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Morphostructural Rhythmics in Developing Cotton Cell-Hairs

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Abstract: The processes of alive cotton hairs growth and lengthening were investigated for the seed-buds of five cotton cultivars. Morphostructural features pointing to the oscillatory, periodic character of the process of cellulose biosynthesis and coordinated uniformity of its arrangement in cotton hairs have been found out. Collective pulsing lengthening and coordinated nonmuscular movement of developing hairs and also wavy character of their arrangement in lobule volume of raw cotton are believed to be the reflection of real existence of oscillatory biochemical processes in cotton fruits-bolls.

Key words: Gossipium, fiber, structure, biosynthesis of cellulose, oscilllatory biochemical processes, mechanism of growth, morphostructure of hairs, plasmalemmma

INTRODUCTION

As it is known, the rhythm of biological processes is the inherent property of alive systems (Solberger, 1965, Byunning, 1961; Shemyakin and Mihalev, 1938). It is showed (Byunning, 1961) that the rhythm behavior of biological systems is characterized by difference period from several second up to several years. The interest of researchers to oscillating processes in living systems in particular to plant take place during last 100 years and specially increased when the periodic changes have been found in the rates of chemical reactions in homogeneous systems (Jabotinsky, 1974). In the work of Kulaeva and Klyachko (1967) the rhythm of protein synthesis was investigated in the cells of tobacco leaf under constant external conditions of cultivation and under constant chemical influences. The periodicity of biological processes influences on different aspects of plant vital functions. It is observed in the movement of cell cytoplasm (Kamiya, 1959), water exchange of plants in the case of sharp change external conditions (Karmanov and Sarin, 1967), protein synthesis (Kulaeva and Klyachko, 1967; Hurgin *et al*., 1967) and synthesis of other phytohormones during glycolysis (Selkov, 1968, 1971) and also in changes of electrophysical parameters of cellular structures, accompanying endogenous processes (Scott, 1964). The periodicity, or regular oscillations in the rates of biochemical processes, is related to fast biochemical intracellular rhythms, caused by oscillatory conformational processes of microstructures and macromolecules under real conditions of an alive cell (Zaguskin *et al*., 1984; Shnol, 1967). According to a hypothesis of Zaguskin *et al*. (1984), high-frequency oscillations of microstructures (up to picoseconds periods) are natural background conditions of the cells.

It is known that cotton hairs are single cells of epidermis, which are abnormally lengthened on the seed-buds. The quantity of such hairs on a surface of ripened cotton seeds is great. For some middle-fibre cotton cultivars, sown under the conditions of Uzbekistan, the number of hairs on one square millimeter of a seed surface can reach from 853 to 1190 hairs (Krakhmalev and Paiziev, 2004, 2006). The number of hairs in downiness for the average statistical seed dimensions can change from 110000 to 155000. In recalculation on cotton boll, in a ripening cotton fruit there can be several millions of the cells-hairs.

Holding the point of view, that any structure is a consequence, result of previous kinetics of biochemical intracellular processes, it is naturally to expect that the oscillatory processes of cellulose biosynthesis should influence the morphological and structural features of the hairs. However, it is quite not simple to see in each concrete case, how a kinetics forms a structure (Molchanov, 1967). But we suppose that the wavelike behavior of numerous hairs and the features of their structure in closed volume of a fruit-boll reflects the character of the occurring processes of cellulose biosynthesis in cotton cell-hair.

In this connection Brown drew attention to the role of the other important organelle in the process of cotton cellulose synthesis. He has shown for the first time that

