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STRUCTURE AND DYNAMICS OF THE ROOT CAP OF AVENA SATIVA L.*

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SUMMARY

Both pulse and continuous labelling experiments were performed to study cap genesis in the primary root of *Avena sativa* L.

The cap meristem comprises one tier of initial cells situated immediately distal to the root cap junction. In the periphery of this tier, the cells have lost their initial character. Compensation for this loss of initial cells is provided by anticlinal divisions of more centrally situated initial cells.

Pulse labelling experiments indicated an average cycle time of the cap initials of 10 to 12 hours. From this cycle time and the number of cells in the central rows of the cap it was inferred that for *Avena* it takes at most 5 to 6 days before a derivative cell is sloughed off.

The results are compared with data from CLOWES (1971) and PHILLIPS & TORREY (1971, 1972).

1. INTRODUCTION

In the present paper a part is reported of a detailed study on the structure and dynamics of the apical meristem of the primary root of *Avena sativa.*

To understand the functioning of apical meristems, it is necessary to study both their structure and the kinetics of their cells. A root apex is a suitable system for such a study because its structure is relatively simple. Unlike the open type, in the closed type of root apex organization (VON GUTTENBERG 1960) the root cap meristem is separated from the meristem of the root by a cell wall layer called the root cap junction. Moreover, the various subpopulations of the meristem, viz., the meristematic cortex, stele, and root cap, are clearly delimited.

For this study the closed apical meristem of *Avena sativa* was used. The structure and dynamics of the root cap were investigated first, because they seem simple in comparison with the meristem of the root proper.

Recently, CLOWES (1971) and PHILLIPS & TORREY (1971) reported data on the cell kinetics of the root cap meristem of the closed apical meristem of *Zea mays* and *Convolvulus arvensis*, respectively. Phillips & Torrey found that in roots pulse-labelled with ^{3}H-thymidine the movement of labelled cells through the root cap columella took 6 to 9 days before the cells were sloughed off. Clowes reported, however, that the root cap is completely renewed every day.

This discrepancy and the unexpected report of daily renewal of the root cap in a grass species led the present author to study cap genesis in *Avena sativa*, using a refinement of the pulse-labelling technique of Phillips & Torrey.

* Dedicated to Professor Karstens on the occasion of his retirement.

2. MATERIAL AND METHODS

Grains of *Avena sativa* L. 'Seger I' obtained from Allmänna Svenska Utsades A.-B., Svalöf, Sweden, were mounted with paraffin (melting point 42 °C) on the inner wall of hollow cylinders (height: 2.6 cm, diameter: 2.7 cm, wall thickness: 2 mm) bored from bricks. The cylinders were placed on culture tubes (*fig. 1*) filled with a 2.10^{-4} M $CaSO_4$ solution according to Bange (personal communication). The distance from each grain to the medium was the same, and the medium was aerated. Germination and growth occurred in the dark at 25 °C.

In pulse and continuous labelling experiments, methyl-^{3}H-thymidine (The Radiochemical Centre, Amersham, specific activity 18.9 Ci/mM) was supplied at a final concentration of 5 μCi/ml and 0.5 μCi/ml, respectively. Forty-hour roots (timed from the first contact with the $CaSO_4$ solution) exposed to a 30-min pulse of ^{3}H-thymidine were fixed either directly or after rinsing in cold thymidine and were then transferred to a fresh $CaSO_4$ solution for 24 and 72 hr prior to fixation. In the continuous labelling experiments 48-hr roots were supplied with ^{3}H-thymidine for 12 hr and fixed immediately.

