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RESEARCH ARTICLE

Molecular Analysis of Three Local Silkworm Breeds (Alaca, Bursa Beyazı and Hatay Sarısı) by RAPD-PCR and SDS-PAGE Methods [1]

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Summary

In this study, PCR-based RAPD method was used to determine the genetic variation in three Turkish local silkworm breeds (Alaca, Bursa Beyazı and Hatay Sarısı) and SDS-PAGE method was used to analyse the sericin proteins of their cocoons. Three local breeds were analysed by randomly chosen 40 primers and 68 total RAPD bands observed with 7 of them. Percentages of polymorphic loci were determined higher in Hatay Sarısı (55.88), lower in Bursa Beyazı and Alaca (44.12). Nei's genetic distance was determined 0.0637 between Bursa Beyazı and Alaca, 0.1012 between Bursa Beyazı and Hatay Sarısı, 0.0793 between Alaca and Hatay Sarısı. In SDS-PAGE sericin proteins of three breeds was yielded a single band of 200 kDa.

Keywords: *Bombyx mori, RAPD-PCR, Genetic polymorphism, SDS-PAGE*

Üç Yerli İpekböceği Irkının (Alaca, Bursa Beyazı ve Hatay Sarısı) RAPD-PCR ve SDS-PAGE Yöntemleri ile Moleküler Analizi

Özet

Bu çalışmada, Türkiye'nin üç yerli ipekböceği ırkında (Alaca, Bursa Beyazı ve Hatay Sarısı) genetik varyasyonu belirlemede PCR-temelli RAPD yöntemi, bu üç ırkın kokonlarında bulunan serisin proteinlerinin analizinde ise SDS-PAGE yöntemi kullanıldı. Üç yerli ırk rasgele seçilen 40 primer ile analiz edildi ve bunlardan 7 tanesi ile toplam 68 RAPD bandı gözlendi. Polimorfik lokusların yüzdesi, en yüksek Hatay Sarısı'nda (55.88) en düşük ise Bursa Beyazı ve Alaca'da (44.12) belirlendi. Nei'nin genetik mesafesi Bursa Beyazı ile Alaca arasında 0.0637, Bursa Beyazı ile Hatay Sarısı arasında 0.1012, Alaca ile Hatay Sarısı arası 0.0793 belirlendi. Her üç ırkın serisin proteinlerine ait SDS-PAGE analizinde 200 kDa'lık tek bant görüldü.

Anahtar sözcükler: *Bombyx mori, RAPD-PCR, Genetik polimorfizm, SDS-PAGE*

INTRODUCTION

The domesticated silkworm [1], *Bombyx mori,* an economic silk secreting insect, comprises a large number of varieties in temperate and tropical countries [2,3]. The tropical varieties (nondiapausing) produce poor quality of silk while the temperate varieties (diapausing) produce good quality of silk [2,3].

Recently, genetic markers have used in animal and plant improvement programmes for varietal and parentage identification, construction of linkage maps and evaluation of polymorphic genetic loci affecting quantitative economic traits [4]. Development of molecular markers is important in the silkworm for construction of linkage map and fingerprinting of strains for breeding [4]. Recently, PCR-based tecniques have been widely used to detect the polymorphic genetic markers in the silkworm [4,5]. Random amplified polymorphic DNAs (RAPDs) is one of the PCR-based tecniques used as a tool for genetic mapping and strain identification [2,4,6-9]. Because of its relative simplicity, RAPD method is being extensively used in genetic analysis [5].

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The *Bombyx mori* cocoon consists of fibroin and sericin proteins [10]. Sericin is a group of glue proteins produced in the middle silk gland (MSG) of the silkworm. It surrounds fibroin fibers and binds them to each other in the cocoons [11]. Recently, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method, has frequently used to determine the molecular weight of sericin protein [10,11]. SDS-PAGE is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility (a function of length of polypeptide chain or molecular weight). In this study, SDS-PAGE method was also preferred to determine the molecular weight of sericin proteins.

Turkish local silkworm breeds are inbreeded in Bursa Provincial Directorate of Agriculture. Alaca and Bursa Beyazı are diapausing genotypes (temperate genotype; high-quality silk) but Hatay Sarısı is non-diapausing genotype (tropical genotype; low-quality silk). Bursa Beyazı have a higher silk quality than others.

The aim of this study was to estimate the genetic polymorphism between three Turkish silkworm breeds by using RAPD-PCR method and to analyze the sericin proteins of three breeds by SDS-PAGE.

MATERIAL and METHODS

Collection of Silkworm Stocks

Individuals of three Turkish silkworm breeds and cocoons were obtained from Bursa Silkworm Breeding Research Institute.

