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# Microscopic Analysis of Cotton Fiber Under Drought and Well Watered Conditions at Difference Developmental Stages

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## ABSTRACT

By using convenient optical microscopy technique cotton fiber convolution parameters of draught and well-watered plants measured. It is shown that content of convoluted fibers for irrigated cotton significantly more than of drought cotton. Correlation between quality parameters (convolution characteristics, HVI (High Volume Instrument) parameters) two variety *Gossypium barbadense* and *Gossypium hirsutum* under draught and well-watered conditions observed.

It has been shown that during different development stages dissolution of primary cell wall take place in cuprum-ammonium solvents more slowly than secondary cell wall. It is connected with structural transformation of cotton cell wall cellulose layers depend on stage its development.

## INTRODUCTION

After ripening of cotton boll all cotton fibers inside of boll becomes dry and collapses into twisted ribbonlike formations. Convolutions in a cotton fiber are developed during the collapse of the tubular fiber as it dries. A convolution is defined as a border between two bobbinlike segments. Usually, twisting of cotton fiber after ripening characterized by density of convolutions, convolution degree and distance between neighboring convolutions [1-3]. There have been several studies on characterizing convolutions of cotton fibers and relating convolution characteristics to fiber quality. According to [1] authors calculated density function of convolutions by using optical microscope. Authors [2-4] found correlation between strength of bundle of cotton fibers and convolution angle and fibrilar orientation. Thibodeaux and Evans [5] used image analysis to measure cotton fiber maturity by measuring convolution characteristics.

Cotton fiber cell wall consist on the cuticle, the primary wall and the secondary wall. The first layer of secondary cell wall named winding layer [6]. The secondary wall mostly consists of pure cellulose. During cotton fiber grow and cellulose deposition on secondary cell wall many reversals takes place [7] and in result of twisting of cotton fibers we can observe convoluted structure of dried fiber.

So, convolutions appears mainly at secondary cell wall deposition stage. But this stage overlap elongation stage too. In this connection it is important to examine convolutions at difference developmental stages. In first it is shown that approximately one of four epidermal cells on each ovule differentiates into a single-cell trichome [8,9] and fibre initials appear on the day of anthesis as spherical protrusions on the ovular surface [10]. Differentiation begins near the chalazal end of the ovule, progressing later toward the micropylar end [8]. On the first and second days post anthesis (DPA), the spherical fibre initials begin to expand lengthwise, bending and growing toward the micropyle. The elongation of the fibre occurs during the 25-30 days after flowering and the rate of fibre elongation increases [11] and reaching a maximum around 15 DPA [12] and at that the length of the elongation period is highly dependent upon environment conditions [13]. Regard to secondary cell wall deposition the transmission electron microscopy have demonstrated that the secondary cell-wall thickening phase overlaps with the elongation phase by up to 10 days [13].

Cotton fibers exhibit three overlapping stages of development: initiation, elongation, and secondary wall thickening, followed by a maturation phase that ends with fiber death. Not all cells of the epidermis develop into fibers, with data ranging between 10% and 25% of epidermal cells becoming fibers. The factors that regulate the number of fibers produced per ovule remain unclear.

Fiber initiation coincides with flower anthesis. Developmental cues for fiber production are present over a long period of time and thus there may be a very long "window of opportunity" over which fiber development may be affected. Genetic techniques have expanded our understanding of the events leading to fiber development and have identified a pre-anthesis stage where epidermal cells undergo changes in gene and protein expression in preparation for fiber expansion. Epidermal cells on pre-anthesis ovules are "primed" for fiber development, awaiting hormone signals associated with anthesis. Several studies indicate that fiber initiation is influenced by physiological changes in the plant. During secondary cell wall deposition suggestive cellulose layer deposition occurs of difference spiral angle and reversal points. After drying namely lamellar structure leads to convolutions of cotton fibers. Therefore to reveal inner lamellar structure and difference cell wall layers need to use cellulose solvents for suggestive dissolution this layers and its visualization. Due to complex and multi-scale structure of cotton cell wall, the mechanisms of swelling and dissolution of difference areas of dried cotton fiber are not homogeneous [14]. According to [15] swelling mostly takes place in transverse direction in certain swelling agent, the radial expansion of the cellulose in the secondary wall causes the primary wall to burst. As the expanding swollen cellulose pushes its way through these tears in the primary wall, the latter rolls up in such a way as to form collars, rings or spirals which restrict the uniform expansion of the fibre and forms balloons" **(Figure 1)**. All the authors [14] assume that the ballooning phenomenon has structural origins, i.e. linked to morphological variations between the different walls.

