MULTICELLULAR ROOT HAIRS ON ADVENTITIOUS ROOTS OF *KALANCHOE FEDTSCHENKOI*[1]

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In the course of examining root tips of various greenhouse plants, freehand sections were made of the stiff, pink-colored aerial adventitious roots of *Kalanchoe fedtschenkoi*. Upon examining sections of these roots, structures resembling multicellular root hairs were observed. Since we were unable to discover an account of multicellular root hairs in recent botanical literature or a textbook of botany, we decided to look into the matter further.

Dittmer (1949) states that he has never seen a septate root hair although he examined "many millions of root hairs on hundreds of plants". He does mention that some of the earlier workers reported septate root hairs but that the structures described were judged by "many workers since" to be fungal hyphae. A rather thorough survey of the literature failed to yield the name of a single specific plant for which multicellular root hairs are claimed.

We at first speculated that the structures which superfically looked very much like aerial adventitious roots of *Kalanchoe* might in reality be abnormal leafless branches with trichomes. However, if they turned out to be adventitious roots we surmised that they might possibly have arisen exogenously as do the adventitious roots of *Radicula aquatica*, in which case the hairs might also be out-growths of cells of the epidermis of the stem. In water cress, epidermal cells of the stem in the vicinity of the exogenous root primordium become meristematic and form a continuous layer of epidermis over the entire root primordium. Both of these possibilities were investigated even though we found no epidermal hairs of any kind on the smooth stems of *Kalanchoe fedtschenkoi*.

MATERIAL AND METHODS

Adventitious root primordia and many sizes of adventitious roots including branched roots seven or more inches long were studied. Fresh freehand sections as well as microtomed transverse and longitudinal sections were prepared. Pieces of aerial adventitious roots preserved in FPA (5 ml. formalin, 5 ml. propionic acid, 90 ml. 50 percent ethyl alcohol) were dehydrated in an ethyl alcohol series and embedded in a special paraffin embedding mass (Popham, 1947) using toluene as the vehicle for infiltration. Microtomed sections were cut at 10 μ and were stained with safranin and anilin blue according to a procedure described by Popham *et al.* (1948).

ROOT APEX ZONATION

An examination of transverse sections of the aerial adventitious roots revealed a rather large pith, xylem (usually 10 to 15 areas) and phloem in radial polyarch arrangement, a pericycle of 1 to 3 cell layers, a uniseriate endodermis, 3 to 5 layers of cortical parenchyma, a uniseriate hypodermis which is poorly differentiated from cells of the cortical parenchyma, and a uniseriate epidermis. Neither stelar cambium nor cambium initials were observed. Anthocyanin was clearly visible in cells of the cortex of fresh, freehand transverse sections.

