

FIBER DEVELOPMENT CHARACTERISTICS OF COTTON MUTANTS: *fuzzless-lintless*, *Ligon lintless* and *fuzzless-lint*

Mehmet KARACA

Akdeniz University, Faculty of Agriculture, Department of Field Crops, Antalya, Turkey
mkaraca@akdeniz.edu.tr

Abstract

The main objective of this research was to compare fiber initiation properties of 3 different cotton mutants; *fuzzless-lintless* (*fl*), recessive mutant which does not produce fuzz and lint fibers, *Ligon lintless* (*Li₁*), a monogenic novel dominant mutant producing only short fibers (short fuzz and lint fibers) and *fuzzless-lint* (*f-142*) which only produces lint fibers. In this research, a Scanning Electron Microscopy (SEM) was used to monitor the changes in the fiber initiations. Studies indicated that the fiber initiation of *Li₁* and *f-142* at 0, 1 and 3 days post anthesis (DPA) were very similar with that of the normal cotton, Texas Marker 1, (TM₁). However, at later stages fiber elongation stopped in the *Li₁* ovules at about 8-10 DPA, whereas it continued in the TM₁ and *f-142* ovules. The *fuzzless-lintless* ovules failed to produce both fuzz and lint fibers. The use of *fuzzless-lint*, *fuzzless-lintless* and *Li₁* mutants clearly indicated that fuzz fibers and lint fibers initiate at the same time but they are controlled by different genes. Present study also suggested that *Li₁* gene is active during the latter stages of fiber development, probably during the later elongation phase.

Keywords: fiber, cotton, SEM, fuzz and lint fibers

Pamuk Lif Mutantlarının Gelişim Özellikleri: *lintersiz-lintsiz*, *Ligon lintsiz* ve *lintersiz-lint*

Özet

Bu çalışmanın ana amacı üç farklı mutant pamuğun; linter (hav) ve lint üretmeyen resesif mutant *lintersiz-lintsiz* (*fl*), kısa lifler veren ve tek bir gen tarafından kontrol edilen, seçkin bir dominant *Ligon lintless* (*Li₁*) ve yalnızca lint üreten *lintersiz fuzzless-lint* (*f-142*) lif oluşumunu başlatma özelliklerinin karşılaştırılmasıdır. Çalışmada bir Tarama Elektron Mikroskop (TEM) kullanılarak lif oluşumundaki değişiklikler izlenmiştir. Sonuçlar *Li₁* ve *f-142*'nin çiçeklenmeden 0, 1 ve 3 gün sonraki lif gelişiminin normal Teksas Markır (TM-1) pamuğuna çok benzediğini göstermiştir. Ancak ileriki dönemlerde lif uzaması mutant ovüllerinde çiçeklenmeden 8-10 gün sonra dururken normal TM₁ ve mutant *fuzzless-lint*'de devam etmiştir. *Lintersiz-lintsiz* mutant ovüllerinde ise linter yada lint gelişmemiştir. *Lintersiz-lintsiz*, *lintersiz-lint* ve *Li₁* mutantlarının kullanılması açıkça göstermiştir ki linter ve lint lifleri aynı zamanda gelişmeye başlamakta ancak bunlar farklı genler tarafından kontrol edilmektedir. Bu çalışma yine mutant *Li₁*, geninin ileriki dönemlerinde, tahminen ileri lif uzama döneminde aktif olduğunu göstermiştir.

Anahtar kelimeler: Lif, Pamuk, TEM, Linter ve Lint Lifleri

1. Introduction

Cotton (*Gossypium* spp.) is the world's most important textile fiber crops and the second most important oil seed source (Anonymous, 1994, 1984). Competition from synthetic fibers and challenges to improve fiber quality are the two major economic forces driving the current global cotton market. However, records indicated that improvement in fiber yield and qualities using agronomic techniques have reached recently at a plateau stage.

The high value per hectare of cotton, and the demands in the global market for increased uniformity, strength, length, and high quality of fibers clearly justify the

importance of new and innovative approaches including molecular techniques toward evaluating and understanding controlling mechanisms of fiber qualities (Orford and Timmis, 1998; Karaca *et al.*, 2002; Saha *et al.*, 2003; Ji *et al.*, 2003; Zhu *et al.*, 2003; Sakhanokho *et al.*, 2004).

Cotton fibers are single-celled seed hairs developed from the outmost layer of ovules. After anthesis, not all but some of the modified fiber cells begin to expand and undergo rapid elongation (usually 1000 to 3000 fold in length), followed by overlapping stages of secondary cell wall synthesis and finally dehydration (Basra and Malik, 1984; Applequist *et al.*, 2001; Kim

and Triplett, 2001).