In this paper light microscopy image analysis developed to quantify morphological characteristics of convolutions in a single cotton fiber. Cuproammonium solvents has been used to show swelling and dissolution of cotton fibres. Cotton fibres were harvested at difference growth stages 2 weak post anthesis (WPA), 4 WPA and mature fibers.

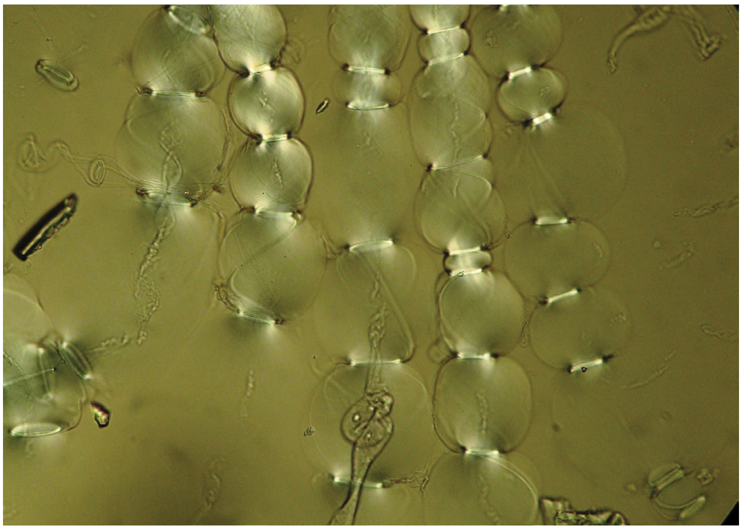

**Figure 1.** Heterogeneous ballooning of mature cotton fiber #60 x20.

# MATERIALS AND METHODS

## Materials

**Cotton production:** Cotton, *Gossypium hirsutum* (G.H.) and *Gossypium barbadence* (G.B.) plants were grown in Israel Laboratory Plant Science Hebrew University at difference irrigation regime. Mature fibers two cotton variety G.B. and G.H. (G.B. F-177 and G.H. H-23 parent genotype, and G.B. 1-1.4, G.H. 3-2.2 of their derivatives) of two irrigation regime (control (normal) irrigation and drought conditions has been prepared. Unopened cotton bolls of same plants were collected on plants at various growth stages (2 week post anthesis (2 WPA), 4WPA and drayed.

**Fiber preparation and convolutions measurement:** The bundle of single fibers was mounted on a microscope slide glass to capture a longitudinal image of the cotton fiber in parallel fashion. Because pulling the cotton fiber may change the shape of convolutions, the ends of fibers were glued to the slide without any tension. Three images were captured from each fiber for convolution measurements-from the bottom, middle and top parts of each fiber. Percentage of convoluted fibers (%) and number of convolutions per mm along fiber in the field of view of microscope scanned across bundle was counted. After the image analysis all samples were sent to HVI analysis of quality parameters of mature cotton fibers.

## Methods

To visualize outside morphological structure of cotton fiber binocular optical microscope "Olympus" CH equipped by ocular micrometer and Axioplan (Zeiss, West Germany) with magnification 5×, 10×, 20×, 40× and 100× was used. Ocular magnification 1,25×, 2,5×, 2× and 1.6×. Image captured by digital photo camera Canon PowerShot G6 (7.1 Mps) and transferred to PC. Mature cotton fibers arranged in parallel on the slide class and covered by slide.

To reveal microstructure of cotton cell wall and to examine cotton wall layer resistivity to cellulose dissolution agent the cuproamonium solution (Sweytserov reagent) has been prepared. Optical image of treated cotton fiber has been captured for difference exposition time after treatment and observed dissolution dynamic of cotton cell wall.

The developmental stages, which are defined by the age of the boll in the number of days post-anthesis (DPA) include primary wall formation, primary-to-secondary wall transition, secondary wall thickening, and maturity. A total of 54 bolls from 12 plants were used. Fiber properties were measured on the middle sections of fibers sampled from the middle section of the most developed ovules or seeds at the specific development stages.

Cotton fibres taken at elongation stage were studied under the optical microscope. To facilitate the isolation of the fibres, which are wet and adhering one to another and only about 100 Fm long, they were dried on a blotting paper and separated in small clusters.

