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# KINETICS OF MERISTEM CELLS IN SUB-POPULATIONS OF THE ROOT APEX OF AVENA SATIVA L.

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SUMMARY

The kinetics of proliferating cells in sub-populations throughout the root meristem of *Avena sativa* were studied by means of labelling-techniques and cell-flow experiments. Estimates of the mean duration of the cell cycle and its constituent phases $G_1$, S, $G_2$ and M were derived from the 'fraction of labelled mitoses' curves. Progressive reduction in cell cycle duration, largely brought about by a decrease in $G_1$, were found in cortex and stele with increasing distance from the tip. Proliferation fractions, calculated from the ratio: duration of cell cycle/cell-doubling time, reveal that most of the sub-populations exclusively consist of cells in the cell-division cycle. The reduction in proliferation fraction found in the proximal sub-population of the meristematic cortex points to a substantial proportion of non-proliferating cells due to the cessation of proliferative activity at the margin of the meristem before it begins to differentiate. We concluded from continuous-labelling experiments that all cells in the quiescent centre progressed through the cell cycle, which accords with the high proliferation fraction predicted from radial transit rates of cap initials by HARKES (1976).

## 1. INTRODUCTION

Apical meristems in higher plants are self-perpetuating growth and construction centres of roots and shoots. We have only a very limited knowledge of the rules that govern cell production in these meristems. Labelling-techniques involving specific DNA precursors and cell-flow experiments using a stathmokinetic agent have made it possible to determine parameters for cell kinetics in populations of proliferating cells. Numerous estimates of average cell-cycle times in root meristems are available. However, to understand the co-ordinated proliferative activity in these meristems, we need data about the kinetics of proliferating cells in sub-populations throughout the meristems. Only a few reports provide data on the duration of cell cycles and their constituent phases, and on proliferation fractions in the meristematic epidermis, cortex, stele and root cap (BARLOW & MACDONALD 1973; CLOWES 1975 a; MACLEOD 1976). Some years ago we started an investigation on cell-population kinetics of root-apical meristems of *Avena sativa* L. in order to understand the cell-production rules of these particular meristems. Data on cell kinetics in the cap meristem, the simplest part of the root meristem of this species, were reported earlier (HARKES 1973, 1976). In this paper we present data on kinetics of proliferating cells in sub-populations throughout the root meristem.

## 2. MATERIALS AND METHODS

### 2.1. Sampling

The seedling culture of *Avena sativa* L. var. Seger I was described in detail in HARKES (1973). In labelling-experiments 40-h seedlings with root length of approx. 1.5 cm were exposed to methyl-$^3$H-thymidine (spec. act. 18.6 Ci $mM^{-1}$) at an activity of 5 $\mu$Ci $ml^{-1}$ for 30 min, rinsed in cold thymidine and transferred to fresh medium. Samples were taken with 2-h intervals after the end of the pulse. For microautoradiography the root tips were processed as described in HARKES (1976).

In cell-flow experiments, 40-h seedlings were treated with a 0.1% solution of colchicine and sampled after 1, 2, 3 and 4 h. The roots were sectioned longitudinally, stained after Feulgen and counterstained with Astra Blue FM (Harkes, 1973).

### 2.2. Fraction of labelled mitoses

Cells engaged in DNA synthesis during the length of the pulse were labelled by $^3$H-thymidine. The passage of these labelled cells through mitoses (metaphases, anaphases, and telophases) was scored as a function of time. For each time six roots were examined. Estimates of the duration of the cell cycle (T) and its constituent phases $G_1$, S, $G_2$ and M were derived from the 'fraction of labelled mitoses' (FLM) curves using the boundary method, 0.5 level (QUASTLER & SHERMAN (1959). The parameters T, $G_2+0.75$ M, and $S+\tau$ (where $\tau$ represents the duration of the pulse) were obtained by measuring the horizontal distances between the intersections of the curve with the 0.5 level of FLM. The distance from the ordinate to the first intersection represents the duration of $G_2+0.75$ M, because prophases, which make up ca. 50% of the mitotic figures in *Avena*, are not involved in the analyses. The duration of M was calculated from the mitotic index (MI) and the cell-doubling time ($T_D$) by the equation

$$M = \ln(MI+1)/r$$

(where $r = (\ln 2)/T_D$), which can be derived from the age distribution for exponential growth (CLEAVER 1967). The cell-doubling time was determined in a separate set of roots. By subtracting $S+G_2+M$ from T, the unknown $G_1$ can be calculated.