All root tips (1–2 mm) were fixed in FAPA (50 ml 40% formalin, 250 ml 96% ethanol, 15 ml propionic acid, 15 ml acetic acid conc., and 170 ml distilled water) for 18–24 hr. They were then embedded in Pegosperse 100S (Glyco Chemicals, New York) containing a mixture of diethylenglycolstearate, diethylenglycol, and stearin (melting point 46–54 °C), after a modification of STEEDMAN (1960). Serial longitudinal sections were cut at 6 and 10 μm on a rotary microtome and mounted on slides with Haupt's adhesive (SASS 1958). For micro-

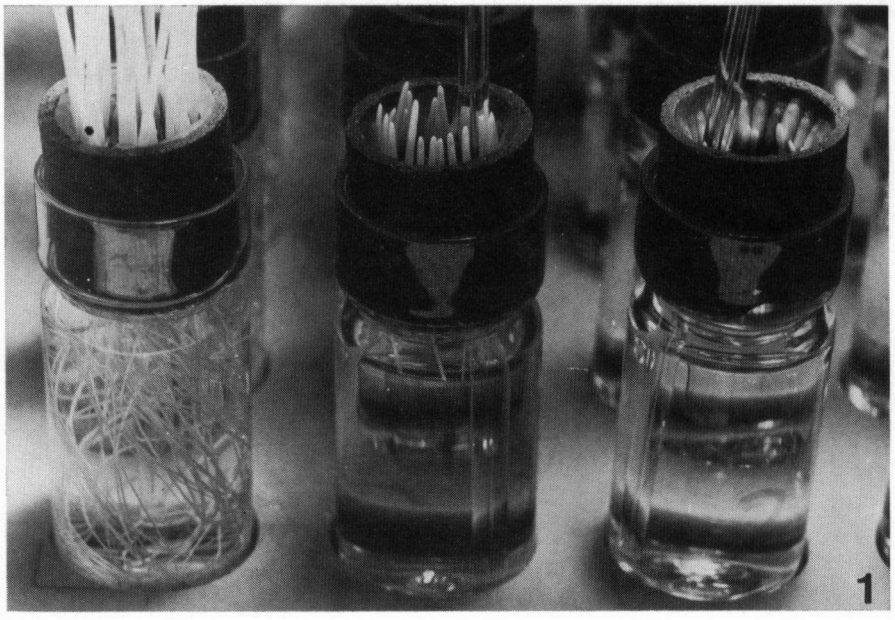

Fig. 1. Culture set-up.

autoradiography the slides were coated with chrome alum gelatin (0.5% gelatin and 0.05% chrome alum). The sections were stained after Feulgen (DEN TONKELAAR 1963) and some of them were counterstained with Astra Blue FM (Chroma, Stuttgart; 0,5% Astra Blue in 2% tartaric acid). For microautoradiography the stained sections were covered with stripping film (Kodak AR 10), exposed for 8–40 days at 4°C, developed with Kodak D-19, dried, and mounted in Eukitt (Kindler, Freiburg).

3. RESULTS

3.1. Light microscopy

The structure of the apical meristem of the primary root is shown by the median longitudinal section in *fig. 2*. The central, late metaxylem vessel consisting of a row of cells as shown in this figure is only present in a true median longitudinal

