Scanning Electron Microscope of Fiber Initiation and Effect of Organic Matter on its Development in Egyptian Long and Extra-Long Staple Cotton Cultivars (*G. barbadense*)

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ABSTRACT

Ovules removed from Egyptian cotton cultivars Giza 45 (Extra-long staple) and Giza 89 (long staple) were examined with a scanning electron microscope at different developmental stages. One day pre-anthesis, the anatropous ovule had many anomocytic stomata, especially on the chalazal end. At anthesis, fibers first appeared on the crest of the funiculus and then around the lateral circumference of the ovule. Fiber initials were delayed for a few hours at the chalazal cap and for 3 or 4 days at the extreme end of the micropylar region. The morphological differentiation of fibers began when an epidermal cell rounded up and protruded. After a brief period of diametric expansion, fiber initials began elongation toward the micropylar end. Two or three days post-anthesis, fiber initials were segregated into small groups and began spiral growth. No differences were observed between long and extra long staple cultivars used at the previous stages. Fiber density was about 3.3 fibers per mm^2 and the ratio of fiber initials to total epidermal cells was 1:3.7 at anthesis. Nutrient flow from leaves to flowers through phloem was blocked to study the effect of organic matter supply on both ovule growth and fiber development. Differences were noted at two stages of ovule growth, pre-anthesis and post-anthesis. No obvious differences were observed in ovules at anthesis. Pre-anthesis ovules showed no fiber initials and a smaller size of both stomata and epithelial cells. Post-anthesis ovules were characterized by clumps of fibers with wider and blunter ends.

Introduction

The great economic importance of Egyptian cotton (*Gossypium barbadense*) and other cultivated *Gossypium* species has stimulated extensive study of fiber and seed development. The Egyptian cotton culture and industry started to develop in Egypt in the 1820s. Recent advancements in the fields of molecular biology and genetic engineering make it possible to adapt this technology for Egyptian cotton fiber quality and yield improvement and also to improve cotton high quality cultivars to maintain fiber quantity and yield when cultivated in newly reclaimed areas in Egypt.

The classic book by Balls (1915) and later work by Farr (1931; 1933), Baranov and Maltzef (1937), Anderson and Kerr (1938) and Lang (1938) laid the basic foundation for current knowledge of the cotton fiber which is the purest form of naturally occurring cellulose (94 % to 98 %). Cellulose is the most abundant biopolymer in the world, being present in all higher plants. The developing cotton fiber has attributes that recommend it as an experimental system of choice for investigation of physiological and biochemical changes accompanying plant cell elongation and / or maturation (Basra and Malik, 1984; Delmer, 1987).

It was established that cotton fibers are single cell protrusions from the epidermal layer of the ovule. Cotton fiber development is characterized by four overlapping distinct developmental stages, initiation, elongation, secondary cell wall thickening and maturation (Basra *et al.* 1984; Graves and Stewart, 1988). Fiber initiation starts at anthesis, the cells enter into elongation immediately and continue linear primary growth for about 20 days during which fiber cells elongate to > 2.5 cm (primary wall stage). The primary cell wall has been reported to contain 90% polysaccharides (50% cellulose along with pectinaceous and waxy materials) and 10% proteins (Meinert and Delmer, 1977; Zeronian, 1985).

When the elongation period comes to an end secondary deposition of cellulose begins. The secondary wall consists of nearly pure cellulose arranged in spiral microfibrils and in homocentric layers. One layer is formed each day (Verschraege, 1989). Cellulose comprises > 90% of the dry weight of a mature cotton fiber (Delmer *et al.*, 1992).

Our knowledge of cotton ovule development has been extended by more recent studies of ovule morphology and embryology (Jensen 1968; Joshi W. and Jori, 1967; Pondir, 1972) and transmission electron microscope studies of fiber development have been reported (Berlin and Ramsey, 1970; Ramsey, 1972). Beasley (1975) presented a general description of the developmental morphology of cotton flowers and seeds with scanning electron microscope (SEM). Also using SEM, Stewart (1974) presented abstracts and

descriptions for cotton ovule (*Gossypium hirsutum*), fiber initiation and development at different stages. This paper presents a detailed account of fiber initiation development on the ovule surface of the Egyptian cotton (*Gossypium barbadense)* at different fiber initial developmental stages and the effect of nutrient flow on fiber initiation as analyzed with the SEM.