### 2.3. Cell-doubling time and proliferation fraction

Assuming that some constant proportion of the cells of a sub-population should remain capable of division, the doubling time ($T_D$) for cells is simply (ln2)/r. The specific growth rate (r) was determined from the accumulation of metaphases in cells blocked with colchicine. We assume that after one-hour colchicine treatment the specific growth rate is related to the metaphase index by the equation:

$$rt_1 = \ln(MeI_1+1),$$

where t, represents the time from the moment that the colchicine blocks the cells at metaphase. After an incubation of 2 h, $r(t_1+1) = \ln(Me_2+1)$ etc. It follows that, if the slope (r) of the straight line through the values for $rt_1$, $r(t_1+1)$,

$r(t_1 + 2)$, $r(t_1 + 3)$ is known, $T_D$ can be calculated.

The proliferation fraction (PF) gives the proportion of cells that are contributing to the increase in cell number. This fraction was estimated by comparing the mean duration of the cell cycle for the proliferating cells of a population with the cell (population)-doubling time ($PF = T/T_D$).

## 2.4. Continuous-labelling experiments

To study the proliferative activity in the quiescent centre, the root meristems were exposed continuously to $^3$H-thymidine at an activity of 0.5 or 1.0 μCi ml$^{-1}$ for various times up to 60 h. Because TAKATS & SMELLIE (1963) reported that a concentration of $6.10^{-6}$M $^3$H-thymidine was completely depleted in about 8 h by root tips of *Vicia*, the medium was replaced every 6 h.

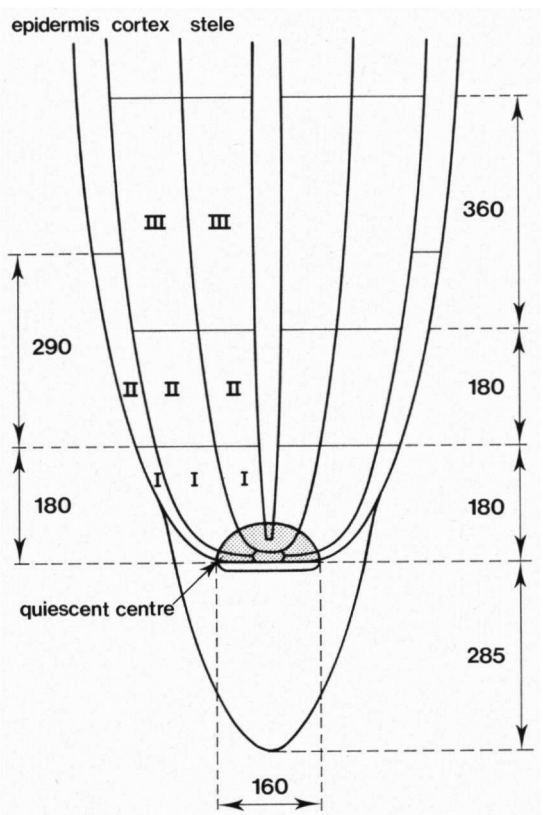


Fig. 1. Median longitudinal section of the root apex of *Avena sativa*, showing the separate regions and their dimensions (μm) of which the cell kinetics were studied. The quiescent centre overlaps the epidermis I, cortex I, and stele I for a small part.