## RESULTS AND DISCUSSION

**Table 1** shows the average content of convoluted mature cotton fibers in micropilar, middle and chalasal part of cotton seed. One of the reasons in evaluating alteration of fiber convolution characteristics is connected with an asymmetric its distribution along seed surface. The examination of individual regions on a single seed revealed the same asymmetric fiber length distributions [15,16]. Although each seed region has an asymmetric fiber length frequency distribution, the mean fiber length is shorter at the micropylar end of a seed than at the chalazal end [17]. It is connected with fact that at anthesis, fibers first appear at the crest of the funiculus. Fiber initials are delayed for a few hours at the chalazal region, and for 3 or 4 days at the micropylar region [8]. Some bolls were harvested 2 and 4 weeks after flowering to assess fiber growth and development. This seeds were removed from bolls opened manually and allowed to air dry. **Table 1** shows that distribution of convolution segments is not homogenous along cotton fibers grown at chalasal, middle and micropilar part of cotton seed **(Figures 2 and 3)**. Content of convoluted mature cotton fibers increase along direction micropile -middle-chalasa. Perhaps cotton fibers on chalasal part are more mature what leads to pronounced convolution degree of cotton fibers in comparison with micropilar end. This distinctions of convolution degree in the micropylar and chalazal regions of the seed could be explained by the duration of the elongation stage of fiber development. Since fibers in the chalazal region begin elongation before those in the micropylar region, one would expect fibers in the chalazal region to be more mature than fibers in the micropylar region.

**Table 1.** Average content of convoluted mature fibers in micropilar, middle and chalasal part of cotton seed (%).

| Variety | Micropile | | Middle | | Chalasa | |
|---|---|---|---|---|---|---|
| | Irrigated | Dryland | Irrigated | Dryland | Irrigated | Dryland |
| GB, F-177 | 46.25 | 30.25 | 55.50 | 36.75 | 57.5 | 35.00 |
| GB, NIL 1-4 | 57.50 | 13.75 | 56.25 | 15.00 | 76.14 | 19.75 |
| GH, Siv'on | 70.00 | 35.00 | 72.50 | 48.75 | 70.00 | 36.00 |
| GH, NIL 3-2 | 57.50 | 31.25 | 70.00 | 37.25 | 67.50 | 38.25 |