Material and Methods

Plant material

Seeds of *Gossypium barbadense* (Giza 45 and Giza 81) were grown in a greenhouse in 25 cm pots containing a mixture of soil, sand and peat moss in a ratio of 1:1:1 and watered once a day.

Ovule preparation for SEM

Ovules were collected from ovaries at 3 different stages, Pre anthesis, Anthesis and Post-anthesis. The ovules were preserved in glutaraldehyde for six hours, after which they were transferred to a buffer solution containing 40 % formaldehyde, 25% glutaraldehyde, 0.07 M $NaH_2PO_4.H_2O$ and 0.006 M NaCl , pH 7.2-7.4 and osmolarity 176. Ovules were dehydrated in different concentration of ethanol starting from 30% and rising to 100 % at intervals of 5 minutes each and then dried in a critical point dryer. The dried ovules were then attached to support stubs using carbon or silver paste and coated with gold to ensure the electrical conductivity of the ovule surface. Ovules were examined and photographed.

Nutrient flow control from lateral floral buds

The leaves supplying nutrients to a number of lateral floral buds were detached. Ovaries were collected at the same ages and stages as the normally developed ovaries. Ovules were collected, examined and photographed under SEM.

Results and Discussion

Ovule morphology and shape of fiber initials

The anatropous nature of the cotton ovule was reported by Balls (1915), Baranov and Maltzev (1937) and Joshi *et al.* (1967). However, the depth of field afforded by the SEM permits a more comprehensive view of intact cotton morphology than was obtainable by conventional microtechniques. Figure 1A illustrates the ovule external morphology with the flattened chalazal end and the narrow micropylar end. The presence of stomata on cotton ovules was noted and discussed by Ayyangar (1948) and Joshi *et al.* (1967). According to Stewart (1974), stomata are anomocytic in type (with two guard cells but no subsidiary cells) and their ontogeny is aperigenous. They begin to develop at least one week before anthesis. However, in the case of Egyptian cotton (*Gossypium barbadense)* ovules, anomocytic stomata were not apparent earlier (6 days before anthesis). They were only observed 1 or 2 days pre-anthesis, (Figure 1C) and subsequently differentiated over the entire surface. Many more stomata then begin to form on the chalazal end at anthesis, (Figure 1D). Before initiation, the epidermal cell surfaces are rectangular to irregular but when fiber initial development begins, the differentiated cells become rounded and enlarge (Figure 1E). Both long and extra-long cultivars showed the same pattern of fiber initial development. Figure 1F illustrates a one-day pre-anthesis ovule showing anomocytic stomata with aperigenous development of guard mother cells.

Fiber initials development

Fiber initials first appeared one or two days pre-anthesis at the crest of funiculus and then around the lateral circumference of the ovule (Figure 2A). At anthesis, fiber initials gradually developed from the chalazal end towards the micropylar end (Figure 2B). Evidently, fiber initiation occurs last in the micropylar region. Fiber initiation at the apex of the chalazal end does not occur until a few hours after initials appear on the side of the ovule. The reason for the delay of initiation on the chalazal cap is not known but it may be associated with the extensive differentiation of stomata that occurs on the chalazal cap (Figure 2C). Growth is more rapid on the chalazal side of the cell and elongation is more or less tangential to the ovule in the direction of the micropyle (Figure 2D). Because fiber initiation is progressively delayed in cells nearer the micropyle, initials in various stages of development are found in this area for a number of days after anthesis when fiber initials became diametrically expanded above the epidermis and begin to extend in the direction of the micropyle (Figure 3A). Figure 3B illustrates fiber initials in all stages of development at the micropylar end of an ovule three to four days post-anthesis. During the second day after anthesis, fiber elongation becomes more evident and a distinct area from which the directional growth radiated, developes at the crest of the funiculus (Figure 3C). This is the same area where fiber initials first appeared on the ovule. According to Stewart (1974) fiber initiation at the apex of the chalazal end is delayed until a few hours after initials appear on the sides of the ovule. However, in the case of *Gossypium barbadense*, fiber initials remain scarce until 2-3 days after anthesis (Figure 3D). Thus far, the fiber initials have developed independently with blunt ends (Figure 3E) but as their length increases, they begin to adhere to adjacent fibers and with continued growth, become spiral with tapered ends (Figure 3F).

