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Morphology and Helical Growth Mechanism of Plasmalemma in Cotton Fiber

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The mechanisms of the living hair growth of cotton cultivars at early stages of their development have been studied. It has been found that a plasmalemma of cells-hairs makes spiral movement during their lengthening. Similar rotation of a plasmalemma takes place at the hair growth of uncultivated cotton sorts. Non-muscular form of mobility is initiated by biosynthesis of cellulose as microfibrils, produced by terminal complexes located in plasmalemma of cells-hairs. As a result the whole skeleton of apical part of a cellulose cell is involved into the movement along spiral line, providing subsequent corkscrew-like external form of a hair at drying.
Key words: cotton fiber, plasmalemma, helical growth, cell wall.

1. INTRODUCTION

Among wide variety of the celluilar forms of vegetative and animal organisms, cotton cell-hairs are characterized by their extremely large relative length [1,2]. Cotton hairs of some cultivars reach 50-60 mm lengthwise at microscopically small own diameter (from 18 till 25-32 microns). The ratio of the hair's length to its diameter can reach 2000-3000 [3].Cotton hairs are developed from some cells of the external epidermis of the developing seed-buds included into ovary inside a cotton boll [4]. The quantities of hairs, including delint, lint and the hairs of fibre, depend on a type and sort of cotton. According to our recent data, the downiness on every mature cotton seed can make the value (for example, for middle-fibre cultivars) from 10-12 to 15 thousands of grown hairs [5]. According to numerous studies on the base of optical and electronic microscopes, a cotton hair is the anisotropically lengthened single cell, it consists of cuticular oily-waxen layer, primary, secondary cellulose walls and channel. Following elements are revealed on the cross and longitudinal microscopic sections: the thickness of plasmatic membrane, nucleus with little nucleus, vacuoles, , Goldzi apparatus, mitochondrion, plastids, chromosomes, and ribosomes [7,8]. Narrow periplasmatic space, filled with the structureless liquid is frequently observed between primary and secondary walls [7]. Among all mentioned elements of the cotton cell structure its lifeless excretory part, being the cellulose cellular wall, is the most investigated [6,9,10]. However, almost in all monographs devoted to cotton and its fibre, nothing is told about the importance and morphological structure of such important intracellular organoid as plasmalemma. And also nothing is reported about its mobility and its role in functioning of the cytoplasm in living hairs, though its active surface is greater by the factor of 1.5-2.0 then the surface of the whole hair due to the intussusceptions. And it is important for the metabolic processes of a cell.

With the use of optic-television devices it has been shown [11], that the plasmalemma in living hair at early stages of its development is not a motionless organoid. Its surface makes wavy oscillations, pushing the cytoplasm towards the tip of growth. We shot all these non-muscular periodic movements of plasmalemma on the videotape. Moreover, we have found, that in apical part of the lengthening hair the plasmalemma rotates, twisting around a spiral sometimes from the right to the left, sometimes in the opposite direction. So the goal of the present work is to describe a technique of the detection of this phenomenon and its influence on a structure of cotton cell.

2. OBJECTS AND TECHNIQUE OF RESEARCHES

The objects under investigation were the living hairs of five middle-fibre cotton sorts: *Tashkent-1, 108-F, Namangan-77, Margelan-3 G. Hirsutum L.* and the coarse-fibre sort of the variety of *G. arboreum L.*, so-called *Turfan Guza*. Among them the hairs of the cotton types *Namangan-77* and *Turfan Guza* were studied in more detail. To exclude the hair damages during their preparation, the living hairs on the surface of just opened boll - on the surface of so-called lobe - were investigated. A lobe is rather hard formation of 5-7 developing seed-buds and their numerous interlaced hairs. However, the hairs begin to dry up and to displace during the observation with optical microscope. On the other hand, it is impossible to study for a long time a living cotton lobe with optical microscope under a powerful light flux, in open air because of the drying the surface under investigation. So we used the electron-microscopic technique intended for obtaining the print (replica) of a cotton lobe surface. For this purpose we prepared thin dried layers of gelatin on the slides, using its 10-15% solutions. To obtain a replica of a lobe we slightly softened the gelatin above the pairs of boiling water and immediately pressed the lobe from just opened cotton boll against a slide without force application. 5-10 seconds later the lobe was separated from the gelatin surface. Clear replica of the great number of hairs, forming cotton lobe, remained on a slide, in the layer of softened gelatin. The obtained constant preparation could be studied in detail, especially by reflected light. The prints of fibres lay in one plane that is very convenient for microscope focusing. Perfect microphotos of hair

