ABOUT ARRANGEMENT OF THE HAIRS ON THE EPIDERMIS OF COTTON SEED

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ABSTRACT

The character of an arrangement of the hairs on seeds of five cotton cultivars has been investigated. Unknown feature has been found in the processes of the cells-hairs appearance from the epidermis of ovule-seed. It is shown that from the beginning of certain moment of cotton ovule development, two closely located adjoining epidermis cells, which just have come out of mitotic process, are more often simultaneously differentiated into the hairs. Arising cells-hairs form various geometrical figures on a surface of a ovule epidermis: lines, arches, circles. The process of differentiation into pairs is simultaneously characteristic not only for the epidermal cells, which initiate hair appearance, but also for the cells which form stomata on the ovule epidermis.

KEY WORDS: Gossypium, ovule, epidermis, hairs, differentiation.

Introduction

Cotton fibre is the basic material for a textile industry and this fact explains the increased interest to the fine points of the process of fibre formation on the ovule or seeds of various cotton cultivars. An end goal of such investigations is to find the possibility of cotton productivity increase, to improve the quality of cotton raw, to study the influence of agricultural features and inorganic nutrition upon the land productivity and cotton quality. So the key problem is to investigate the differentiation of epidermal cells into the hairs and their development for various cotton cultivars. Many problems of fibre formation were studied relatively well [1-5, 9-12, 14-18, 21-24]. Bowman's papers, published in 1823, have layed the foundation for study of fibre formation on cotton seed [2]. He observed the developing hairs during first days after fertilization of the cotton seed-buds and he has wrongly concluded that the fibre on the seed-buds is formed of the cells of second layer of external epidermis.

W.Balls has brought the clearness into this problem [1]. Studying the development of all the layers of seed shell, he has found that the hairs arise from separate cells of external epidermis of the seed-buds. On the subject of duration of fibre formation W. Balls has assumed at first that the differentiation of epidermal cells into the hairs occurs only at the day DPA. However later he has specified, that the fibre formation on a surface of developing seed occurs during several days after its fertilization.

The other researches made farther definitions of the terms of hair occurrence depending on cotton kind and conditions of its cultivation [4, 16]. In the main they were related to the duration of fibre formation. A.Gulati, experimenting with three cotton kinds (G. indicum L., G. hirsutum L., G. barbadense L.), has established, that the mitotic division of epidermal cells and differentiation of cells into the hairs do not stop to 10-th day DPA [4]. T.N. Singh on the basis of his researches believes that fibre formation takes place during all the period of seed development [16]. A question is: when the hairs of a fibre and the hairs of an underdown appear? Is their appearance divided in time?

The initiation of fibre formation depends on a site of epidermal cells location on developing seed-bud. It has been established that independently of cotton kind the process of fibre formation starts on its extended, halasal part, passes progressively to an average part and, at last, it is activated on narrowed, micropilar part of a seed [14, 20, 21].

It should be noted that almost all observations of fibre formation on cotton seeds were obtained during investigation of a structure of cross and longitudinal cuts of developing seed-buds [13, 19]. The information about the appearance of the hairs from separate epidermal cells was obtained on the basis of such researches. The preparation of the cuts of cotton seed-bud is related to a number of manipulations, namely: removal of a seed-bud from cotton boll, its fixing in spirituous solutions of coloring matter, placing of the seed-bud into the waxes or hardening polymeric mixes. After a series of chemical treatment and fixing in solid mass the cuts of the seed-buds of required thickness are prepared with the use of microtome or sharp razor and observe with optical or electron microscopes. In the obtained cuts various artefacts are often observed. However these investigations have proved, that the hairs arise from separate single epidermal cells and they are located along cut line by random manner. Since then the researchers were interested in the problem: why all the cells of external epidermis of a seed-bud are not differentiated into the hairs, but only some of them? What is the distinguishing feature of such cells?