Effect of nutrients on ovule growth and fiber initials development

Upward transport occurs almost exclusively in the xylem. From autoradiographic investigations of the phloem after administration of labelled materials and by the aphid technique, it was possible to determine the nature of the substances transported in the phloem. Quantitatively, carbohydrates predominate, the most important being sucrose. There are also small amounts

of oligosaccharides such as raffinose, stachyose and verbascose. The rest of the sieve tube sap contains amino acids, amides, nucleotides, nucleic acids, phytohormones, the controversial flowering hormone and inorganic ions in addition to the phosphates of various hexoses. Cellulose, the main constituent of fibers, is highly affected by blocking the flow of nutrients (mainly glucose) in the phloem. Poor nutrition brought about by the removal of leaves supplying the nutrients, caused decreased thickening of the secondary cell wall. The most significant impact on fiber quality by genetic and environmental factors occurs during fiber elongation. The influence of these factors on the rate and duration of fiber elongation directly affects fiber properties such as length, fineness, uniformity and to some extent fiber strength. Fiber length is highly controlled by the genetic code passed along by the plant parents. But length also can be influenced by the environment. Stress during this period, especially a moisture shortage, may cause fibers to be shorter than normal.

A cotton fiber is like a hollow tube. The amount and pattern of cellulose growth determines its strength, fineness and maturity. Fiber quality traits are controlled by the genetic makeup of a cultivar, influenced by environment and management factors. Unfavourable growing conditions during the cellulose "filling" period may result in weak, immature fibers with low micronaire. This was observed in pre-anthesis ovules that showed no fiber initials compared to normally developed ovules (Figures 4A and 4B) as well as a decrease in size of stomatal and epidermal cells (Figure 4C and 4D). However, marked differences in ovules at anthesis were not noted.

The normal pattern of fiber development in the case of post-anthesis ovules differed extensively, the fibers tending to clump together forming bundles (Figure 4E and 4F). Obviously, any decrease in the supply of nutrients, especially carbohydrates, markedly affects fiber cellulose, thereby weakening resulting fibers.

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Figure 1. Ovule morphology. (A), anatropous ovule of *Gossypium barbadense* before fiber initiation (100x). (B), ovule showing fiber initials (50x). (C), chalazal end of ovule 1-2 days before anthesis showing stomata (350x). (D), chalazal end of ovule at anthesis showing increased number of stomata (arrows) (350x). (F), one day pre-anthesis ovule showing anomocytic stomata with aperigenous development of guard mother cells (500x).

