INSOLUBLE CARBOHYDRATES AND RNA IN ROOT APICAL CELL OF *CERATOPTERIS THALICTROIDES**

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Abstract: Changes of both insoluble carbohydrates and RNA are described in the root apical meristem of *Ceratopteris thalictroides* (L.) Brongn. The apical cell exhibits more carbohydrate granules than its neighboring cells in the actively growing root. It is almost missing in the apical cell of the old root. There are no marked differences in RNA content between the apical cell and its derivatives in both young and old roots. The division role as well as their physiological roles of the apical cell, based upon the high amount of insoluble carbohydrates in the growing root, compared to the low amount in old root, were discussed.

INTRODUCTION

The presence of a tetrahedral apical cell in the root apical meristem of pteridophytes is well known. This apical cell is considered to be the initial cell of all the tissue in root. The significance of the apical cell for the tissue development has been widely recognized (Bierhorst, 1977; Bower, 1923; Chiang, 1972; Chiang and Gifford, 1971). Most of the early studies centered at analysis of the pattern of cell lineages. Although application of the histochemistry on the shoot apical meristem of pteridophytes have been the subject of numerous investigations during the past decade, the histochemical study on the root apical meristem has been made in only a few plants (Avanzi and D'Amato, 1967, 1970; Chiang, 1979; D'Amato and Avanzi, 1965; Gifford, Polito and Nitayangkura, 1979; Gifford and Kurth, 1982). As in most of the other pteridophytes, the lateral roots of *Ceratopteris thalictroides* originate endogenously in one of the endodermal cell of the parent root (Chiang, 1971; Chiang and Gifford, 1971; Lachmann, 1907; Mallory, Chiang, Cutter and Gifford, 1970). The xylem of it is diarch. The lateral root primordia form opposite the incipient protoxylem poles, and are arranged orderly in two rows (Fig. 1). This specific order of arrangement provide one able to observe a subsequent series of different stages (or ages) of developing lateral root primordia in the same longisection of a root (Chiang, 1971). In other words, the younger the primordia the closer are they located to the apical cell of the parent root. The developmental pattern of the lateral root of *C. thalictroides* is basically similar to that found in its parent root. The earlier studies of the present author have illustrated that there is a correlation between the mitotic activity of root apical cell and the developmental stage (or length) of the root (Chiang, 1972; Chiang and Gifford, 1971). The purpose of this study is to confirm that the histochemistry of the apical cell in younger root must be closely correlated with that of the apical cell in older root, in both main and lateral roots.

MATERIALS AND METHODS

Ceratopteris thalictroides (L.) Brongn. was cultivated in the greenhouse of the Botany Department of National Taiwan University under natural light. Several adventitious roots arise from the abaxial surface of the basal part of each petiole (Chiang, 1971). The materials

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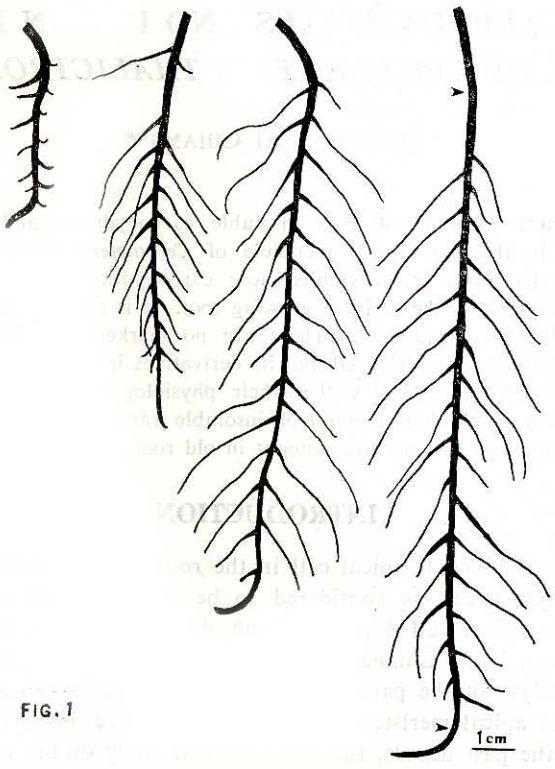