surfaces and pictures of their superdense packing were made from a replica (Fig.1a).

The method of replica preparation from the fibres allows revealing the morphological structure of their plasmatic membranes (Fig.1b). For this purpose it is necessary to soften a layer of dried gelatin at the larger depth, than for the first case, and slightly increase the time of pressing to glass. The topography of a surface becomes clear, with contrast deflections of plasmalemma. The most easily a plasmalemma of hairs is revealed at early stages of their development. In this period the primary wall is extremely thin. It is shown in [12], that the primary wall consists of two layers of the thinnest microfibrils of cellulose covered with thin layer of oily-waxen substances. Under transmitting light beams the primary wall is very transparent. Moreover, when the hairs are pressed against the softened gelatin, this layer of microfibrils fits snuggly against the intussusceptions of plasmalemma, repeating its waviness. According to [13,14] the formation of a wavy surface is characteristic for a plasmalemma of plants in general that is verified by living cotton hairs observed in transmitting light beams (Fig.1g,d). However, in this case the resolution of a picture of a structure is worse, than on the prints. The use of a print technique allows revealing the fineer structures in cotton hairs, than the surface waviness of their plasmalemma (Fig.1v,e). The spheroidal particles of 0.14 microns in diameter, closely related to the plasmatic membrane, are revealed on a surface of plasmalemma, especially at the tops of corrugations. It is supposed that every such microscopic formation is an association, block of many terminal complexes numbering in their structure some tens of row [15]. The character of their arrangement on a surface of developing hair influences the direction of microfibril packing [16].

The cotton boll selection for study was made taking into account the days of their development. Cotton flowers were labeled at the days of their opening. The hairs, a surface of living lobe and also the prints-replicas were studied by reflected light using the universal optical microscope Neophot-2. Shooting of cotton-fibre structure was carried out on a high-sensitivity photographic film with the size of 9x12 sm at the greatest possible optical magnification (~×910).

3. RESULTS AND DISCUSSION

Earlier we have shown [11], that the process of living hair growth has oscillatory, rhythmic character that was clearly seen by the movement of the growing tips of separate cells-hairs. Their images were displayed on the screen of the monitor of optic-television system with the magnification of 3700. Rhythmically lengthening apexes of the hairs were observed simultaneously with auto-oscillations of a plasmalemma's surface and with the movement of cytoplasm to a tip of cell growth. Similar apical parts of the hairs on gelatin prints had the characteristic periodic transverse and rare longitudinal structures (Fig.2). The transverse dark lines correspond to deepenings, surface intussusceptions of a plasmatic membrane. The longitudinal lines - bars of microfibrils, composed of lines of globular formations (Fig.2a, b) - are of the greatest interest. The bars arranged in parallel to the growth axes only at early stages of hair development (1- 3 days). To five and more days of their development the rows of globular particles take an inclined position to a hair axis. Respectively, the rectilinear lines were changing their orientation (Fig.2a, b). On a tip of growth they took an inclined position repeating the coils of spirals in their further course. Such phenomenon indicated that a tip of hair growth made helical rotation around a direction of lengthening. The angles of rotation, measured by this inclined bars of microfibrils for the hairs of different sorts, were 3°-5° at certain parts of an apex, on the other parts they varied from 10°-15° up to 40°. An apex of a lengthening hair periodically changes a direction of its rotation from the right to the left and contrariwise.