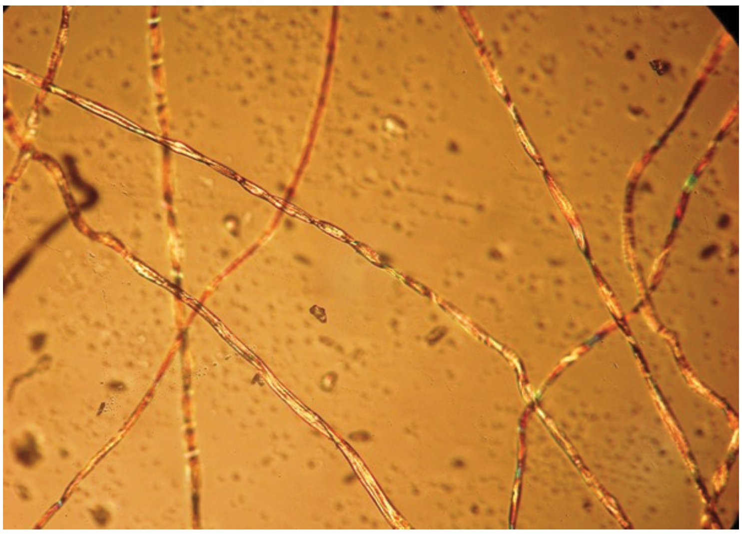

**Figure 2.** Mature cotton fibers 59 G.B.1-1.4 dry. Optical microscopy image.

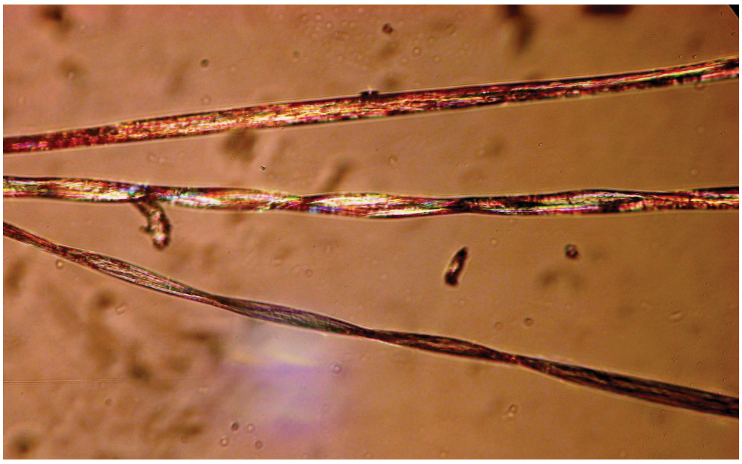

**Figure 3.** Example of difference kind of cotton morphology along length (convoluted and no convoluted single fibers) N15 5.3-2.2 wet.

Electron microscope image of cotton fiber convolution is shown in **Figures 4-6**. Here we can see regular convolutions **(Figure 4)** and reversal **(Figure 5)**. Within the wall, cellulose microfibrils are helically oriented around the fiber wall. In the secondary walls of cotton fibers, microfibrils routinely reverse their gyre. This is a unique occurrence found only in cotton fibers and has been referred to as a reversal point. Reversal points are thought to create weak areas in the secondary wall where fibers will probably break. Reversal frequency increases during fiber development [18].