Fig. 1. Root morphology of various developing stages; arrows showing proximal (upper) and diatal (lower) clear areas.

used in this investigation were the adventitious roots emerging from the petiole base. In order to receive the same light intensity, all the roots collected were submerged in water but not rooted in mud. The materials were collected in July, 1979. The root tips for sectioning were (1) embedded root (embedded in petiolar tissue); (2) 5 cm; (3) 10 cm; and (4) 15 cm in length. Ten to twelve roots for each group were harvested. Immediately after collection, they were fixed in FPA (formalin-propionic acid-ethanol), followed the traditional paraffin embedding method and stained with PAS (periodic acid Schiff's stain) for carbohydrates detection (Jensen, 1962), and pyronin Y coupled with RNAse for RNA detections (Tepper and Gifford, 1962),

RESULTS

Insolube carbohydrates:

The roots of 5 cm, 10 cm and 15 cm in length, represent young (at early period of growth), medium (at middle period of growth) and old (at the later period of growth) roots respectively. Besides, the embedded roots are named as root primordism in the present report. The following description is based on the results observed on the median longisections through the apical cell. Observations were centered mainly on the PAS particles which were located inside the cells, though the cell walls were also well stained. The PAS granules are definitely distributed in the protoderm and periblem in all the root tips examined (Figs. 2, 3) These

granules were found to be starch as tested in KI-I_2 solution. A distinct single apical cell is constantly recognized located at the extreme tip of the meristematic region (Figs. 2, 3). The PAS granules exhibit very few in the apical zone except the apical cell, or/and its immediate derivative in the younger roots. The apical cells of a young root always contains more PAS particles than that in the apical cell of an older root (Figs. 2, 3). All the roots used in this study originate from the basal petiole. One of the cell in petiolar tissue enlarged, became vacuolated and followed a series of sequential cell divisions to give rise to a root (Figs. 4, 5). Numerous PAS granules were present in the root primordium (embedded stage), but it does not develop PAS granules when it was still in one cell stage (root initial, Fig. 4). The insoluble carbohydrates in the single initial cell was very scanty, and once the root primordium has organized they became distributing throughout the whole apical cell except the nucleus (Fig. 4). The PAS content of the apical cell in the root primodium immediately before the protrusion from the petiole surface was always more abundant than that in other stages of the growth. As the root primordium became emergent, the PAS content decreased slightly then these granules continued to decrease in number as the root grew. Figures 5, 2 and Figure 3 show the sequential changes of the PAS content in the apical cells of a series of the growing roots. Almost no cytoplasmic insoluble carbohydrates could be seen in the apical cell of the old root (Fig. 3).

The root cap tissue is well established in the young root (5 cm in length). The number of the cell layers in root cap are 6 to 8 in the roots of yonng, medium and old stages (Figs. 2, 3). The PAS particles, or starch particles in cap cells were abundant in the young root, and decreased gradually as the root grew. Only a few PAS particles could be identified in the root cap of the old root (Fig. 3). The starch granules in the cap cells of the young root appeared mainly in two to three of the inner layers. The starch granules in the cells of the outermost layer were missing.

As shown in Figure 1, all the roots, either young or old, exhibit two clear areas free from the lateral roots. They are the areas that bear no lateral roots. One is the distal tip of the root of about 1 to 5 cm in length, the other the proximal portion of the root i. e., the region closest to the petiole base. But the lateral root initials are constantly formed inside the distal clear area, even in the roots which have ceased to grow. Apparently the lateral root primordia initiated in the very old stage would die prematurely. Observations on the changes of PAS content in the apical cell of the developing lateral root were made mainly on the young root (5 cm in length). The lateral root initial originated very closed to the apical cell of the parent root (Fig. 8). PAS granules in the early developmental stages of the lateral root were paucity, and increased in number gradually with increasing in age. They reached to the maximum content in the apical cell of the lateral root slightly before the penetration of the outer tissues of the parent root. As that is the same in the parent root, the apical cell was always the cell accumulated more PAS granules than any other cells in the meristematic zone of the developing lateral root (Fig. 6). After emergence, carbohydrated granules in the apical cell became fewer and fewer. The apical cells of the laterals in the old root (15 cm in length) always contained fewer PAS granules as compared with those of the laterals located in the same level in the young root (Compare Fig. 6 with 7).