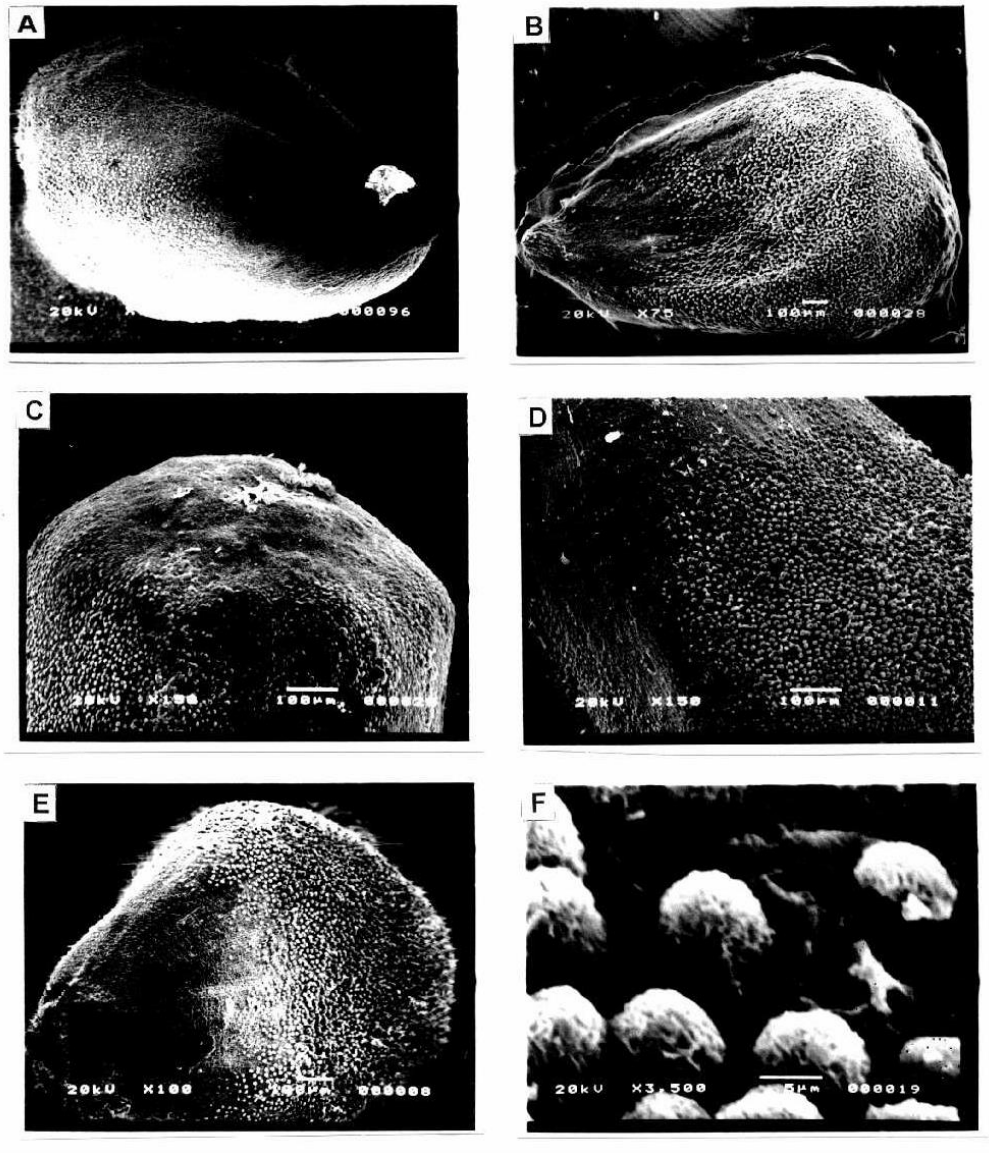

Figure 2 . Fiber initiation. (A), one day pre-anthesis ovule: fibers begin to initiate at the chalazal end and then around the lateral circumference (100x). (B), ovule at anthesis showing fibers initiated in all areas except the micropylar end (75x). (C), chalazal end showing delayed fiber initiation at the tip, note the numerous stomata (arrows) (150x). (D), Fiber initials as they appear on the sides of the ovule at anthesis (150x). (E), ovule obtained 2 days after anthesis showing fiber initials in all areas except the micropyle (100x). (F), fiber initials 1-2 days after anthesis, elongation is towards the micropyle (350x).