When the structure of a mature hair surface was studied with the electronic microscope, such longitudinal bars, as in Fig.2v,g should be observed along the full length of a hair, that did not prove to be true. It means, that the fibrillar beams forming at the apex at early stages of growth are unstable and they undergo changes in the process of their moving away from a point of growth and maturing of a fibre or disintegrate with time.

It is necessary to note, that the occurrence of beams of longitudinal microfibrils at a tip of growing living hair and, in particular, the orientation of microfibrils in the direction of spiral twisting, then subsequent disappearance and reconstruction - all these processes are also observed under the protruding of such eukaryotes cells as amoeba, human nervous cells in culture, fibroblasts, leucocytes [17]. The main feature of such cells at the non-muscular movement is the ability to form the pseudopodium with subsequent contraction.

When we obtained the prints of the hairs of a lobe, which was pressed against the softened amorphous gelatin, we often observed the insignificant displacement and spreading of a hair primary wall relatively to a plasmatic membrane (Fig.2d). It was often seen that the cross tucks and longitudinal lines of spheroidal particles on the tips of growth of living hairs were located only on the plasmalemma (Fig.2b). In the spread part of a primary wall such rows of lines are not present, though the presence of longitudinal microfibril beams, which thickness lies in the range of electronic microscope resolution, is not excluded.

It is impossible to observe the rotation of plasmatic membrane of a hair by 360° using the method of prints. However, there are convincing proofs of this phenomenon. If the ripened cotton hairs are processed in a Sveyserov's solution [21] and the process of swelling and dissolution of cellulose of primary and secondary walls is interrupted in proper time, the amazing pictures of plasmalemma appear (Fig.3). In microphotos it is possible to observe a layered structure of swollen cellular hair wall, thin spiral-like course of fibril beams of a primary wall and the plasmalemma, consisting of almost identical small coils, resulting from the apex rotation during growth. The analysis shows that for the middle-fibre cotton sorts (*Namangan-77, Tashkent-1, 108-F, Fergana-3, Turfan Guza*) the number of such coils-rotations of plasmalemma varies from 15-18 to 30-38 per 1 mm of the swollen hair length. This number depends on the conditions of chemical treatment, time of the cellulose walls dissolution and interrupting the reaction course.

Thin fibril beams of a primary wall, spiral-likely surrounding a hair, known character of cellulose layer deposition in the secondary wall, twistedness of plasmalemma testify that during the hair growth and lengthening all components of its cellular wall are in the movement. It is possible to assume that a plasmalemma rotates faster of all. Twistings of plasmalemma have the same folding character of a surface, as at early stages of cotton hair development (Fig.3g, d).

Taking into account the obtained data it was interesting to check up, whether a plasmalemma rotates in the hairs of uncultivated cotton sorts? In our opinion, the investigation of this phenomenon is not of only scientific-cognitive interest. So the hairs of uncultivated cotton sorts *G. mexicanum L.* and *G. raimondii Ulber* were studied in a Sveyserov's solution. The structural analysis has shown that a plasmalemma of the hairs of uncultivated cotton sorts also rotates (Fig.3d, e).

The fact was surprising that the quantity of plasmalemma twistings per 1 mm of the straight hair length for uncultivated cotton sorts was almost the same, as for white investigated hairs. For the hairs of *G. mexicanum* this quantity is 14-30 twistings per 1 mm of length, and for hairs of *G. raimondii* it was 12-28 twistings/mm. Thus, within the 5% limits of error the quantities of plasmalemma twistings of the hairs of middle-fibre cultivars and the hairs of uncultivated cotton sorts coincide, though the external form and hair length on their seed-buds are different.