Genomic DNA Isolation

Genomic DNA was isolated from adult's tissue. Firstly, tissue was pulvarized in liquid nitrogen with mortar and pestle. Then powder was placed in a 1.5 ml micro-centrifuge tube and resuspended in TE buffer. Fermentas Genomic DNA Purification KIT® was used in DNA isolation. Lysis solution ($KHCO_3$, NH_4Cl, 0.5 M EDTA, pH: 8.0) was put into sample and incubated at 65°C for 10 min. Chloroform was added and gently emulsified by inversion and centrifuged. The upper phase containing DNA was transfered to a new tube and precipitation solution was added. Supernatant was removed and DNA pellet was dissolved in 100µl of 1.2 M NaCl solution by vortexing. Cold ethanol was added and DNA was precipitated at -20°C for 10 min. Then ethanol was poured off and pellet was washed once with 70% cold ethanol and DNA was dissolved in sterile deionized water by gentle vortexing. DNA concentration was measured by spectrophotometer and diluted to 5 ng/µl.

Amplification and Seperation of DNA

PCR reaction in a 25 µl volume contained 0.2 u/µl Taq DNA polymerase, 1X PCR buffer, 3 mM $MgCl_2$, 1.25 mM dNTP, 0.6 µM primer. For this study 40 primers were chosen arbitrarily *(Table 1)*. The reactions were initiated at 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 34°C for 30 sec and 72°C for 45 sec. Final extension time was 72°C for 10 min. The amplified products were seperated on 1.7% agarose gel in 1X TBE buffer. The products were run at 100 V for 2.5 h. Amplified products were detected under UV light by staining 0.5 µg/ml ethidium bromide. DNA bant profiles were evaluated with 100 bp DNA marker.

Table 1. *Primer base sequences and the ratio of guanine-cytosine bases (G-C)*

Tablo 1. *Primerlerin baz dizileri ve G-C bazlarının oranı*

Primer	Primer Sequence (5'---3')	G- C (%)
1	CTGGGGACTT	60
2	CTGAGACGGA	60
3	AGCGTCCTCC	70
4	TGGGCGTCAA	60
5	GGGCGGTACT	70
6	AGCCTGAGCC	70
7	ACCACCCACC	70
8	ACAACGCCTC	60
9	GGCGGTTGTC	70
10	GGGACGTTGG	70
11	ACTGAACGCC	60
12	ACAACTGGGG	60
13	GTCAGAGTCC	60
14	TCGGCGGTTC	70
15	GAGGTCCACA	60
16	GTCAGTGCGG	70
17	GTCCGGAGTG	70
18	GTGACCGAGT	60
19	ACGCAAGAGG	60
20	AGACGATGGG	60
21	GGGTCGCGGT	80
22	GGTCGATCTG	60
23	AGTCGCCCTT	60
24	GGGCCAATGT	60
25	GTGGAGTCAG	70
26	TGAGGGTCCC	70
27	AGCCGTGGAA	60
28	GTGTCGCGAG	70
29	CTCTGGAGAC	60
30	GGGAATTCGG	60
31	TGTCATCCCC	60
32	AAGCCTCGTC	60
33	GGACTGGAGT	60
34	GGTGACGCAG	70
35	CCTTGACGCA	70
36	TTCCCCCGCT	70
37	AAGCTTGATTGCC	50
38	AAGCTTCGACTGC	50
39	AAGCTTTGGTCAG	50
40	AAGCTTCTCAACG	50

Data Analysis

Data were analysed using POPGENE Software (POPGENE VERSION 1.31 Microsoft Window-based Freeware for Population Genetic Analysis) program. Polymorphism was detected according to presence or absence of bands. Absence of band was recorded as 0 and presence of band was recorded as 1. Percentage of polymorphic loci, number of polymorphic loci, observed number of alleles (na), the gene diversity (h=the mean percentage of heterozygous per locus), the diversity within the populations (Hs), the magnitude of differentiation among the populations (GST), Nei's genetic distance [12] (accounts for multiple mutations per locus in populations) was determined.

Preparation of the Silk Sericin Protein

The cocoon samples (450 mg) cut into the pieces and washed repeatedly with hot water. Then it was immerged in 3 ml of distilled water overnight. It was put into a high-pressure boiler at 120°C for 1 h. The degumming solution was filtered to remove precipitates. The resulting solution with yellow color due to the higher molecular weight of the sericin peptide [10].

SDS-PAGE Analysis

Sericin proteins of three local breeds were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method previously by Laemmli [13,14] with 12% acrylamide gel and 5% condensing gel. Then gel was stained with 0.25% Coomasie Brilliant Blue R-250 [10].

RESULTS

RAPD-PCR: In this study 40 arbitrarily primers were used and 61 polymorphic loci were produced with only seven of them. These seven primers and RAPD-DNA bands are given in *Table 2*.

