BIOLOGIA PLANTARUM (PRAHA)

21 (5) : 355—360, 1979

The Cytological Analysis of Causes Affecting the Root Cell Length

MÁRIA LUXOVÁ

Institute of Experimental Biology and Ecology of the Slovak Academy of Sciences, Bratislava*

Abstract. The final length of the root cell is the result of a series of processes which represent a transition of the growing cell from its origin up to the completion of its elongation. These processes are associated on the one hand with cell proliferation, or, after the termination of proliferation, with the proceeding DNA synthesis, and with cell elongation on the other. The group of properties characterizing the growth region of the root as a cytologically heterogeneous complex, is at the same time the group of causes which affect the length of root cells. The individual cases documented in the paper point out the fact that the mechanisms regulating growth processes, have a locally limited action, often only within one single cell, and that they are simultaneously subordinated to the regulatory mechanism which controls the growth of the root as an entirety.

On determining gradients in the length of vessel members of length-ways maize roots, a certain variation range was always observed within short root segments (LUXOVÁ and KOZINKA 1973). Mature cells isolated by maceration did not allow one to draw further conclusions from this finding, since the positional relations of vessel members in the longitudinal column, which represents a vessel, as well as the relations among vessels may be investigated only within an intact organ, or on longitudinal section. Moreover, mature tissues are then only the resultant of formative processes, so that the regularities of their formation must be searched for at their initiation.

In the paper which dealt with the kinetics of growth of the *Zea mays* root, the length of successive cells in longitudinal columns was measured in the growth region in three tissue types (LUXOVÁ, in preparation). The analysis revealed that the elongation of cells in columns has, besides the exponential course, an oscillatory character. The present paper aims at analysing cytologically the causes affecting the length of root cells.

MATERIAL AND METHODS

The primary roots of *Zea mays* (inbred line A 15 and the hybrid CE 380) of 5—10 cm in length served as experimental material. Root apices were fixed in Navashin's solution, and following dehydration by the alcohol

Received March 27, 1979; accepted April 24, 1979

**Address:* Dúbravská cesta 26, 885 34 Bratislava, Czechoslovakia.

series they were transferred through methylsalicylate in paraffin. Longitudinal sections 5—7 μm thick were stained with Heidenhain's haematoxyline. For the photodocumentation the apparatus Zetopan (Reichert) was employed.

RESULTS

In a lengthways generally very short growth region of the root a whole series of factors may participate in cell growth, which affect their length. These factors are associated on the one hand with cell proliferation, or, after the termination of proliferation, with the proceeding DNA synthesis, and on the other with cell elongation.

From the point of view of cell proliferation, the processes affecting the cell length are identical with a group of properties which characterize the root meristem as a highly organized, cytologically heterogeneous complex. In this respect certain properties of the root meristem were analysed in our previous papers (LUXOVÁ and MURÍN 1973, MURÍN and LUXOVÁ 1974, LUXOVÁ 1975, MURÍN and LUXOVÁ 1978).

Cell enlargement already occurs in the meristematic region: cells extend both in width and length. The vessel members of metaxylem are strikingly large, endodermal cells, pericyclic cells and adjacent layers are smallest. The differences in the cell size of individual tissue types point to the fact that the tissues of various types have a different, genetically determined growth potential. The proliferation capacity of the individual regions of the root meristem is of various duration, so that cell division does not stop at one level. The extent of the meristematic region is species specific, and within the species it is affected by the morphologic character of the root and its developmental stage. The cells originating in the course of successive cell cycles, form into longitudinal cell columns groups by two, four, eight, *etc.* cells according to the number of cycles accomplished, or by reduced or increased cell numbers in the case when all cells have not yet gone through the division of a certain cycle, or, on the contrary, when a stimulation of division occurs. The boundary of these groups into longitudinal columns is made expressive by the size of the end cells which are usually somewhat narrower and longer (Fig. 1). The greater number of cycles the cells go through, the relatively shorter they are owing to this. In the maize root *e.g.* small cells of the internal cortical region and those of the peripheral stelar region undergo 5 to 6 cell cycles and maintain their proliferation capacity approximately to the distance of 1500 μm, whereas the metaxylem vessel members go through only 3 to 4 cell cycles and their mitotic activity ceases already at a distance of approximately 400 μm above the boundary between the root cap and root meristem. Within the growth region of the root an equilibrium must be maintained between cell division and elongation. The individual meristem regions have a mitotic cycle of different duration; with an increasing distance of cells from the quiescent centre in the basipetal sense their mitotic cycle shortens. Another cause of a different cell length is an unequal cell division, as well as the change in the polarity of cell division, in which the transverse cell division is substituted by a longitudinal one (Figs. 2, 3). A certain part in the fluctuating length of cell elements is taken by a transient or permanent cessation of the proliferation of some cells in the meristematic region. If the transverse division does not occur, it is com-