As mentioned above, carbohydrates granules always accumulated in the apical cell of young and of the developing lateral root primordium in the young root (Figs. 2, 4, 5, 6, 7). In some slides, both the apical cell as well as its immediate derivative contained of about the same amount of PAS granules as that exhibited in the apical cells. This case was found in both meristematic zone of both the parent root and the lateral root primordium (Figs. 5, 6).

RNA:

The RNA was evenly distributed throughout the meristematic zone (Fig. 8). However,

RNA content of the protoderm as well as the inner layer of the root cap cells was higher than that of the other regions. It did not show a significant differences in the apical cell from that in its surrounding cells. The same is also true for the apical cell in the developing lateral root of the young parent root which usually did not contain a denser color of RNA stain than the other cells of the lateral root primordium (Fig. 9).

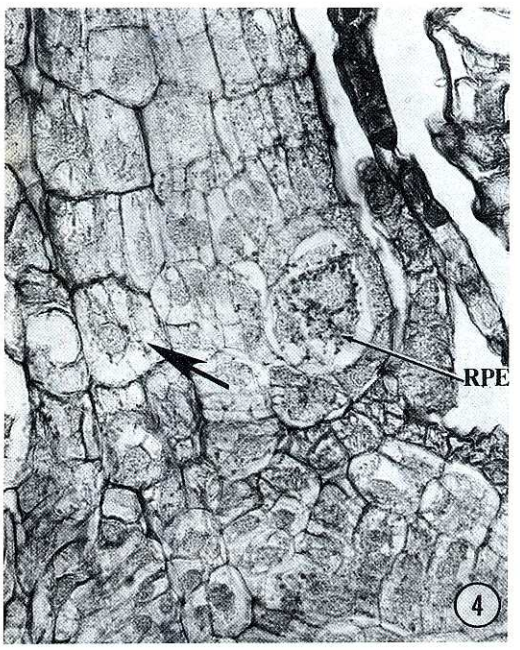
DISCUSSION

In previous reports, it was revealed that the apical cell in the actively growing roots of *Ceratopteris* divided more frequently than its adjacent cells (Chiang, 1972; Chiang and Gifford, 1971). It was also shown that the shoot apical cell of *Adiantum capillus-veneris* possessed more carbohydrates particles in its growing season (Chiang and Lin, 1979). As would be expected, the present results obtained with PAS-stained technique support fairly the earlier studies with cytohistological observations. The active cells possess more starch granules. The presence of a quiescent center in the root have been mentioned in some preridophytes (D'Amato and Avanzi, 1965, 1970). But no indication of such a center exists in *C. thalictroides* and some others as observed by both cytohistological and cytochemical studies (Chiang, 1972; Chiang and Gifford, 1971; Gifford *et al.*, 1979; Gunning, Hughes and Hardham, 1978; Gifford and Kurth, 1982; Nitayangkura and Gifford, 1980).

Starch accumulation is obvious in the apical cell of the young root which is observed to be at its growing period. In most case, in the meristematic zone, the apical cell is always the only cell exhibits more PAS granules than the cells surrounding it. But still in some other specimens both apical cell and its immediate derivative contain almost the same amount of the PAS granules. This fact, i. e., both apical cell and its immediate derivative have PAS granules, could be interpreted that these two cells have recently divided from a common mother cell (previous apical cell). The starch granules in this newly formed immediate derivative has not yet disappeared. After being formed, the starch granules would decrease gradually in the derivative. As mentioned in 'Materials and Methods', all the roots collected were kept to receive the same amount of sunlight. Thus the sunlight is unlikely to be responsible for the amount of the starch granules in the present investigation.

