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THE ULTRASTRUCTURE OF THE ROOT CAP IN CULTURED ROOTS OF CONVOLVULUS ARVENSIS L.[1]

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ABSTRACT

The ultrastructure of root cap cells of cultured roots of *Convolvulus arvensis* L. was studied in order to relate sequential changes in the fine structure with known changes in morphology and physiology determined earlier by light microscopy and autoradiography. The rapid structural changes observed in columella cells formed from the root cap initials distal to the quiescent center can be related to the function of these cells as sensors of geotropic stimuli. Even more rapid structural changes in cells of the root cap periphery can be ascribed a functional role in protecting the columella and initial cells as the root is displaced through the soil. Mechanisms are discussed whereby cell division and differentiation in the root apex may be controlled by hormones in the apex.

EXPERIMENTAL and ultrastructural research on the root cap of intact seedling roots has shown that sequential changes occur in the differentiation of geotropically sensitive cells of the root cap columella and that these cells differ physiologically from other cell populations in the root cap (Clowes and Juniper, 1964; Griffiths and Audus, 1964; Northcote and Pickett-Heaps, 1966; Dauwalder, Whaley, and Kephart, 1969; Iversen, 1969; Juniper and Barlow, 1969; Juniper and French, 1970).

Previous experimental research in this laboratory has confirmed that physiologically distinct cell populations occur in the root cap of cultured roots of *Convolvulus* (Phillips and Torrey, 1971a, 1972). In addition, in the development of cells of the columella from the root cap initials one of the early events appears to be the termination of any further DNA synthesis (Phillips and Torrey, 1971a). Studies on the amounts of DNA within cells of the root cap of intact roots of *Zea* support the idea that DNA synthesis is terminated early in cells derived from the root cap initials (Clowes, 1968; Barlow, 1970).

A study of the ultrastructure of the root cap of cultured roots of *Convolvulus* was initiated to determine the cellular events involved in the differentiation of geotropically sensitive cells and to compare these cells with other differentiating cell populations in the root cap and in the root proper.

MATERIALS AND METHODS—Root tips of cultured roots of *Convolvulus arvensis* L. were grown and prepared for electron microscopy as described earlier (Phillips and Torrey, 1971b and 1974). At the time of fixation, the roots were growing in liquid medium and showed the typical geotropic tip curvature described by Tepfer and Bonnett (1972). Fixation was rapid, but no special precautions were taken to assure exact localization of organelles within the root cap cells.

The different cell populations examined in the root cap were the root cap initials and their derivatives, the root cap columella, and the root cap periphery (see Fig. 1 in Phillips and Torrey, 1974). The root cap initials included those cells of the first tier which are located immediately distal to the root cap junction and which give rise to the cells of the central columella. The columella starts at the second tier distal to the root cap junction and extends as linear files of cells through the central portion of the root cap to the root tip. The portion of the root cap periphery discussed in this paper denotes the initial cells and the derivatives that form the peripheral portion, starting at the level of the apical initials and distal to the root cap junction.

RESULTS—*Root Cap Initials*—The cells of the quiescent center and the root cap initials are separated by a cell wall layer, designated the root cap junction (rcj), which is considerably thicker than other walls in the area (Fig. 1). The thickness of this wall indicates that cell division among the cells of one region does not contribute cells to other regions.

The root cap initial cells in the first tier immediately distal to the root cap junction are large and rectangular. The longitudinal walls (lw) are slightly thicker than the transverse walls (tw) of adjoining, successive root cap cells. The cells of the next three, more distal tiers have longitudinal walls that are slightly thicker than the transverse

