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Molecular Genetic Analysis of Three Turkish Local Silkworm Breeds (Bursa Beyazı, Alaca and Hatay Sarısı) by RAPD-PCR Method

Demet EROĞLU[1] Şükran ÇAKIR ARICA[2]
Kırıkkale University, Faculty of Arts and Science, Department of Biology, 71450 Yahşihan, Kırıkkale-TURKEY

[*]**Corresponding Author**
e-mail: demeteroglu2002@yahoo.com

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ABSTRACT

In this study PCR-based Randomly Amplified Polymorphic DNA (RAPD) method was used to determine the genetic variation in three local silkworm breeds (Bursa Beyazı, Alaca and Hatay Sarısı) of Turkey. These three breeds were analysed with 23 RAPD primers. 34 polymorphic loci were observed with 5 primers of them. Percentage of polymorphic loci were varied between 17.65 and 55.88. Nei's genetic distance was 0.0822 between Hatay Sarısı-Alaca and 0.1106 between Bursa Beyazı -Alaca. The diversity within the populations (Hs) was found 0.1334 and the magnitude of differentiation among the populations (G_{ST}) was found 0.2968.

Key Words: *Bombyx mori*, silkworm, RAPD-PCR, genetic polymorphism.

INTRODUCTION

The silkworm, *Bombyx mori*, is one of the genetically well-characterized insect as a Lepidopteran molecular model system [1]. The silkworm genetic stocks maintained around the world contain geographical races, inbred lines and mutants. These stocks carry morphological, developmental, behavioural and biochemical characters [2]. In Asia and Europe more than 3000 silkworm genotypes have been estimated. The tropical varieties (nondiapausing) produce poor quality of silk while the temperate varieties (diapausing) produce good quality of silk [3]. The classical silkworm breeding studies have been used to increase the silk productivity of these genotypes [3,4].

Recently, the silkworm linkage map is based on morphological and biochemical markers [3].

These markers are used to estimate the genome polymorphism [2]. Development of these molecular markers is important in the silkworm for construction of linkage map, marker-assisted selection and fingerprinting of strains for breeding [5].

Polymerase chain reaction (PCR) [6] tecnique, provides alternative strategies for generating molecular markers [3]. Random Amplified Polymorphic DNAs (RAPDs) [7-9] is one of the PCR-based tecniques used as a tool for genetic mapping, strain identification, systematics and population studies [3].

The aim of this study is to estimate the genetic polymorphism between three Turkish silkworm breeds by using RAPD-PCR method.

MATERİALS AND METHODS

Collection of Silkworm Stocks

Individuals of three Turkish silkworm breeds 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 microcentrifuge 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 65º 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 -20º 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 5ng/µl.

Amplification and Seperation of DNA

PCR reaction in a 25 µl volume contained 0.2 u/µl Taq DNA polymerase, 1X PCR Buffer, 3.5 mM $MgCl_2$, 1.25 mM dNTP, 0.4 µM primer. 10-mer 23 primers (OPA-01, OPA-02, OPA-05, OPA-18, OPA-20, OPB-8, OPB-10, OPB-13, OPB-20, OPC-05, OPC-06, OPC-08, OPC-12, OPC-13, OPD-02, OPD-09, OPD-15, OPE-02, OPE-04, OPF-05, OPF-06, OPG-02 and OPK-20) were chosen arbitrarily from Operon Technology®. For PCR amplifications, Thermo Hybaid Cycler was used and programmed. Initial denaturation was 94 ºC for 2 min, followed by 40 cycles of 94 ºC for 1 min, at 35 ºC for 1 min and at 72 ºC for 2 min. Final extension time was 72 ºC for 10 min. 1.7 % agarose (Sigma ®) gel in 1X TBE buffer was used in seperating of amplified products. The products were run at 100 V for 2.5 hours. Amplified products were detected under UV light by staining 0.5 µg/ml ethidium bromide. DNA bant profiles were evaluated with 100 bp MBI Fermentas ® SMO321 DNA marker.

Data Analysis

Data were analysed with 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.

RESULTS

In this study 23 arbitrarily primers were used and 34 polymorphic loci were produced with only five of them. These five primers and RAPD-DNA bands were given in Table 1.

Table 1. Approximate size of alleles (bp) in silkworm breeds.

Primers	Approximate base pairs (bp)
OPA-O2	200-2000
OPA-20	350-1200
OPC-O5	400-750
OPE-O4	250-1031
OPF-O5	250- 400

The primer called OPA-02 produced 250,300,400,500 bp DNA bands in all individuals of these three breeds. But some individuals were polymorphic for 200,225,550 bp DNA bands. In some individuals of Hatay Sarısı, 600,800,900,1031,1200,2000 bp DNA bands were also observed. DNA band profile obtained with OPA-02 was given in Figure 1.