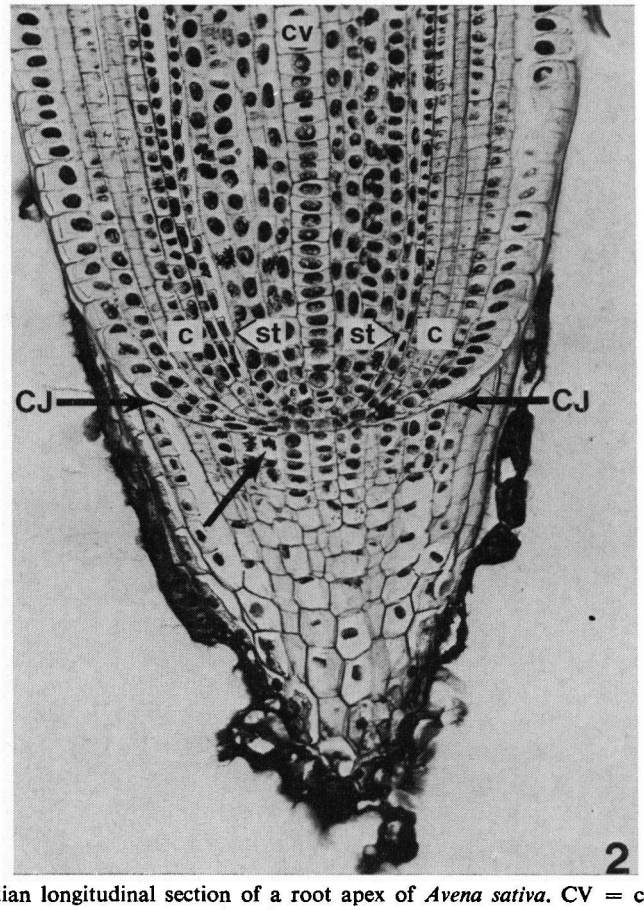

Fig. 2. Median longitudinal section of a root apex of *Avena sativa*. CV = central vessel, ST = stele, C = cortex, CJ = cap junction. The blind arrow points to a periclinally dividing nucleus just to the right of a nucleus dividing anticlinally. (× 83)

section. This is an important aid in the selection of median longitudinal sections for the study of the organization of the apex.

The cap junction separates the root cap from the cells of the root proper. In the cap itself there are different planes of division (*fig. 2*). Most of the daughter cells lie in a longitudinal direction, but divisions with daughter cells in a lateral direction are also important. Both periclinal and anticlinal divisions are found along the root cap junction, but at the extreme periphery only periclinal ones.

Over 90% of the divisions occurred in the first tier of cells immediately distal to the root cap junction. The others (less than 10%) occurred in the second and third tiers.

The progeny of cells dividing periclinally appeared in rows reflecting the activity of the initial cells.

3.2. Microautoradiography

Microautoradiographs of median longitudinal sections of 40-hr root tips supplied with ^{3}H-thymidine for 30 min showed that about 50% of the nuclei in the root cap initials were labelled (*fig. 3*). In the periphery of the root cap there were also some heavily labelled nuclei, most of them in a region without cell division activity in the distal part of the cap.

In pulse labelled roots transferred to a fresh $CaSO_4$ medium, the initially labelled cap initials produced labelled derivative cells by repeated periclinal divisions. *Fig. 4* shows a microautoradiograph made 24 hr after the pulse. Most of the initial cells had divided twice. *Fig. 5* shows a microautoradiograph made 72 hr after the pulse. Labelled derivative cells of the initial cells are progressively displaced through the root cap. It is clear that the nucleus of the first derivative cell, which is now farthest from the initial cell, has the strongest labelling of the row of derivative cells, the initial cell the weakest.

Microautoradiographs of median longitudinal sections of root tips supplied with ^{3}H-thymidine for 12 hr (*fig. 6*) show that two tiers of cells immediately distal to the root cap junction were labelled. In the 12-hr period most of the initial cells had divided once. The occurrence of rows with one labelled cell may be due to anticlinal divisions of the initial cell, such that the labelled nucleus of one of the daughter cells is present in another plane.

If we compare *fig. 3* with *fig. 6*, we see that the labelled nuclei in the periphery of the cap shown in *fig. 3* are absent in *fig. 6*. The only difference between these roots was that a 30-min pulse was supplied to the 40-hr roots in *fig. 3* and a 12-hr continuous labelling period to the 48-hr roots in *fig. 6*.

4. DISCUSSION

The observation that most mitoses occurred in the first tier immediately distal to the root cap junction is in full agreement with previous work (WAGNER 1939 in *Hordeum*, *Triticum*, *Avena*, and *Zea*; BUVAT & LIARD 1953 in *Triticum*; HAGEMANN 1956 in *Hordeum*). Our data and those in the literature suggest that the cap initials occupy a single tier of cells.