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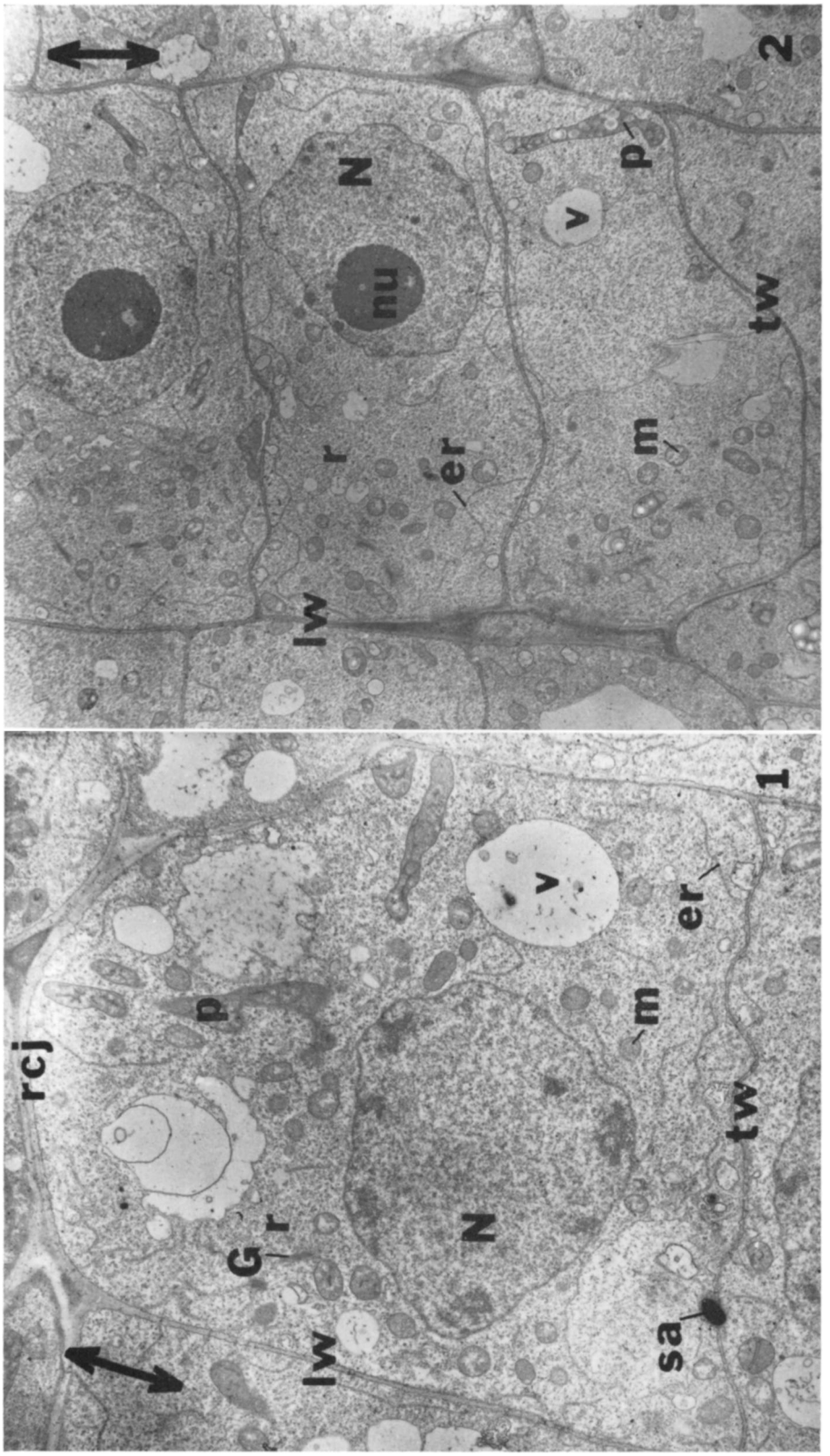

Fig. 1–2. Electron micrographs of root cap initials and derivatives. **1.** Large initial cell in the first tier of the root cap. Note the abundant mitochondria (m), the undeveloped plastids (p), free ribosomes (r), the fragmentary endoplasmic reticulum (er), the heterochromatic regions of the nucleus (N), the relatively thin longitudinal (lw) and transverse (tw) cell walls, and the thickened root cap junction (rcj) separating the root cap initials from the quiescent center. *G*, Golgi body; *N*, nucleus; *sa*, stain artifact; *V*, vacuole. The double-headed arrow indicates the direction of the longitudinal axis of the root. × 6970. **2.** Cells of the root cap columella found in the second through fourth tiers distal to the root cap junction. Note the relative absence of vacuolation in these cells and the formation of starch grains in the plastids as well as the abundant ribosomes and mitochondria, the sparse endoplasmic reticulum, the lightly staining nucleolar vacuoles of the nucleolus (nu), and the Golgi bodies. Note also the slightly thicker longitudinal walls. See Fig. 1 for key to labels. × 4120.

walls (Fig. 2). Plasmodesmata occur in both the longitudinal and transverse walls of these meristematic cells but cross the thick root cap junction only infrequently.

The cytoplasm of the cells in the first four tiers contains abundant free ribosomes (r), some endoplasmic reticulum (er), undeveloped plastids (p), mitochondria (m), and Golgi bodies (G). The enlarged initial cells in the first tier are more highly vacuolated than the cells in the next three tiers, which contain only a few, small vacuoles (v).

