ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XL — nr 2
1971

Organization of the root apical meristem in *Linum usitatissimum* L. grown at 25°C and 7°C

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(Received: February 1, 1971)

Abstract:

In the first days of intensive growth of the *Linum usitatissimum* root, the central part of the apical meristem exhibits usually a 4-tier organization. When growth ceases reorganization of the cell arrangement occurs. It starts by periclinal division of the subprotodermal initials, whose derivatives are forming the secondary columella in the central part of the root cap.

INTRODUCTION

The apical meristem of the root of *Linum usitatissimum* L. was described by several authors as having four tiers of initials (Janczewski 1874; Eriksson 1876; Crooks 1933, Heydel according to Guttenberg 1960; Deschamps 1967). The root apex organization in *Linum usitatissimum* is different from the common pattern in roots of dicotyledons, because it has two tiers of cortical initials, instead of the single one. The outer layer of cortical initials is continuous with the subprotodermal layer. Outside the cortex initials, there is a tier of rootcap-protoderm initials and inside a tier of central cylinder initials (Fig. 1, 2).

Recently Byrne and Heimisch (1968) investigated the structure of the root apical meristem in 13 species of the genus *Linum*. In ten species, including *Linum usitatissimum*, they found four-tiered organization. According to their observations, there are variations in the number of tiers of cortical initials; either an increase to three or a decrease to one may occur when the roots are elongating. However, these variations were interpreted as not leading to reorganization of the root apex.

In this paper we present observations made on the radicles of ripe seeds and on the primary roots of various length from *Linum usitatissimum* seedlings grown at temperatures of 25° C and 7° C.

MATERIALS AND METHODS

After germination on moistened paper, seedlings of *Linum usitatissimum* were placed on plastic nets and grown in containers in pond water for up to 22 days at 25°C (Table 1) or at 7°C (Table 2). Root tips from ripe seeds and from seedlings were cut off and fixed in CRAF' (0,5—1—20). Sections were stained with safranin and fast green.

RESULTS

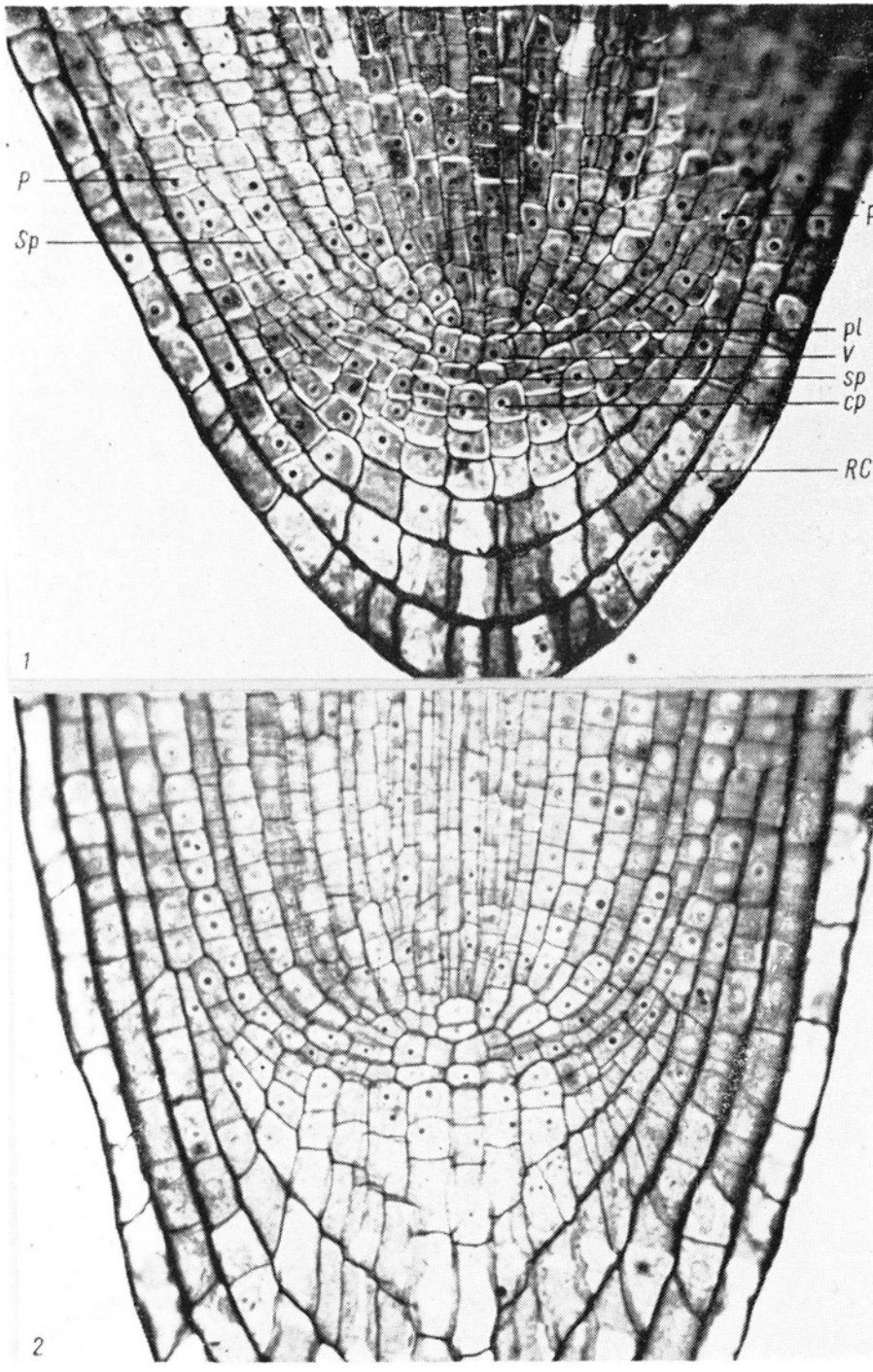
Seedlings with 0.8 cm roots were cultured at 25° C. On the first day, the roots elongated 1—1.5 cm; in the following days 2—2,5 cm daily. After five days of culture roots reached a length of 10—12 cm. From the sixth day, the speed of growth suddenly decreased, and the side roots began to develop concurrently. After 8—10 days of culture, the main roots ceased to elongate altogether. At the temperature of 7°C the roots grew for about ten days with a speed of 3—4 mm per day and later even slower, reaching eventually a length of 5,5 cm after 22 days.