pensated, as in the preceding case, by increased cell length. The length of cells is also affected by whether the cells stop their mitotic activity at the G_1 or G_2 phase of the cell cycle, or whether the synthesis of nuclear DNA continues and an endoreduplication takes place (Fig. 4).

As far as cell elongation is concerned, the changes in the overall growth rate of the root, and the changes in growth rate as caused by the gradient lengthways of the root growth region, come into consideration. The overall

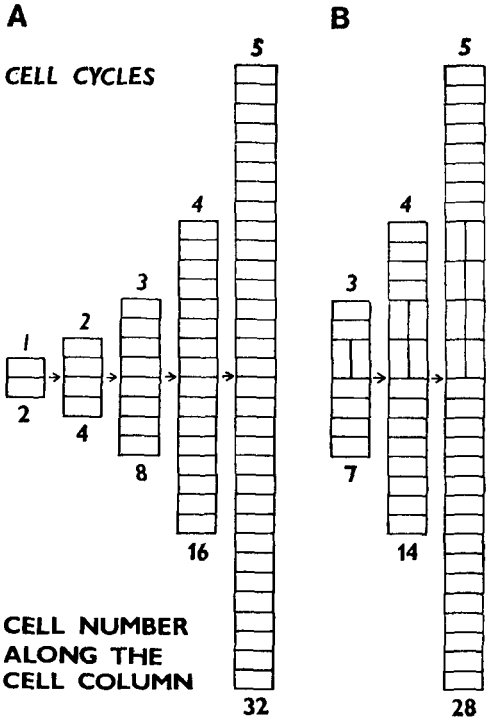

Fig. 2c. A diagram of cell division of a derivative cell which undergoes five cell cycles. A — a continuous process occurring in a longitudinal cell column is demonstrated as individual successive cycles. B — the situation on a change in polarity of the cell division in one cell of the third cycle.

rate of root growth as related to time represents, in the course of ontogenesis, a sigmoid curve with an exponential, linear, and logarithmic phase. External conditions may induce irregular changes of this general programme. The elongation growth lengthways in the growth region is not uniform. As to the position it represents a single-peak curve with low values in the proximal proliferation region, with basipetally proceeding increase in the rate of elongation growth up to its maximum, and its successive decrease up to complete cessation. The gradient of the growth rate lengthways in the root growth region may result in a differential growth within individual cells with differences in the elongation rate of their proximal and distal regions (Fig. 5). This may be the case especially in longer cells, *e.g.* in vessel members which, in the maize roots analysed, attain a length of up to 1.5 mm in their mature state. In accordance with the gradient mentioned, the proximal part

of the cell elongates more rapidly, then the increase in growth rate passes to its distal part. Another case of a differential growth is brought about by small differences in the growth rate of adjacent longitudinal cell collumns. They become evident by a differential growth of opposite side walls of the column, which forms a boundary between the region of faster and slower growth. On one side of the column the common walls grow faster, on the other slower. The end cell walls in the corresponding segment of this column become slanting in consequence of this (Fig. 6). If the increase in growth rate later passes to the region originally growing more slowly, they may again become oriented transversely. On the basis of the material analysed one cannot decide, if greater differences in the growth rate occur also in adjacent regions where symplastic growth is substituted by a sliding one.