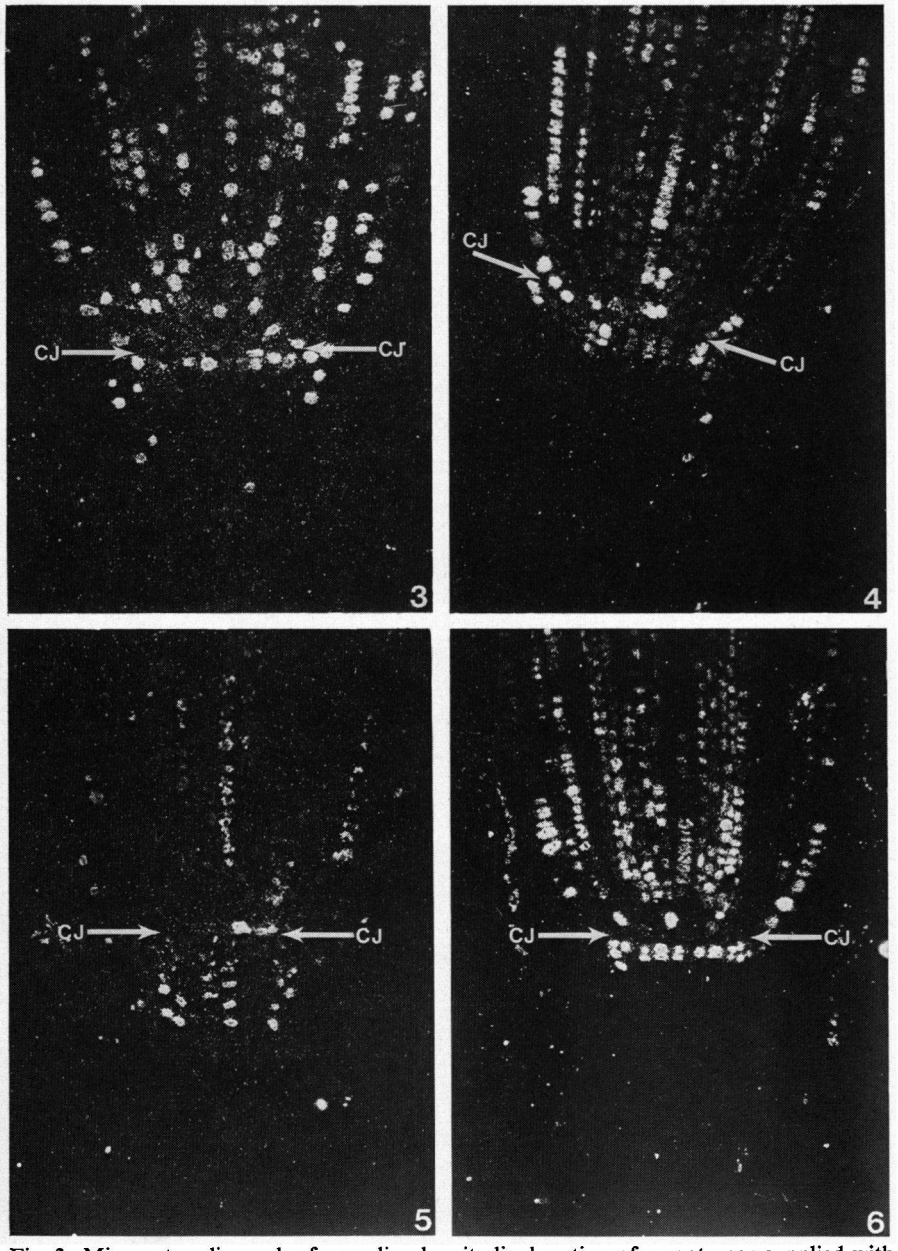

Fig. 3. Microautoradiograph of a median longitudinal section of a root apex supplied with ^{3}H-thymidine for 30 min, showing silver grains associated with incorporation of the label into nuclei of the root cap initials and of peripheral cells of the cap. CJ = cap junction. (× 122, dark-ground illumination)

Fig. 4. Microautoradiograph of a median longitudinal section of a root apex supplied with ^{3}H-thymidine for 30 min before transfer to fresh medium for 24 hr. (× 122)