Table 1

Organization of apical meristem in roots growing at 25°C

Time, days	Number of roots	Length cm	Number of tiers		Beginning of reorganization	Reorganization	
			4	5		one tier initials of cortex	two tiers initials of cortex
Embryo roots	44	—	38	2	4	—	—
0	10	0,8	9	1	—	—	—
1	10	2.0	8	1	1	—	—
2	16	4.5	12	3	1	—	—
3	15	7.0	15	—	—	—	—
5	27	10—12	24	2	1	—	—
6	12	10—12	3	4	—	1	4
10	16	11—12	4	—	—	4	9
12	14	10—12	2	—	—	3	9
22	18	10—12	3	1	—	1	13

The great majority of embryo roots and seedlings roots grown during 5 days at 25°C displayed a four-tiered organization of the apical meristem. Only a few roots (about 7%) had five tiers of initials in the embryos and seedlings (Table 1, Fig. 3). Roots with five tiers of initials in the apical meristem had three tiers of cortex initials, a single tier continuous with the subprotoderm and two upper tiers connected with the inner cells of the cortex.



Central longitudinal section through the apical root meristem of *Linum usitatissimum*

Fig. 1. Root apex of an embryo in a ripe seed. Four-tiered apical meristem
Fig. 2. Apex of an 8 cm root, four-tiered apical meristem grown at 25°C, × 450