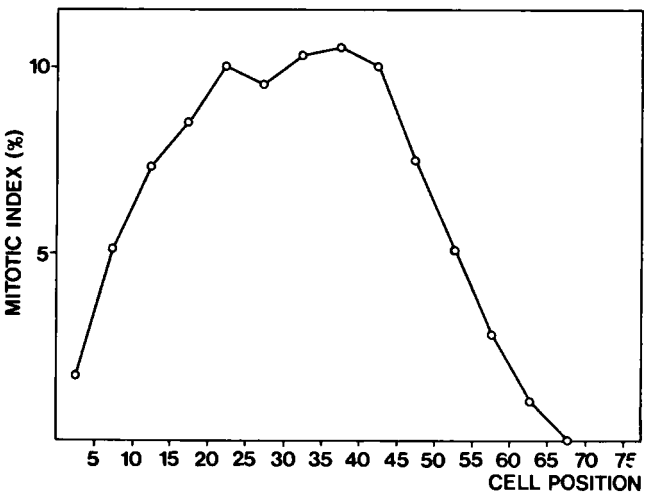


Fig. 2. Mitotic index per cell position in the epidermis of *Avena sativa*. The cells adjacent to the apical initials are numbered 1. Based on a sample of 72 roots.

## 3. RESULTS

### 3.1. Delimitation of sub-populations of meristem cells

Each of the various sub-populations of meristem cells that were distinguished corresponded with a part of a histogen, except for the quiescent centre, which comprised the initials of epidermis, cortex and stele as well as adjacent cells (*fig. 1*). In the epidermis, the terminal divisions were asymmetric, producing small distal cells (trichoblasts) and proximal 'normal' epidermal cells. Only few of those latter cells divided once more. The MI per cell position (the cells adjacent to the initials are numbered 1) showed a rapid decrease after cell 45 (*fig. 2*) as a result of an increasing proportion of cells (trichoblasts and 'normal' epidermal cells) that were permanently out of the cell-division cycle. Therefore to exclude non-dividing cells, the proximal boundary of the meristematic epidermis was fixed at cell 40 (470 μm). In the cortex and stele the mitotic activity ceased at a distance of 500–1200 μm from the cap junction. *Fig. 1* shows the separate regions and their dimensions (μm) of which cell kinetics were studied.

### 3.2. The cell cycle

The cell-cycle parameters T, $G_2+0.75$ M, and $S+\tau$ were extracted from the smooth curves drawn from the fractions of labelled mitoses (*table 1*) found at different times following a 30 – min pulse with $^3$H-thymidine in the various sub-populations of meristem cells. Since in epidermis I and the quiescent centre a second wave in the FLM curve was lacking, no satisfactory analysis of the curve could be made. *Table 2* presents the duration of the cell cycle (T) and its constituent phases $G_1$, S, $G_2$, and M as well as the corresponding values for MI.

Table 1. Fraction of labelled mitotic figures in sub-populations of root-meristem cells of *Avena sativa* at time intervals following a 30-min pulse with tritiated thymidine.

| sub-population | hours | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 |
| epidermis I | 0 | 0 | 0.875 | 0.952 | 0.895 | 0.562 | 0.500 | 0.118 | 0 | 0.045 |
| epidermis II | 0 | 0 | 0.743 | 0.783 | 0.773 | 0.405 | 0.382 | 0.347 | 0.210 | 0.564 |
| cortex I | 0 | 0.039 | 0.981 | 0.986 | 0.903 | 0.091 | 0.148 | 0.169 | 0.305 | 0.516 |
| cortex II | 0 | 0.079 | 0.846 | 0.919 | 0.687 | 0.045 | 0.264 | 0.381 | 0.491 | 0.617 |
| cortex III | 0 | 0.067 | 0.779 | 0.900 | 0.353 | 0.048 | 0.605 | 0.677 | 0.632 | 0.286 |
| stele I | 0 | 0.648 | 1.0 | 0.549 | 0.113 | 0.043 | 0.451 | 0.567 | 0.428 | 0.352 |
| stele II | 0 | 0.800 | 1.0 | 0.532 | 0.178 | 0.344 | 0.754 | 0.656 | 0.485 | 0.184 |
| stele III | 0 | 0.713 | 1.0 | 0.489 | 0.029 | 0.370 | 0.840 | 0.402 | 0.311 | – |
| quiescent centre | 0 | 0 | 0.833 | 1.0 | 0.909 | 0.571 | 0.333 | 0 | 0 | 0 |