The cytological heterogeneity of the root growth region indicates the heterogeneity of its metabolic activity. The documentation of the cases mentioned gives evidence in this respect of a considerable autonomy not only of the individual root region, but also of cell columns and individual cells, or their parts. On applying Mitchison's growth model (MITCHISON 1974) to our material, the final length of the root cell may be characterized as the result of a series of processes representing the transition of the growing cell from its origin to the completion of its elongation. The cytological analysis pointed out the character of the processes mentioned which may participate in the final length of the root cells variously represented. The processes associated with cell proliferation and elongation have, as has been convincingly demonstrated by several authors, their own regulatory mechanisms. The results obtained suggest that the mechanisms regulating growth processes have a locally limited action and simultaneously they are subordinated to the regulatory mechanism which governs growth of the root as an entirety.

DISCUSSION

Far less attention has been paid as yet to the cell length of plant organs during their primary growth than to the cell length of secondary tissues, especially to the secondary xylem. Even in the well-studied field of qualitative xylotomy the approach of the evaluation of element length in relationship to the rate of elongation of the organ analysed is quite unique, though a long time has elapsed since this was stated by DINWOODIE (1961).

The processes which affect the cell length during the primary growth of the root are associated not only with cell enlargement, but also with their proliferation. There must be an equilibrium between cell proliferation and enlargement within an organ. From the point of view of cell proliferation, the processes affecting the cell length are identical with the cytological heterogeneity of the root meristem. In this respect some of its properties in relationship to the cell length deserve special attention.

An indirect correlation exists between the number of cycles the cells go through and the cell length. This relationship is shown not only between individual tissue types (or individual cell columns), but it also holds within a cell column. If it is assumed that all cells in the meristem are mitotically active, their number in the populations of successive cycles should increase with geometrical progression. In reality, this is not the case, for, as found out by BALODIS and IVANOV (1970), one part of cells in the proximal half

of the region of the mitotic activity which should be approximately the region of the last mitotic cycle, does not enter mitosis any more. CLOWES (1975) observed a decline in the mitotic activity towards proximal and distal margins of the root meristem. He proved that the decline in both regions is not brought about by an increase in the duration of the cell cycle, but by a reduction of the fraction of proliferating cells. DAVIDSON (1977) refers to the fact that the cell which, in consequence of a differential mitotic division stops proliferating, grows slower than its sister cell which goes on proliferating. However, we observed that the cells whose mitotic activity ceases earlier, are eventually longer also owing to a non-realized transverse division. The same tendencies of the growth of cells in columns are referred to by BALODIS and IVANOV (1970). The authors emphasized that no sliding growth took place. On the contrary, according to DAVIDSON (1977) the fact that cells in the meristem grow at a varying rate, gives evidence in favour of the presence of the sliding growth. A long discussed question, as to whether or not the sliding growth exists in roots, which SINNOTT and BLOCH (1939) and BRUMFIELD (1942), when studying the cell growth in living roots, answered negatively, is thus again brought up for discussion. In spite of the fact that we were able to register a varying growth rate of laterally located cells, the differences were small enough, so that in the cell column only a slanting of end cell walls occurred. However, it is not clear whether in some cases the mutual position of cells changes, as occurs if the sliding growth is concerned.

The relationship between the cell growth and the proceeding synthesis of nuclear DNA, as observed in certain root regions after the cessation of mitotic activity (TSCHERMAK-WOESS 1956), is of interest. The possibility of a continuous and thus faster growth in endocycles was pointed out by STEBBINS (1965). In this respect the interest was focussed first of all on the role of endocycles in the ontogenesis of vessel members (LIST 1963, INNOCENTI and AVANZI 1971, INNOCENTI 1975, LAI and SRIVASTAVA 1976).