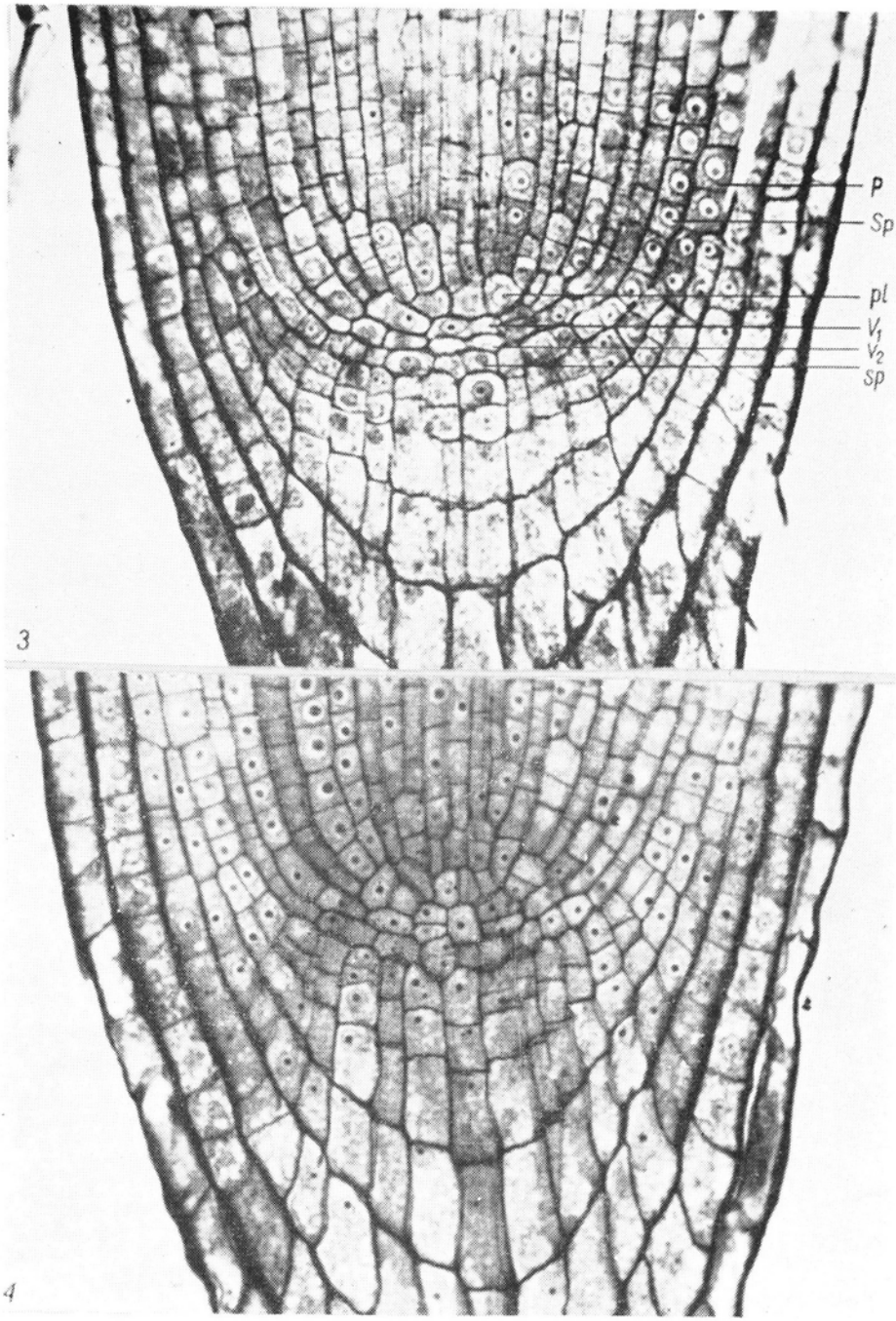


Fig. 3. Apex of a 4,5 cm root, five-tiered apical meristem with three tiers of cortex initials

Fig. 4. Apex of a 10 cm root, a cell in the subprotoderm initials tier has divided grown at 25°C, × 450

