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RESEARCH ARTICLE

A Scanning Electron Microscopic Study on Some Morphological Properties of Three Local Silkworm Breeds (Alaca, Bursa Beyazı and Hatay Sarısı)

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Summary

In this study the cocoon, egg and silk gland of three local silkworm breeds were analysed by the scanning electron microscopy (SEM). The egg and cocoon morphology of Bursa Beyazı was observed as different from other breeds. The cocoon fibers were tightly packed in the cocoons of Bursa Beyazı which have a higher silk quality whereas the fibers were loosely packed in the cocoons of Alaca and Hatay Sarısı. In addition, the motif structure on Alaca's egg surface was irregular. Interestingly, there was a regular motif structure on the Bursa Beyazı's and Hatay Sarısı's egg surface.

Keywords: *Silk, Cocoon, SEM, Silk gland, Bombyx mori*

Üç Yerli İpekböceği Irkının (Alaca, Bursa Beyazı ve Hatay Sarısı) Bazı Morfolojik Özellikleri Üzerine Bir Elektron Mikroskop Çalışması

Özet

Bu çalışmada üç yerli ipekböceği ırkının kokon, yumurta ve ipek bezleri taramalı elektron mikroskobu (SEM) ile incelenmiştir. Bursa Beyazı'nın yumurta ve kokon morfolojisinin diğer ırklardan farklı olduğu gözlenmiştir. Yüksek ipek kalitesine sahip Bursa Beyazı'nın kokon fiberleri daha sık paketlenirken Alaca ve Hatay Sarısı'nın kokon fiberleri daha gevşek paketlenmiştir. Ayrıca Alaca'nın yumurta yüzeyindeki motif yapısı oldukça düzensizdi. İlginç olarak Bursa Beyazı ve Hatay Sarısı'nın yumurta yüzeyinde düzenli bir motif yapısı vardı.

Anahtar sözcükler: *İpek, Kokon, SEM, İpek bezi, Bombyx mori*

INTRODUCTION

The silk of lepidopteran insects secrete a solid fiber, the silk [1]. The silk is secreted from labial silk glands. In the domestic silkworm *Bombyx mori* [2], the silk fibre core, which is secreted in the posterior section of the silk glands (PSG), consists of heavy-chain fibroin (H-fibroin) [1], light-chain fibroin (L-fibroin) and protein p25. The middle silk gland sections (MSG) secrete several sericins that sticky coating of the silk [1]. Fibroin is the water insoluble core of the silk fiber whereas sericin is the water soluble proteinaceous glue [3]. The structure and properties of the silk filaments of wild silkworms has been studied by a number of workers [4].

There are three local silkworm breeds are inbreeded in Bursa Provincial Directorate of Agriculture. Alaca and Bursa Beyazı are diapausing genotypes but Hatay Sarısı is nondiapausing genotypes. Bursa Beyazı have a higher silk quality than others.

In this study the cocoon, silk gland and egg morphology of three local silkworm breeds studied by the Scanning Electron Microscopy (SEM).

MATERIAL and METHODS

Collection of Samples: Cocoons, larvae and eggs of three Turkish silkworm breeds were obtained from Bursa

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Silkworm Breeding Research. The silk glands were obtained from the last instar larvae of three local silkworm breeds.

Preparation of the Silk Gland, Cocoons and Eggs for the SEM: The silk glands and cocoons were fixed in 3% gluteraldehyde solution buffered in 0.2 M phosphate buffer for 1 h; post fixed in 1% osmium tetraoxide [5] in the same buffer for 1.5 h. For scanning electron microscopic observations dehydrated through 50%, 60%, 70%, 80%, 90%, 99% and 100% series of alcohol. Then the samples were dried in the room for the night and gold coated for the SEM [6]. The eggs were gold coated and observed under the SEM [7].

Preparation of Degummed Silk Fibers: Two different method were used to remove the sericin layer. In the first; the fibers were washed in boiling distilled water for 1 h. Then the samples were dried in the room and observed by the SEM. In the second method; the fibers were processed by extraction of sericin by using an aqueous solution containing 0.02 M Na_2CO_3 and white soap powder for 60 min at 90°C as previously described [8]. Dehydrated through 50%, 60%, 70%, 80%, 90%, 99% and 100% series of alcohol. Then the samples were dried in the room for the night and gold coated for the SEM [6].

RESULTS

The cocoon filaments and degummed fibers of three breeds were examined under scanning electron microscope. The morphological structure of cocoon filaments were different in silkworm breeds. The cocoon fibers were tightly packed in the cocoons of Bursa Beyazı which have a higher silk quality whereas the fibers were loosely packed in the cocoons of Alaca and Hatay Sarısı. Another difference was observed on the surface of the silk fibers. There were more voids on the fibers of Bursa Beyazı than fibers of Alaca and Hatay Sarısı *(Fig .1).*

The sericin layer wasn't removed by washing with distilled water *(Fig. 2).* This glue-like layer was removed by washing with Na_2CO_3 and white soap powder as previously described [8] *(Fig. 3).* It's clear that this sericin layer occurs breed-special fiber design.