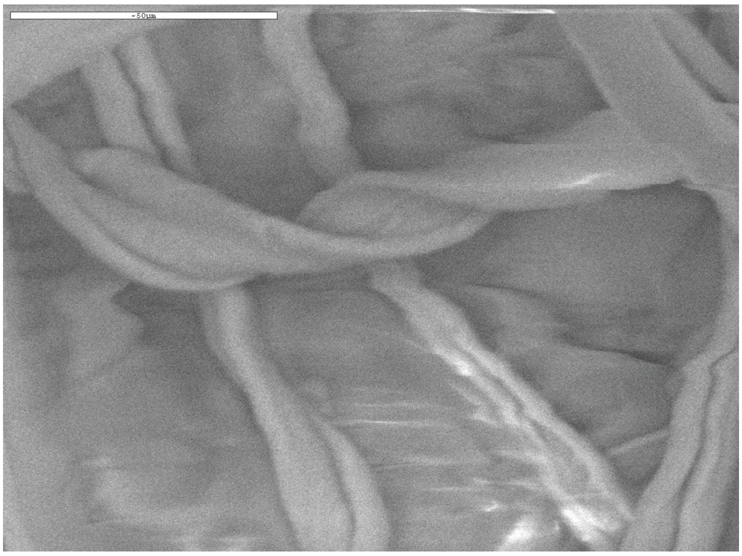

**Figure 4.** Mature cotton fibers 59 G.B.1-1.4 dry. Electron microscopy image.

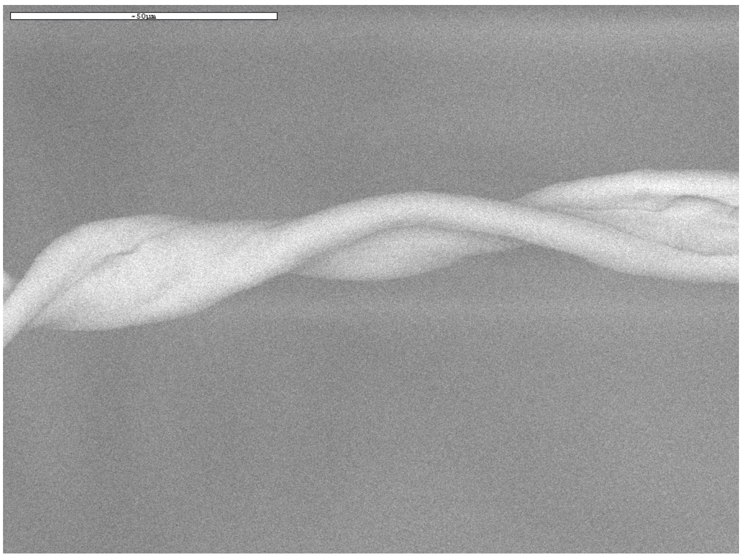

**Figure 5.** Example of reversal point mature fiber. Electron microscopy image. (54 G.H. H-23 wet).

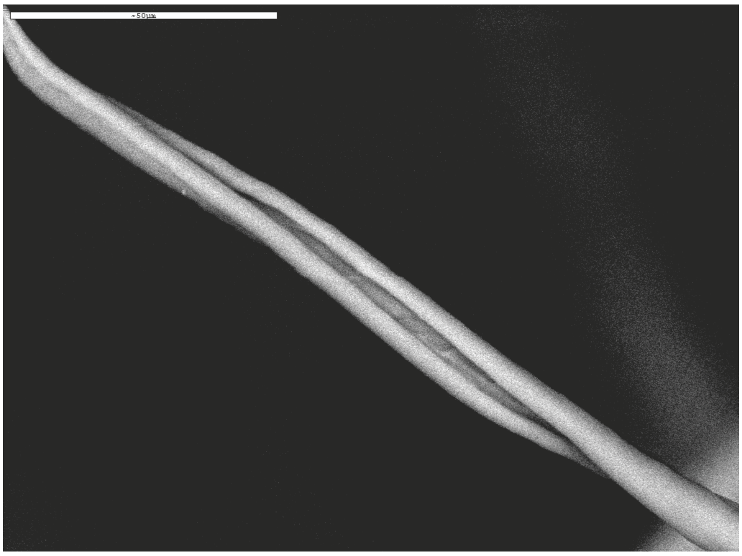

**Figure 6.** Electron microscopy view of banana like shape of dried mature cotton fiber. 25 G.B. F-177 dry.

But any quantity measurements of convolution parameters based on optical microscopy image analysis. Irrigate regime had shown that cotton fibers of well-watered cotton have significantly pronounced convolution degree than fibers in dryland conditions for all measured cotton varieties **(Table 1)**. **Table 2** shows content of convoluted fibers along their length in direction basal-central-distal part scratched in parallel fibers **(Figure 3)**. As we can see here is not any regularity in distribution of convolutions. But here observed predominance of irrigated fibers than dryland one regard content of convoluted areas in the field of view of microscope like **Table 1**.

**Table 2.** Average content of convoluted parts of mature fibers along their length in basal, middle and micropilar parts (%).

| Variety | Distal | | Central | | Basal | |
|---|---|---|---|---|---|---|
| | Irrigated | Dryland | Irrigated | Dryland | Irrigated | Dryland |
| GB, F-177 | 45.00 | 18.57 | 53.75 | 26.22 | 63.75 | 35.00 |
| GB, NIL 1-4 | 37.50 | 27.50 | 56.25 | 15.50 | 58.75 | 12.50 |
| GH, Siv'on | 73.75 | 33.75 | 80.00 | 30.00 | 78.75 | 41.25 |
| GH, NIL 3-2 | 58.75 | 33.75 | 82.50 | 42.50 | 83.75 | 36.25a |

It is found that along cotton fibers average number of convolution per millimeter (N/mm) is not equal for difference varieties and irrigation regimes **(Table 3)**. Accordingly [19] the number of convolution segments per millimeter is connected with length of convolution step (L) as N=1000/L. It is shown, that N increase from dry to irrigated cotton all varieties and N for G.B. is more than for G.H. Especially this trait pronounced for dryland fibers **(Table 3)**. This feature correlate with strength measurements of fibers **(Table 4)** where we can observe similar behavior of fiber strength especially expressed for dryland fibers. Length of step ranged from 106 mkm to 164 mkm for all varieties. **(Table 3)**.

**Table 3.** Average number of convolutions per mm (N/mm) and content of cellulose in mature cotton fibers.

| Variety | N/mm | | Cellulose | |
|---|---|---|---|---|
| | Irrigated | Dryland | Irrigated | Dryland |
| **GB, F-177** | **8.69** | **8.02** | **95.75** | **96.88** |
| **GB, NIL 1-4** | **9.32** | **8.39** | **98.09** | **96.59** |
| **GH, Siv'on** | **6.98** | **6.38** | **95.5** | **97.25** |
| **GH, NIL 3-2** | **8.06** | **6.16** | **96.16** | **97.66** |

**Table 4.** HVI parameters.

| Variety | Elongation | | Fineness | | Strength | |
|---|---|---|---|---|---|---|
| | Irrigated | Dryland | Irrigated | Dryland | Irrigated | Dryland |
| GB, F-177 | 8.68 | 9.38 | 3.68 | 4.20 | 42.03 | 40.05 |
| GB, NIL 1-4 | 10.50 | 10.53 | 3.55 | 4.41 | 39.08 | 40.25 |
| GH, Siv'on | 8.03 | 7.30 | 3.85 | 4.25 | 34.25 | 29.33 |
| GH, NIL 3-2 | 7.25 | 6.70 | 3.35 | 4.05 | 35.90 | 31.48 |