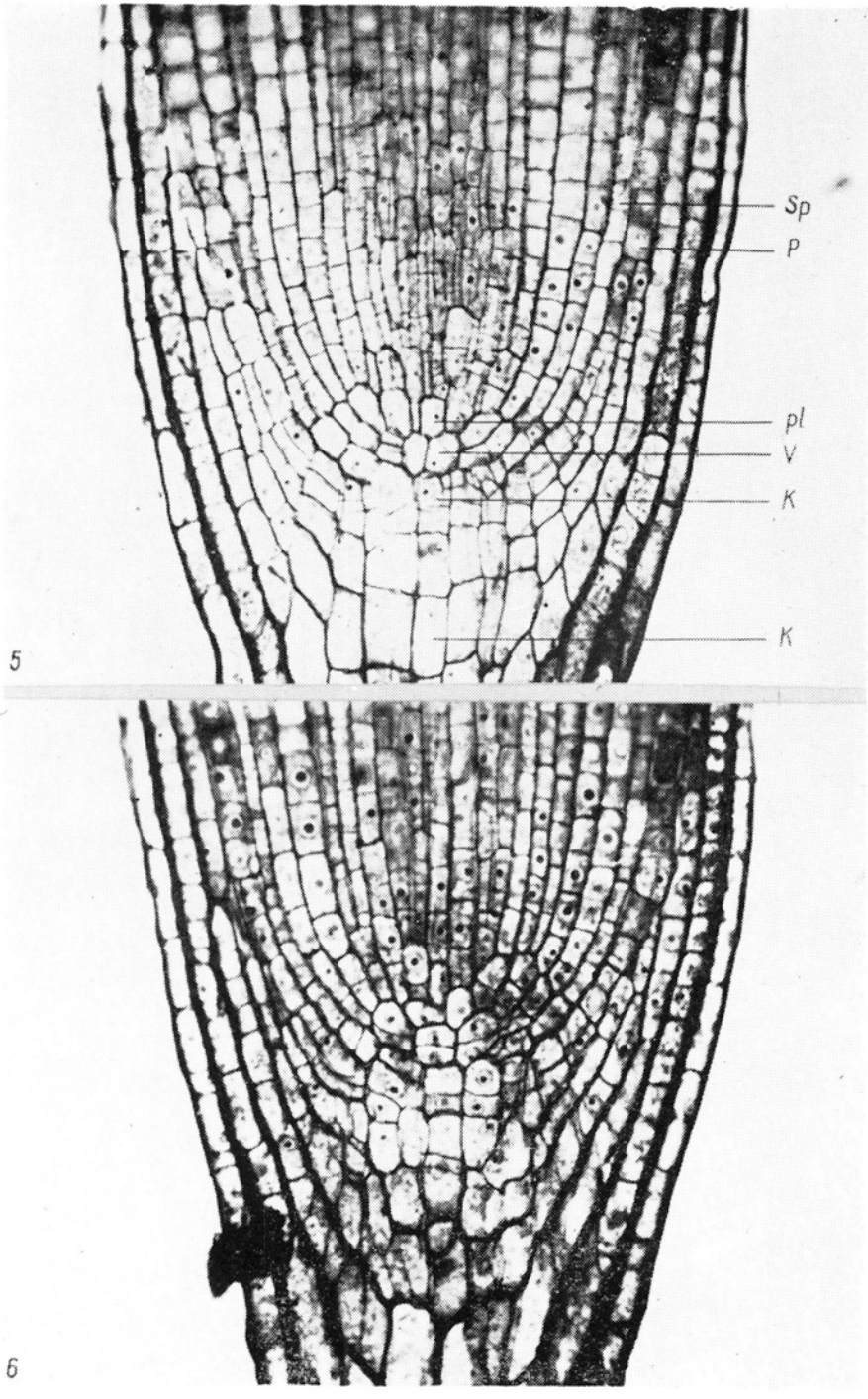
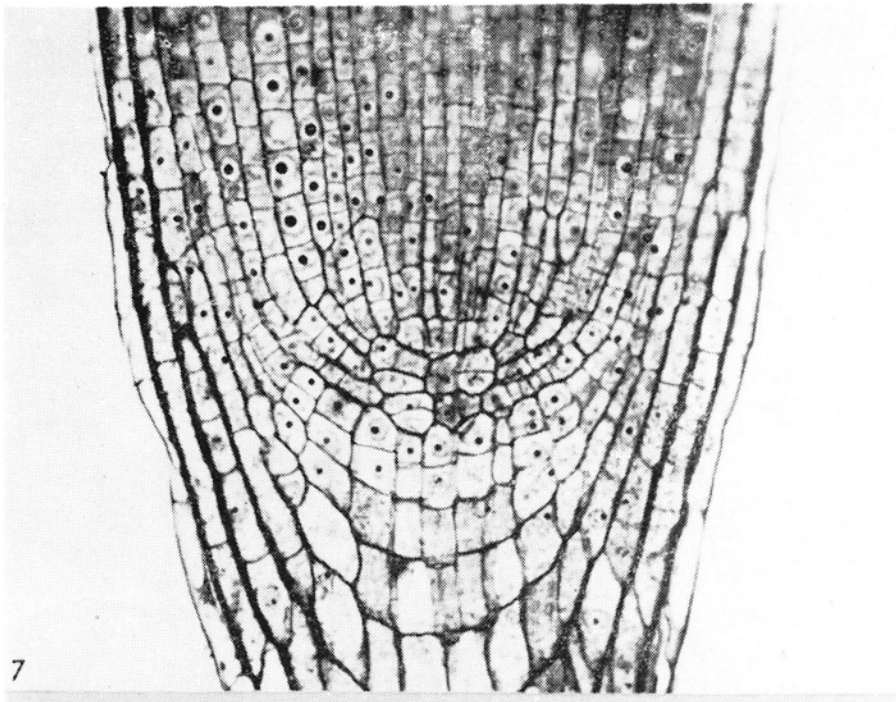