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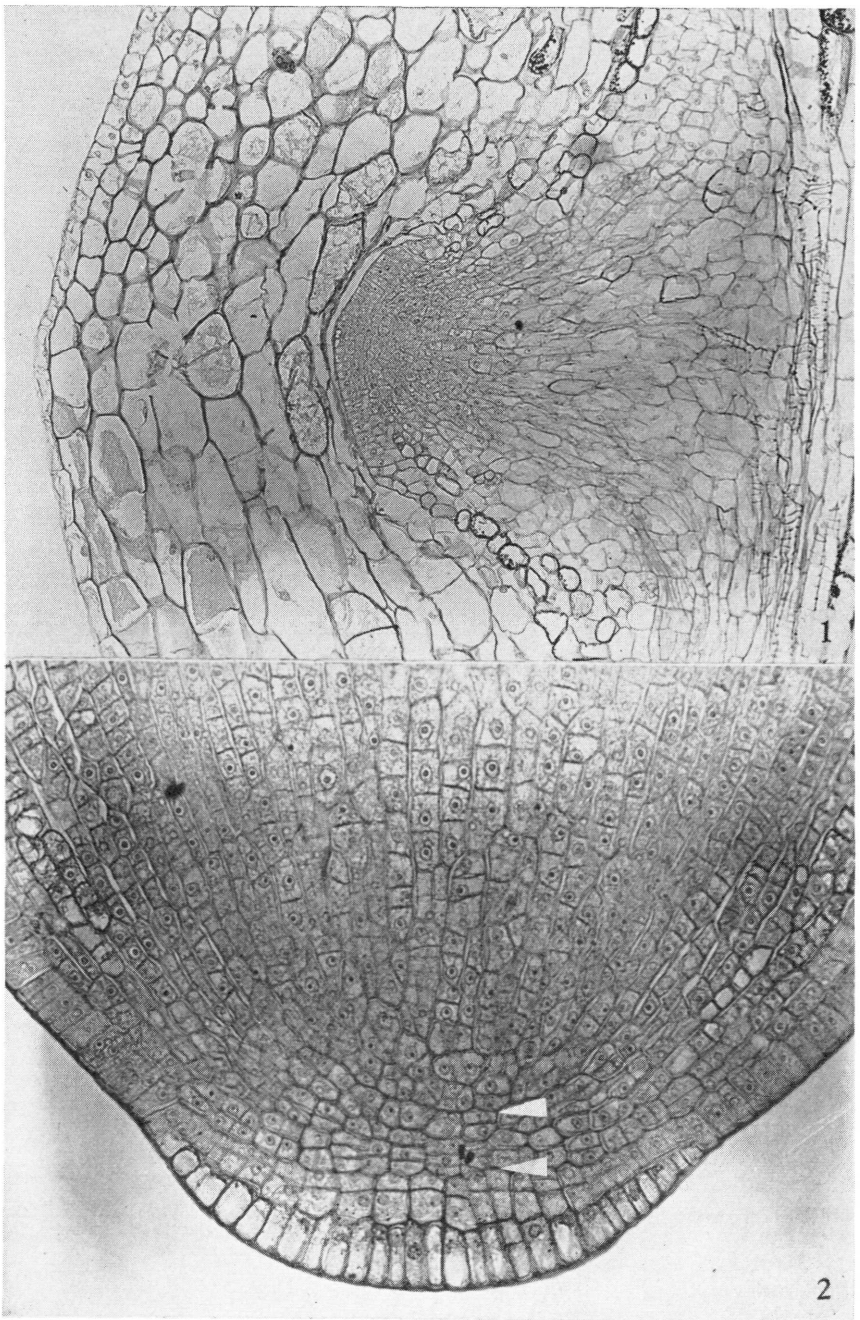

FIGURE 1. Longitudinal section of stem and aerial adventitious root illustrating the endogenous origin of the latter. X120.

FIGURE 2. Median longitudinal section of aerial adventitious root tip illustrating Janczewski's type III, *Convolvulus* sub-type, of zonal organization in the promeristem. The plerome lies just above the top indicator marker, the several-layered periblem lies between the two markers, and the dermato-calyptrogen lies just below the lower marker. X330.

In median longidutinal sections of the apex we found an unmistakable root apex organization (fig. 2). Three histogens[2] were observed: (1) a plerome, (2) a periblem, and (3) a dermato-calyptrogen. This zonal pattern is, in general, the same as that frequently designated as the common dicotyledenous type, Janczewski's (1874) type III. However, the cortex of *Kalanchoe fedtschenkoi* originates from two or more (usually more) layers of cells (periblem) lying between the plerome and the dermato-calyptrogen (fig. 2). The hypodermis does not originate from a separate histogen.

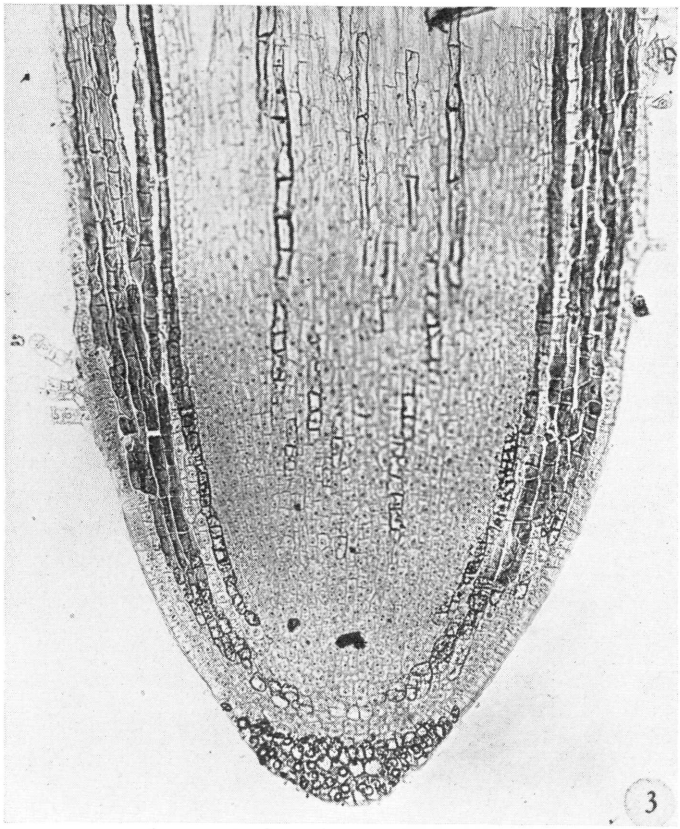

FIGURE 3. Longitudinal section of aerial adventitious root tip with multicellular root hairs within 380 μ of the tip cell of the root. Notice that a root hair in an early stage of formation is farther from the tip than those composed of many cells. X130.