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so-called terminal complexes, placed on the surface of plasmalemma, are the catalyst of cotton cellulose synthesis (Brown and Saxena , 2000). In next work of this authors (Feng and Brown, 2000) the growth of submerged cotton (*Gossypium hirsutum* L.) fibers from cultured ovules has been investigated. The results indicate that cotton cell morphology have helical pattern. This experiments have shown that the morphology of the artificially grown cotton fibre and its physical-chemical and qualitative properties are essentially different from those for natural fibres. According to Krakhmalev and Paiziev (2004, 2006) spiral structures in cotton cell results from the superposition of the rotary movement of cellulose microfibrils which are being deposited on the internal cellular surface and the forward movement of the cell hair during its development. In this connection it is very important to understand influence of a kinematics factors of cellulose deposition on cellular wall, initial stages of spiral twisting of the apical part of cotton cell, mechanisms of wavy arrangement of cotton cells-hairs in cotton boll, rhythmic processes of cell-hair growth, a nature of non-muscular movement of a plasmalemma in growing cotton hair and others. All these questions are directly related to the spatial-time organization of native cotton fibre and they require the development of new *in vivo* methods of alive cotton cell growth observation and using the method of the prints-replicas preparation.

Therefore the morphstructural description of separate developing cotton hairs and coherent behavior of very many number of cells in developing cotton boll is the basic matter of the given work.

MATERIALS AND METHODS

Alive hairs of the seed-buds of following cotton sorts: Tashkent-1, 108-F, C-4727 *Gossypium hirsutum* L. C-6030, C-6524 *G. barbadense* L. and also coarse-fibered form *Turfan Gusa G. herbaceum* L. were investigated. Opened flowers were labeled every day at the same time (approximately at 10 o'clock in the morning on local time) and studied with universal optical microscope Neophot-2 (K.Zeiss, Jena) under large magnification (×910). In order to observe the beginning stage of cotton cell-hair protruding, the seed-buds of ovaries were also investigated some hours before cotton flowering. In all cases labeled elements of the fruits were cut off together with a significant part of a branch. In a fold of green cotton boll a small hole (~1-1.5 mm^2) was punctured with special device. Through it *in vivo* observations were carried out of cell-hairs in cotton boll. Under such way of preparation the hairs were not damaged and microenvironment around the cells-hairs was maintained for a long time.

To display fine structure of primary wall of alive hair and plasmalemma, the method of prints-replicas was also used, which allowed to reproduce precisely in that given moment the features of the structure of alive hair and seed-bud surfaces at different stages of their development (Shimmel, 1972). The method of prints-replicas is widely used in the practice of electron microscopy. It is especially convenient for prompt prints of alive cotton hair structure and seed-bud surface. Preparation in this case is carried out by application of polymeric films to the objects under observation (for example, from water solution of gelatin), which are closely adjacent to moist surfaces of the hairs and seed-buds. After separation of dried film the hair prints were investigated with optical or electronic microscopes. Similar results were obtained and in the case of fast, short-time pressing of the hairs with seed-buds to a slide, covered with thin hardening gelatin layer from its water solutions.

The mechanism of occurrence of the first hairs on epidermal surface of the seed-buds, dynamics of their lengthening, growth and formation of volumetric structure of raw cotton lobules, cytoplasm movements and auto-oscillations of plasmalemma in the cells-hairs were photographed on the highly sensitive film with the size of 9×12 cm under the magnification adjusted for maximum (×910). Simultaneously the patterns of cotton hair and seed-bud structures were projected onto the screen of Video-Control Device (VCD) with total magnification of ×3700 and were recorded on a videofilm. Such high magnification of the structure images allowed measuring with high accuracy of the process of rhythmical lengthening of apical parts of alive hairs. Error in measurement of pulsing lengthening of hair tip (for example, 0.5 mm on VCD screen) gave the possibility of guaranteed determination of the hair movement rate with the accuracy equal to 0.135 m km. It is impossible to obtain such accuracy of determination within fixed time interval, for example, 10 or 20 sec on the base of traditional methods used in plant physiology.

This study has been conducted by using alive developing cotton plants in the field at Institute of Electronics Uzbek Academy of Science harvested in 2004-2005 season.

RESULTS AND DISCUSSION

First cell- hairs occur on halasal part of the seed-buds, where a maximum quantity of the stomata is placed (Krakhmalev and Zakirov, 2000). Depending on cotton kind and sort, the number of stomata on halasal part of the seed-buds can be equal from 300 to 400 hairs per 1 mm^2 of epidermal surface. Some hours before flowering the cells of external epidermis of the seed-buds become more active, convex and microscopic protrusions with the

dimensions of ~1 m km^2 occur on some of them closer to the centre. The protrusions gradually stretch and by the moment of flowering and during following hours they protrude, firstly taking the form of hemispheres and then getting the spherical form with 18-22 m km in diameter.

