0.2142	****	0.7799	0.7525	0.7404
Karayaka	0.2070	0.2485	****	0.6647	0.6791
Sakız-local	0.4709	0.2844	0.4084	****	0.9577
Karayaka-local	0.3903	0.3006	0.3870	0.0432	****

Estimated pair-wise distances in Phylip software were used to construct a neighbour joining tree (Figure 3.2). Since Phylip constructs the tree using not the dataset but the estimated distances, bootstrapping of the data was not possible. This neighbour-joining tree was shown that Bafra, Sakız and Karayaka are in a group, Karayaka-local and Sakız-local are in another group.

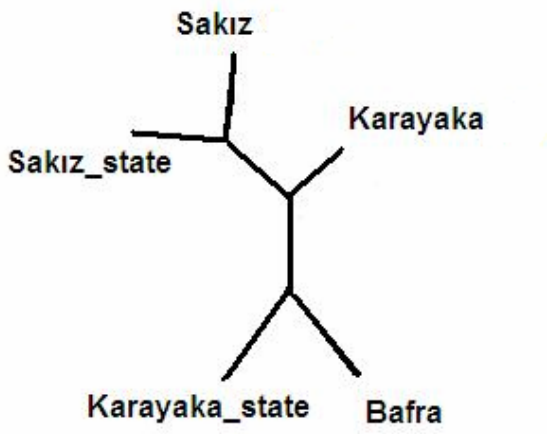

Figure 3.2: Neighbour-joining tree of the Turkish sheep breeds, based on estimated genetic distances in Phylip software.

CONCLUSION

RAPD-PCR assay has been used extensively for various purposes which include determination of genetic diversity [14, 16, 26], identification of sex specific markers [17], supporting for taxonomic identification [15], conjecture of genetic relationship [18, 26], estimation of inbreeding [27]. in addition, this method has been used successfully for livestock animals such as sheep, goat, chickens, buffalo [14, 15, 16, 17, 26, 27, 28]. this study produced results which corroborate the findings of a great deal of the previous researches.

Although this method is sensitive to minor changes in the reaction conditions, some researchers pointed out that PCR products are useful and reproducible when reaction repeat the same reaction conditions [14, 18, 26]. In our study, experiment was carried out since the same results were obtained for band patterns and repated at least three times for each primers.

The current study found that the breed specific band which was OPC05-1250 bp, observed for Bafra sheep breed. Breed/population specific primers were reported by some researches in this field [20, 27]. The findings of this study have a number of important implications for future practises, because it can be used for discrimination of Bafra breed from the others. We hope that this specific DNA marker will be used in sheep improvement program.

Uzun et al. [29, 30]studied some Turkish sheep breeds that were Tuj, Hemsin, Karayaka, Akkaraman and Morkaraman. They observed that Karayaka was certain differently from the other breeds. They reported that it showed quite a high variability for microsatellite loci. This result supported the findings of some primers for Karayaka sheep breed in our study.

Cushwa et al. [17], screened 131 RAPD primers in different sheep breeds. They reported that three primers linked with Y chromosome among the total primers. These markers are OPA20-1230, OPD02-550, OPE04-1107. However, the findings of the current study do not support the previous research for the same primers.

Kantanen et al. [10]applied this method in order to investigate genetic variability in cattle and sheep breeds. They pointed that sheep breeds have more homogenity than cattle breeds. In this study, homogenity was observed within and among the three Turkish sheep breeds for some primers.

One unexpected finding was that there were deviations found between two populations of the same breed; one taken from local farmers and the other one taken from state farms. A possible explanation for this might be that in state farms, the breeding individuals were recruited within the flock. Thus, there can be differentiation from the genetic pool of the breed easily due to genetic drift, when the population size is relatively small and there is inbreeeding and if there is no male and/or female breeding individuals introduced to the flock.

In conclusion, this study shows that genetic diversity exists among the breeds. But our study is initial work in the Turkish sheep breeds. This results will be supported with other molecular techniques such as microsatellite analysis.

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