In the cells in tiers 2–4, the plastids begin forming small starch grains (Fig. 2). In cells in the fifth tier and in tiers further displaced distally, the starch grains occupy most of the space within the plastids.

The nuclei of these cells contain small regions of heterochromatin, primarily near the nuclear periphery. Each nucleus (N) has a prominent, darkly stained nucleolus (nu) or nucleoli which contain many small nucleolar vacuoles and regions with lightly stained material, as well as an associated karyosome. Cell division figures are present only in the first four tiers of cells which give rise to the root cap columella. The highest frequency of cell division is in the first tier.

Root Cap Columella—The cells of the central columella are rectangular and elongated along the longitudinal axis of the root up to four times the original length of the initial cells from which they were derived (Fig. 3). Both the longitudinal and transverse walls are relatively thin. Plasmodesmata are more frequent in the transverse walls.

Although more so than cells of the first four tiers, the columella cells are not highly vacuolated. The cytoplasm stains much less intensely and is less finely granular than the cytoplasm of the initial cells, indicating that these cells contain far fewer ribosomes per unit area. Golgi bodies and mitochondria are the main cytoplasmic organelles other than the large plastids. Very little endoplasmic reticulum is present in these cells. The plastids are packed with darkly stained starch grains (s) and are displaced distally in the cell. The nucleus of each cell occupies only a very small portion of the cell volume.

Columella cells further displaced and located near the distal end of the root cap have evenly thickened walls (Fig. 4). These distal columella cells have the thickest walls of any cell in any region of the root apex other than those located outside of the columella near the surface of the root cap periphery. Plasmodesmata (single arrows) are numerous on the transverse walls, but very few occur in the longitudinal walls. In addition, the cells appear to be secreting a non-staining material (mu) between the plasmalemma and the inner cell wall which is similar to previously reported polysaccharide mucus (Northcote and Pickett-Heaps, 1966; Juniper and Pask, 1973).

The cytoplasm of distal columella cells stains somewhat more intensely than that of central columella cells. Mitochondria, Golgi bodies, and strands of endoplasmic reticulum are found in the cytoplasm along with abundant ribosomes. As in the central columella cells, the most conspicuous organelles are plastids packed full of starch grains and typically displaced toward the distal end. These cells contain several small vacuoles. The nucleus continues to have darkly stained heterochromatin areas at its periphery.

Root Cap Periphery—The cell files considered as part of this region are oriented more or less perpendicular to the longitudinal axis of the root. The terms "longitudinal" and "transverse" therefore are used to denote the relative orientation of the cells with respect to this axis.

Initial cells of the root cap periphery are located adjacent to the root cap junction and surround the initial cells of the columella at the same level (Fig. 5). Whereas initial cells distal to the quiescent center give rise by periclinal divisions to cells which constitute the root cap col-