Fiber quality is of paramount importance for textile industry, and quality characters are mostly controlled by genetic factors, genes, and environment (Kim and Triplett, 2001; Paterson *et al.*, 2003). Several spontaneous mutants defective in fiber development were found during cotton breeding; however, none of the mutated genes has been cloned, partly because of the large genome size of cotton and limited information available at molecular level (Zhu *et al.*, 2003).

One of the major limitations in the genetic improvement of cotton fiber is the paucity of information at the molecular level about genes controlling fiber development. This is specifically surprising since a large number of mutants are available specific to the fiber traits. The identification and characterization of the genes that affect the phenotypic expression in fiber will provide valuable tool in the genetic improvement of cotton fiber as well as yield.

Understanding of fiber development is the most important step to understand and improve the fiber properties in cotton. A mutant specific to fiber development is very important for plant breeders and molecular geneticists. Griffee and Ligon first discovered the *Ligon Lintless* (Li_1) mutation in 1929 and Kohel (1972a) first documented its genetic characteristics. Li_1 is a simply inherited, dominant mutant characterized by short fiber (~6 mm long) and distorted plant growth in the leaves, stems and flower (Kohel, 1972a). The Li_1 locus causes several pleiotropic effects suggesting that it is a regulatory gene (Karaca *et al.*, 2002). The contorted leaves are first visible in the cotyledons and the mutant phenotype persists throughout the remainder of the plant growth and development (Kohel, 1972a).

A developing fiber passes through four discrete but overlapping phases, namely, initiation, elongation (primary cell wall deposition), secondary cell wall synthesis, and maturation at harvest. It is widely accepted that primordial fiber cells (epidermal cells on the surface of the cotton ovules) that initiate elongation on the day of anthesis (DPA) or 1 day before anthesis to

4-6 DPA are destined to become lint fibers (Basra and Malik, 1984). Fuzz fibers are initiated at 4 to 10 DPA but never obtain lengths greater than 10 mm (Basra and Saha, 1999). Although Li_1 has been studied intensely there is limited research comparing Li_1 with normal cotton and other fiber mutants at specific development levels using SEM analysis.

In the present study, in addition to the Li_1 mutant, two other fiber mutants; *fuzzless-lintless* and *fuzzless-lint* were utilized. They are specifically important considering the fact that fiber price in textile industries is dependent on the content of short (fuzz) versus long (lint) fibers in a variety. Genetic information about *fuzzless-lintless* and *fuzzless-lint* is limited although they have been known for a decade. Preliminary studies indicated that both *fuzzless-lintless* and *fuzzless-lint* are controlled by 4 recessive alleles (Field trails at Akdeniz University, 2003). All four recessiveness results in *fuzzless-lintless* phenotypes, *fuzzless-lint* phenotype is, on the other hand, produced when there is one dominant allele in out of the four alleles: Further analysis is, therefore, required to confirm this preliminary results.

A study conducted to determine fiber properties is very critical for understanding the cotton fiber development. Therefore, this study was undertaken to characterize cotton fiber initiation and elongation using 3 different fiber mutants and one normal cotton, TM₁ which is a standard cotton line for comparative studies.

2. Materials and Methods

Cotton (*Gossypium hirsutum* L. cv Xu-142) (Zhu *et al.*, 2003) plants were first selfed and in the progeny, two different phenotypes were selected, *fuzzless-lintless* and *fuzzless-lint* (Zhang and Pan, 1991). *Ligon Lintless*, Li_1 mutant of cotton and these two mutants were grown in a greenhouse at the fields of Western Akdeniz Agricultural Research Institute, Antalya.. Development of the cotton bolls from each mutant along with Texas Marker 1 (TM₁) were monitored and tagged on the day of

anthesis which was called 0 days post anthesis (0 DPA) and at various developing stages 0, 1, 3 and tagged as 0, 1, 3 DPA.

Scanning Electron Microscopy analysis (SEM) were used to compare the fiber initiation among a normal cotton, Texas Marker 1 (TM₁) which produced both short (fuzz) and long fibers (lint) and a mutant cotton *Ligon Lintless*, *Li*₁ which only produced short fibers. Ovules (0, 1 and 3 DPA) were collected from TM₁, *Li*₁, *fuzzless-lintless* and *fuzzless-lint* flowers and fixed in half strength Karnovsky's fixative, pH 7.2, overnight at 4°C.

Specimens (collected ovules) were rinsed, post fixed in 2% osmium tetroxide (OsO₄), dehydrated and critical point dried. Samples were then mounted on aluminum stubs, coated with gold and imaged in a LEO S 360 SEM using accelerating voltage of 15 kV. Images were recorded on Polaroid Type 55 P/N films. The pictures of ovules at later stages were also taken using a digital camera for analysis.