By this time each cell-hair has short basis-pedestal under itself, which is bound with primary epidermal cell. The nuclei of epidermal cells move into the forming hairs and they as though oscillate around the centre of spherical formation. In that period the directed circular movement of cytoplasm in arising hairs was not observed. The occurrence of the cells-hairs is avalanchely increased from the moment of fertilization of a flower's ovary. The density of arising hairs on halasal part of the seed-buds becomes so high (for the seed-buds of C-6524 cotton sort it is about 2200 mm^{-2}), that they come in contact with each other. And this is, apparently, the signal to the beginning of occurrence of local microprotrusions on their surfaces (Fig. 1a), which are rhythmically increased up to the given genetic fibril diameters. Pulsing, rhythmical growth of the protrusions and widening of their bases are repeated continuously up to achievement of the hair length limits. *In vivo* observations of large groups of the cotton cell-hairs show, that the formation of local, plastically deformed parts on a surface of apical part of developing hairs and their protruding occur almost synchronously (Fig. 1b). The evidences of such mechanism of alive hair growth are well observed not only on the screen of videocontrol device at early stages of cotton cell development, but also later, for example, during the period of 20-40 days from the date of flowering. As an example it is enough to observe with microscope the alive lobules of raw cotton from non-opened boll (Krakhmalev and Paiziev, 2004). The lobules are organized by densely overlapping hairs of seeds, put in one nest of ovary and they keep their form long time enough to be observed under microscope. On a surface of the lobules the great number of growing hair tips are visible with characteristic

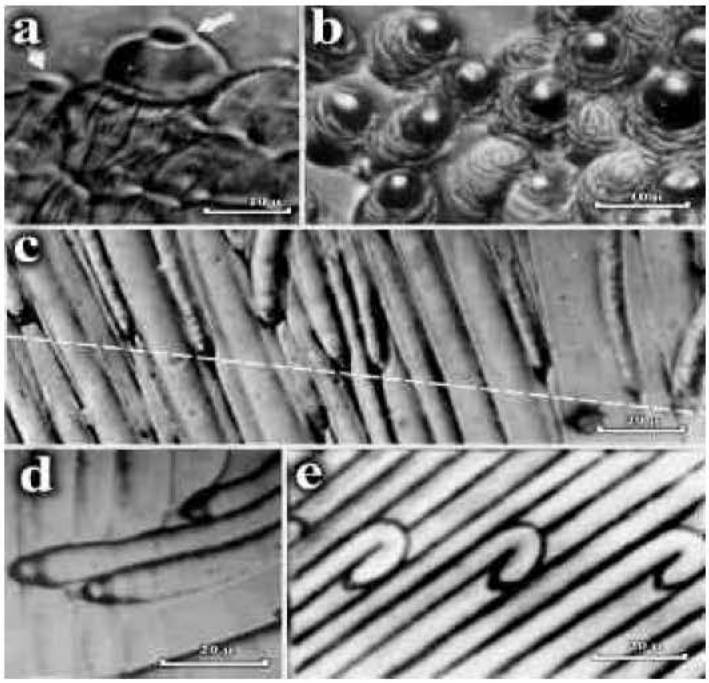

Fig. 1: Features of the mechanism of hair growth and development on the cotton seed-buds: a- local microprotrusions on epidermal cells of the seed-buds at the moment of hair occurrence (arrows). Cotton sort is 108Φ *G. hirsutum* L. Some hours before cotton flowering, b- simultaneity in occurrence of microprotrusions on appeared hairs of the seed-buds. Cotton sort-*Tashkent-1 G. hirsutum* L., second day from the date of flowering, c-synchronous growth of apical tips of cotton hairs on a lobule surface of cotton boll. Cotton sort C-6030 *G. hirsutum* L. twenty five days from the date of flowering, d- microprotrusions on the tips of growth of 45-day alive cotton hairs of *Turfan Guza*, e- and synchronous, coordinated turning of hair growth direction by 180° and less during the formation of raw cotton lobules. Cotton sort-*Tashkent*-1, 35 day from the date of flowering