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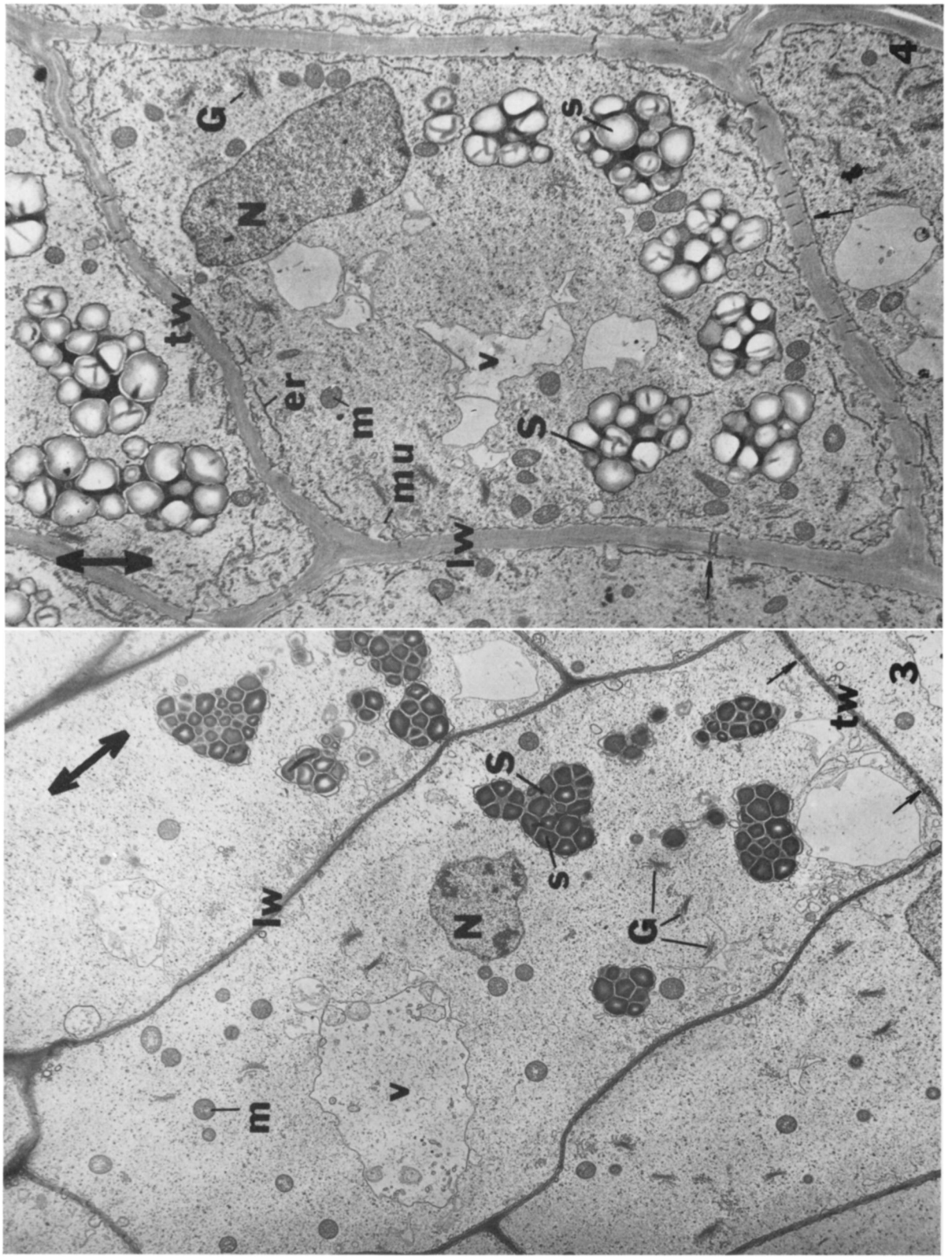
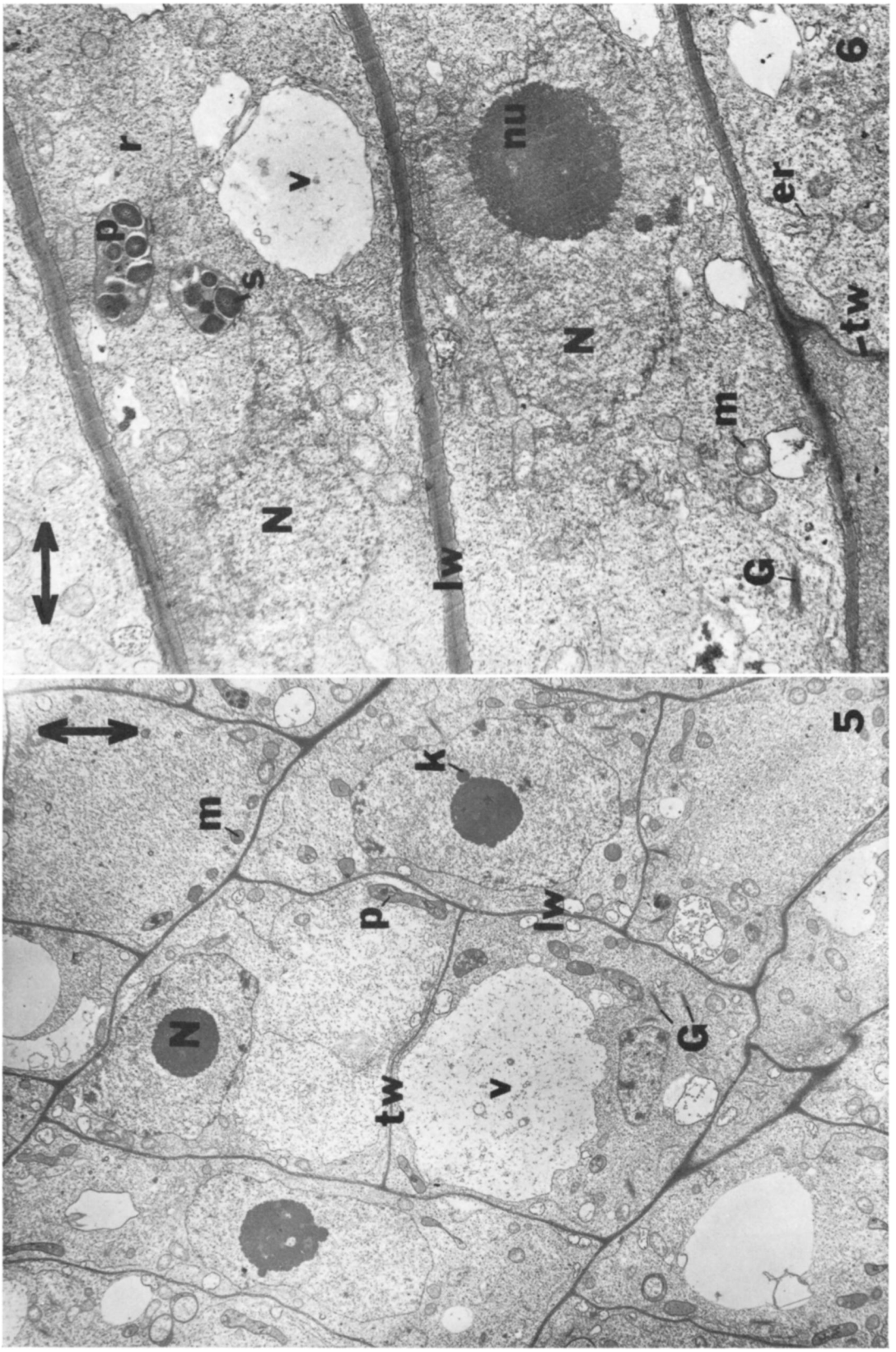
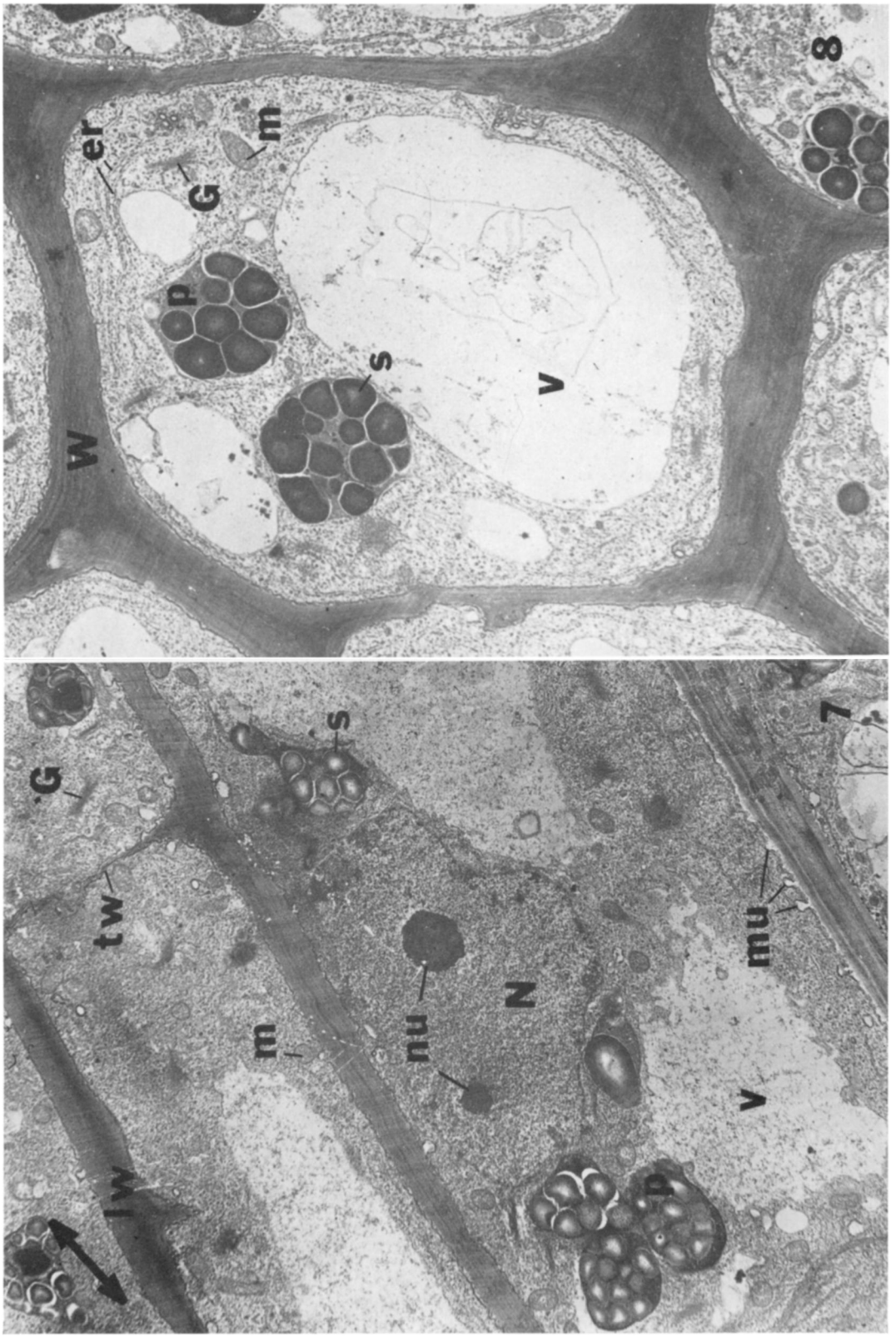
Fig. 3–4. Cells of the root cap columella. **3.** Highly elongated cell of the central columella of the root cap. Note the lack of cytoplasmic staining, the numerous Golgi bodies, and the large plastids, or statoliths (S), packed full of starch grains (s) and located at the distal end of the cell. Also note the relatively thin cell walls. Single arrows, plasmodesmata. See Fig. 1 for key to labels. × 4440. **4.** Root cap columella cell located near the distal end of the root cap. Note the highly thickened cell walls and the greater number of plasmodesmata (single arrows) crossing the transverse walls. Also note the increased cytoplasmic staining, the numerous Golgi bodies, the sparse endoplasmic reticulum, the large plastids with starch grains, and the sites of accumulation of polysaccharide-rich material (mu) between the plasmalemma and the cell wall. See Fig. 1 for key to labels. × 5095.