These and other questions are stated in detail in the works of N.A.Vlasova [20, 21]. The development and structure of seed peel, and also the processes of fibre formation were investigated for various kinds of cotton (G. hirsutum L., G. barbadense L., G. davidsonii Kell., G. trilobum Skovsted, G. herbaceum etc.). Particularly, the detailed study of 108-F G. hirsutum L cultivar has shown that a day before flowering and at the day of flowering all nuclei of epidermal cells of a seed-bud are similar by their structures. The epidermal cells are mitotically divided and there are no signs of fibre formation. And only 24 hours DPA in these concrete experiences the developing fibrils have appeared in the form of rounded protruded hemispheres. Studies of cytonuclear relations of mitotically active and differentiated epidermal cells of the seed-buds have shown that not any random cells of epidermis are differentiated into the hairs, but the cells, which have lost their mitotic activity. Before a flower blossoming and at the day of fertilization such cells had the nuclei volume of ~ 150 micrometer^3, that was twice as large as the average volume of nuclei (65 micrometer^3) of those cells of the same epidermis, which were still mitotically active. 2-3 days later the volume of nuclei of epidermal cells is decreased, from some of them (with the volume of nuclei of 27 micrometer^3) the fibrils of underdown appeared. As a result: kinetics of the appearance of the fibrils of fibre and underdown has shown that these cells are mixed with mitotically active cells [20, 21]. Their spatial localization has not been found. But occurring first fibrils have certain localization on a seed-bud. The process of fibril appearance during the differentiation of epidermal cells occurs from lateral part of a halasa to micropilar part [21]. The rate of moving

is different for some kinds and forms of cotton, that is generically caused and it can vary under the influence of environmental factors. First fibrils of the underdown appear on micropilar part and at centre of halasa, then from lateral part of a halasa and then the process moves to the middle part of a seed-bud.

In conclusion we shall note once more that the information about the process of fibre formation was obtained only on the basis of the analysis of seed-bud cuts. It has been shown that the hairs are differentiated from separate cells of an epidermis. In a plane of cut of epidermal cells the formed hairs are placed separately or close to each other. At further stages of the seed-bud development (in a day after blossoming, in two days, etc.) the appeared hairs on the cuts are found as a dense row and they often are tangled among themselves. Whether the above-stated observations of the differentiation of epidermal cells into the hairs reflect all features of the process of fibre formation? Apparently, it is not so, as the behaviour of the nearest epidermal cells, located around lengthening hair, is unknown. In order to observe the behavior of neighboring and distant cells at the given moment, it is required to photograph the surface of alive seed-bud around a cell of external epidermis, which has been differentiated into a hair. Attempt to answer the arisen question makes the contents of our paper.

MATERIALS AND METHODS

The surfaces of alive cotton seed-buds and the hairs appearing on them were investigated in the period from one day before and also from 1 to 10 days DPA. The observation were carried out on Gossypium hirsutum L., on the sorts Tashkent-1, Fergana-3, 108-F; G. barbadense L., C-6524, on coarse-fibered form Turfan Guza G. herbaceum L., and also on blackseed fibre-free cotton form A-720 G. hirsutum with the seeds bound together. Opened flowers were daily labeled at the same time (approximately at 9 hours 30 minutes on local time). Required elements of the fruits with the seed-buds, lying inside them, were cut off together with a significant part of a branch. After careful opening of the elements of the fruits the alive seed-buds from a ovary were placed on a layer of gelatine, softened in vapors of boiling water, to obtain their prints during several minutes. Thin dried gelatin layers were prepared before from its 10%' water solution by applying to the slides [7]. Then the prints of the seed-buds were observed with universal optical microscope Neophot-2 at various magnifications. At low magnifications the panoramic photographs were made of the seed-bud surfaces, with the large number of formed hairs, at high magnifications the relative positions and the details of the structures of epidermal cells and the apexes of arising hairs were investigated [8].

In addition to the surfaces of alive developing seed-buds, the surfaces of mature seeds of the same cotton sorts with mature hairs of the fibre and underdown on them were investigated. For this purpose the method of microsections was used [6]. The feature of the preparation of such mature cotton seeds, having hard seed shell and covered with abundant downiness, in our case is that the microsections of seed peel with the bases of various possible hairs prepared from the interior side of a seed. The hairs covering mature seeds were cut off by scissors, so the length of downiness became 4-6 mm. After that such seeds were placed into epoxy resin with a hardener, and the hardened resin fixed firmly the bases of the hairs on a seed peel. After final resin hardening the seeds were cut between the hairs into halasal, micropilar and middle parts using steel fretsaw. The flat pieces of each part of the seeds were fixed on adhesive backing in rows so that the interior side of a seed shell would be glued. The pieces of peel were bordered with steel tubes with 2-25 mm in diameter and 10-15 mm in height. The tubes-holders were filled inside with the epoxy resin with hardener. After solidification the holders were turned over, and the pieces of seed peel were found on top in the plane of steel holder processing. The holders were polished with diamond dust (~1micrometer) in order to remove only the seed shell and reveal the bases of mature hairs, grown on the seeds. The microsections of internal sides of a peel, made in this way, allowed to calculate the real surface density of the hairs on various parts of the seeds of each sort, [9], and also to register the features of their arrangement in the process of appearance and development on the surface of external epidermis.