protrusions, which are frequently arranged in a line (Fig. 1c). Protrusions on the hair apexes have the identical linear dimensions and morphology (Fig. 1d). This fact also proves their synchronous growth. Both for such hairs on a surface of formed lobules and for individually lengthening cells-hairs the pulsation, rhythm of their growth rate under the action of turgor pressure are characteristic. Thus, the rate of cotton hair growth periodically changes: at first it increases, then decreases. Following interesting fact is found out: the period of oscillation of growth rate for alive hairs is close to 4.4-5.0 min that is characteristic for earlier investigated other vegetative, chemical and animal objects (Byunning, 1961). However, the amplitude of changes in hair growth rate depends first of all on their age and on a place of initial occurrence of a hair on epidermal surface of a seed-bud. So, the processed images of the process of three-day hair growth on halasal part of a seed-bud have shown that the rate of hair lengthening is, on average, 0.0144 m km sec^{-1} (or 51.84 microns h). For one day such hairs could lengthen by 1.24 mm. For five-day hair the increase was 0.015 microns sec^{+} or 54.1 microns/h, for 10 day hair the increase on the average was already 0.039 microns sec^{-1}, for 20-day this value was about 0.0436 microns sec^{-1}. Relatively, five-day hairs could grow for one day by 1.3 mm, ten day by 3.37 mm and 20 day by 3.767 mm. The calculated values of growth rates satisfactorily coincide with experimental data of works (Popova, 1975; Vlasova, 1976) for various cotton sorts.

The rhythm in hair growth processes is not disturbed even in the case, when all hairs in the given layer of a lobule simultaneously, according to some signals change the direction of their lengthening. Most impressive are the patterns of synchronous change in direction of hair growth on the opposite one or on more significant angle of rotation (Fig. 1e). Thus it is possible to visually demonstrate that the hairs, occurring at different parts of the seed-bud surfaces and in different time, forming a layer in a volume of lobule, have the identical linear dimensions of their bent tips of growth. Hence, the cellular wall protrusions at all tips of growth occurred simultaneously and grew with the same rate. As it is possible to believe, endogenous biochemical processes control occurring morphostructural reorganizations of the hairs. But at the same time it is not clear, why growing cell-hair suddenly and sharply change a direction of its lengthening by 180° or less. What is the nature of such behavior of the hairs and what are the forces causing this sharp bend of a hair cellular wall? Pulsing, rhythmical character of the rate of alive hair growth inside closed volume of cotton boll is well seen underobservation of the processes of cell-hair lengthening with the optical-television system (Fig. 2).

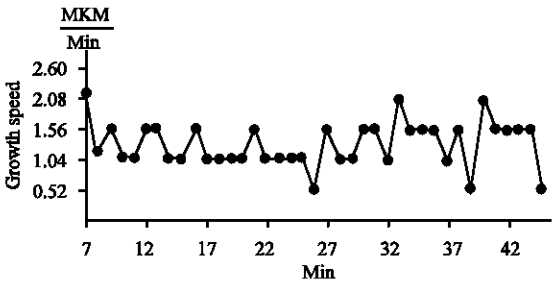

Fig. 2: Growth rate of active group of alive hairs of a seed-bus for cotton sort 108-F. The rate was measured on the VCD screen of optical-television system in time intervals of 60 sec. The beginning of observation time: nine hours seven minutes from the date of flowering

The transparency of primary wall of alive hairs at early stages of their development allows seeing a structure of plasmalemma of the cotton cells (Fig. 3a). As it is seen from figure, it is not smooth and it has periodically located dark and light zones oriented perpendicularly to an axis of hair growth. The flexures and protrusions of plasmalemma surface are in accordance with them and they have average width about 2.5 m km.