Table 2. Mitotic index (MI) and the time parameters of the cell cycle (hours) in sub-populations of root-meristem cells of *Avena sativa*.

| sub-population | MI | $G_1$ | S | $G_2$ | M | T |
|---|---|---|---|---|---|---|
| epidermis I | 0.059 | – | 7.3 | – | – | – |
| epidermis II | 0.088 | 3.8 | 5.5 | 2.7 | 1.6 | 13.6 |
| cortex I | 0.055 | 5.1 | 5.1 | 2.8 | 1.1 | 14.1 |
| cortex II | 0.068 | 3.9 | 4.9 | 2.7 | 1.3 | 12.8 |
| cortex III | 0.067 | 0.4 | 3.8 | 2.7 | 1.4 | 8.3 |
| stele I | 0.069 | 4.4 | 3.8 | 1.2 | 1.1 | 10.5 |
| stele II | 0.092 | 2.9 | 3.8 | 1.1 | 1.2 | 9.0 |
| stele III | 0.083 | 2.6 | 3.8 | 1.1 | 1.2 | 8.7 |
| quiescent centre | 0.037 | – | 6.9 | – | – | – |

Table 3. Fraction of metaphase figures in sub-populations of root-meristem cells of *Avena sativa* following a 1, 2, 3 and 4-h treatment with colchicine, and the corresponding specific accumulation rate (r), cell-doubling time ($T_D$), and proliferation fraction (PF).

| sub-population | hours | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | $r(h^{-1})$ | $T_D(h)$ | PF |
| epidermis II | 0.0420 | 0.1063 | 0.1600 | 0.2160 | 0.05109 | 13.6 | 1.0 |
| cortex I | 0.0360 | 0.0914 | 0.1417 | 0.1997 | 0.04852 | 14.3 | 0.99 |
| cortex II | 0.0432 | 0.1001 | 0.1494 | 0.2138 | 0.04983 | 13.9 | 0.92 |
| cortex III | 0.0460 | 0.0999 | 0.1355 | 0.2076 | 0.04627 | 15.0 | 0.55 |
| stele I | 0.0609 | 0.1196 | 0.1926 | 0.2741 | 0.06124 | 11.3 | 0.93 |
| stele II | 0.0548 | 0.1562 | 0.2454 | 0.3227 | 0.07532 | 9.2 | 0.98 |
| stele III | 0.0458 | 0.1217 | 0.2083 | 0.2724 | 0.06627 | 10.5 | 0.83 |

The figures show that in the cortex the duration of the cell cycle decreased progressively with the distance from the tip; a 41.1% reduction in T was found. This decrease was due to a shortening of the duration of $G_1$ from 5.1 to 0.4 h and, to a lesser extent, to a shortening of the duration of S from 5.1 to 3.8 h (*table 2*). In the stele, however, the reduction in T was considerably smaller, viz., 17.1%, largely as a result of a decrease in the duration of $G_1$.

Besides differences in phase durations within histogens, differences occurred also between histogens. In comparing the sub-populations of cortex and epidermis with those of the stele, we found that in the cortex and epidermis $G_2$ lasted more than twice as long as in the stele.

### 3.3. Proliferation fraction

The proliferation fraction of a particular population was calculated from the ratio: cell-cycle time (T)/ cell-doubling time ($T_D$). Cell-doubling times were calculated from the specific growth rates (r) which were determined from the fraction of colchicine-metaphase figures following a 1, 2, 3 and 4-h treatment with colchicin in sub-populations of meristem cells. The PF's thus obtained are summarized in *table 3*.