RESULTS AND DISCUSSIONS

The investigations of alive seed-buds at the stage of two-three days before DPA show, that their internal surface in the process of development consists of epidermal cells, divided into the areas, which are bordered with weak, slightly deepened edges. These are the areas of clone reproduction (fig.1,a). By the day of cotton flowering, the cells of external epidermis of the seed-buds acquire slight protrusion, with precisely defined dimensions. It is especially characteristic for halasal parts of the seed-buds, where the mitotic processes are the most intensive [21]. It is characteristic for all investigated cotton sorts that in the day of DPA the cells of epidermis begin their differentiation into the hairs. The investigations demonstrate the unusual fact, that not separately observed epidermal cells are mainly differentiated into

the hairs, but closely located cells with adjoining cellular walls (fig.1,b). Such cells are usually located within the area of clone reproduction (fig.1,a) and, apparently, they just have got out from the process of mitosis. During the day and night such paired and single differentiating cells sharply increase their dimensions (fig.1,c), transforming into hemispheres with epidermal cell as a basis. Simultaneously due to the growth and increasing seed-bud dimensions, they look like moving off from each other.

It should be noted, that the mass differentiation of epidermal cells of a seed-bud into the hairs veils, shades clear features of appearance of the hairs as paired and single protrusions. Only the technique of prints from surfaces of alive developing seed-buds allows avoiding this defect in study of initial stages of originating hairs. It is possible to prepare panoramic photographs almost from a half of total surface of future seeds (fig.1,d). Thus, if on the microphotograph of a seed-bud (fig.1,d) the arbitrarily chosen area with the dimensions of 1 cm^2 42-45 cells-hairs are observed (taking into account optical magnification), then on the panoramic image about 2000 appeared hairs are visualized. It is clearly seen in fig.1,d that halasal and middle parts of a seed-bud are entirely covered with differentiated cells. Only micropilar part of a seed-bud is free of protruding cells. Under high optical magnification on the obtained prints-replicas both single, and paired protruding of epidermal cells on a surface of developing seed-bud are clearly observed. Moreover, there is the tendency to form various geometrical figures