Such structure of plasmatic membrane allows its increasing active surface more than 1.5-1.7 times in comparison with smooth cylindrical form repeating hair morphostructure. The studies of alive hair structures at early stages of development using gelatin prints under large magnifications show, that goffered, wavy surface of plasmalemma has thin structure. The flexures and protrusions of hair plasmalemma are organized of packages of more fine similar structures (dimension from 1.05 to 1.37 microns). The larger the hair age, the coarser cross structure of plasmalemma; single elementary zones, grouped by 4-6 pieces in packs, deflect into cytoplasm. In alive hairs cross structure of plasmalemma is in oscillatory movement. It is well observed on transparent young cells-hairs, especially on the VCD screen of optical-television system at 3700-multiple magnification. With the microscope (and on VCD screen) it is observed, that the protrusions and concaves of hair's plasmalemma are smoothly replaced and move together along a cell towards a growth tip due to continuous rhythmical oscillations of a surface of plasmatic membrane. Under observation of a group of close-fitting hairs with microscope it is found that the arrangement of flexures and protrusions on their plasmalemmas coincide (Fig. 3b). It is possible to say that the original effect of Bayersdorf (Usmanov and NIkonovich, 1962) is repeated, which is observed on electron microscopic photos of thin structures of cellulose microfibrils: dense crystal areas of one microfibril precisely coincide with the same ordered areas of another

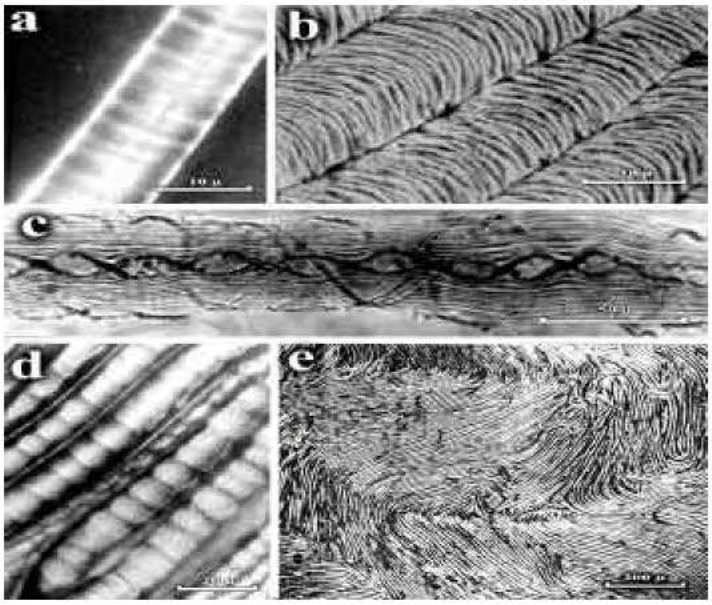

Fig. 3: Periodicity of a thin structure of alive cotton hairs, a- structure of five-day alive cotton hair of *C*-6524 *G. barbadense* L. cotton sort, observable by reflected light at the moment of wavy movement of plasmalemma, b-structure of plasmalemma surface of closely adjoined hairs (cotton sort is 108-F *G. hirsutum* L., fifteen days from the date of flowering), laying on a surface of a lobule of raw cotton, c- structure of mature cotton hair of *Turfan Guza G. herbaceum* L., swollen in a cupric-ammoniac solution. The ordered layer structure of cellulose deposition and spiral-like twisting of plasmalemma are seen, d- the surfaces of adjoining cotton hairs of *Tashkent*-1 *G. hirsutum* L., forming a mosaic structure of volumetric structure of raw cotton lobule, some days before a fruit-boll opening. Influence of synchronous occurring intracellular processes upon the external form of the hairs and e-oscillatory, wave character of package of the hairs, forming a volumetric mosaic structure of raw cotton lobule

microfibril. The same coordination of cross structure arrangement is also observed in the case of plasmalemmas of adjoining hairs, which are packed in layers of cotton lobule during their growth.