Fig. 5–6. Cells in the root cap periphery. **5.** Cells located in the third through fifth tiers distal to the root cap junction (located to the left of this picture) in the root cap periphery. Note the large vacuoles, the abundant free ribosomes, the abundant mitochondria, Golgi bodies, the thin cell walls, the sparse endoplasmic reticulum, and the lightly stained nucleolar vacuoles and the karyosome (k) of the nucleolus. See Fig. 1 for key to labels. × 3780. **6.** Portion of a highly elongated cell located in the root cap periphery. Note the thickened longitudinal walls, the thin transverse walls, the abundant ribosomes, the small vacuoles, the lobed nucleus with a large nucleolus, and the plastids with small starch grains. See Fig. 1 for key to labels. × 7000.







umella, these initial cells form a separate population of cells which is found in the root cap periphery. Some of these cells are derived from periclinal divisions and others from anticlinal divisions. The transverse walls of the cells in the first two tiers of the root cap periphery are slightly thicker than the longitudinal walls, although both are relatively thin. In cells further displaced, the relative thickness of the walls depends on the plane in which further division occurs. In general, the walls in the first five tiers are relatively thin (Fig. 5), and the cells in these tiers are generally quite highly vacuolated. Mitochondria, Golgi bodies, numerous free ribosomes, and undeveloped plastids are found in the cytoplasm. The nuclei are similar in morphology to those of the columella initials.

As cells are displaced further into the root cap periphery, they become as long as columella cells. The longitudinal walls become much thicker, whereas the transverse walls show little or no change in thickness (Fig. 6). The granularity of the cytoplasm in these cells indicates that they are rich in free ribosomes; their nuclei often appear lobed. The only other major change is the production of starch grains in plastids. Unlike those in the root cap columella, the plastids in this region appear to be randomly scattered throughout the cells.

Cells located several layers from the surface of the root cap show a major thickening of the longitudinal walls (Fig. 7, 8). The transverse walls still remain relatively unthickened. The plastids are enlarged because of the continued production of starch grains, but they remain randomly scattered. The cytoplasm is rich in ribosomes even though the cells contain large vacuoles.

DISCUSSION—In *Convolvulus* the root cap is separated from the root proper by a thick cell-wall boundary, the root cap junction. The presence of such a boundary indicates that little or no interchange of cells occurs between the root cap and the main part of the root. A few plasmodesmata crossing the root cap junction can be observed in micrographs, suggesting some intercellular association across the junction. Leech, Mollenhauer, and Whaley (1963) noted in *Zea mays* the occurrence of intercellular connections, either individually or in groups, between cells in the root proper and in the root cap. Clowes and Juniper (1964) also reported the presence of a thick root cap junction between the two regions in *Zea mays*. Profiles of endoplasmic reticulum seemed to be associated with these intercellular connections.