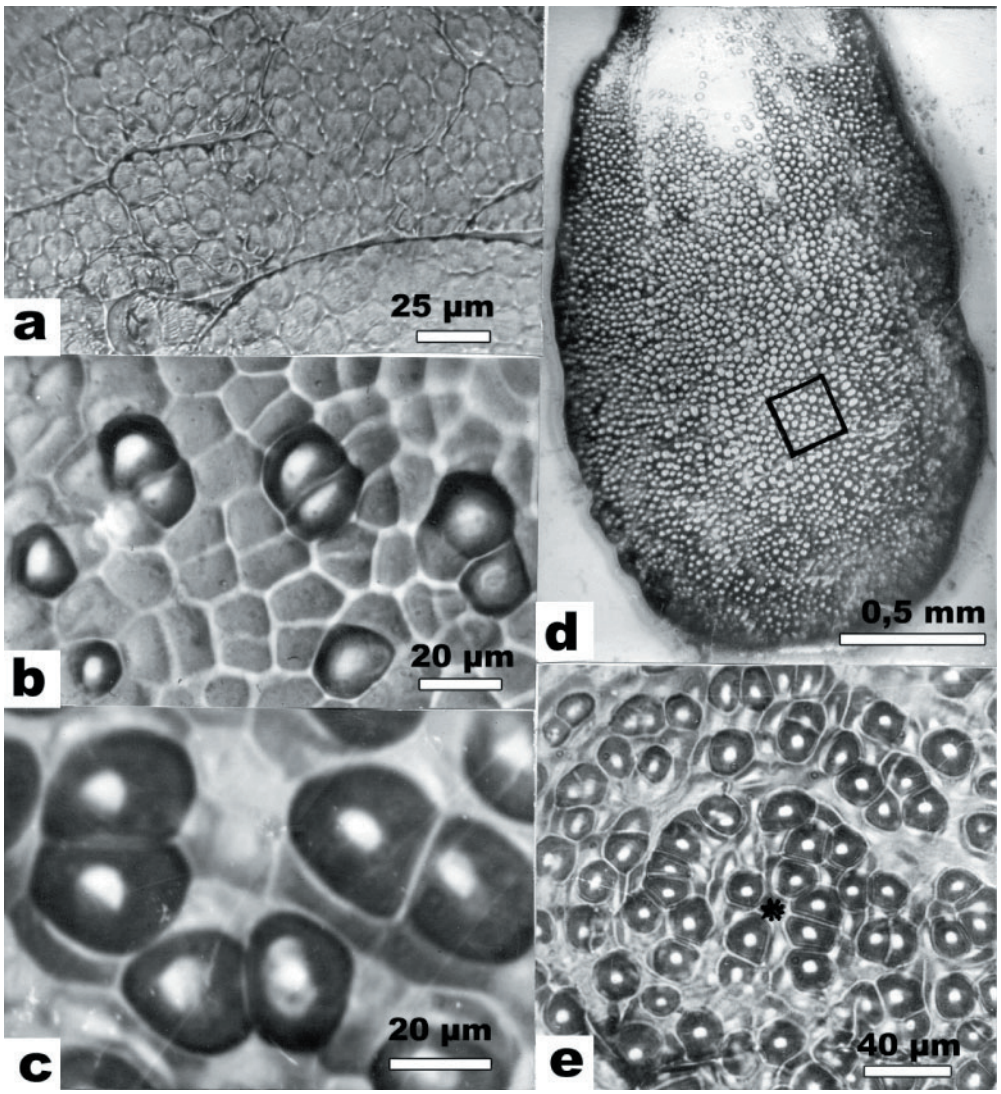

Fig.1. Microphotographs of alive cotton seed-ovule at difference stage of developing (explanations in the text body)

of differentiated cells, namely: direct lines, extended arches, circles. It is evidently demonstrated in fig.1,e. the protruded cells-hairs are grouped as though round the circumferences about the point, which is conditionally placed at the centre of microphotograph fig.1,e and designated as the asterisk.

Further the protruded epidermal cells are lengthened, occupying free space inside a fruit-boll, reach its internal surface, and turn back in the opposite direction, being packed into the layers and forming complex volumetric structure of cotton lobule [12]. A lobule of raw cotton depending on kind and sort can consist of 5-7-9 and 11 developing seed-buds and the hairs appear on each of them according to a scheme, found out by us. During intensive fibre formation and cyclic lengthening, 5-7 and more days DPA, it is practically impossible to obtain any information about the character of hair bases arrangement [7,8]. However, it is possible to clear up the final character of an arrangement of the hairs on a surface of well developed seed-bud using the method of cotton seed preparation described in the corresponding section of our paper [6].

The question itself on final character of an arrangement of the hairs on well developed cotton seed is of fundamental importance. It can corroborate or supplement the information about the features of epidermal cell differentiation into the hairs at early and subsequent stages of seed-bud development, and also substantiate