Fig. 5. Microautoradiograph of a median longitudinal section of a root apex supplied with ^{3}H-thymidine for 30 min before transfer to fresh medium for 72 hr. (× 122)

Fig. 6. Microautoradiograph of a median longitudinal section of a root apex supplied with ^{3}H-thymidine continuously for 12 hr. (× 122)

From analyses of the structure and labelling pattern of the root cap we may conclude that the following phenomena contribute to a steady state in cap genesis:

1. The activity occurs in one tier of initial cells immediately distal to the root cap junction.
2. Repeated periclinal divisions of the initial cells produce rows of differentiating non-dividing cells.
3. The loss of the initial character of the cells at the periphery of the cap is compensated for by anticlinal divisions of more centrally situated initial cells.
4. The sloughing off of the root cap cells takes place all over the surface of the cap. (See diagram in *fig. 7.*)

From the semi-conservative replication of DNA we might expect a dilution of the label present in the initial cells as a result of their division activity. The rows of labelled progeny provide information about the average duration of the

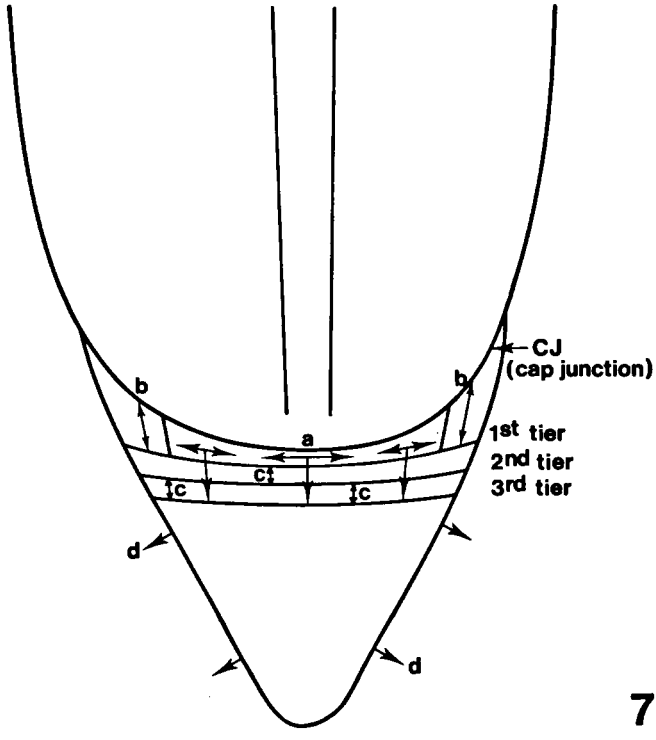

Fig. 7. Diagram of a median longitudinal section, showing distributional areas. In the area below a (thick arrows) lies the first tier in which the initial cells are localized. In the area below b (thin arrows) is the peripheral part of the first tier, in which the cells (occasionally showing periclinal divisions) have lost their initial-cell character. c: the second and third tiers, in which a low percentage of periclinal divisions sometimes occurs. d: surface of the cap of which root cap cells are sloughed off.

cycle of the initial cells and the age of the derivative cells in the root cap when they are sloughed off. Over periods of 24 and 72 hr (*figs. 4* and *5*) there are about 3 and 8 labelled ones per row, respectively. From these data we can calculate an average cycle time of the initial cells of 10–12 hr, which is very close to the 10.4 hr we found (HARKES, in preparation) with the pulse chase method after QUASTLER & SHERMAN (1959) and to the values found by CLOWES (1971) for *Zea* (10.4 hr) and by PHILLIPS & TORREY (1972) for *Convolvulus* (13 hr).

Although the average cycle times of the meristematic cap cells in *Zea* and *Convolvulus* were approximately the same, Phillips and Torrey found that in *Convolvulus* it took 6 to 9 days for a derivative cell of a cap initial to be sloughed off, while Clowes reported that in *Zea* the root cap is completely renewed every day. From the length of the central rows, which comprise approximately 12 cells, and from the average 10.4 hr cycle time of the cap initials we may infer that in *Avena* at most 5–6 days elapse before a derivative cell is sloughed off. This is rather close to the 6 to 9 days Phillips and Torrey found in cells moving through the columella of cultured *Convolvulus* roots.

