THE ESTABLISHMENT OF THE ANATOMICAL ZONATION IN THE ROOT APEX OF *PHASEOLUS RADIATUS*[1]

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Abstract: This study emphasizes the fact that dynamic changes occur in the apical meristem of the primary root in ***Phaseolus radiatus*** Linn. during its early stages of development which are associated with germination. The root apical meristem is composed of a group of common initial cells in its early stage. The diameter of these initials average from 24.70 to 31.20 μ in their different developing stages. Twelve hours after germination, it is gradually reorganized into central initials (transverse meristem) and peri-initials. The former gives rise to the stele and columella by transverse division, and latter gives rise to the cortex, epidermis and cap cells other than the columella by various planes of cell division. Slightly before the reorganization of the meristematic region, the root cap cells appear identifiable zonation, *i. e.*, the formation of a columella.

INTRODUCTION

The structure and development of the root apical meristems of angiosperms have interested botanists for many years. Studies on these aspects of leguminous plants have been made by Eriksson (1878), Guttenberg (1947), Popham (1955), Hayat (1963) and Hayat & Heimsch (1963). But none of these studies were made by studying serial stages of the root, during its early development. The ontogenetic changes in the root apical initials, and the relationship between the meristematic cells and its derivative tissues as well as the mature structure were studied in *Ipomoea* by Seago (1971), and *Glycine* by Sun (1957).

Occurrence of the anatomical zonation in the root apical initials is known in many angiospermous roots including the leguminous ones. Guttenberg (1960) stated that in some angiosperms all root tissues arise from a common meristematic group of cells in the embryonic root, whereas in other groups the root tissues can be traced back to separate initials at a later stage of root development. He termed the former an open and the latter a closed-type. The studies of Seago (1971) and Sun (1957) also showed that the anatomical modifications of the apical initials and their immediate derivatives, by mitotic activity, commenced about 12 hr after germination. This shows that the organization of root apical initials in the radicle is different from that at a later stage.

This investigation was initiated to explore the dynamic zonal changes of the apical organization in the *Phaseolus* root. Observation of the cellular arrangement, mitotic activity of the derivative regions and the differentiation of them which differ from that described for other plants during the early stage of germination are reported.

MATERIALS AND METHODS

Seeds and seedling of *Phaseolus radiatus* Linn. were used. Seeds were obtained

(1) This work was supported by a grant of the Biological Research Center, Academia Sinica.
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from the market, and seedlings were grown from these seeds. Seeds were soaked in the Petri-dishes on several layers of wet filter paper. Distilled water was added every day for retaining moisture. The Petri-dishes were put in a culture room under continuous fluorescent light intensity of around 1,200 lux. Temperature was adjusted to 22±1°C.

The root tips were collected at specified times after sowing. The materials were killed and fixed in FPA (formalin-propionic acid-alcohol), dehydrated in TBA (t-butanol) series, embedded in tissuemat, sectioned at 5-8 μ, and stained with safranin, tannic acid and orange G combinations (Sharman, 1943).

RESULTS

Outer Morphology at Varying Stages of Growth.

Four hours after germination, the root tip became visible at the suture of the ruptured coat. Upon the rupture of the seed coat, the primary root (originally radicle) increased in length (Teble 1). The first indication of hairs occurred at the 12 hr stage when the root was of about 0.643 cm in length. Lateral roots appeared 60hr after germination. Though the length of the primary root increased gradually, its diameter increased only duriug the early stage of germination but decreased gradually from the 8 hr stage onwards. The root reached its minimum diameter at the end of this experiment (127 hr after germination). It was of about 300 μ in diameter and 9.953 cm in length (Table 1).

Tabel 1. Characteristics of the root tissues at selected stages of growth

	Time after germination (in hrs)									
	Embryonic root	4	8	12	24	48	60	72	96	127
width of apical initials (μ)	24.70	25.48	31.20	32.50	35.10	39.78	44.20	37.96	44.98	54.08
diameter of columella (μ)	-	-	(44.72) *	48.10	58.50	59.98	60.06	53.30	61.62	72.80
height of root cap(μ)	192.00	241.28	247.96	301.60	322.40	330.20	353.28	359.28	362.44	369.20
diameter of root (μ)	593.25	858.90	885.15	610.05	606.90	470.40	402.15	333.90	330.75	300.30
length of root (cm)	-	-	-	0.643	1.293	1.843	2.065	3.411	4.983	9.953

* ( ) indicates the region with columella-like arrangement of cap cells

The Organization of Root Tissues.