3. Results and Discussion

With the exception of *fuzzless-lintless* all the outer epidermal layer of ovules started to produce fiber at the day of anthesis (0 DPA). Both types of fiber initiations (fuzz and lint fibers) appeared first at the chalaza part of the ovule (Figure 1) and continued progressively towards the microphyle where the first fibers initials were observed at least 24 to 48 hours after anthesis (Basra and Malik, 1984).

The mutant *fuzzless-lintless* did not produce any fibers at 0 DPA, 1 DPA, 3 DPA and at later stages. There were no differences among the mutants *Li*₁ and *fuzzless-lint* and normal TM₁ for fiber initiations at 0 DPA, 1 DPA and 3 DPA. However, there were two kinds of fibers; fuzz-fibers and lint-fibers in TM₁ ovules. Fuzz and lint fibers looked morphologically different as they can be seen in Figure 2. The *fuzzless-lint* ovules, on the other hand, produced only one kind of fibers, lint-fibers, TM₁ and *Li*₁ produced two kinds of fibers. The fiber of *Li*₁ both looked distorted. Lint and fuzz fibers of *Li*₁ could elongate up to 6

cm long. This observation indicated that there was no difference in the fiber initiation at earlier development stages between the normal TM₁ and the *Li*₁ mutant. However, at 3 DPA, *Li*₁ fibers appeared to be more contorted than that of the TM₁. Although the *Li*₁ locus caused several pleiotropic effects including contorted leaves, cotyledons,

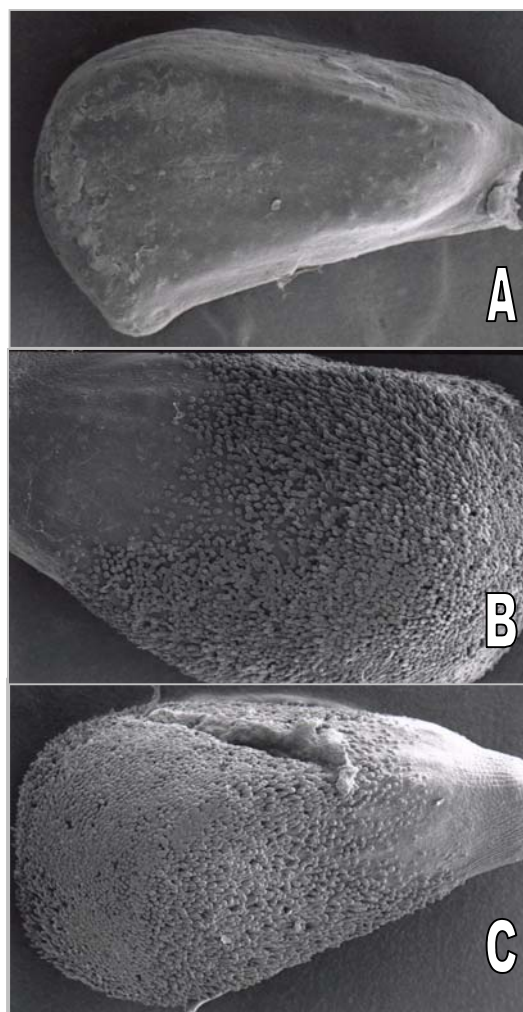


Figure 1. Pictures A, B, C are 1 DPA ovule fibers of mutants, namely, *fuzzless-lintless*, *Ligon lintless* and normal standard cotton line, TM₁, respectively. Note that both types of fiber initiations (fuzz and lint fibers) appeared first at the chalaza part of the ovules as expected. Ovules of the *fuzzless-lint* mutant (not shown) also appeared as the same as that of the *Ligon lintless*.

stems and flowers, no information on contorted fiber was previously reported (Kohel, 1972b). This was a strong indication that Li_1 gene is a regulatory gene, capable of affecting several traits.

Analysis of ovules at later stages (up to 19 DPA) revealed that at the 8-10 DPA fiber elongation on the ovules of Li_1 almost stopped but the fiber elongation of the normal ovules of TM_1 continued as it can be seen in Figure 2. At harvesting stage TM_1 produced both long lint and short fuzz fibers, whereas Li_1 only produced very short fibers. These observations suggested that fiber initiation in the outer epidermal cells of Li_1 ovules were alike normal plant's fiber initiating at the same time perhaps under similar genetic control mechanism (s).

Results of the present study indicated that there were differences between TM_1 and Li_1 at fiber initiation indicating that the

genetic control of fiber initiations were same between the two lines. These results suggested that perhaps both fuzz fibers and lint fibers initiated under the same genetic control mechanism, however, it is the elongation factor(s) that ultimately make(s) the differences (Basra and Malik, 1984).