To study developmental stages of cotton boll some convolution parameters of mature and no mature (2 WPA, 4 WPA) cotton fiber has been examined. We can see that content of convoluted fibers for no mature cotton (4 WPA) is smaller **(Table 5)** than for mature fibers **(Tables 1 and 2)**. For dryland cotton 4 WPA fibers number of convoluted fibers close to mature one, but for irrigated cotton 4 WPA fibers number of convoluted fibers significantly lower than mature irrigated one. For irrigated cotton (4 WPA) content

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of convoluted fibers for *G. barbedense* (F-177, NIL 1-4) more than for *G. hirsutum* (Siv'on, NIL 3-2) **(Table 5)**. This trend correlate on the single fiber strength and crystalline structure of two cotton fiber species, *G. hirsutum (Texas Marker 1) and G. barbedense (Pima S7*), at varying developmental stages from 20 days post-anthesis (dpa) to maturity [20]. It was shown that as fibers develop beyond 30 dpa, the single fiber breaking force and tenacity of *G. barbedense* fibers are higher than those of *G. hirsutum* fibers.

**Table 5.** Content of convoluted fibers for non-mature (4 WPA) cotton fibers.

| Variety | 4 WPA | | Seed Avg | | Rel change | |
|---|---|---|---|---|---|---|
| | Irrigated | Dryland | Irrigated | Dryland | Irrigated | Dryland |
| GB, F-177 | 18.63 | 22.50 | 53.08 | 34.00 | 3.10 | 1.58 |
| GB, NIL 1-4 | 34.25 | 14.75 | 66.51 | 16.17 | 1.93 | 1.58 |
| GH, Siv'on | 16.25 | 27.75 | 70.83 | 39.92 | 4.70 | 1.56 |
| GH, NIL 3-2 | 21.25 | 20.00 | 65.00 | 35.58 | 3.58 | 1.84 |

Measured HVI parameters shown increasing of strength and correspond elongation parameters for transition from dryland cotton to irrigated cotton **(Table 4)**. This tendency correlate with behavior of parameters N and L **(Table 3)**. This confirms the observation of Merdith [2] that the mean breaking load of the fibers was higher for the highly convoluted fibers. There are correlation between elongation and strength parameters for *G. barbedense* (F-177, NIL 1-4) and *G. hirsutum* (Siv'on, NIL 3-2) **(Table 4)** too. There are distinct structural differences between the fibers of *Gossypium barbadense* and *Gossypium hirsutum* [21]*.* We can observe it for instance for N **(Table 3)**, elongation and strength parameters **(Table 4)** and percentage of convoluted fibers for mature **(Tables 1 and 2)** and no mature fibers **(Table 5)**. Especially this phenomena pronounced for dryland fibers **(Tables 1-3 and 5)**.

To clarify developmental structural properties of cotton fiber we used other method suggestive dissolution cellulose layers under impact cellulose solvents. Here we used cuproammonium Schweitzer's reagent [22]. It is shown that swelling and dissolution under acting Schweitzer's reagent occurs mainly from inside of cotton fiber when solvent penetrate to inner cannel. In first swelling and ballooning take place and next its dissolution. **Figure 7** shows dynamic of mature cotton fiber ballooning and dissolution after every 5 minutes after starting solvent action. Black line is border of cover class glued to microscope slide with fiber mounted between them. Samples were observed between two glass plates by optical microscopy in transmission mode with a Zeiss microscope equipped with a photo camera Canon. The solvent contained in a pipette was injected by capillarity between the two glass plates for mature and no mature (2WPA, 4WPA) fibers. We can see ballooning near border of slide where flow of solvent inside of cotton fiber lumen is blockaded. After 15-20 min cellulose cell wall dissolute in full **(Figure 7)**. It is shown that for mature fibers mainly take place ballooning before dissolution **(Figure 1)**. But for no mature fibers **(Figure 8)** we can observe heterogeneous swelling before dissolution. Character of dissolution for irrigated and non-irrigated cotton fibers same. Cotton fiber all variety at elongation stage (2WDA) do not show swelling and ballooning what is connected with resistance of primary cell wall to dissolution in solvent **(Figures 9 and 10)** because at this developmental stage fiber have primary cell wall only. These results show that the primary wall of cotton fibres is very resistant to dissolution in solvents. This is fully in agreement with the common explanation which attributes the balloons to the swelling of the secondary wall causing the extension and the bursting of the primary wall. Without secondary wall, there are no balloons. It is shown that S1 layer is very resistant to dissolution for all variety what may be connected with high crystallinity of S1 layer. **Figure 11** shows resistant S1 layers and residues of thirty layer after dissolution secondary cell wall. This result shows that the balloons are indeed linked to the existence of a secondary wall layer under the primary wall. The remaining fragments were solid (most probably very crystalline) rodlike pieces, elongated in the fibre direction **(Figure 11)**. This shows that the weak areas, most probably corresponding to less crystalline parts, were also oriented in the fibre direction. In case of no mature fibers (2 WPA and 4WPA) the dissolution process occurred without ballooning **(Figures 8-10)** and the fragments eventually dissolved totally. The outer layer dissolved more slowly. With the appearance of the S2 wall, an important phenomenon is observed. The inside of the balloons dissolves by fragmentation. A fraction of the cellulose chains inside of the balloons is dissolving and balloons are growing due to the intake of solvent (osmotic pressure). The fragmentation is the result of the dissolution of the inner part of the fibre. After the total dissolution of the balloons, the unswollen sections and remainings of the primary wall **(Figure 11)** will also dissolve. The sequence of dissolution is thus the following: first the inside of the fibre (S2 wall) by fragmentation, then the S1 wall, then the unswollen sections and remainings of the primary cell wall.