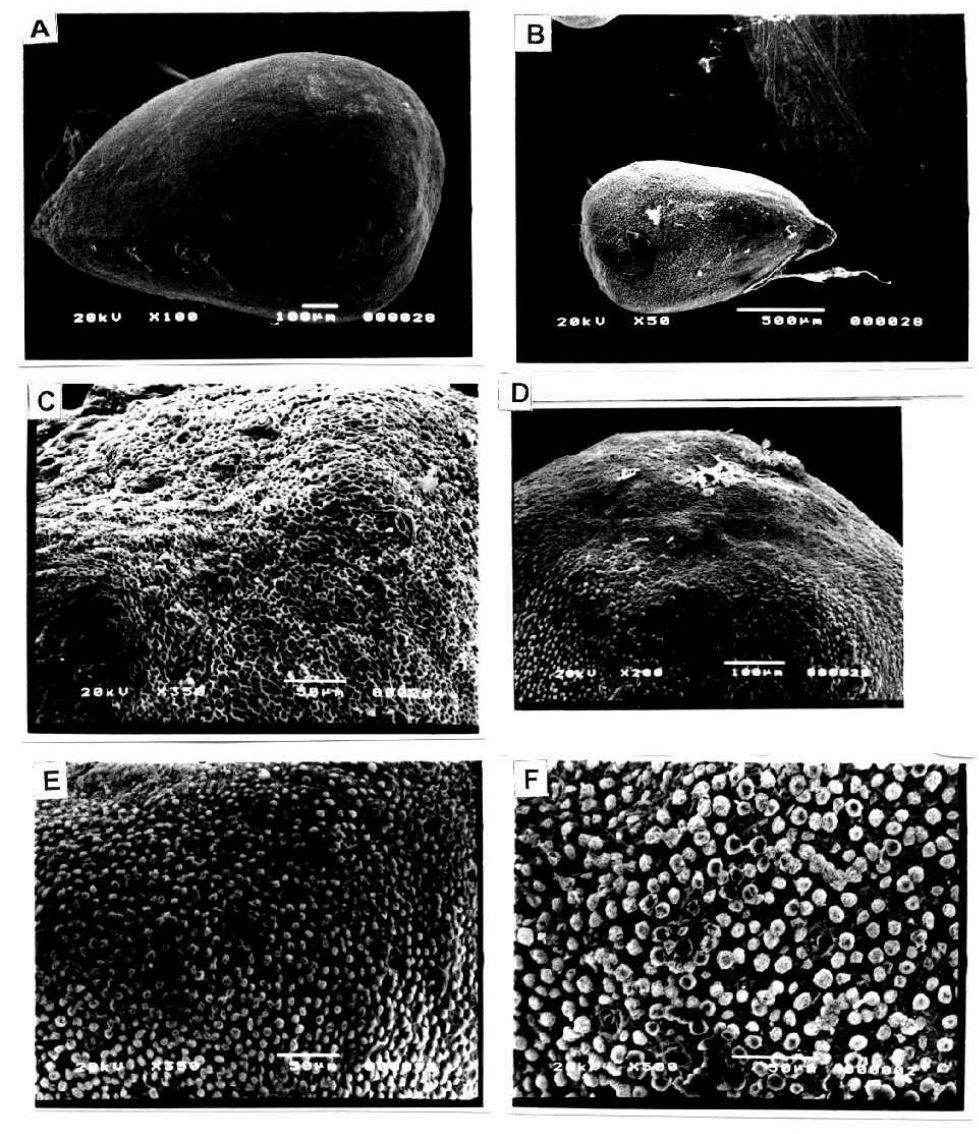

Figure 3. Fiber expansion and orientation. (A), two day post-anthesis ovule, except in the micropylar region, fiber initials are expanded showing a slight inclination towards the micropyle (50x). (B), fiber initials in all stages of development at the micropylar end of an ovule 3-4 days after anthesis (200x). (C), two day post-anthesis ovule showing the crest of the funiculus from which directional growth radiates (100x). (D). three days post-antheis ovule showing delayed fiber development at the chalazal cap (200x). (E), fibers developing independently 3 days after anthesis, note the relative blunt ends (500x). (F), six day post-anthesis ovule showing fibers elongating in spiral manner with tapering tips (350x).