The primer called OPN-05 produced 900 bp DNA bands in some individuals of Bursa Beyazı and Alaca. But 900 bp DNA bands weren't observed in Hatay Sarısı. DNA band profile obtained with OPN-05 is given in *Fig. 1*.

Table 2. *Approximate size of alleles (bp) in silkworm breeds*
Tablo 2. *İpekböceği ırklarından elde edilen allellerin yaklaşık büyüklükleri (bç)*

Primers	Approximate Base Pairs (bp)
OPL12	200-1500
OPL18	225-900
OPN05	350-1100
OPU19	200-1500
OPY11	200-800
OPY13	250-425
OPY15	275-1000

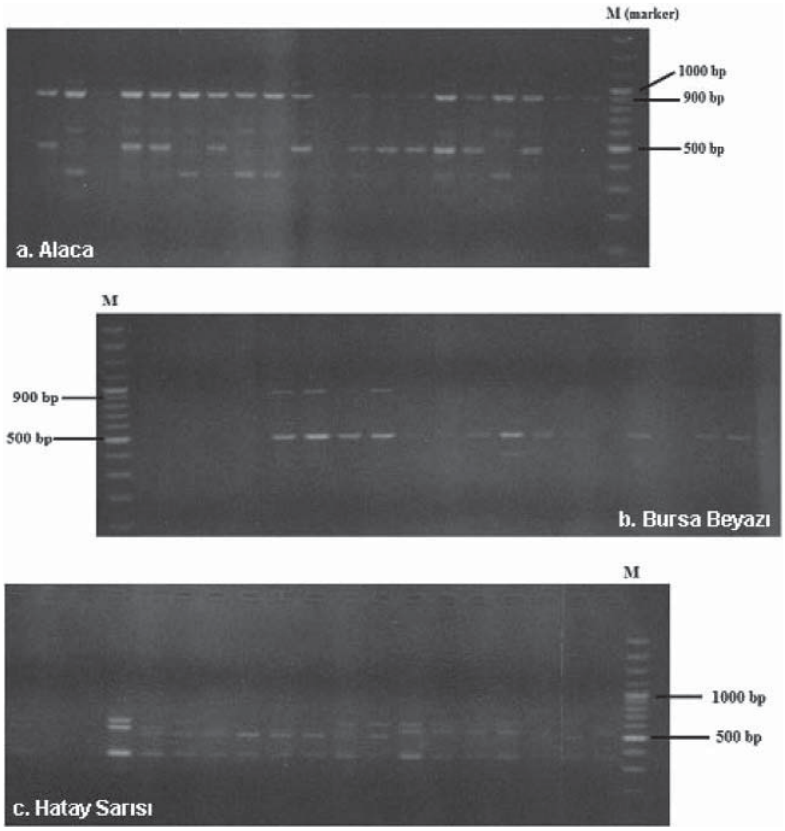

Fig 1. RAPD bands obtained with OPN-05 primer

Şekil 1. OPN-05 primeri ile elde edilen RAPD bantları

OPU-19 produced 650 bp DNA bands in some individuals of Hatay Sarısı. DNA band profile obtained with OPU-19 is given in *Fig. 2*.

POPGENE program was used to analyze data. According to these data, percentage of polymorphic loci varied between 44.12 (Bursa Beyazı and Alaca) and 55.88 (Hatay Sarısı). Number of polymorphic loci was the highest in Hatay Sarısı but the lowest in Bursa Beyazı and Alaca. Na, ranged between 1.4412 and 1.5588. The highest gene diversity was recorded in Hatay Sarısı (0.1396) and the lowest gene diversity was observed in Bursa Beyazı (0.1176). Hs was 0.1299 and the GST was 0.2586. Nei's genetic distance was 0.1012 between Bursa Beyazı - Hatay Sarısı, 0.0637 between Bursa Beyazı - Alaca and 0.0793 between Alaca - Hatay Sarısı. According to these data, Bursa Beyazı and Alaca are the closest breeds but Hatay Sarısı and Bursa Beyazı are the most divergent breeds as genetic.

SDS-PAGE: In SDS-PAGE sericin proteins of three local breeds yielded a single band of 200 kDa *(Fig. 3)*.

DISCUSSION

In this study three Turkish silkworm breeds were analysed by RAPD-PCR method to obtain molecular DNA markers. For this analysis, 40 arbitrarily primers were used and 61 polymorphic loci were obtained from only seven of them. Diapausing (Bursa Beyazı and Alaca) and non-diapausing (Hatay Sarısı) local breeds were seperated by these primers successfully.