Fig. 5. Apex of a 12 cm root with the secondary three-tiered apical meristem. The initials of the subprotoderm give rise to the secondary columella; one tier of inner cortex initials is present

Fig. 6. Apex of a 12 cm root with the secondary four-tiered apical meristem; two tiers of inner cortex initials are present

grown at 25°C, × 450



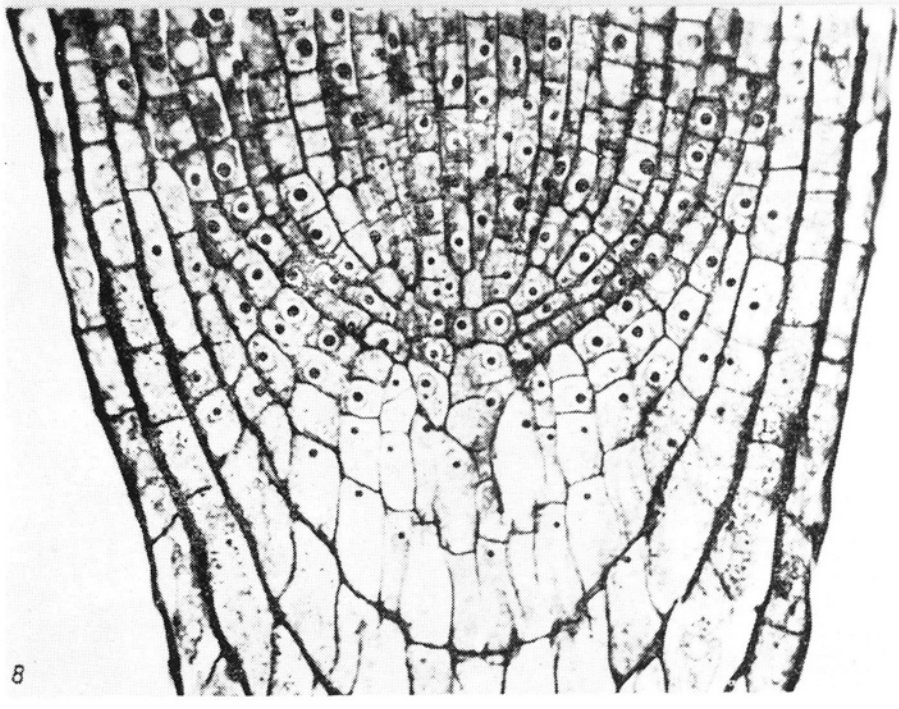


Fig. 7. Apex of a 1,5 cm root. One cell divided periclinally in the tier of the subprotoderm initials

Fig. 8. Apex of a 2,5 cm root. Subprotoderm initials give rise to the secondary columella. Secondary three-tiered apical meristem
grown at 7°C, × 450