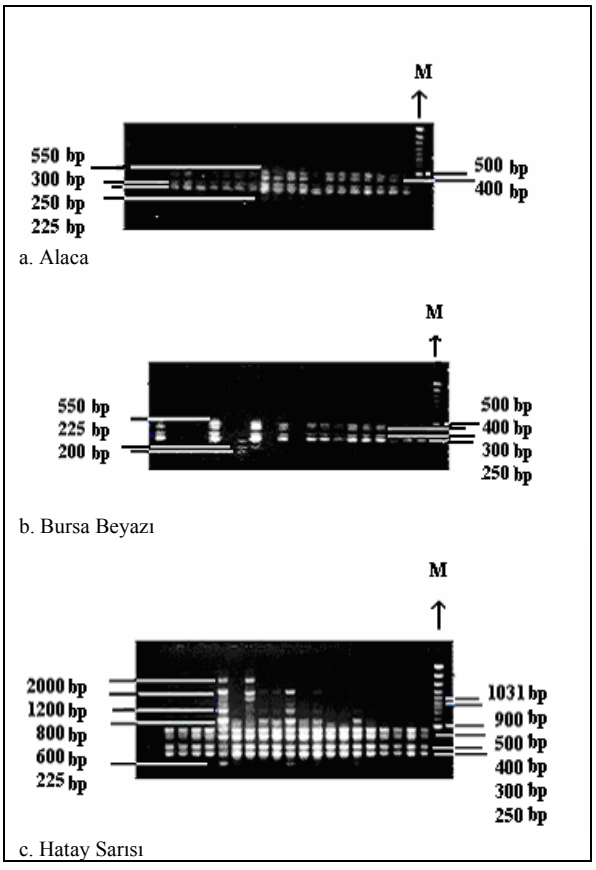

Figure 1. RAPD bands obtained with OPA-02 primer (a) in Alaca, (b) Bursa Beyazı (c), Hatay Sarısı, (M: marker).

The primer called OPA-20 produced 350 and 600 bp DNA bands in all individuals of Bursa Beyazı and Hatay Sarısı. But produced 400 bp band in only some individuals of these two breeds. Some individuals of Hatay Sarısı were polymorphic for 900,1031 and 1200 bp DNA bands. In all Alaca individuals 400 and 600 bp DNA bands were obtained. Interestingly, 350 bp band was not observed in Alaca individuals. DNA band profile obtained with OPA-20 was given in Figure 2.

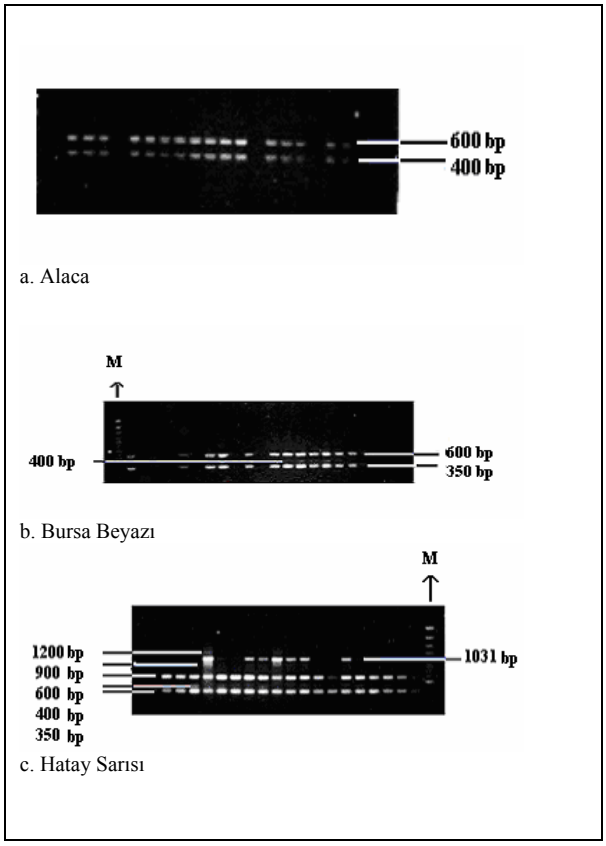

Figure 2. RAPD bands obtained with OPA-20 primer (a) in Alaca, (b) Bursa Beyazı, (c) Hatay Sarısı, (M: marker).

Other three primers produced 14 common markers for three silkworm breeds. DNA band profile obtained with OPE-04 was given in Figure 3.