The egg morphology of three breeds were different. The motif orientation on Alaca's egg surface was irregular. Moreover, shape of all motifs and the distances between motifs were different. Interestingly, there was a regular motif orientation on the Bursa Beyazı's egg surface.

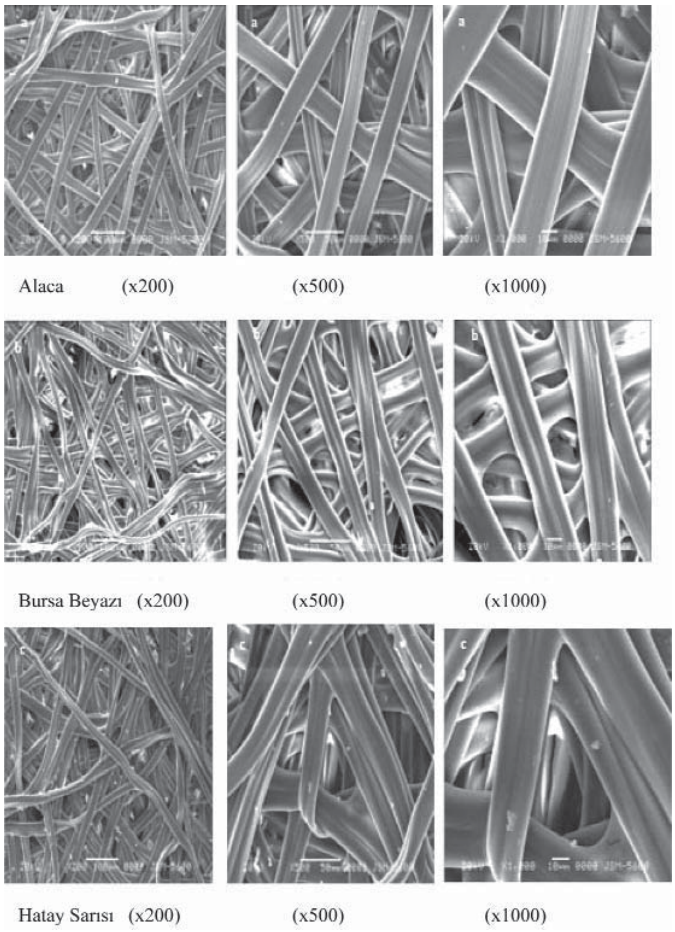


Fig 1. The morphology of cocoon in local silkworm breeds

Şekil 1. Yerli ipekböceği ırklarında kokon morfolojisi

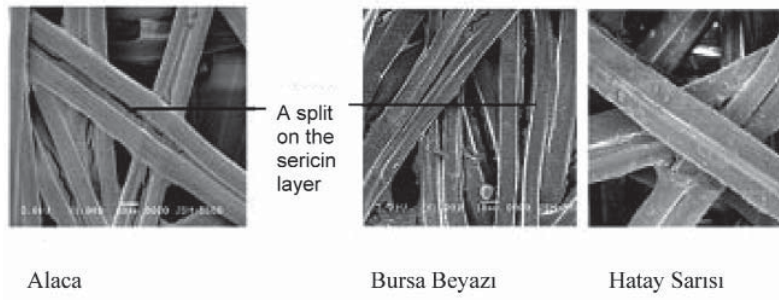
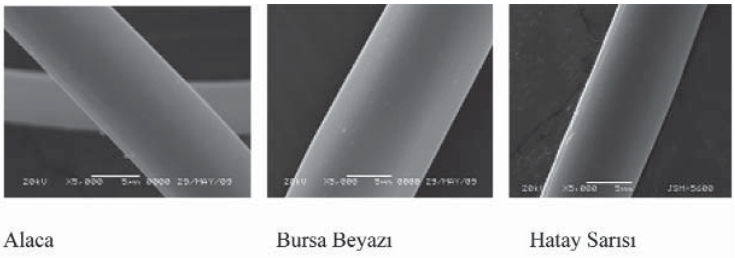


Fig 2. Degummed silk fiber surfaces of local silkworm breeds by washing with distilled water (X1000)

Şekil 2. Yerli ipekböceği ırklarının distile su ile yıkanarak soyulmuş ipek fiber yüzeyleri (X1000)

Fig 3. Degummed silk fiber surfaces of local silkworm breeds by washing with Na_2CO_3 and soap powder (X5000)

Şekil 3. Yerli ipekböceği ırklarının Na_2CO_3 ve sabun tozu ile yıkanarak soyulmuş ipek fiber yüzeyleri (X5000)