The following statements can be proposed on the reasons of non-muscular mobility of plasmalemma in the cotton hairs in the process of their growth. It was shown in the numerous scientific works, that the cellulose is synthesized on a surface of plasmalemma [2, 15, 16, 18, and 19]. The terminal complexes, rosettes, corresponding to them, hexagonally oriented aggregates, associates or blocks of them are directly responsible for the synthesis of cellulose chains and the formation of microfibrils. A distance between the rows of fermentative complexes in plasmalemma corresponds to the distance between microfibrils of cellulose in cellular wall. The fixation of the ends of depositing microfibrils in a matrix of the cellular wall creates the great total repulsion forces towards the surface of plasmalemma, and it (not the terminal complexes) starts the helical movement. The direction of movement, its continuance are apparently determined by the orientation of the rosette rows on the internal side of plasmatic membrane controllable by a direction of microtubules of the hairs situated in cytoplasm [16].

Proceeding from experimental data it is easy to understand now, why in the process of cellulose biosynthesis in a cotton cell the spiral-like cellulose layers are deposited on its secondary wall, why the natural form of cotton hairs becomes corkscrew-like shape during their drying and why the twistings of form arise along all hair length. It is also possible to assert, that in the vegetative fibres of bast, jute, wood, ambary, hemp, in which the spiral packing of cellulose fibrils in cellular walls is observed, a rotation of plasmalemma also takes place [20]. The hairs of a poplar should be especially noted, as their mechanism of growth represents the evident analogue to the cotton hair [22].

4. REFERENCES

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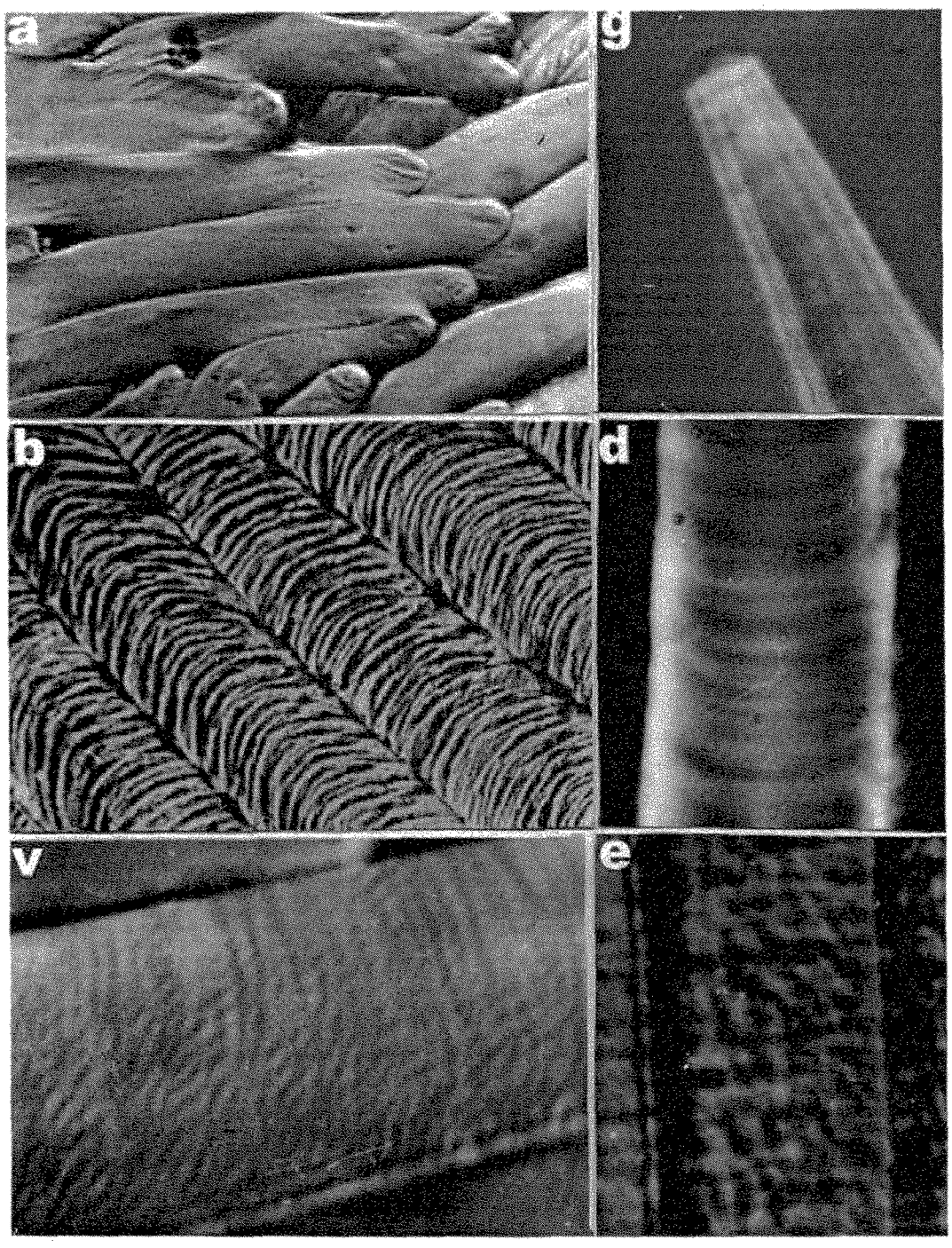