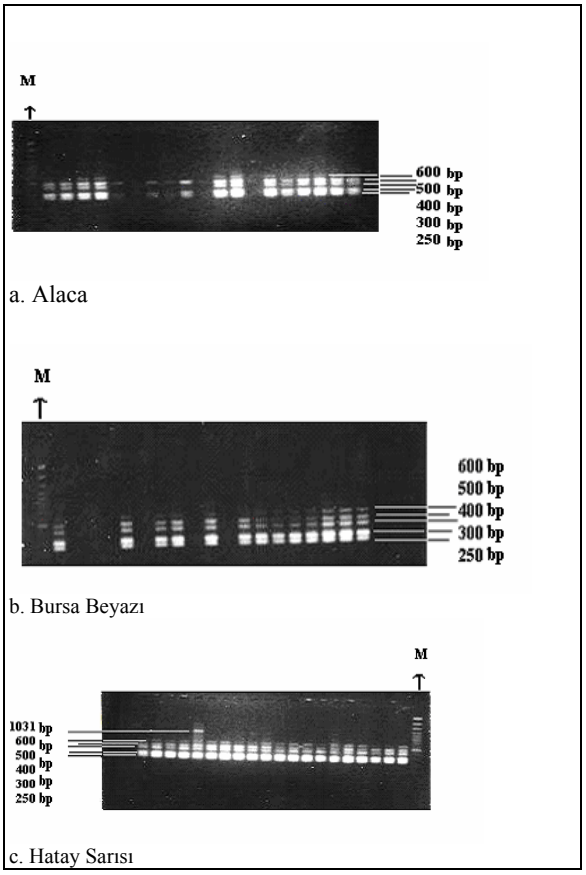

Figure 3. RAPD bands obtained with OPE-04 primer (a) in Alaca, (b) Bursa Beyazı, (c) Hatay Sarısı, (M: marker).

These markers were OPE-04 (250, 300, 400, 500 ve 600 bp), OPC-05 (400, 450, 500, 600 ve 750 bp) and OPF-05 (250, 300 ve 400 bp). No polymorphism was detected with these primers.

POPGENE program was used to analysed data. According to these data, percentage of polymorphic loci was varied between 17.65 (Alaca) and 55.88 (Bursa Beyazı). Number of polymorphic loci was the highest in Bursa Beyazı and the lowest in Alaca. Observed number of alleles (na) was varied between 1.5588 and 1.1765. The highest gene diversity (h) was observed in Bursa Beyazı (0.2370) and the lowest gene diversity was observed in Alaca (0.0659). The diversity within the populations (Hs) was 0.1334 and the magnitude of differentiation among the populations (G_{ST}) was 0.2968. Nei's genetic distance was 0.0822 between Hatay Sarısı-Alaca, 0.1106 between Bursa Beyazı –Alaca and 0.1083 between Bursa Beyazı-Hatay Sarısı.

According to dendogram, Hatay Sarısı and Alaca breeds are the closest but Bursa Beyazı and Alaca breeds are the most divergent to each other (Figure 4).

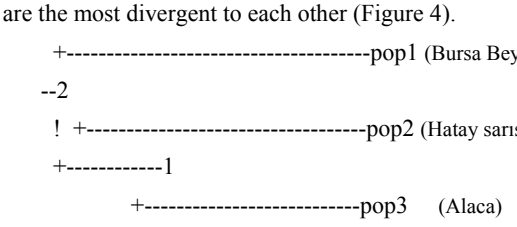

Figure 4. Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA Modified from NEIGHBOR procedure of PHYLIP Version 3.5.

DISCUSSION

Recently, DNA-markers obtained from molecular genetic analysis have been used in improvement programmes [5] of farm animals. PCR-based tecniques have been widely used to detect the polymorphic genetic markers [6]. So, molecular tecniques are important to have a body of knowledge about national gene resources. Today, PCR-based tecniques are also frequently used for characterization of silkworm breeds and genetic mapping studies [5].

In this study three Turkish silkworm breeds were analysed by RAPD-PCR method to obtain molecular DNA markers. This is the first DNA analysis of Turkish silkworm breeds to estimate the genetic polymorphism. For this analysis, 23 arbitrarily primers were used and 34 polymorphic loci were obtained with only five of them. Sizes of the products ranged from approximately 200 bp to 2000 bp.

One of the primers was OPA-02 which produced 250, 300, 400 and 500 bp DNA bands in all individuals of these three breeds. Some individuals were polymorphic for 200, 225 and 550 bp DNA bands.

In addition, 600, 800, 900 ,1031, 1200 and 2000 bp DNA bands were also observed in some individuals of Hatay Sarısı with OPA-02 primer. Nagaraja and Nagaraju (1995) had used the same tecnique for some silkworm breeds. They reported that, 216 products had been generated by using 40 random primers in 13 silkworm genotypes. The size of the amplified products had ranged from 200 bp to 3000 bp. Diapausing and nondiapausing genotypes had been distinguished by 5 specific markers. OPA-02 was also used by them. The marker OPA-02 $_{(800)}$ had been observed in only nondiapausing genotypes. Similarly, in our study, the marker OPA-02 $_{(800)}$ was observed in Hatay Sarısı which is a local nondiapausing genotype of Turkey.

Nagaraju et al. [6] had used RFLPs, RAPD-PCR, ISSR-PCR and SSRs techniques for genetic analysis of 13 silkworm strains. Diapausing and non-diapausing varieties had been seperated by them successfully. This is the first study for molecular genetic analysis of three Turkish silkworm breeds. The other molecular techniques should be applied to get more genetic information for characterization of them. These preliminary results can be potentially utilized in improvement programmes of Turkish silkworm strains in future.

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