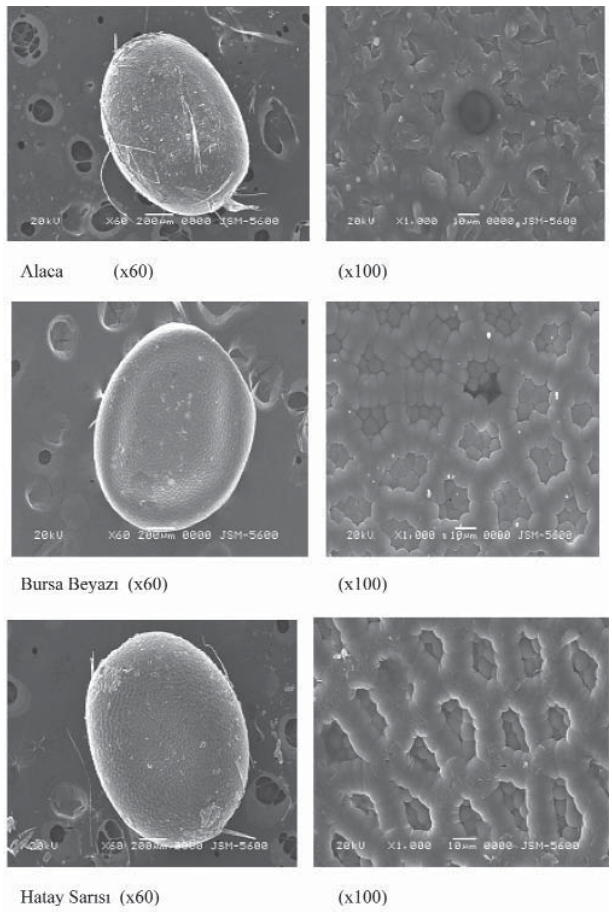


Fig 4. The morphology of egg in local silkworm breeds
Şekil 4. Yerli ipekböceği ırklarında yumurta morfolojisi

Because the dimension of motifs were the same and the distances between motifs were equal. In addition, each motif had more than one honeycomb structures. There was a regular motif orientation on the Hatay Sarısı's egg surface, too. The egg morphology of Hatay Sarısı was resemble to Bursa Beyazı. But the honeycomb structure in motifs wasn't so clear as in Bursa Beyazı *(Fig. 4)*.

There was no difference between local three breeds' silk glands *(Fig. 5)* There were much secretory tubes on the posterior part. Middle silk gland was very smoothy which is a depot part of the silk gland.

DISCUSSION

According to these observations the cocoon morphology of Hatay Sarısı and Alaca were similar in each other. Similarly, Mondal et al.[4] had determined that fibers had tightly packed in pure bivoltine breeds (have a higher silk quality) whereas fibers had loosely packed in pure multivoltine breeds. The number of voids in pure multivoltine breeds had been determined much higher than had found in pure bivoltine breeds. Narumi et al.[9], had studied the cocoon filaments of various wild silkmoth species under a SEM and had found voids in the cocoon filaments. Narumi et al.[10], had reported that the number of voids in male filament was larger than that of female silkworms. Lin et al.[11], had determined that the presence of voids in the cocoon filament is a characteristic of the fine structure of cocoon filament in wild silkworms belonging to the family Saturnidae. Mondal et al.[4] had determined that voids in the cocoon filament are important in the selection process of good quality of silk cocoons for silkworm breeding.

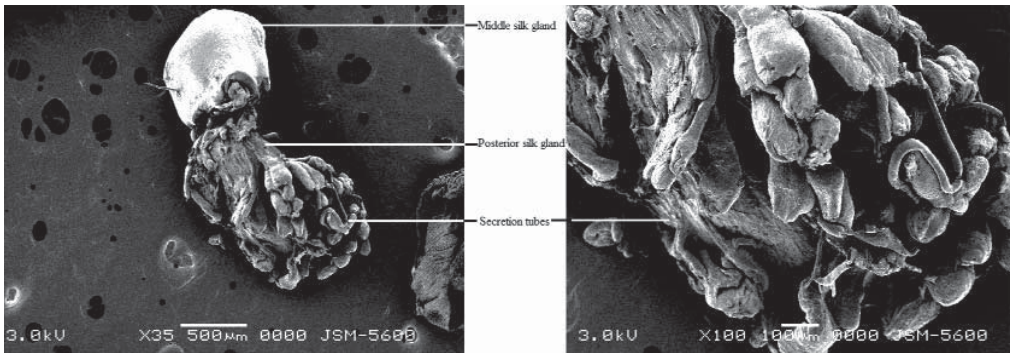


Fig 5. The morphology of silk gland in local silkworm breeds

Şekil 5. Yerli ipekböceği ırklarında ipek bezi morfolojisi

Interestingly, there was a regular motif orientation on the Hatay Sarısı's and Bursa Beyazı's egg surface. The motif orientation on Alaca's egg surface was irregular. The studies on the silkworm eggs are limited. Keino and Takesue [12] had studied morphological changes on the surface of eggs of the silkworm *Bombyx mori* L. during the early developmental stages by scanning electron microscopy.

In this study secretory tubes were observed on the posterior part of the silk gland. These tubes secrete the fibroin fibers and is surrounded by a liquid secretion [13]. In the middle secretory portion, this secretion receives the sericin layers, which envelopes the fibroin fibers [14]. Sasaki and Tashiro [5] had showed two microtubule systems in the posterior silk gland cells, a radial and a circular microtubule system.

This is the first morphological study of three Turkish silkworm breeds. The egg and silk morphology of Bursa Beyazı was different from other breeds. So the data which obtained from this study can be important for characterization of them. These preliminary results can be potentially utilized in improvement programmes of Turkish silkworm strains in future.

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