In describing his type III zonation of roots, Janczewski states that the cortex of *Helianthus* and *Fagopyrum* originates from one or more cells disposed in a *single* layer (periblem). He regarded the root promeristems of *Linum usitatissimum* as representing a sub-type of this general zonal organization. In *Linum* the cortex originates from two layers of cells, the outer of which gives rise to the hypodermis exclusively. Although the general zonal pattern is the same for *Kalanchoe* as for *Helianthus* and *Linum*, the multi-layered condition of the periblem

[2]Some anatomists seem to feel that the use of Hanstein's histogen terminology and Janczewski's typology imply a constancy of root apex zonation for the individual plant and/or the species. Be this as it may, we have used their terms solely as a convenient means of describing what we observed.

places the root of *Kalanchoe* in a separate and second sub-category of Janczewski's type III.

Just such a sub-category was described by Eriksson (1878) and was named the *Convolvulus* sub-type. Janczewski's type III and his *Linum* sub-type were recognized by Eriksson (1878) although he referred to them as type I and the *Linum* sub-type. The *Convolvulus* sub-type, which Eriksson discovered, is characterized by a periblem consisting of three to several superposed layers of cells. The cells of the outer layer are described as dividing anticlinally and only occasionally periclinally. Root apices of the following plants were found by him to exhibit this *Convolvulus* sub-type of zonal organization: *Hoja carnosa* (*Asclepiadeae*); *Abutilon molle*, *Pavonia weldini*, *P. spinifex* (*Malvaceae*); *Villarsia nymphaeoides* (*Menyantheae*); *Convolvulus cneorum* (*Convolvulaceae*); *Begonia nelumbifolia*, *B. ricinifolia*, *B. glacialis* (*Begoniaceae*).

Neither Janczewski (1874) nor Eriksson (1878) recorded observations on a species belonging to the *Crassulaceae*. Adventitious root primordia of *Sedum sieboldtii* and *Bryophyllium calycinum* as well as lateral root primordia of *Sedum acre* and *S. maximowiczii* were studied by Van Tieghem and Douliot (1888). They described them as having the common dicotyledenous type of root zonation with a periblem composed of a single cell or a single layer of cells. The same type of organization was seen by Koch (1874–77) in root apices of *Sedum spurium* and *S. album*. In the root apex of another plant (*Umbilicus horizontalis*) belonging to the *Crassulaceae*, Flahault (1878) observed and described a zonal organization like Janczewski's type III. The periblem in the promeristem of *Umbilicus* is a single layer of cells.

We were unable to discover a description in the literature which claimed Eriksson's (1878) *Convolvulus* type of zonal organization for the root apex of any species belonging to the family *Crassulaceae*.

ORIGIN AND GROWTH OF THE MULTICELLULAR ROOT HAIRS

After thus completely satisfying ourselves that the structures observed on *Kalanchoe* aerial stems were roots, we turned our attention to the problem of ascertaining whether the root hairs arose from a bonafide root epidermis or whether they arose from cells of stem epidermis overlying the root apex proper.

Serial transverse and longitudinal sections were made of portions of stems on the surface of which slight protuberances were observed. After sectioning many such stem pieces, we were able to assemble an intergrading series of longitudinal sections of adventitious root primordia and adventitious roots whose apices had grown several millimeters beyond the epidermis of the stem. All of these sections showed evidence of an endogenous root origin (fig. 1). The adventitious roots probably arise in phloem parenchyma although this point was not surely confirmed. In any event, the origin is internal to the cortex since the layers of cortex are compressed and subsequently punctured as the root primordium enlarges and elongates. In every case where the root apex was found protruding beyond the epidermis of the stem, the epidermis was clearly broken and showed no evidence of continuity with that of the root.

One of the facts that had originally made us doubtful as to whether we were observing bonafide root hairs was their occurrence at levels extremely close to the root tip, even on roots several centimeters long. Many were found as close as 380 μ back of the tip cell of the root cap (fig. 3); some were seen closer to the root tip. Many persisted as long as the epidermis remained firmly attached to the root but hairs composed of living cells were usually found less than 1 cm. from the root tip.