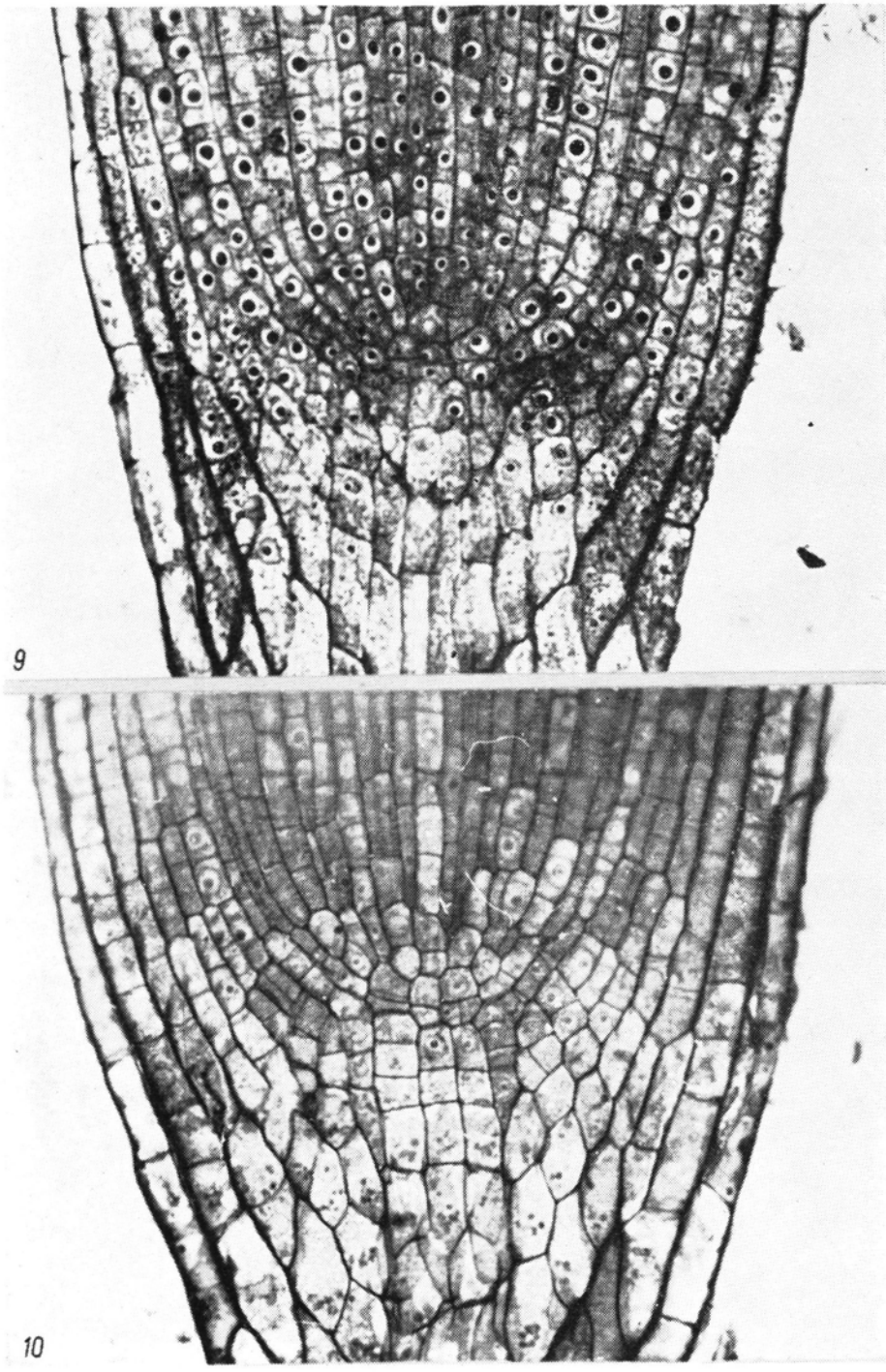
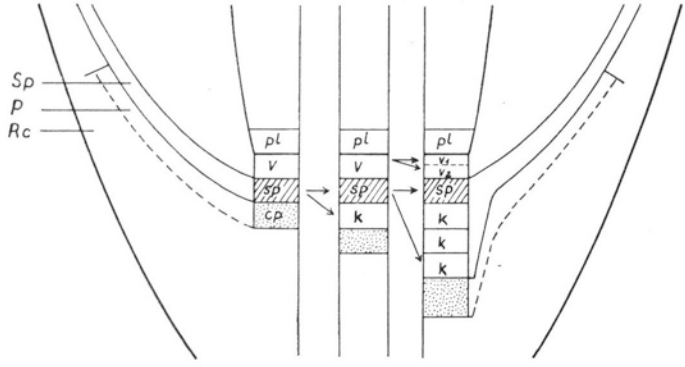


Fig. 9. Apex of a 2,5 cm root, with secondary four-tiered apical meristem

Fig. 10. Apex of a 4,5 cm root with secondary four-tiered apical meristem. The secondary columella originating from subprotoderm initials stretches along the central region of the root cap

grown at 7°C, × 450

Beginning with the sixth day of culture, growth of the roots at 25°C slowed down and stopped. In a large proportion of these roots (73%) changes in the arrangement of apical meristem initials were visible.