The plants of *fuzzless-lintless* mutant, which were isolated from cotton cv Xu-142, had seeds without any kinds of fibers (fuzz or lint fibers) and the plants of this mutation did not show any other phenotypic changes unlike that of the Li_1 mutant plants (Yu *et al.*, 2000; Zhang and Pan, 1991; Karaca *et al.*, 2002; Zhu *et al.*, 2003).

4. Conclusions

In conclusion, scanning electron microscopy revealed that fiber initials were

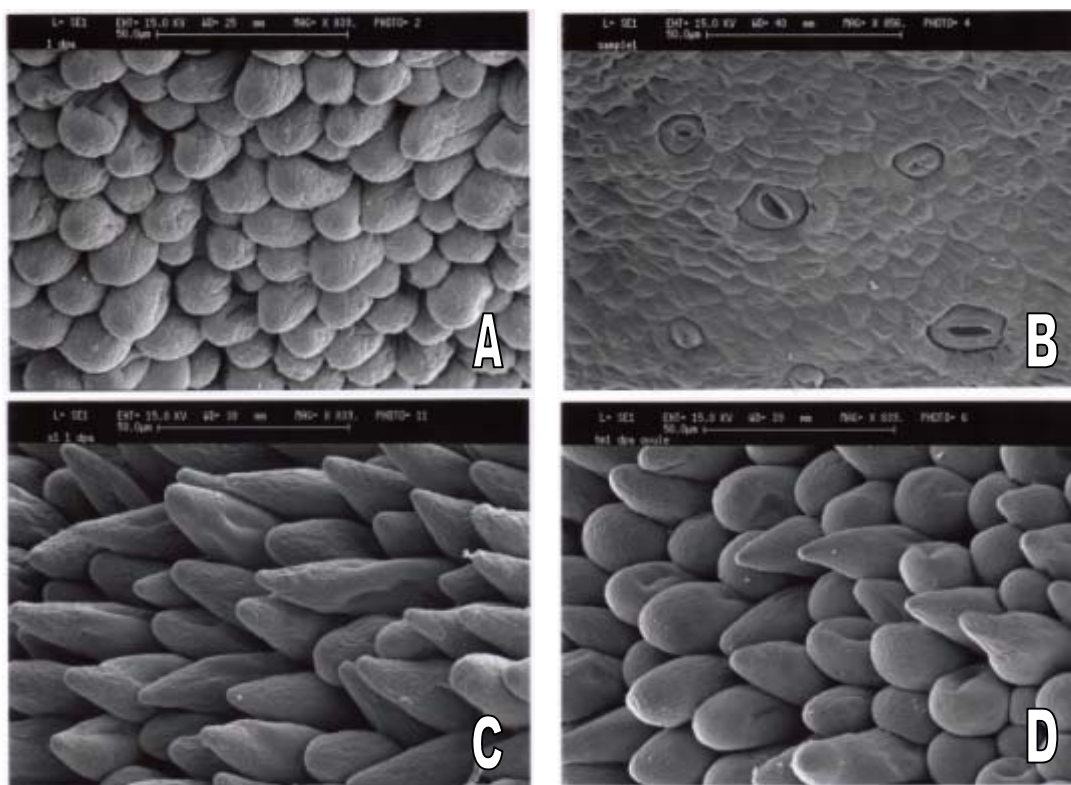


Figure 2. Pictures A, B, C and D are 1 days post anthesis (DPA) stage of *Ligon Lintless* (Li_1), *fuzzless-lint*, *fuzzless-lintless* and Texas Marker 1 (TM_1) ovules, respectively. The *fuzzless-lintless* (*fl*), recessive mutant which does not produce fuzz and lint fibers, *Ligon lintless* (Li_1), a monogenic novel dominant mutant producing only short fibers and *fuzzless-lint* (*f-142*) which only produces lint fibers. The pictures above were from a Scanning Electron Microscopy (SEM).

virtually absent from *fuzzless-lintless* ovules. This clearly indicated that the gene causing the mutation functions in an early stage of fiber cell differentiation.

Comparative scanning electron microscopy studies of fiber development in a normal TM₁ genotype and the near-isogenic *Li*₁ mutant at 0, 1 and 3 days post anthesis (DPA) revealed little differences between the two during early stages of development, suggesting that *Li*₁ gene expression occurs later, probably during the elongation phase. Furthermore, the use of lintless-lint mutant indicated that fuzz and lint fibers start initiation at the same time in cotton. Overall, results of the present study indicated that the *fuzzless-lintless* mutant along with the *Ligon lintless* and *fuzzless-lint* mutants could provide an ideal system for comparative analysis of cotton fiber development. These mutants will be valuable tools for further studies in cotton molecular studies.

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