The first sign of root hair formation was the radial elongation of one of the typically radially elongated cells of the epidermis. Accompanying the radial elongation of the trichoblast was an expansion of the outer end of the cell and

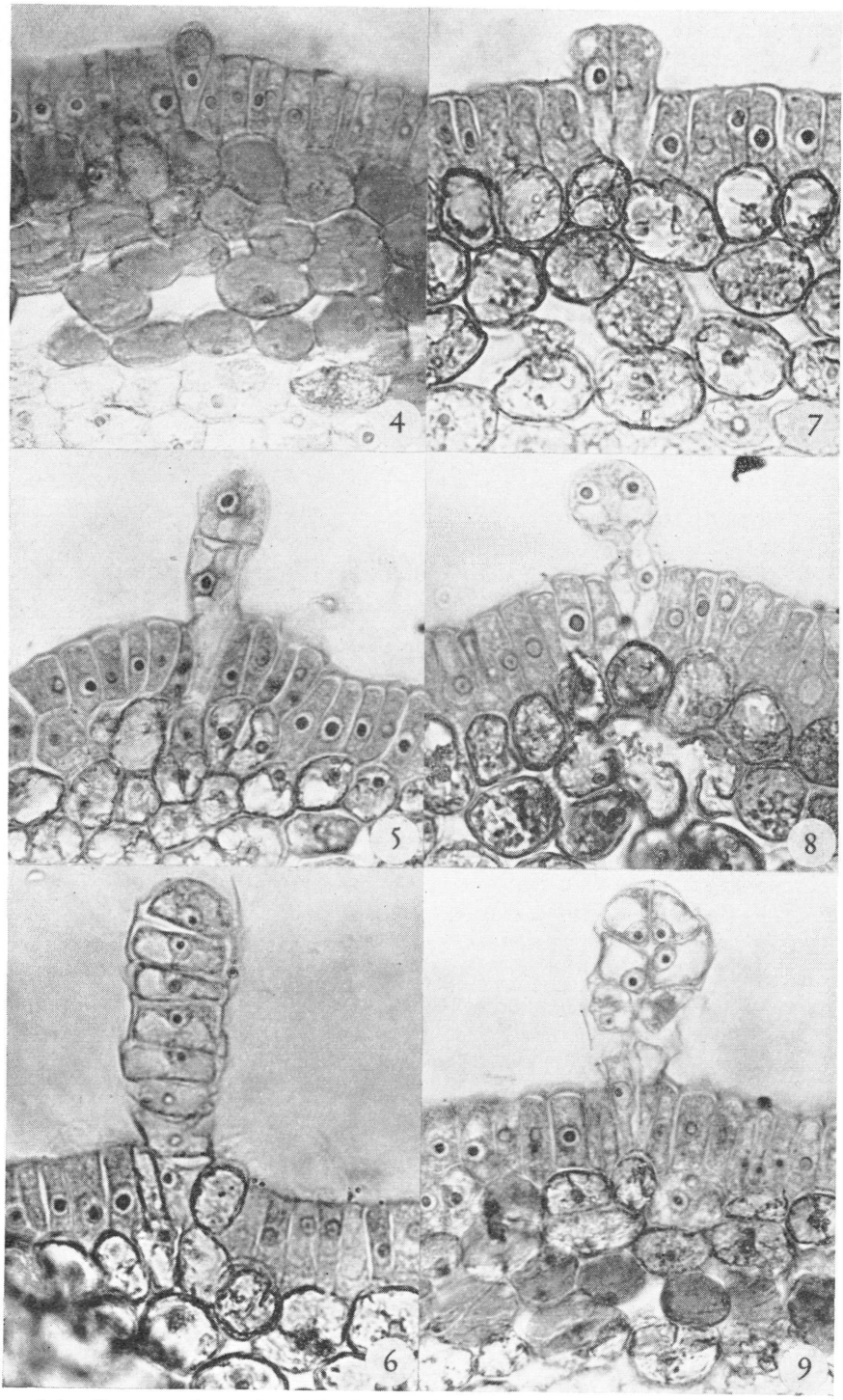

FIGURE 4–9. Cross sections of aerial adventitious roots illustrating stages in the formation of uniseriate and biseriate multicellular hairs. X650.