The previous work has indicated that the cells in whole apical zone (including apical cell and its surrounding cells) undergo cell division (Chiang and Gifford, 1971). However, only few PAS granules are present in the derivative area (Figs. 3, 5). There is no evidence to suggest that the high content of starch granules in these derivative cells is responsible for cell division. It seems to one that the sufficient accumulation of the starch in a cell is not a necessary factor for the induction of cell division. On the other hand, it may be interpreted that the different cells need different amount of starch accumulation for division-induction. The apical cell may be the one which needs the highest concentration for the induction for the induction of cell division. Based on that the different starch content in the apical cell and its closely associated cells, both undergo cell division, the accumulation of starch may not be necessary for cell division. But together with the earlier work, it is shown that the cell division is an important activity in the apical cell of the growing root. One can not ignore to relate these two factors. However, it is also important to note other significant role or roles in addition to the cell division in the apical cell. The changes of the starch

Fig. 2. Median longisection through the apical meristem of the young root (5 cm in length).
Fig. 3. Median longisection through the apical meristem of the old root (15 cm).
Fig. 4 and 5. Longisection of the petiole base through the embedded root primodia, arrow marks the primordium at one celled stage (all in PAS stain). AC—apical cell; E—petiolar epidermis; RPE—root primordium embedded in petiolar tissue.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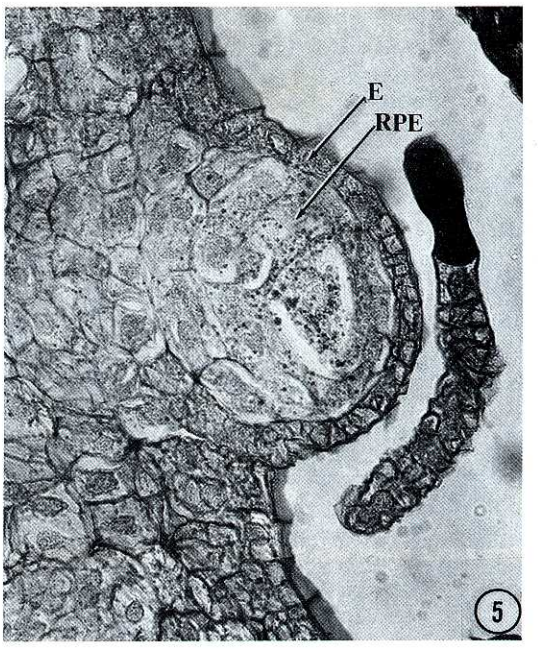
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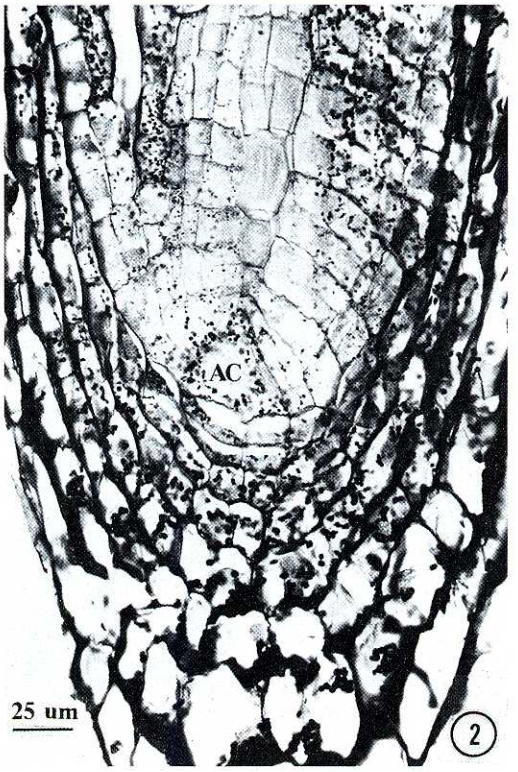
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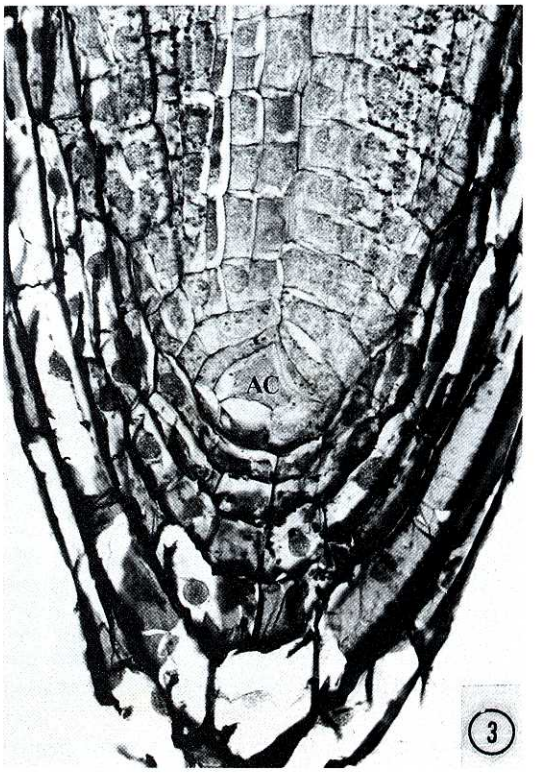
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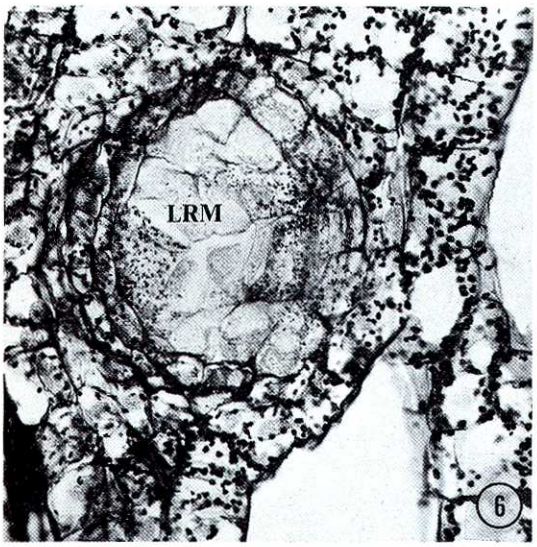
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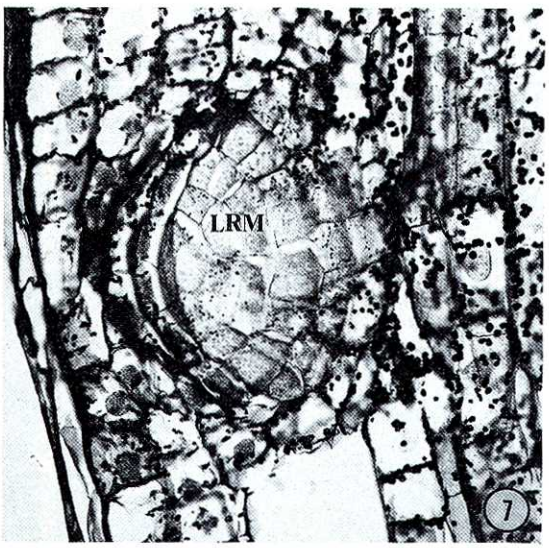
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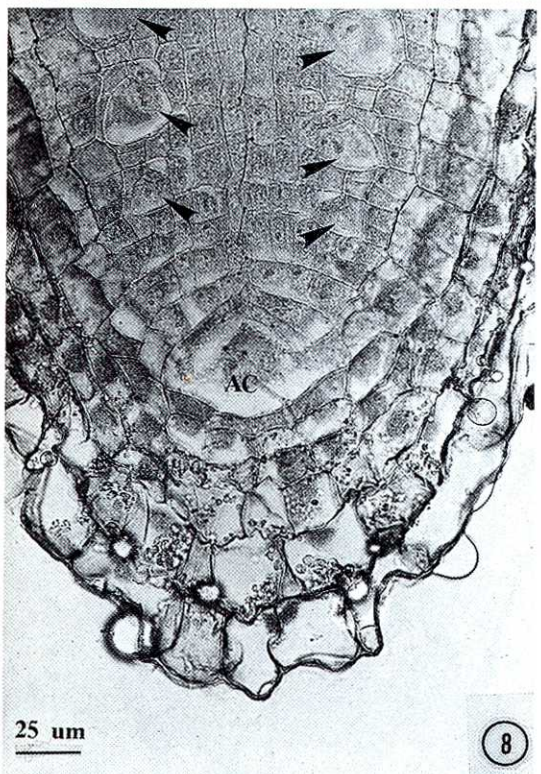
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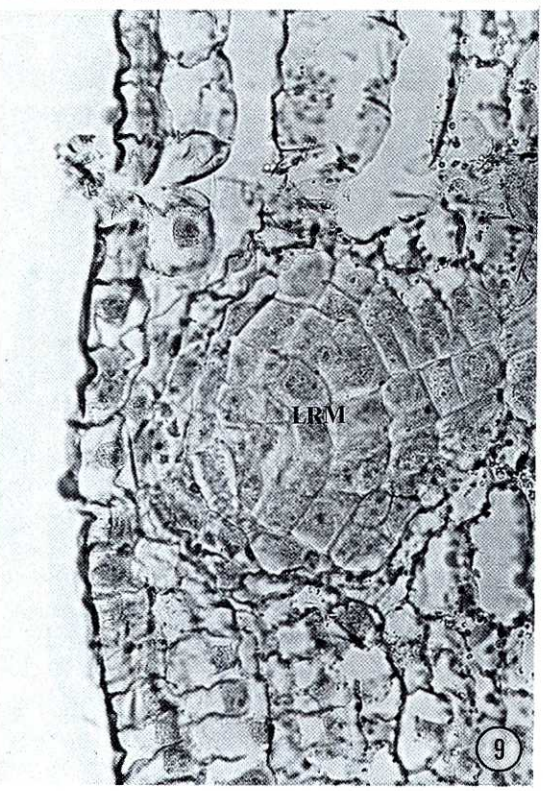
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concentrations in the apical cell of the root is somewhat different from that found in the shoot of *Adiantum* (Chiang and Lin, 1979). The starch granules in the apical cell of the old root was missing, whereas the apical cell of the shoot constantly exhibited a considerable amount of starch granules, even in the stage of resting period, i.e., winter. Apparently the amount of the insoluble carbohydrates in the root meristem is affected by the developmental stage of the individual root, since all the materials were collected in July. Again, the apical cell of the growing root is always the only cell containing more carbohydrates granules. The apical cell showed different properties from its neighboring cells. The evidence presented here supports the idea that whether or not the accumulation of starch is a necessary factor for division-induction, the apical cell of the young root differs from its neighboring cells, and differs from the apical cell of the older root in some unrevealed physiological activities. The concentration of the starch in the apical cell is closely related to these unrevealed physiological activities. There is no evidence to neglect the special meaning of the existence of the apical cell, and to treat the whole meristematic area as a multicellular organization in its growing period.

The rich RNA content in physiologically active cells is quite common (Gall, 1969; Stevenson, 1976). There are no marked differences in RNA concentration between the apical cell and its derivatives. It is quite likely that the RNA plays the different role from the carbohydrates granules during the same developmental stage of the root.

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Fig. 6. Longisection of the young root through the lateral root primordium located 350 μm from the apical cell of the parent root, stained with PAS.

Fig. 7. Longisection of the old root through the lateral root primordium located 350 μm from the apical cell of the parent root, in PAS stain.

Fig. 8. Median longisection of a young root stained with pyronin Y, arrows showing the sequential developing lateral root primordia.

Fig. 9. Longisection of a young root through the lateral root primordium located 350 μm from the apical cell of parent root. (with pyronin Y).

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