Scheme showing the successive reorganization of the apical root meristem
Central longitudinal section

pl — initials of the central cylinder, *v* — initials of the inner cortex, *sp.* — subprotoderm initials, *sp* — rootcap-protoderm initials, $v_1 - v_2$ — cells arising from *v*; *k* — cells of the secondary columella, *Sp* — subprotoderm, *P* — protoderm, *Rc* — rootcap

Beside the roots with rearranged organization of apical meristem, there were roots with the usual four or five-tiered centre of apical meristem (Table 1).

The change of the apical meristem organization began in the tier of the subprotoderm initials. At first, one cell was divided by a periclinal wall and the distal derivative penetrated into the territory of the root cap (Fig. 4). Then the neighbouring subprotodermal initials divided periclinally, forming two-cellular complexes protruding in distal direction and displaying the rootcap-protoderm initials. In this manner, the formation of the secondary columella began. In extreme cases, it was built of perpendicular rows of 5—6 cells. Former rootcap-protoderm initials were displaced acropetally and completely differentiated into rootcap parenchyma cells. As a result of these changes, the original pattern of four initial tiers was replaced by three-tier apical meristem (Fig. 5). There was a tier of central cylinder initials, one tier of the inner cortex initials and one tier of secondary columella initials continuous with the subprotoderm.

The roots of plants grown at 7°C elongated very slowly and after five days reached a length of about 2,5 cm. The rearrangement of the apical meristem began already on the second day of culture, in 1.5 cm long roots. Reorganization of the apical meristem advanced in the course of further days and appeared in many of the roots tested (Table 2). The basic character of the reorganization was identical to that observed in long

Table 2

Organization of apical meristem in roots growing at 7°C.

Time, days	Number of roots	Length cm	Number of tiers		Beginning of reorganization	Reorganization	
			4	5		one tier initials of cortex	two tier initials of cortex
0	10	0.8	10	—	—	—	—
1	10	1,0	10	—	—	—	—
2	16	1.5	10	2	2	1	1
3	18	2,0	9	—	4	4	1
5	22	2,5	4	1	3	6	8
15	12	4,5	2	—	—	3	7
22	32	5,5	4	4	9	1	14

roots kept at 25°C which ceased to elongate. Fig. 7 shows a beginning of reorganization similar to that in Fig. 4. A cell of the initial tier of subprotoderm is periclinally divided, the distal cell of the two-cell complex has penetrated into the rootcap region. Further stages of the secondary columella development are visible in Figs 8 and 9. Three periclinally divided subprotoderm initials are visible in Fig. 8. Their derivatives lie in the rootcap region. The secondary columella grows further, eventually occupying the whole central region of the rootcap. The columella is built of perpendicular rows of cells, each including a cell from the initial layer and its derivatives (Fig. 10).

DISCUSSION

Fast growing roots of *Linum usitatissimum* seedlings and roots of embryos from ripe seeds usually have four tiers of apical meristem initials. In some roots (in 9 out of 122) there is a five-tiered organization with three tiers of cortex initials. Such a structural variation was described by Byrne and Heimisch (1968), though other authors have seen only four-tier initials.

This four or five-tiered organization of apical meristem was changed in most roots, which had ceased growing after several days of culture at 25°C or in very slowly growing roots of seedlings kept at 7°C. The changes appeared in the roots of 10—12 cm and 1.5 cm long roots at 25°C and 7°C respectively. Thus reorganization takes place in roots where growth stopped or greatly diminished.