### 3.4. Proliferative activity in the quiescent centre

The fraction of labelled mitoses for the quiescent centre was scored during a 34-h period. The FLM curve consisted of a single peak and no estimation of the duration of the cell cycle could be made. These pulse-labelling experiments demonstrated that the root meristem of *Avena* included a population of cells with a low division rate as compared to contiguous sub-populations. In continuous-labelling experiments, however, the proportion of cells which were labelled increased from 0.28 after 12 h, to 0.44 after 24 h and to 1.0 after 60 h of continuous exposure to $^3$H-thymidine. If we assume that in the quiescent centre a labelled cell represents a cell in the cell-division cycle, than these figures suggest that the proliferation fraction of this particular population was 1.0.

## 4. DISCUSSION

### 4.1. Kinetics of meristem cells

The only data available on cell-cycle times, phase times, and proliferation fractions for sub-populations in seperate regions along the root meristem were obtained from two sets of *Zea* roots grown under slightly different conditions (BARLOW & MACDONALD 1973; MACDONALD 1974, and CLOWES 1975a), and from lateral roots of *Vicia faba* (MACLEOD 1976).

BARLOW & MACLEOD (1973) found a progressive reduction in T within 1000 μm in the cortex towards the proximal region of the meristem. However, the figures show that in the epidermis and stele, T was approximately constant. In the cortex the reduction in T was attended by a progressive reduction in $G_1$, S and M, whereas in $G_2$ a small increase was found in the proximal regions. In the epidermis and stele the values of $G_1$, S and $G_2$ seemed approximately constant,

although some fluctuations occurred. MACDONALD (1974), using the same experimental data as in BARLOW & MACDONALD (1973), estimated PF's for the epidermis (1.0), cortex (0.91–1.0) and stele (0.84–1.0). For the region of the stele just above the quiescent centre and for the quiescent centre itself MACDONALD found relatively low PF's, viz., 0.54 and 0.50, respectively. CLOWES (1975a) found that within 1200 μm in the stele and the cortex-epidermis complex of *Zea*, T decreased progressively with the distance from the tip. The reductions in T were brought about by shortening of $G_1$ and $G_2$, though both S and M became longer. In the cortex-epidermis complex at 1200 μm from the junction of cap and quiescent centre, M amounted to even more than 50% of T. Proliferation fractions decreased progressively towards the margins of the meristem. From the data of Clowes it can be derived that in the stele the PF decreased from ± 0.83 at 200 μm to ± 0.05 at 1200 μm while in the cortex-epidermis complex a reduction of the PF occurred from ± 0.88 at 400 μm to ± 0.07 at 1200 μm. From the same data it can be derived that ca. 25% of the cells of the quiescent centre were engaged in the cell division cycle. MACLEOD (1976) found that in the lateral root meristem of *Vicia faba* the duration of the cell cycle of the fast-dividing cell population in both cortex and stele, was less between 750 and 800 μm basal to the

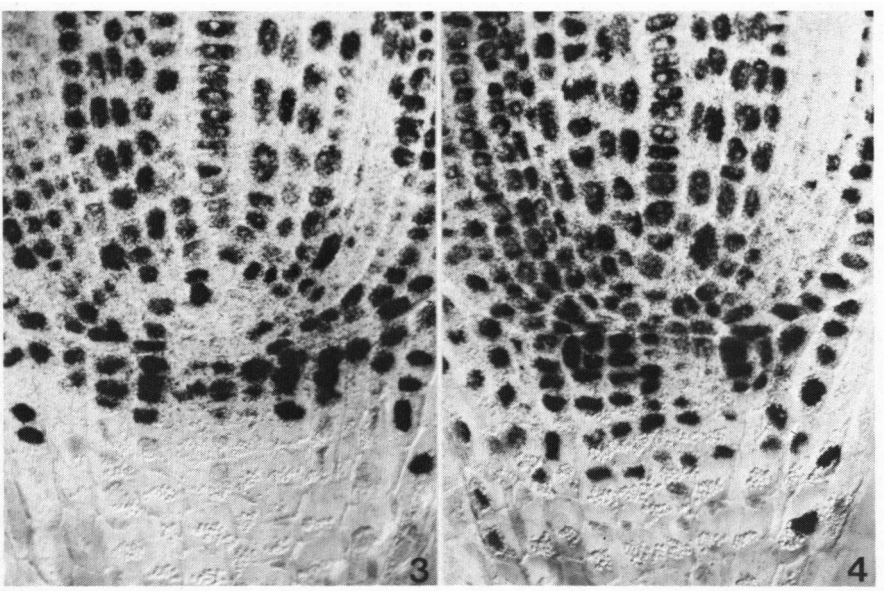


Fig. 3. Micro-autoradiograph of a median longitudinal section of a root apex of *Avena sativa* supplied with tritiated thymidine for 24 h. Labelled nuclei are seen in the 3rd and 4th tier of the cap.