The cytological characteristics of causes which affect the length of root cells is evidence of the complexity of the problem investigated. Since the knowledge of regulation of the synthesis of enzymes and other proteins is considered as a key problem for the understanding of cytodifferentiation (TRUMAN 1976), it is clear on the basis of the present material that the elucidation of the physiological causes of the oscillating length of root cells calls for analyses at the cell level.

REFERENCES

BALODIS, V. A., IVANOV, V. B.: [Study of the cell division in the root.] In Russ. — Tsitologiya **12** : 983—992, 1970.

BRUMFIELD, R. T.: Cell growth and division in living root meristems. — Amer. J. Bot. **29** : 533 to 543, 1942.

CLOWES, F. A. L.: The cessation of mitosis at the margins of a root meristem. — New Phytol. **74** : 263—271, 1975.

DAVIDSON, D.: [Heterogeneity of cell populations in the root meristem.] In Russ. — Fiziol. Biokhim. kul't. Rast. **9** : 3—17, 1977.

DINWOODIE, J. M.: Tracheid and fibre length in timber. A review of literature. — Forestry **34** : 125 to 144, 1961.

INNOCENTI, A. M.: Cyclic changes of histones/DNA ratio in differentiating nuclei of metaxylem cell line of *Allium cepa* root tip. — Caryologia **28** : 225—228, 1975.

INNOCENTI, A. M., AVANZI, S.: Some cytological aspects of the differentiation of metaxylem in the root of *Allium cepa*. — Caryologia **24** : 283—292, 1971.

LAI, V., SRIVASTAVA, L. M.: Nuclear changes during differentiation of xylem vessel elements. — Cytobiologie **12** : 220—234, 1976.

LIST, A. JR.: Some observations on DNA content and cell and nuclear volume growth in the developing xylem cells of certain higher plants. — Amer. J. Bot. **50** : 320—329, 1963.

LUXOVÁ, M.: Some aspects of the differentiation of primary root tissues. — In: TORREY, J. G., CLARKSON, D. T. (ed.): The Development and Function of Roots. Pp. 79—90. Academic Press, London 1975.

LUXOVÁ, M., KOZINKA, V.: Study of the vascular flow in the root segments of *Zea mays* L. — Biológia **28** : 227—234, 1973.

LUXOVÁ, M., MURÍN, A.: The extent and differences in mitotic activity of the root tip of *Vicia faba* L. — Biol. Plant. **15** : 37—43, 1973.

MITCHISON, J. M.: Sequences, pathways and timers in the cell cycle. — In: PADILLA, G. M., CAMERON, J. L., ZIMMERMAN, A. (ed.): Cell Cycle Controls. Pp. 125—142. Academic Press, New York 1974.

MURÍN, A., LUXOVÁ, M.: Heterogeneity of the cell population in the root tip of *Vicia faba* with respect to the mitotic cycle time. — In: KOLEK, J. (ed.): Structure and Function of Primary Root Tissues. Pp. 33—36. Veda, Bratislava 1974.

MURÍN, A., LUXOVÁ, M.: The sequence and duration of mitotic cycles in the apical root meristem of *Vicia faba* L. — Biol. Plant. **20** : 293—298, 1978.

SINNOTT, E. W., BLOCH, R.: Changes in intercellular relationships during the growth and differentiation of living plant tissues. — Amer. J. Bot. **26** : 625—634, 1939.

STEBBINS, G. H.: Some relationships between mitotic rhythm, nucleic acid synthesis and morphogenesis in higher plants. — Brookhaven Symp. Biol. **18** : 204—221, 1965.

TRUMAN, D. E. S.: [Biochemistry of cell differentiation.] Translat. in Russian. — Mir, Moskva 1976.

TSCHERMAK-WOESS, E.: Karyologische Pflanzenanatomie. — Protoplasma **46** : 798—834, 1956.

Figures at the end of the issue.