The cells of the root cap columella are formed by periclinal divisions of the cells in the first four tiers. Cell division occurs at a more rapid rate in initial cells in the first tier than in cells in the next three tiers. Cells further displaced do not undergo further DNA synthesis or cell division (Phillips and Torrey, 1971a, 1972). The major changes that occur sequentially in a cell file are as follows: increases in the thickness of the walls, radial expansion and elongation, formation of starch grains in plastids, decreased vacuolation, and eventually, the accumulation of polysaccharide material between the plasmalemma and the cell wall.

The initial cells have thicker longitudinal than transverse walls. This difference is the result of divisions that produce new, thin transverse walls and do not affect any changes in the longitudinal walls—other than to thicken them slightly. More distal elongating cells in the central columella have longitudinal and transverse walls of equal thickness. Subsequently, both longitudinal and transverse walls undergo major thickening. These observations agree with those of Clowes and Juniper (1964) on *Zea mays*.

Columella cells enlarge primarily in the direction of the longitudinal axis of the root. This elongation begins in the third through the fifth tiers distal to the root cap junction and reaches a maximum in the central portion of the root cap. Changes in cell shape affect the distribution of plasmodesmata on the walls. The ratio of the number of plasmodesmata per unit area on the transverse walls to that on the longitudinal walls increases as cells are progressively displaced through the columella. Griffiths and Audus (1964) reported for roots of *Vicia faba* that the number of plasmodesmata on the transverse walls was greater than the number of plasmodesmata on the longitudinal walls of cells in the root cap columella. In the columella of *Zea* Juniper and Barlow (1969) showed that ten times as many plasmodesmata per unit area are found on the transverse walls as in the longitudinal walls. Therefore, cells are intimately linked in linear files along the longitudinal axis and more or

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Fig. 7–8. Cells of the root cap periphery. **7.** Portion of much elongated cells located within several cells of the surface of the root cap. Note the highly thickened longitudinal walls, the unevenly thin transverse walls, the numerous ribosomes, the randomly scattered plastids with large starch grains, the abundant mitochondria, and the accumulation of polysaccharide-rich material (mu) between the plasmalemma and the cell wall. See Fig. 1 for key to labels. × 6410. **8.** Cross section of a cell located in the root cap periphery very close to the surface of the root cap. Note the intensely staining cell walls (W), the endoplasmic reticulum extending along the inner side of the cell walls, the Golgi bodies, the increased vacuolation, and the large plastids with intensely staining starch grains. See Fig. 1 for key to labels. × 8800.

less isolated physiologically from others—even within the same cell population—in a transverse direction. They suggested that this asymmetric distribution influences in some unknown way the patterns of cell division and cell differentiation. They further showed that the same preferential distribution of plasmodesmata occurs in initial cells and their derivatives in the ground meristem and the procambium. The only region which has no preferential distribution of plasmodesmata is the quiescent center.

Plastids start forming starch grains in the second through sixth tiers of the columella, so that plastids packed almost completely with starch grains are observable from the fifth tier onward. Large plastids with many large starch grains occur in the cells of the central portion of the columella and in the cells further displaced toward the root cap tip. Clowes and Juniper (1964) reported for root tips of *Zea mays* that the development of immature proplastids in root cap initial cells to mature amyloplasts in central columella cells occurred over a distance of 250 μm. In *Vicia faba* and in *Zea mays*, Griffiths and Audus (1964) and Juniper and French (1970) reported, respectively, that the root cap columella cells contained amyloplasts with large, distinct starch grains located at the distal ends. Electron microscope autoradiographic studies by Northcote and Pickett-Heaps (1966) and Dauwalder, Whaley, and Kephart (1969) on the incorporation of labelled glucose in root tips of *Triticum vulgare* and *Zea mays*, respectively, showed that the labelled precursor was incorporated preferentially into starch grains of the central columella. In outer root cap cells, labelled glucose was not incorporated into starch grains but instead almost exclusively into cell walls.