The effect of rhythm in metabolic processes and in particular, in cellulose biosynthesis is, apparently, increased by symmetric, ordered arrangement of associates of terminal complexes on the external surface of plasmalemma of cotton hairs. According to our data (Krakhmalev, Zakirov, 2000), the diameters of associates of mobile fermentative complexes or terminal complexes (deducting the thickness of primary wall) are approximately 0.2-0.3 microns. Such dimensions are quite real, as Giddings *et al*. (1980) observed, for example, on plasmatic membranes of the cells of higher plants the hexagonally oriented nests of rosettes consisting of 175 pieces and synthesizing parallel beams of cellulose microfibrils in secondary cellular wall. Linear density of terminal complexes on plasmalemma surfaces of cotton hairs, investigated by us, is 820 mm^{-1} on the average. Surface density of associates of terminal complexes on plasmalemma is ~106 mm^{-2}. It has been experimentally established (Willison, 1976) that the single terminal complexes are frequently related to the growing tips of cellulose microfibrils, i.e., cellulose is synthesized on a surface of plasmalemma and it is deposited inside the cells on their primary walls. Deposit of cellulose microfibrils in cellular wall and their subsequent growth causes the occurrence of pressure on a surface of plasmalemma and it begins the movement to the direction, which is opposite to the direction of coordinated lengthening of microfibrillar masses. The direction of movement is determined by orientation of the lines of protein granules, the orientation, in its turn, is regulable by the orientation of microtubules. As the process of cellulose biosynthesis has, apparently, the oscillatory character, the intensity of deposition of cellulose microfibrils also periodically varies. As a result of active influence of the coarse tips of synthesized cellulose microfibrils on plasmalemma of the hairs during their development a plasmalemma begins its movement and twisting (Fig. 3c) (Krakhmalev and Paiziev, 2004). This

property influences the regularity in the deposition of cellulose in secondary cellular wall. This inhomogeneity of the process of cellulose microfibril packing is clearly displayed for drying hairs at the end of their development in non-opened cotton boll (Fig. 3d). When every cotton hair is dried up, its cellulose cellular wall reproduces the spiral-like twisted form of plasmalemma on which the cellulose microfibrils were synthesized. As the process of cellulose biosynthesis in every hair has rhythmic character, the whole pattern of drying hairs, which is seen on Fig. 3d, has corresponding symmetric form repeating the from one hair to another. In conclusion it is necessary to tell about unique regularities, observable during the formation of volumetric structure of so-called cotton lobule in cotton boll. Cotton bolls are multinested. Usually they are three-, four- and five nested. From 5 to 7-11 seed-buds develop in each nest of a boll. Great number of the hairs of various lengths occurs on the seed-buds from the moment of ovary fertilization up to the end of their development. Depending on a quantity of the seed-buds in an ovary nest, on the sort and kind of cotton, the number of hairs, which should be packed within strictly fixed volume, in a lobule, formed by the hairs of these seed-buds, can reach one million and more hairs (Krakhmalev *et al*., 2003). The experiments show, that lengthening hairs in a lobule are not interlaced with one another, they do not form the kinks, do not damage each other, though the hair length of a fiber for middle-fibered cotton sorts varies from 31 to 34 mm. Really observable scheme of hair packing in a lobule is unique without exaggeration, it is practical, symmetric (Fig. 3e). The observable external surface of cotton lobule has so-called mosaic structure by analogy with the monocrystals of metals, i.e., it is made of microscopic areas with strongly ordered arrangement of the hairs. More correctly, it is made not of the hairs with their total length, but of small parts of cellular walls of these hairs. If one could make a roentgenogram of such area of a lobule, we could obtain very clear diffraction image, typical for crystal objects. If we'll observe an order of packing of a hair in lobule layer, we'll see that the same hair naturally passes from one area of a mosaic to another, performing oscillation of the direction of its lengthening. And complete pattern of all packages of the hairs into lobule layers has oscillatory, wave character. It is unclear till now, what are the regularities in package of the hairs inside the volume of cotton lobule. But it is clear, that these regularities were developed by a nature for a long time. Thus, a structure, mechanism of cotton hair growth and development profoundly reflect occurring intracellular kinetics of metabolic processes. The explanations become simple, why the cellulose layer deposition in cotton hairs has the spiral-like character, why the morphological form of the ripened cells has corkscrew-like shape and at last, why so-called twisting of fibers arise and impart to cotton fiber its important technological property (Popova, 1975).

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