The vascular stele, cortex, epidermis and root cap in a fully developed root are arranged in the usual manner as in other dicotyledonous roots. Three region ——columella, peripheral cap and middle cap can be identified in the root cap according to their cell arrangement (Figs. 1, 2a). The columella is conspicuous at the central region of the cap. The transverse cell walls of columella are regularly arranged in rows and the longitudinal walls in the same row occur in orderly lines. Apparently they do not divide longitudinally. The peripheral cap cells cover the outer portion of the root cap. The cells located between the columellar and peripheral cap cells are termed middle cap cells. The boundary between the columella and middle cap cells are more conspicuous than between the

middle cap cells and peripheral cap cells. The cell arrangement in the distal region of the columella is more or less similar to that of peripheral cap cells (Fig. 2a).

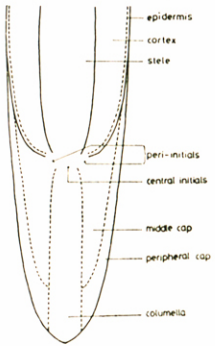


Fig. 1. Schematic drawing of root tip, showing the anatomical zonation in later stage of growth.

The apical organization in a fully developed root shows a regular anatomical zonation (Fig. 2; Table 3). This zonation can be identified simply by their cell arrangement. No clear boundary can be traced between each histogen (Hanstein, 1868). The central initial zone (transversal meristem by Popham, 1955) is wide, consisting of more than seven vertical rows of cells. But it consists of fewer cells in its horizontal direction. The cells in the central zone mainly divide transversely. The derivatives of the central initial give rise to the plerome upward, and central cap initials downward (Table 3). The cell division in the central region of the central cap initials contribute to the columella cells and the peripheral cap initials form part of the middle cap cells, and occasionally peripheral cap cells which are located on the border of the columella.

The initial group located peripheral to the central initial is peri-initial (Figs. 2b, 2c; Table 3). The peri-initial shows a continual pattern around the central initial, due to cell lineage. But the direction of cell division differs from that of the central initials. The cells of peri-initial form new walls somewhat oblique to the root axis (parallel with future pericycle, Fig. 2). The peri-initial consists of 2 to 3 (rarely 4 cell layers) which give rise to both the outer tissues of the root proper (such as: ground meristem and calyptrogen) and the cap cells, except most of the columella (Fig. 2). Therefore the cell arrangement as well as the cell morphology in the columella is very different from that in both the middle cap and peripheral cap cells. The ground meristem and calyptrogen finally result in the formation of the cortex and epidermis respectively.

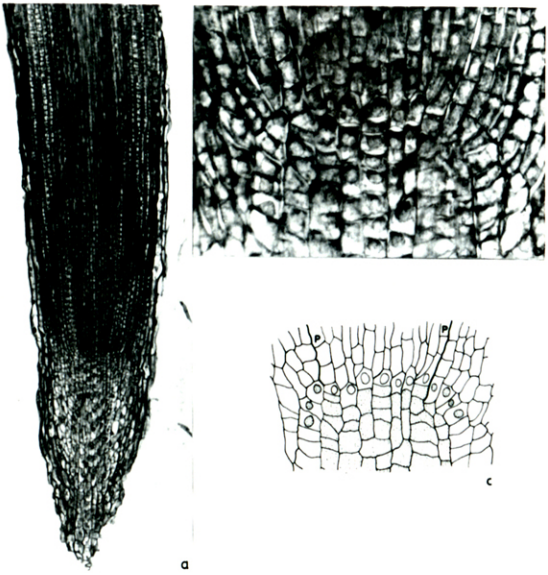


Fig. 2. The median longitudinal section of a root tip from 127 hr. material. (a. × 130, b & c × 700), b. enlarge view of apical initials, c. drawing of apical initials.

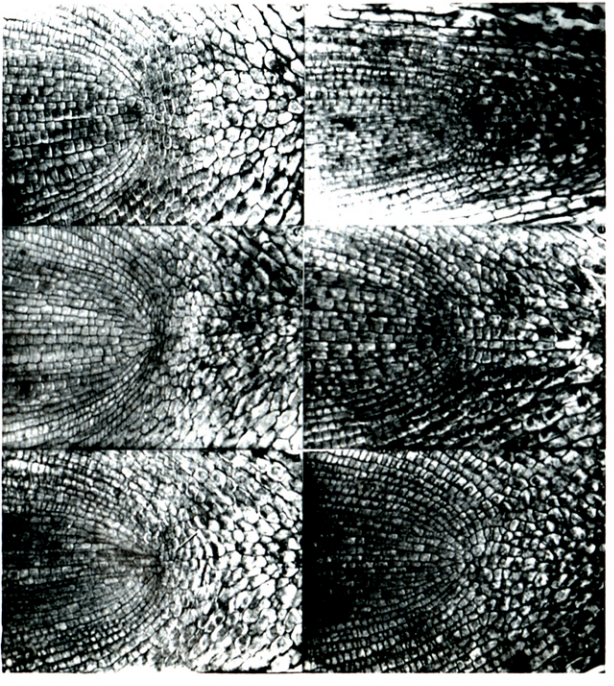


Fig. 3. Microphotographs from the median longitudinal sections of roots showing the apical meristems and their adjacent tissues, all ×230.
(a) dormant radicle, (b) 4hr root, (c) 8hr root, (d) 12hr root, (e) 24hr root, (f) 48hr root.