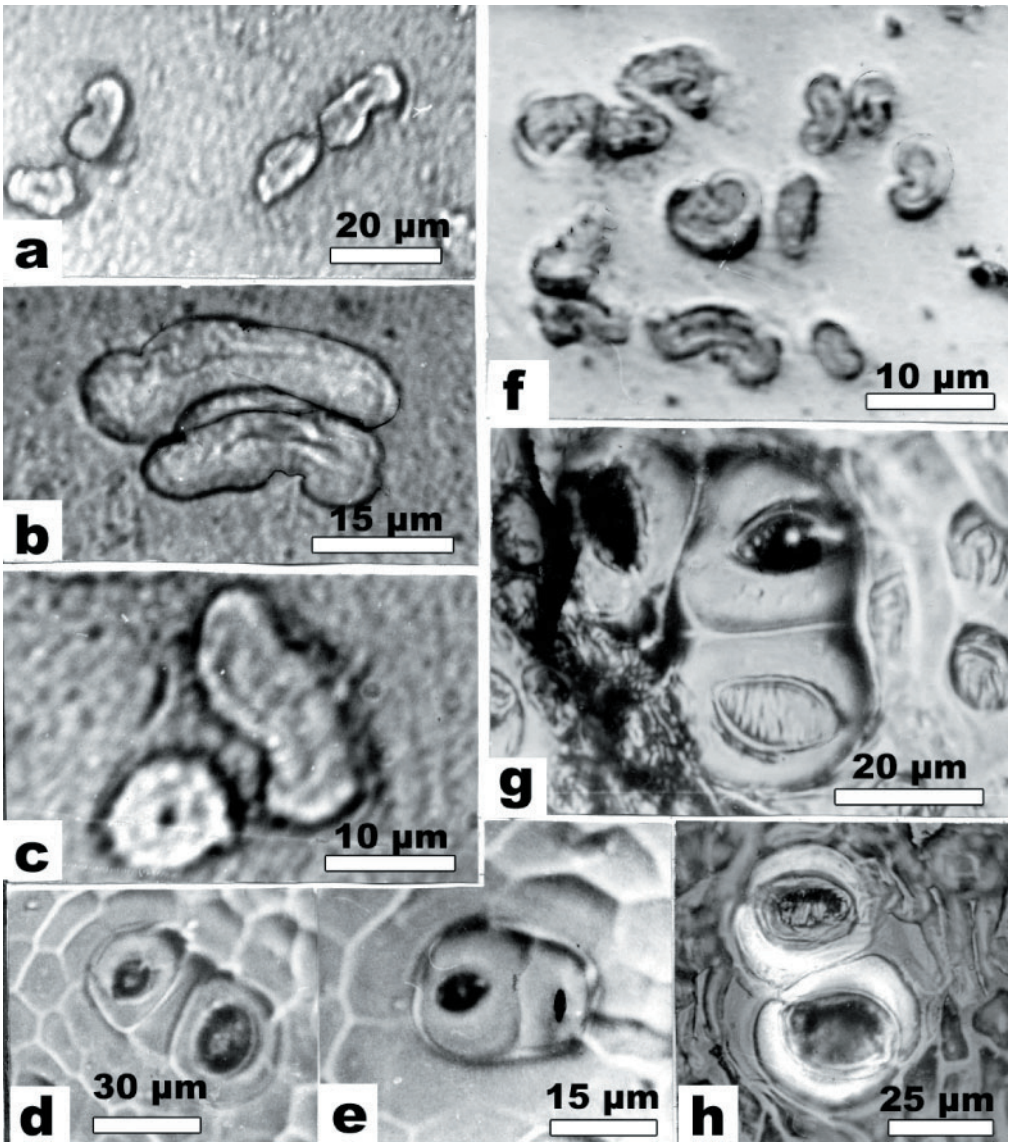

Fig.2. Investigation of mature cotton seed surface by micro section method (explanations in the text body).

in general the data on cotton fibre yield. The results of investigation of the microsections of mature seed peel for all cotton sorts have exceeded all expectations (fig.2). All the data on the phenomenon of formation of both paired, and single protrusions of differentiated epidermal cells (fig.2,a-c) are completely proved to be true. On the microsections of the seed peel the arrangement of the bases of developed hairs in the lines, arches, circles (fig.2,f) is observed. In the most observed cases of such arrangement as visible regular figures, the numbers of the hair's bases in them are divisible by 2. The same tendency – simultaneous differentiation of two cells into the hairs – is also characteristic for the cells of epidermis, from which the stomata should be formed (fig.2,d,e,g,h). The stomata on the epidermis of the seed-buds are known to be the most important part of vegetative organ; they serve as the functions of gaseous-water exchange for the seed-buds in closed volume of a boll [3, 21]. They make a contribution to the intensification of the processes of hair growth and biosynthesis of cellulose in them. Both single and densely adjoined paired stomata are formed out of epidermal cells of the seed-buds. Stomata can gather in small groups of 3-4 and more pieces. The single stomata are mostly arranged arc-wise or by a circle. It has been observed, that the frequency of formation of the paired, or tandem-type stomata increases for hybrid cotton sorts, which are obtained by crossing, especially by crossing of parental forms, which are dissimilar in genetic relation.

The tendency of formation of paired stomata on the seed-buds is the most characteristic for uncultivated cotton sorts, as for low-fuzzy, and for blackseed fibre-free cotton forms. For example, the last A-720 cotton sort (catalogue index of the Institute of Experimental Biology and Cotton Genetics Acad. Sci. of Uzbekistan) with the seeds bound together. On their halasal parts, where the intensive processes of mitosis of epidermal cells are observed, the frequency of formation of paired stomata is 0,08-0,1 of their observable quantity. For cultivated middle-fibered cotton sorts, such as Tashkent-1, 108-F, C-6530, C-6524, Fergana-3 and others the observable frequency of appearance of paired stomata in halasal part of the seeds is small, about 0, 04 and less of found functioning stomata.

It is possible to consider the found out tendency of paired, coupled, tandem differentiation of epidermal cells of cotton seed-buds into the hairs and into the stomata, as natural consequence of the processes of mitosis (division) and specialization of the cells of organisms, continuously proceeding in vegetative space.

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