According to Clowes, 66% of all cap cells of *Zea* are meristematic and 66% of these cells have a short cycle time of 10.4 hr. From these data he calculated that the whole root cap is renewed every 24 hr. He agrees that this seems surprising, but advances in support the findings of JUNIPER et al. (1966) that, after complete removal of the cap, a root of *Zea* can produce a new cap within 36 hr at 28°C.

WAGNER (1939) found that 81% of the mitotic figures in the root cap of *Zea* occur in the first tier of cells, 9% in the second tier, and 10% in the third. This makes it very probable that the root cap meristem of *Zea*, too, comprises one tier of initial cells. Obviously this is not consistent with the high percentage of meristematic cells reported by Clowes. It is a pity that Clowes did not mention the source of his evidence.

His additional argument of root cap regeneration is questionable, since normal cap genesis and cap initial regeneration followed by cap genesis may be two different matters.

The main difference between the labelling technique used by Phillips and Torrey and the technique used in this study is the length of the pulse (14 hr *versus* 30 min). We consider a short pulse to give a better picture of the production of derivative cells by individual initials, which, for example, immediately provided an impression of the average cycle time of the cap initials.

The incorporation of the label by non-dividing cells in the periphery of the cap of 40-hr roots pulse-labelled for 30 min (*fig. 3*) may be due to resumed DNA synthesis which is not coupled with karyo- and cytokinesis. These latter processes evidently stop in most cells at a later stage of root development as shown by the microautoradiographs of 48-hr roots continuously labelled for 12 hr (*fig. 6*).

Studies on the incorporation of ^{3}H-thymidine into root caps immediately after germination of the fruits are in progress.

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REFERENCES

BUVAT, R. & O. LIARD (1953): Nouvelle constatation de l'inertie des soi-disant initiales axiales dans le méristème radiculaire de Triticum vulgare. *C. R. Acad. Sc.* Sér. D. **236**: 1193–1195.

CLOWES, F. A. L. (1971): The proportion of cells that divide in root meristems of Zea mays L. *Ann. Bot.* **35**: 249–261.

GUTTENBERG, H. VON (1960): Grundzüge der Histogenese höherer Pflanzen. I. Angiospermen. *Handbuch der Pflanzenanatomie* VIII(3). Gebr. Borntraeger, Berlin.

HAGEMANN, R. (1956): Untersuchungen über die Mitosenhäufigkeit in Gerstenwurzeln. *Die Kulturpflanze* **4**: 46–82.

JUNIPER, B. E., S. GROVES, B. LANDAU-SCHACHAR & L. J. AUDUS (1966): Root cap and the perception of gravity. *Nature (Lond.)* **209**: 93–94.

PHILLIPS Jr., H. L. & J. G. TORREY (1971): Deoxyribonucleic acid synthesis in root cap cells of cultured roots of Convolvulus. *Plant Physiol.* **48**: 213–218.

— & — (1972): Duration of cell cycles in cultured roots of Convolvulus. *Amer. J. Bot.* **59**: 183–189.

QUASTLER, H. & F. G. SHERMAN (1959): Cell population kinetics in the intestinal epithelium of the mouse. *Exp. Cell Res.* **17**: 420–438.

SASS, J. E. (1958): *Botanical microtechnique*. The Iowa State College Press, Ames, Iowa.

STEEDMAN, H. F. (1960): *Section cutting in microscopy*. Blackwell, Scientific Publications, Oxford.

TONKELAAR, E. M. DEN (1963): *Fotografische colorimetrie*. Thesis Univ. Leiden.

WAGNER, N. (1939): Über die Entwicklungsmechanik der Wurzelhaube und des Wurzelrippenmeristems. *Planta* **30**: 21–66.