The cells of the root cap initial region are more highly vacuolated than the cells of the root cap columella. As cells are displaced into the columella, there is a decrease in staining intensity of the cytoplasm caused by a decrease in the number of ribosomes per unit area. Yet cells further displaced toward the root tip show an increase in cytoplasmic staining. Juniper and French (1970) observed that cells of the central columella in roots of *Zea mays* contained fewer ribosomes than those located elsewhere in the root tip. In addition, they noted that these cells had only a few vacuoles, whereas initials were more highly vacuolated.

Griffiths and Audus (1964) reported that root cap columella cells of *Vicia faba* had extensive endoplasmic reticulum which was concentrated primarily in a mass at the proximal end. They noted that profiles of endoplasmic reticulum were found associated with the nuclei and with plasmodesmata. Juniper and French (1970, 1973) reported that the initial cells of the root cap of *Zea mays* showed sparse profiles of unoriented endoplasmic reticulum, while those of the central columella had endoplasmic reticulum that appeared in units of four membranes without any attached ribosomes. In cells displaced toward the periphery of the root cap, the endoplasmic reticulum was irregularly distributed. Unlike *Vicia faba* and *Zea mays*, columella cells in the root cap of *Convolvulus* contain only fragmentary profiles of endoplasmic reticulum. In addition, Juniper (1972) reported that two varieties of *Pisum sativum* had no endoplasmic reticulum comparable to that found in the root cap columella of *Zea*.

As cells are displaced outward toward the root cap tip, material is secreted between the plasmalemma and the cell wall. In *Zea* Juniper and Pask (1973) described the secretion of a polysaccharide-rich material between the plasmalemma and cell wall and the subsequent movement to and accumulation of this mucus-like material in outer tangential walls near the root tip. They suggested that the function of this material is to coat and protect the outer tangential wall—the wall exposed to the soil—as the root grows.

The same major changes found in the central portion of the root cap also occur in a file of cells formed by initials that give rise to the root cap periphery. These changes, though, occur over a much shorter distance. The cells elongate in a direction oblique to the longitudinal axis of the root—in a manner similar to columella cells. Although plastids form starch grains as in the columella, those in the root cap periphery are randomly scattered throughout the cells, whereas they are displaced toward the distal end of cells in the columella. Changes in cell wall thickness occur, but only the longitudinal walls increase considerably in thickness. The transverse walls remain essentially the same. The major thickening of the longitudinal cell walls is the most distinct change in these differentiating cells. The thick walls protect the geotropically sensitive cells in the root cap columella and the initial and meristematic cells of the apical meristem.

The rapid differentiation of root cap cells is clearly correlated with structural and functional changes in the nucleus and in the cytoplasm, organelles, and walls of these cells. The cessation of DNA synthesis in columella cells formed from the root cap initials is one of the earliest events in the onset of cellular differentiation (Phillips and Torrey, 1971a, 1972). Especially striking are the early and rapid changes in organelle frequency and distribution, especially of the starch-containing plastid population. These rapid changes in the cells of the root cap columella are probably directly related to the sensitivity of these cells to geotropic stimuli. Later changes associated with specific wall thickening and mucus accumulation on the outer walls can be related to the protection

of the root meristem proper by the root cap as the root grows through the soil.

The close proximity of the root cap initials to the quiescent center is important to consider in developing models for control of cell division, enlargement, and differentiation in the root apex even though a root cap junction separates the two regions. As Juniper and Barlow (1969) showed in *Zea mays*, sufficient numbers of plasmodesmata maintain cellular connections between the quiescent center and the root cap, so that substances produced in the root cap cells may influence the activity of cells in the root proper or visa versa. The preferential distribution of plasmodesmata on transverse walls may provide a specialized pathway for preferential flow of materials and account for cell differentiation in linear cell files along the longitudinal axis of the root as well.

Although Juniper and Barlow (1969) provide a model for interpreting differentiation within the root apex, the morphogenetic substances or factors involved in this control and the site of their production are unknown. Phillips and Torrey (1971b, 1972) proposed from quite indirect evidence that the quiescent center itself may be the site for specialized synthesis of hormonal substances active in influencing cell division activity in the root apex. Recent experimental evidence obtained by Webster and Langenauer (1973) in *Zea mays* suggests that the initial cells surrounding the quiescent center may impose restrictions on the activity of quiescent center cells by acting as a site for the production of factors limiting cell division and cell differentiation. Shaw and Wilkins (1973) have proposed that the root cap cells are the source of substances which may serve to control the growth of the root and specifically to influence the geotropic response of roots. Thus, at least three possible sites for hormone synthesis in the root apex have been proposed. It is evident that definitive experiments designed to differentiate these possibilities must be performed.

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