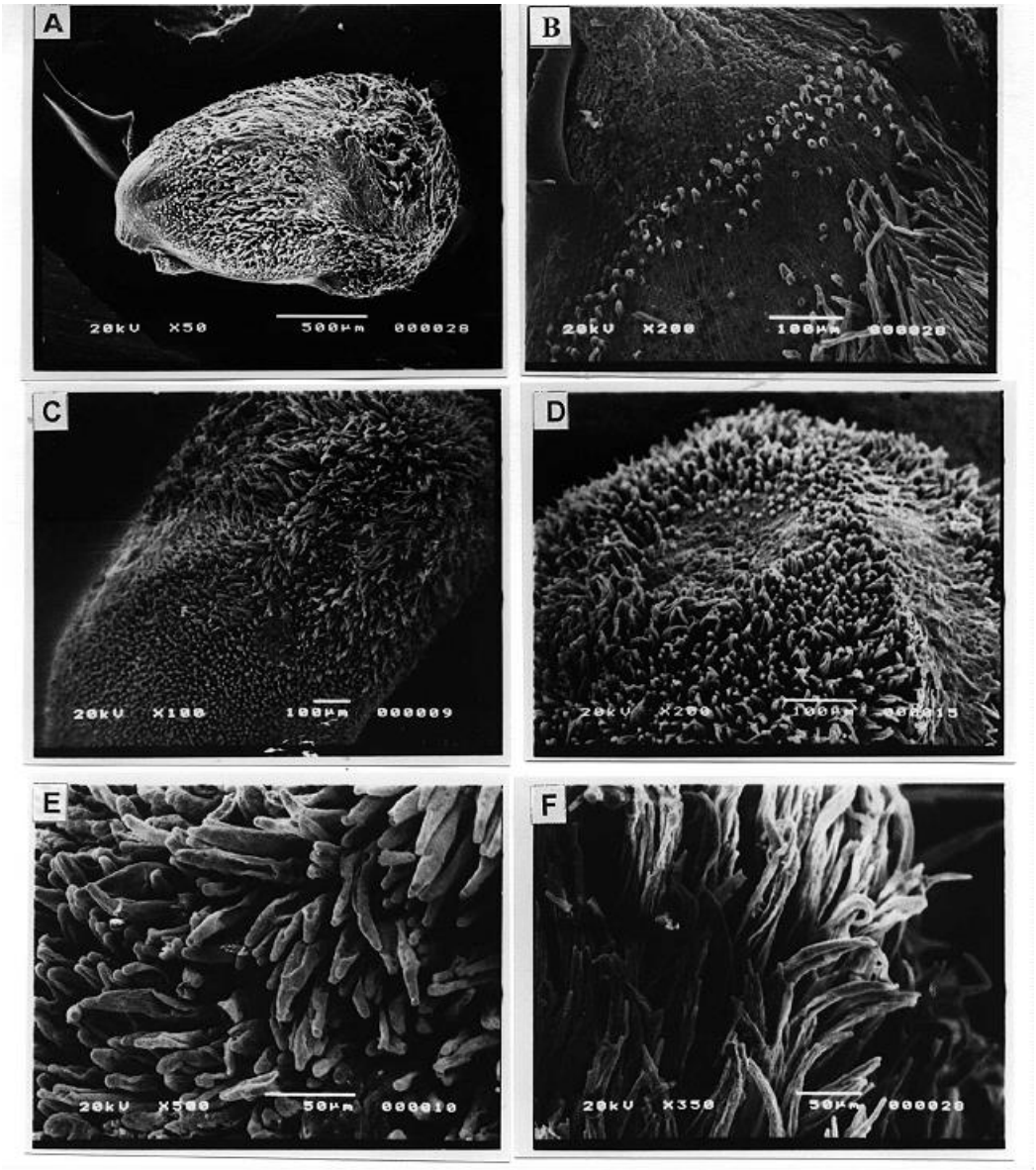

Figure 4 . Effect of nutrient flow on ovule growth and fiber initiation. (A), normally developed ovule 1-2 days pre-anthesis showing fiber initials at the chalazal end and around the lateral circumference (100x). (B), ovule 1-2 days pre-anthesis deprived of nutrients, showing no fiber initial (100x). (C), chalazal end of normal ovule 1-2 days pre-anthesis showing stomata and epidermal cells (350x). (D), chalazal end of an ovule 1-2 days pre-anthesis showing a smaller size of both stomata and epidermal cells (350x). (E), two day post-anthesis ovule showing elongated fibers with relatively blunt ends (350x). (F), two day post-anthesis ovule deprived of nutrients showing narrower fibers clumping together in bundles (350x).