Fig. 1. Pictures of a structure of cotton hair surface for the various sorts obtained by a method of prints on a thin gelatin layer under their study by reflected light:

(a)- character of growing hair packing in living boll. Age of a boll-fruit is 20 days from the date of flowering, ×760; (b)- a picture of a plasmalemma structure of these hairs. The folds-intussusceptions of the adjoining cells-hairs are arranged in coordination, ×1150; original "effect of Bayersdorf" [6]; (v)- the features of the spheroidal particles arrangement on a surface of plasmalemma of a hair. The hair age is 10 days from the date of flowering, ×4700; (g)- apical part of growing hair with characteristic longitudinal beams of the microfibrils. The hair age is 3 days from the date of flowering, ×1860; (d)- structure of hair plasmalemma in middle of their length observable by reflected-transmitted light beams, ×1860; (e)- a structure of a plasmalemma surface of the hairs when the primary wall is moving away. The hair age is 12 days from the date of flowering, ×1900.

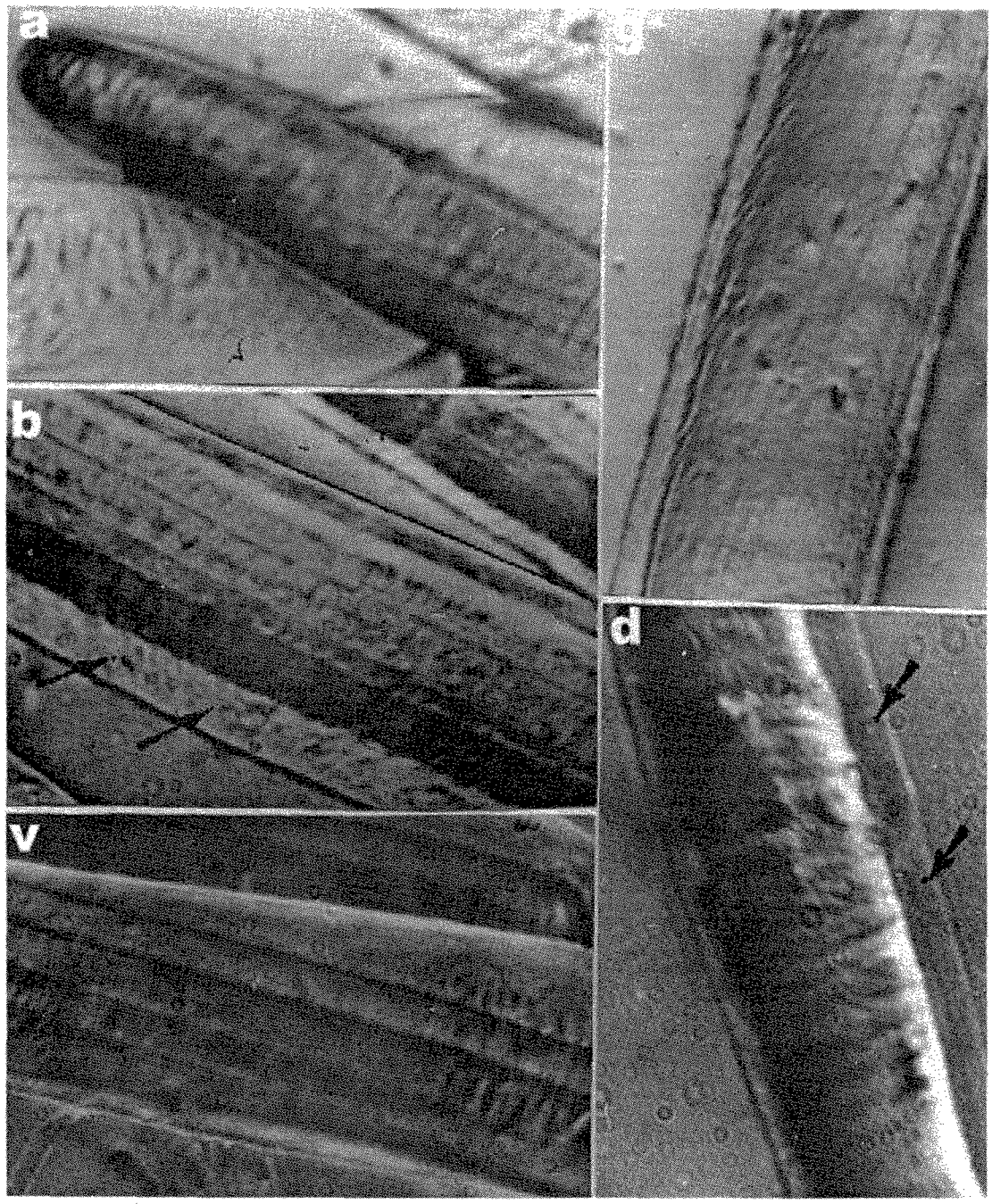

Fig. 2. Pictures of a structure of a plasmalemma surface on the apical parts of growing cotton hairs:

(a)- cotton sort *Tashkent-1*. Age of a fibre is 4 days from the date of flowering. Two inclined lines on a plasmalemma surface are visible, ×1300; (b)- cultivar *Namangan-77*. The hair age is 7 days from the date of flowering. A part of the hair is close to an apex. Longitudinal lines - only on a plasmalemma, ×2500; (v, g)- cotton cultivars 108-F and *Turfan Guza*. The hair age is 10 days from the date of flowering. A series of lines on hair plasmalemma at their rotation along the 10 degrees spiral line around an axis of growth, ×2100; (d)- *Margelan-3* cotton sort. The hair age is 12 days from the date of flowering. Spread primary wall is structureless (two arrows), ×2300.