Fig. 4. Micro-autoradiograph of a median longitudinal section of a root apex of *Avena sativa* supplied with tritiated thymidine for 60h. Labelled nuclei are seen in the 6th and 7th tier of the cap, so at least 5 divisions have occurred. Note that in the quiescent centre all nuclei have incorporated tritiated thymidine.

junction of cap and quiescent-centre than in the corresponding initial cells, largely as a result of a decrease in the duration of $G_1$.

From the present results (*tables 2* and *3*) it can be concluded that in the root meristem of *Avena* a reduction took place in the duration of the cell cycle of the proliferating cells, mainly due to a decrease in the duration of $G_1$, as they were displaced toward the margins of the meristem. The cessation of proliferative activity in the histogens as they began to differentiate was due solely to a reduction in the size of the proliferation fraction as revealed by their cell-doubling times. The values calculated for the PF's of sub-populations within the meristem of *Avena* (*table 3*), based on the ratio of the duration of the cell cycle and the cell-doubling time, reveal that most of the population exclusively consisted of cells in the cell-division cycle.

The discrepancy between the cell-doubling and the average duration of the cell cycle in all dividing cells in various regions of the meristem of *Zea*, led CLOWES (1976) to discuss the nature of a population of non-cycling cells. Relative proportions of proliferating and quiescent-cell fractions in the meristem of lateral roots of *Vicia* based on the lack of similarity between the cell-doubling time and the mean cycle time for all the proliferating cells, were reported by MACLEOD (1976). The consequence of whether certain cells in a population drop out of the cell cycle in reference to the anatomical cell pattern was discussed by GREEN (1976) and made CLOWES (1976) conclude that cells in regions close to the quiescent centre are not permanently out of cycle.

### 4.2. The quiescent centre

The PF in the quiescent centre (1.0), which was derived from the labelling pattern after a 60-h exposure to $^3$H-thymidine, accords with the prediction of a high PF made on account of radial transit rates of cap initials, if we assume symplastic growth (HARKES 1976). It has been demonstrated that changes in proliferative activity occur in different parts of the root meristem of *Zea*, when a series of pulses of $^3$H-thymidine is used at normal labelling concentrations (DE LA TORRE & CLOWES 1974). These authors reported a shortening of cell-doubling times in the quiescent centre brought about by radiation effects of β-decay of $^3$H.

CLOWES (1975b) suggested that anything that reduces the rate of cycling in the normally meristematic cells will increase the proliferative activity in the quiescent centre. Therefore, we studied the lineage of cells of the cap initials after 24 and 60 h of continuous exposure to $^3$H-thymidine (*figs. 3* and *4*). The positions of the labelled cells reveal that the cap initials had divided c. 2–3 and 5–6 times within 24 and 60 h, respectively. These results agree with the lineage of initially labelled cap initials after the same period of growth following a 30-min pulse with $^3$H-thymidine (HARKES 1973), whereas the number of divisions equals the calculated number if we assume that the average cell-cycle time is 11.0 h for the cap initials (HARKES 1976). Thus, up to 60 h, β-decay of $^3$H incorporated in nuclei engaged in the cell cycle did not slow down the division rate in the cap initials. Therefore, we have no indication to assume an increase in proliferative activity in the quiescent centre of *Avena*, during a 60-h exposure to $^3$H-thymidine at 1 μCi $ml^{-1}$.

The substantial difference in PF's between the quiescent centres of *Zea* and *Avena* may be explained by differences in the organization and activity of their root-cap meristems (HARKES 1976).

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