Similarly, Thanananta [15] used RAPD-PCR and seperated five morphological races to diapausing and non-diapausing varieties. Nagaraja and Nagaraju [2] used 40 random primers in 13 silkworm genotypes. Diapausing and non-diapausing genotypes were

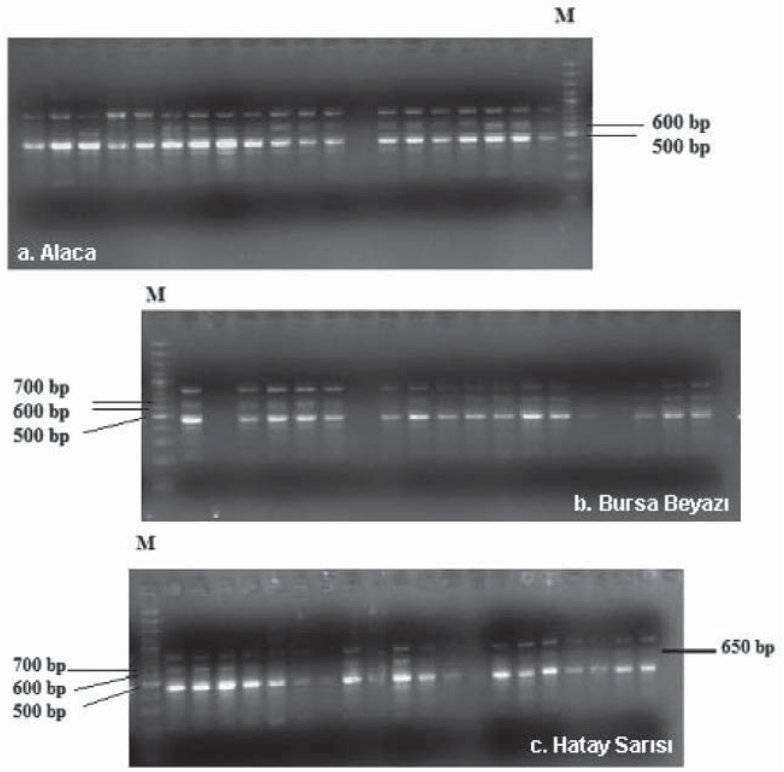

Fig 2. RAPD bands obtained with OPU-19 primer

Şekil 2. OPU-19 primeri ile elde edilen RAPD bantları

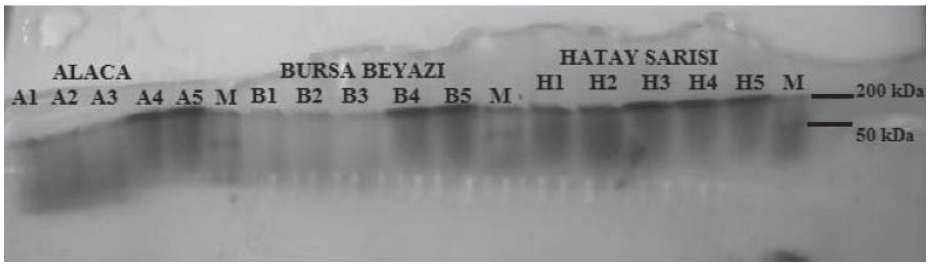

Fig 3. Band profiles of sericin proteins in SDS-PAGE

Şekil 3. SDS-PAGE'de serisin proteinlerinin bant profilleri

distinguished by 5 specific markers. The marker OPA-02 (800) was observed in only non-diapausing genotypes. Similarly [16] the marker OPA-02 (800) was observed in Hatay Sarısı which is a local nondiapausing genotype of Turkey. OPN-05 (900 bp), OPU-19 (650 bp), OPA-02 (800 bp) can be used in characterization of three local breeds.

Sprague [17] reported the presence of more than 15 sericin polypeptides that have molecular masses ranging from 20 to 220 kDa. Takasu et al.[11] found three polypeptides with molecular masses of the 400, 250 and 150 kDa estimated by SDS-PAGE. Zhang et al.[10] characterized the sericin protein with the wide range of molecular weight from about 50 kDa to over 200 kDa. Dash et al.[18], characterized the sericin of the tropical tasar silkworm, *Antheraea mylitta* by the SDS-PAGE. A single band of approximately 200 kDa was detected. Similarly in our study, sericin protein of three breeds yielded a single band of 200 kDa by the SDS-PAGE.

There is no sufficient study about molecular characterization of local silkworm breeds. In this study, three Turkish silkworm breeds were examined by RAPD-PCR and SDS-PAGE. Local silkworm breeds were seperated as diapausing (Bursa Beyazı and Alaca) and non-diapausing (Hatay Sarısı) varieties by RAPD-PCR method. OPN-05 (900), OPU-19 (650) RAPD bands and 200 kDa of sericin protein band can be used in characterization of local breeds. This and other molecular studies are important to have a body of knowledge about national gene resources. In conclusion, the results obtained from this study can be used for improvement programmes of Turkish silkworm breeds in future.

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