The Apical Organization In The Radicle.

Only one group of common initial cells is observed in the root tip of the radicle (Figs. 3a, 4a). The width of this initial group ranges from 23 to 25 μ (Table 1), and consists of 4 to 5 cells in a horizontal direction (Fig. 3a, 4a). No layered organization is seen. The primary meristem can be recognized at the region beside

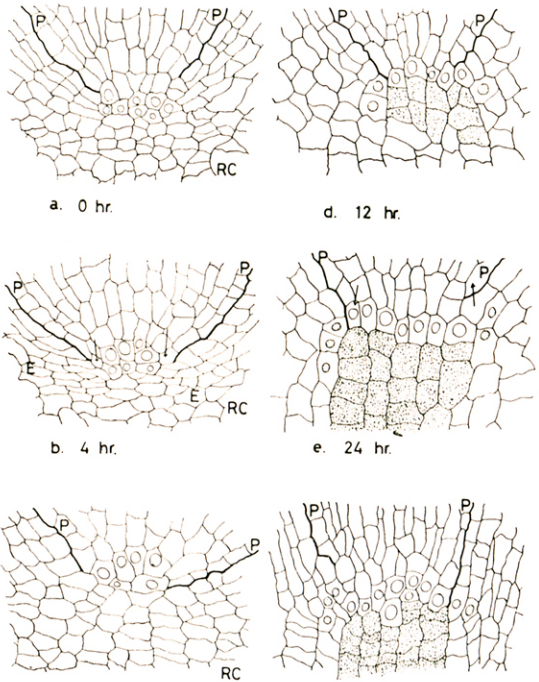


Fig. 4. Camera lucida drawings showing the cellular lineage in apical initials, interpreting the details of Fig. 3.

the common initial cells, e. g., plerome, periblem and calyptrogen. Most common initial cells divide transversely, and the cells in plerome divide longitudinally, rarely transversely. The boundary between the central stele and cortex is distinctly marked by the deeply stained cells, probably together with intercellular substances. Both longitudinal and transverse divisions occur in the periblem.

The indication of columella-like structure is present in the root cap of radicle. But newly formed tranverse walls of the cap initials lying close to the common initial is obvious (Fig. 3a, arrow). The cell arrangement in the central cell arrangement in the central cell group (or columella-like region) differs from that in the other cap cells, but no clear boundary separates them from each other. The cells in the central cell group are often stained lighter than the rest of the cap cells. Mitotic figures have not been seen in this stage.

Table 2. Zonal construction of apical initials and tissue differentiation at an early stage of root growth (from 0 hr to 12 hr after germination)

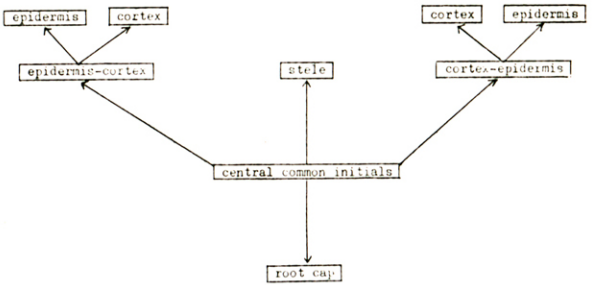
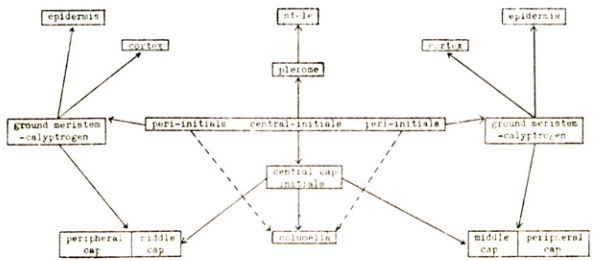


Table 3. Zonal construction of apical initials and tissue differentiation at a later stage of root growth (12hr after germination)



The apical organization of the root 4–8 hr after germination

The most conspicuous change during this stage was the change of the width of the central initials (Table 1, Figs. 3b, 3c, 4b, 4c,) and the specialization of the future columella cells. The cell number of the central initials did not increase (Figs. 4a–4c). Apparently the increase of central initials was caused mainly by the enlargement of the cells during this stage. The new transverse cell walls in the future columella region were numerous. These new transverse walls occurred in the areas very near the calyptrogen as well as at the region some distance from the central initial group (Fig. 3c).