the contraction of the end of the cell adjacent to the hypodermis (fig. 4). During these early phases of root hair formation the nucleus retains an approximately central location in the cell (fig. 4, 7). In contrast to typical root hair formation during stages of which a *portion* of the free wall bulges, the *entire* free wall of the multicellular root hair initial was forced out into a dome. Concomitantly the nucleus moved outward toward the bowed surface of the cell. After becoming conspicuously more radially elongated and vacuolated than neighboring epidermal cells, the trichoblast divided either transversally (fig. 5) or longitudinally (fig. 7). In either event enlargement of the two cells formed was followed by one or several successive transverse divisions in the one (fig. 6) or the two (fig. 8 ,9) tiers of cells. Transverse wall formation in adjacent cells nearly always occurred at the same level and simultaneously resulting in two tiers of nearly equal size corresponding cells. It was inferred that growth of the hairs was due to divisions occurring in the terminal cell of each tier although confirming mitotic figures were not observed. On older portions of roots, hairs consisting of two tiers of cells sometimes split throughout one-fourth to three-fourths of their length. This appeared to be due to the dissolution of the middle lamellae holding cells of the two tiers together although no microchemical tests were conducted to verify this thesis.

Few hairs attain lengths greater than 75 μ. Due to the short maximum length and due to the fact that the maximum length is rapidly attained, zones composed of root hairs of decreasing lengths and ages, such as those often seen at root tips, are not evident in *Kalanchoe fedtschenkoi* (fig. 3).

HAIRS OF AERIAL VS. SUBTERRANEAN ADVENTITIOUS ROOTS

It appears somewhat incongruous that multicellular root hairs have not been found to occur on adventitious as well as on primary and lateral roots of plants having multicellular stem hairs. Most botanists will probably agree that anatomical form is controlled by and is the result of the combined influences of genes and the environment to which cells are exposed. If we wish to assume that the gene complements of cells of roots and stems of a plant are identical, differences in stem vs. root epidermal hair structure for any particular plant must be attributed to differences in the environment to which the cells are exposed. It would seem that occasionally, at least, epidermal cells of roots would be exposed to environmental conditions identical, or nearly so, to those of stem epidermal cells. It is therefore interesting that this first documented occurrence of multicellular root hairs is reported for *aerial* adventitious roots whose initiating epidermal cells are exposed to environmental conditions similar to those of stem epidermal cells. Hairs of the aerial roots of *Kalanchoe fedtschenkoi*, whether original adventitious roots or lateral roots arising from them, are multicellular. It is equally noteworthy, but not astonishing, that roots of the adventitious root system growing in the *soil* were found to have *unicellular* hairs. We did not observe multicellular hairs on any subterranean root, although we did not conduct an exhaustive search for them.

The adventitious root system of *Kalanchoe fedtschenkoi* would seem to afford experimental material for a new avenue of approach to the solution of a fundamental problem of living organisms, namely, what combination of environmental factors is responsible for the initiation of the process of mitosis?

SUMMARY

The zonal organization of the aerial adventitious root apex is described as conforming to Eriksson's *Convolvulus* sub-type of Janczewski's common dicotyledonous type III. A plerome, a several-layered periblem, and a dermatocalyptrogen are clearly defined in the root promeristem.

It was found that true multicellular root hairs arise from epidermal cells of aerial adventitious roots of *Kalanchoe fedtschenkoi*. The hairs were found to be uniseriate or biseriate. They persist until the epidermis separates from the root, although hairs composed of living cells are usually found only on the tip 1 cm. of the roots.

Hairs of adventitious roots growing in the soil are most commonly or always unicellular and look very much the same as hairs of many other roots.

It is suggested that the adventitious root system of *Kalanchoe fedtschenkoi* may provide suitable material for a new approach to investigations aimed at discovering the combination of environmental factors responsible for the initiation of mitosis.

Since completing this paper, we have found multicellular root hairs, similar to those described, on the aerial adventitious roots of *Kalanchoe somaliensis*, *K. daigremontiana*, and an unidentified *Kalanchoe* species.

In addition, two papers (Haberlandt, 1915 and Jurišić, 1934) dealing with glandular hairs ("Drüsenhaare") of roots were found. Diagrams accompany the former but not the latter paper. Descriptions of the hairs indicate that the structures which these authors saw on adventitious roots of several plants [*Bryophyllum pinnatum* and *B. crenatum* (Haberlandt, 1915); *Bryophyllum proliferum*, *B. verticillatum*, *B. daigremontianum*, and *B. pinnatum* (Jurišić, 1934)] are very similar to those described in this paper. These two papers have apparently been generally overlooked.

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