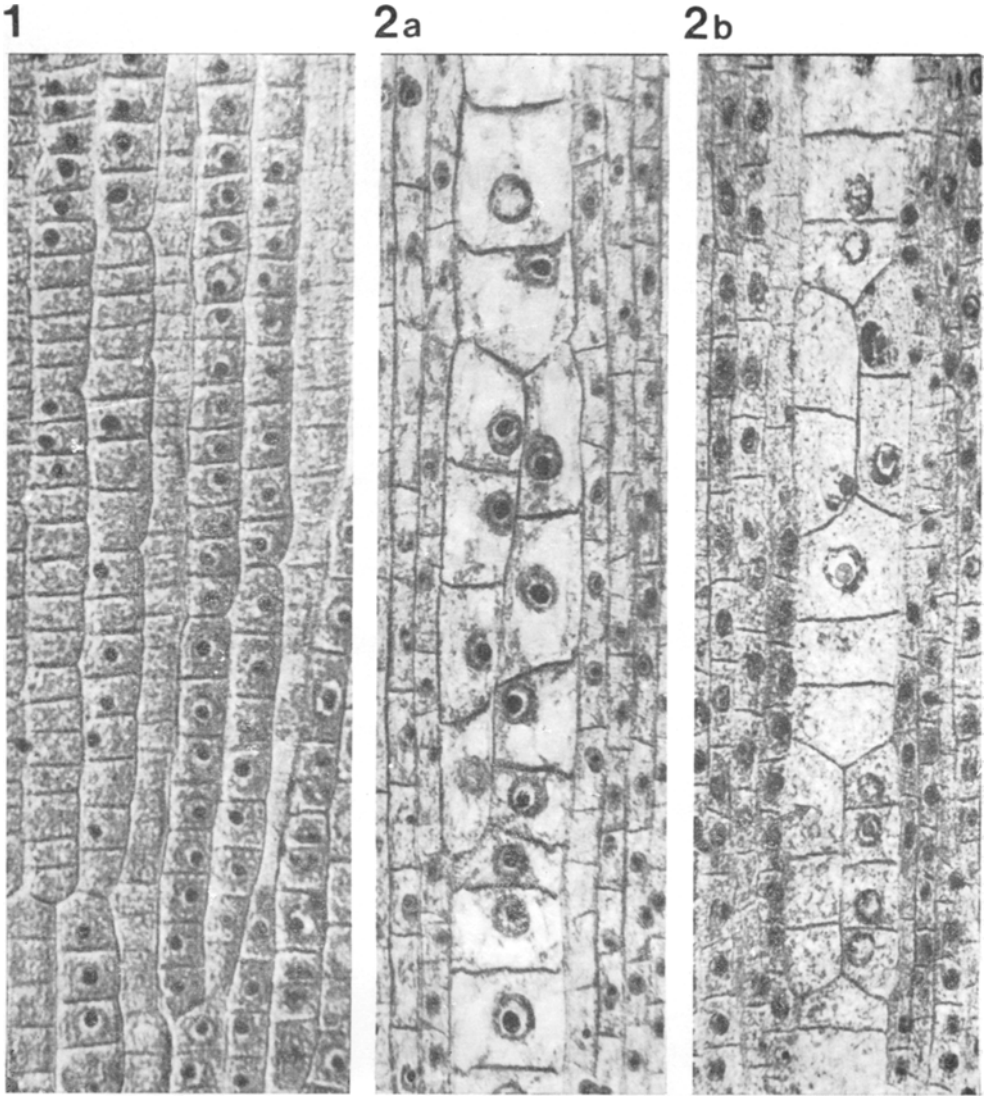

Fig. 1. The groups of cells originating during successive cell cycles are expressive by the shape of their end cells, which are somewhat longer and narrower, so that in the column they form a notch. × 500.
Fig. 2a. The change in polarity of cell division in vessel members of the large metaxylem which normally divide only transversely. Narrower, but longer sister vessel members resulting from the longitudinal division divide further transversely until the programme of their division is exhausted. In this way a locally limited doubling of the cell column occurs. A reduced cell number along the column is compensated by their length.
Fig. 2b. A longer cell originates also in the case when transverse division does not occur (indicated with an arrow). × 350.