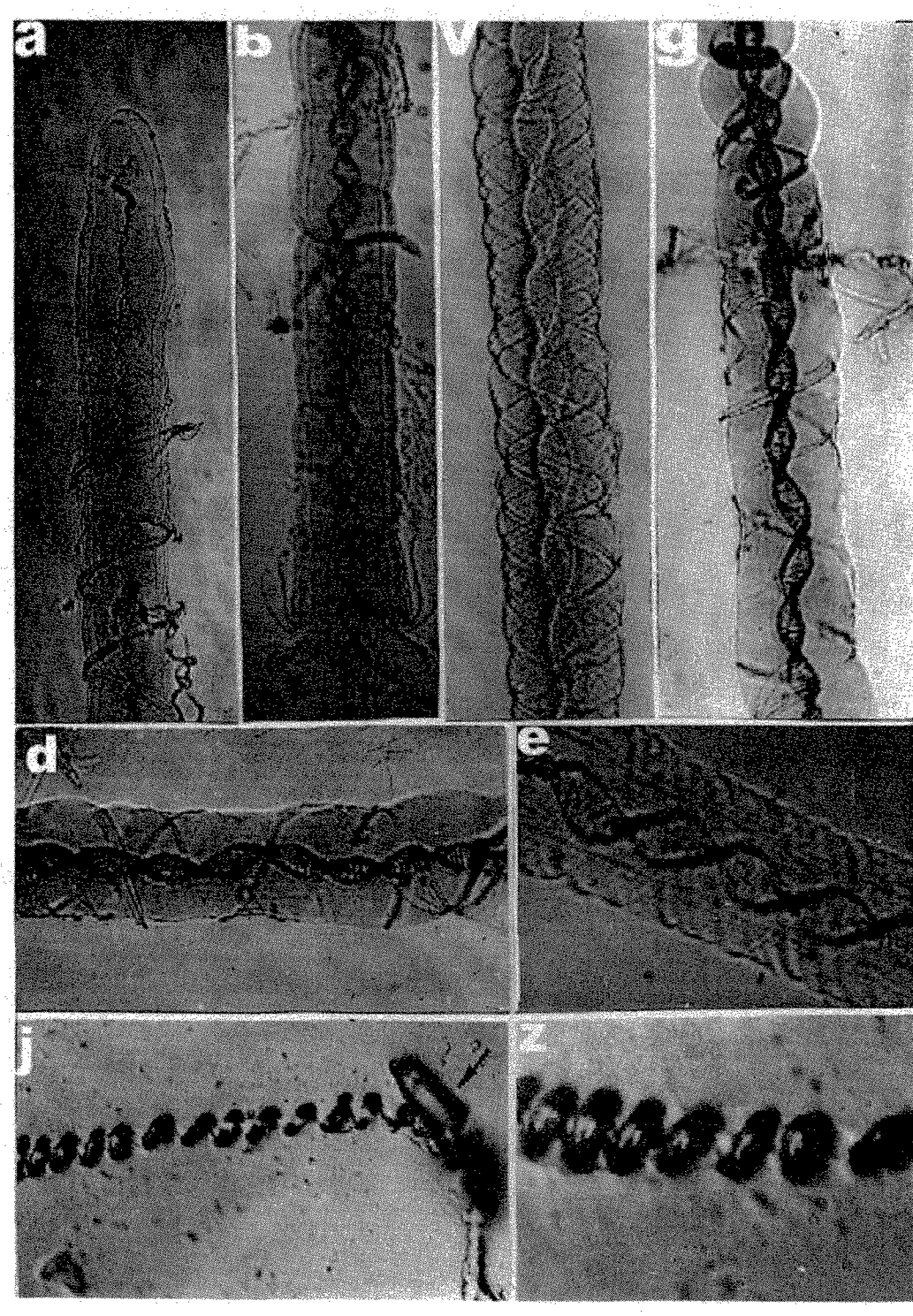

Fig. 3. Helical twisting of plasmalemma in the hairs of various cotton sorts, observable at the hair processing by a cupreous-ammoniac solution:

(a)- apical part of a growing hair of *Turfan Guza*. The hair age is 20 days from the date of flowering, ×220; (b)- *Fergana-3* sort. The hair age is 30 days from the date of flowering, ×270; (v)- *Namangan-77* cultivar. The hair age is 35 days from the date of flowering, ×460; (g)- *Tashkent-1* sort. The hair age is 38 days from the date of flowering, ×275; (d)- mature hair of the uncultivated cotton *G. Mexicanum L.* , ×460; (e)- mature hair of the uncultivated cotton *G. Raimondii Vlber.* , ×520; (g)- plasmalemma of the *108-F* cotton hair, stretched from an living hair by the age of 10 days from the date of flowering. On the right is the rest of a tip of hair growth, ×650; (z)- the same picture, but at the greater optical magnification, ×1500.