The apical organization in the later stage of growth

The first indication of a columella formation appeared earlier than the modification of central initial group. Both shifting of common central- and peri-initial groups, and establishment of columella in root cap can be observed in the root 12 hr after germination (Figs. 3d, 4d). The central initial region widens by the increase in cell numbers. The filing of the columella cells began in the area surrounded by the central initial group and peri-initials (Fig. 4d). The well established central initials, peri-initials and the regularly filed columella (except the base of columella) could not be identified until 24 hr after germination (Figs. 3e, 4e).

The future stele, central initials and the young columella formed a continuous pattern of cell lineage as the root grew (Figs. 2, 3a, 4a). The width of the central initials varied from 35.10 to 54.08 μ in all the stages from the 24 hr root up to the end of this experiment (127 hr root). The diameter of columella and of the root in these stages corresponds with that of the apical initials. The height of the root cap increases rapidly in the early stages of growth (zero hr to 12 hr root), the increase of cap height slowed as the root developed at later stages (Table 1). Though the width and cell number in the apical initial group and other categories were variable in the different developing stages, the cell arrangement and the pattern of cell lineage in the apical meristem remained constant in all the materials from about 12 to 24 hr after germination, and on up to the 127 hr stage (Fig. 2).

DISCUSSION

Both "the open and closed types" of Guttenberg (1960) are present in the root apical meristem of *Phaseolus radiatus*. The apical organization in the embryonic root and in the early stage of development showed the "open type" in which all the root tissues arise from a common meristematic group of cells whereas they later turned to a "closed type" to which two initial groups (central initial group and peri-initial group) can be traced (Table 2, 3). This dynamic change occurred at about 8 to 24 hr after germination. A structural change that occurred in the root tip was that of the columella formation in the central part of root cap. The columella formation appeared slightly earlier than the rearrangement of apical initial group. Though the authors have studied numerous radicles of developing embryos, no central apical cell (Guttenberg, 1940) was seen in any of the stages examined. However it is certain that the central initial group occupies a small area in the very young root, consisting of only a few cells (Figs. 3a, 3b, 4a, 4b). The so called "central apical cell" could be present in the developing radicles of younger materials than the authors collected. Apparently it is unlikely to find a central apical cell in plants which possess a layered meristem or "closed type".

Though the fundamental structure in each stage in the apical meristem differs from that in *Ipomoea* and *Anoda* as well as in many plants, the fact that the reorganization in the meristematic group of cells associated with germination agrees with that demonstrated in the earlier work by Byrne and Heimsch, (1968); Guttenberg, Burmeister and Brosell, (1955); Hayat, (1863); Seago, (1971); Seago and Heimsch, (1969). This work adds one more evidence to the earier work to show that the ontogenetic modifications occur in developing primary root growth. This anatomical change occurred in the roots of about 5 mm in lengh in *Ipomoea*, but 6 to 13 mm in the present material (Table 1). The cell number in each initial group might vary in different stages of growth after 24 hr but the basic organization always showed a somewhat identical pattern in all the stages of later development.

The presence of a columella in the root cap is very common in many seed plants. The colunella has separate initials from the central initials in some plants (Pillai, 1963; Popham, 1955; Seago, 1971; Sun, 1957), whereas in others both columella initials and central initials have common origin, which is the case in the present material (Clowes, 1950; Hayat, 1963; Seago & Heimsch, 1969). Upon the establishment of initial groups in the central part of the meristem, the central initial group increased gradually in width (Table 1). As in *Cassia* (Hayat, 1963) and *Pisum sativum* (Popham, 1955) the width of the columella in the root cap increased as the central initial group broadened. Because of the intimate continuity of the central group and future columella, the diameter of columella was closely correlated with that of the central initial group. And the height of the central initial group could not be measured for the same reason. The rapid increase of the root diameter in the early stage of growth was mainly due to the enlargement of cells in a horizontal direction rather than the formation or widening of the columella. Because the increase was completed before the formation of columella.

The most conspicuous change in the root is the increase in root diameter in its early stage of growth, and then its gradual decrease at a later stage. It reached maximum diameter in the 8hr root, and became narrower in later stages of development. The narrowest root was obtained at the end of this investigation (127 hr root). Apparently this accompanying decrease in root diameter was caused neither by the reduction of the diameter of the central initirals nor by the reduction of the columella. It is obvious from the data in Table 1, the central initials as well as the columella widened as the root grew. The decrease in root diameter was caused by the reduction of cell size in later structures of both the root proper and the root cap, and also by the shift of the orientation of the long axis of the cells in this area. This phenomenon also occurs in some other plants (Clowes, 1950; Hayat, 1963; Pillai, 1963; 1964). That the number of cell layers in the root cap did not vary is evidently associated with the root growth. They retained rather constant cell layers throughout their growth. The increase of the height of the root cap was caused by the elongation of cap cells (especially the columella cells) in the direction parallel to the root axis.

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