Histological changes consisted of periclinal divisions of the cells in the subprotodermal initial tier. New rows of cells arose and formed the secondary columella occupying the central part of the rootcap. The common initials of the rootcap and protoderm were displaced distally

and differentiated into ordinary parenchymatous rootcap cells. As a consequence of the reorganization, a new pattern of apical meristem developed, which may be either secondary, three- or four-tiered. The three-tiered apical meristem was formed, when in the original four-tiered apical meristem, subprotodermal initials divided and the ensuing secondary columella displaced the tier of rootcap-protoderm initials. Then there were three tiers of initials in the apical meristem; the first tier is composed of central cylinder initials, the second of inner cortex initials and the third of the secondary columella initials.

It seems that the secondary 4-tiered pattern of growth originates: a) from the secondary 3-tiered one through periclinal divisions of the tier of cortical initials V in V_1 and V_2 (Fig. 6), b) from the five-tiered pattern of growth, when the outermost tier of the apical meristem is displaced by the secondary columella formed by periclinal divisions of the subprotoderm initials (Fig. 10).

The reorganization of the growth pattern of root apical meristem has been observed in experimental conditions after X-ray treatment of *Zea mays* roots (C l o w e s 1963). In treated roots, growth and mitotic activity in the meristematic region were stopped. In the quiescent centre, however, where in normal conditions mitoses are rare or nonexistent they appeared after X-ray treatment. Divisions in the quiescent centre led to an irregular dearrangement of the apical meristem. Treatment with 2,4-D also brought about the initiation of mitotic actvity in the cortex initials of the apical meristem in roots of rice (K a u f m a n n 1955).

In the case described by us, the rearrangement led to the formation of new growth patterns of the apical meristem. These changes occurred in non-growing long roots at 25°C and in very slowly growing roots at 7°C.

Similar changes of the growth pattern of the apical meristem were also described in the normal growth of roots of *Helianthus* annuus and *Anoda triangularis* (v. G u t t e n b e r g, B u r m e i s t e r and B r o s e l l 1955) and particularly in *Elodea canadensis* and *E. densa* (A. R. K a d e j 1966).

SUMMARY

After 5 days of growth at 25°C, roots of *Linum usitatissimum* seedlings were 10—12 cm long. During this period of active growth there was a 4-tiered organization of the apical meristem and in about 7% 5-tiered roots, with 3 layers of cortex initials. The outermost layer of cortex initials was always continuous with the subprotoderm layer. In about 73% of non-growing, long roots, there occurred a reorganization of the growth pattern of the apical meristem during the following 17 days of culture. Similar rearrangemant was observed in very slowly growing roots at 7°C. Reorganization led to new patterns of growth: a secondary three-tiered and four-tiered pattern. It began with the periclinal divisions of the subprotoderm initials which gave rise to the formation of the secondary columella displacing the rootcap-protoderm initials and occupying the central part of the rootcap (scheme).

The authors express their thanks to Dr B. Rodkiewicz for his advice and to Professor H. Teleżyński for his interest for this investigation.

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Organizacja merystemu wierzchołkowego korzenia Linum usitatissimum rosnącego w temperaturze 25° i 7°C

Streszczenie

Korzenie *Linum usitattissimum*, hodowane w temperaturze 25°C, rosną przez 5 pierwszych dni równomiernie osiągając długość 10—12 cm. W tym okresie intensywnego wzrostu centrum merystemu wierzchołkowego korzeni wykazuje 4-piętrową organizację; dolne piętro kory pierwotnej tworzy z subprotodermą ciągły pokład. Tylko nieliczne korzenie (7%) mają budowę 5-piętrową z trzema piętrami komórek inicjalnych kory. Po zaprzestaniu wzrostu u większości korzeni długich (73%) zaobserwowano zmianę w układzie komórek centrum merystemu. Podobna reorganizacja występuje w krótkich, bardzo wolno rosnących korzeniach hodowanych w 7°C. Zmiany w układzie komórek centrum merystemu prowadzą do przekształcenia pierwotnego, 4-piętrowego wzoru wzrostowego merystemu we wtórny 3 i 4-piętrowy (schemat). Reorganizację rozpoczynają podziały peryklinalne w komórkach inicjalnych piętra subprotodermy. W wyniku tych podziałów wykształcają się kompleksy komórek tworzące wtórną kolumellę. W krańcowym przypadku jest ona rezultatem 4—5 podziałów peryklinalnych i zajmuje środkową część czepka.