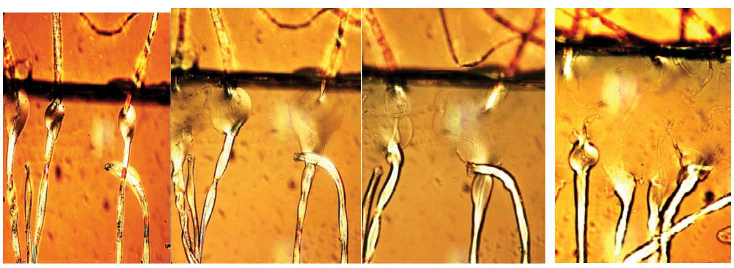


**Figure 7.** Dynamic of mature cotton fiber ballooning and dissolution (N47 mature 47 G.B. F-177 wet) after every 5 minutes after starting solvent action.

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**Figure 8.** Homogenous swelling of no mature cotton fiber (4 WPA) #60 x20.

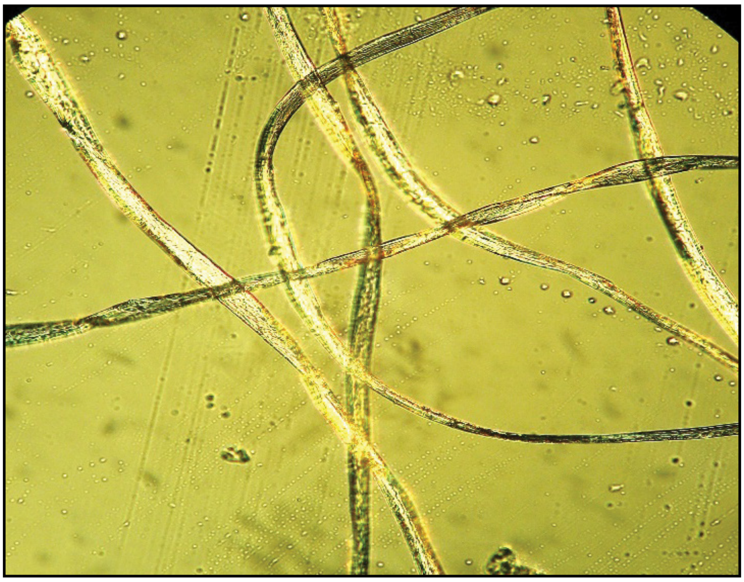

**Figure 9.** No mature cotton fiber (2 WPA) before treatment.

**Figure 10.** No mature cotton fiber (2 WPA) after treatment.

**Figure 11.** Full dissolution of cellulose cell wall and visualization of winding layer and residues of S3 layer. (# 60 19-37 after treatment).

## CONCLUSION

- Convolution parameters of mature cotton fibers may be serving as easy and convenient morphological method to distinguish fibers what grown under stress (drought) conditions.
- Dissolution and swelling of mature and no mature cotton fibers may be used to estimate developmental parameters and maturity cotton fibers.

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