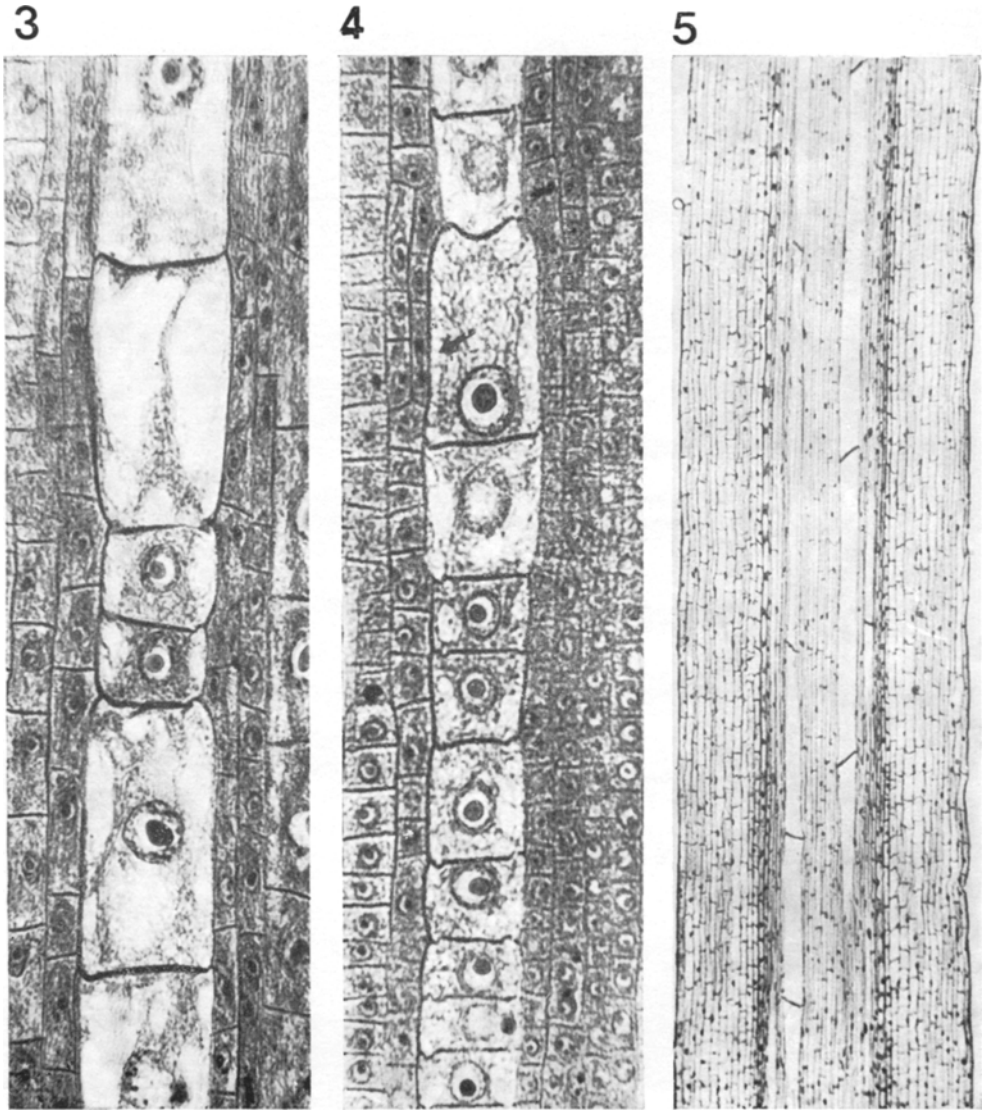

Fig. 3. A detail of the column of vessel members otfhe large metaxylem. The size of the nucleus in the lower member suggests a somatic endopolyploidy. In two short members the development was evidently inhibited. × 350.
Fig. 4. The differential growth of the vessel member whose proximal part elongates faster. In the adjacent cell (indicated with an arrow) unequal cell division may be observed. × 350.
Fig. 5. The differential growth of common side walls of a cell column, which grow faster on the external side. In consequence of this the end walls become slanting. × 50.