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Effects of water soluble oncostatic fraction from *Rheum officinale* Baill. rhizomes on *Allium cepa* root meristem

II. Meristem length and mitotic activity distribution

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Abstract

Incubation in 5 and 12.5 per cent extract from *Rheum officinale* rhizomes causes disturbance of the dynamic equilibrium between the number of dividing cells and the number of those passing to the elongation zone. The zone of meristematic cells is shortened to 2/3 and the zone of mitoses to 1/2 after 24-h incubation in 5 per cent extract. 12-h incubation in 12.5 per cent extract does not reduce the zone of meristematic cells, although it shortens the mitosis zone to 1/5. This suggests that a high concentration of the inhibitor arrests elongation growth.

Mitotic activation of the meristem in the beginning of postincubation period occurs on a wide area since the last mitotic cycle runs in the cells of the basal part of the meristem. During further postincubation (48 and 72 h after 5% and 72 h after 12.5% extract) the meristematic zone is greatly shortened and the zone of highest mitosis frequency shifts in apical direction. The mitotic activity in the apical sector much higher than in the control suggests, that the quiscent centre takes part in the reconstruction of the meristem.

INTRODUCTION

The length of the meristematic zone in bulb roots growing at a constant rate is constant (Lopez-Saez et al., 1975). The stability of the meristem length expresses the state of dynamic equilibrium between the number of dividing cells and the number of those passing to the elongation zone. Preliminary observations demonstrated that during incubation in the extract of *Rheum officinale* rhizomes and during postincubation the state of equilibrium is disturbed. Part of the

cells instead of entering the new mitotic cycle begin to elongate, owing to which the apical meristem becomes shorter. This problem was investigated in detail in the present study.

MATERIAL AND METHODS

Adventitious roots of onion (*Allium cepa* bulbs) of 3-cm length initially, growing in darkness at room temperature were the object of the study. The experimental conditions are described in detail in Part I of the present paper (Dawidowicz-Grzegorzewska, 1976). Material was collected after incubation for 6, 12 and 24 h in 5 per cent extract and after 6- and 12-h incubation in 12.5 per cent extract. The root apexes were fixed in CrAF (percentual composition 0.5—0.5—20), longitudinal microtome sections 6.6 μm thick were prepared and stained with iron haematoxylin and counterstained with fast green.

The length of the meristematic zone was measured and the distribution of mitoses in it analysed each time on the 3 middle sections of two roots. The diagrams show arithmetic means of the measurement results. As zero level in the apical part of the root was considered the border between dermatogen initials and the root cap initials. The criterion according to which the given cell in the root apex was qualified as meristematic was adopted after Gonzalez-Fernandez et al. (1966). According to these authors, the nuclear diameter in meristematic onion cells is longer than or equal to 1/2 of the cell length measured along its long axis.

RESULTS AND DISCUSSION

The mean length of the meristematic zone (measured from 0 level in basal direction) in control roots changes slightly oscillating between 2200 and 1920 μm. In control roots the mean range of the meristematic zone was shorter than the initial value by about 30 μm after 96 h of the experiment. Changes in the length caused experimentally differ significantly from the control data. Dermatogen, periblem and plerome in 5 per cent extract have after 6 h of incubation a level of 1800 μm, this indicating that their length is reduced by about 300 μm (Fig. 1) as compared with the corresponding control data. After 24 h of incubation in 5 per cent extract the length of these histogens is still more reduced, on the average by 700 μm (Fig. 1). These data indicate that under conditions of considerable mitotic depression (Dawidowicz-Grzegorzewska, 1976) the cells undergo elongation growth.

During postincubation a further shift of the meristematic zone occurs in apical direction (Fig. 1, 24 and 48 h of postincubation). In the latter case after 48 h od postincubation the meristem length returns to the

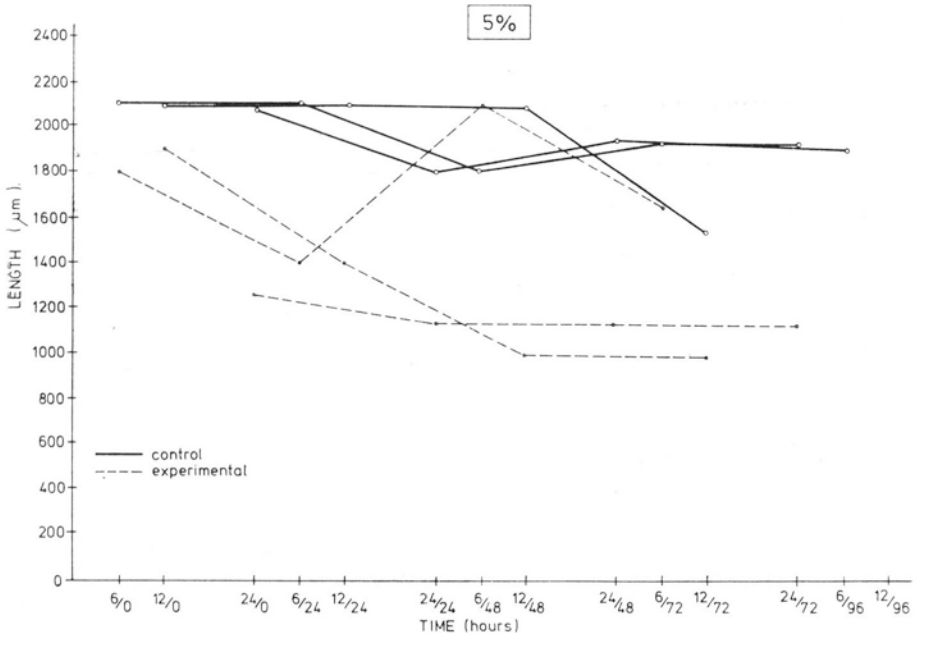

Fig. 1. Length (in μm) of periblem in root meristematic zone during incubation in 5 per cent extract and during postincubation in water. Particular curves denote data for 6, 12 and 24 h of incubation and following postincubation

control level. In the remaining cases further shortening of the meristematic zone was observed, on the average by 900 μm as compared with the control, this constituting an almost twofold shortening of the meristem length. The above described changes occur similarly in he particular histogens.

Incubation under sublethal conditions (i.e. 12 h in 12.5% extract) does not produce a shortening of the meristematic zone (Fig. 2). This suggests that under sublethal conditions the inhibitory effect involves not only cell division, but also their elongation. During postincubation elongation growth gradually involves the basal part of the meristem which is reduced after 96 h to 600 μm that is to 1/3 of the control.

Changes in the length of the meristematic zone are accompanied by changes in distribution and frequency of mitoses along the root axis. These changes were investigated by counting the mitoses in 11 successive 210-μm transverse sectors, each time for 3 longitudinal middle sections of two roots (Fig. 3).

In control roots mitoses occur in 10 sectors that is on a length of 2100 μm. Mitosis distribution along the meristem axis is nonuniform. The highest frequency of division is found in the subapical part of the meristem on a length of 840 μm comprising secors II, III, IV and V with maximum frequency in sector IV.

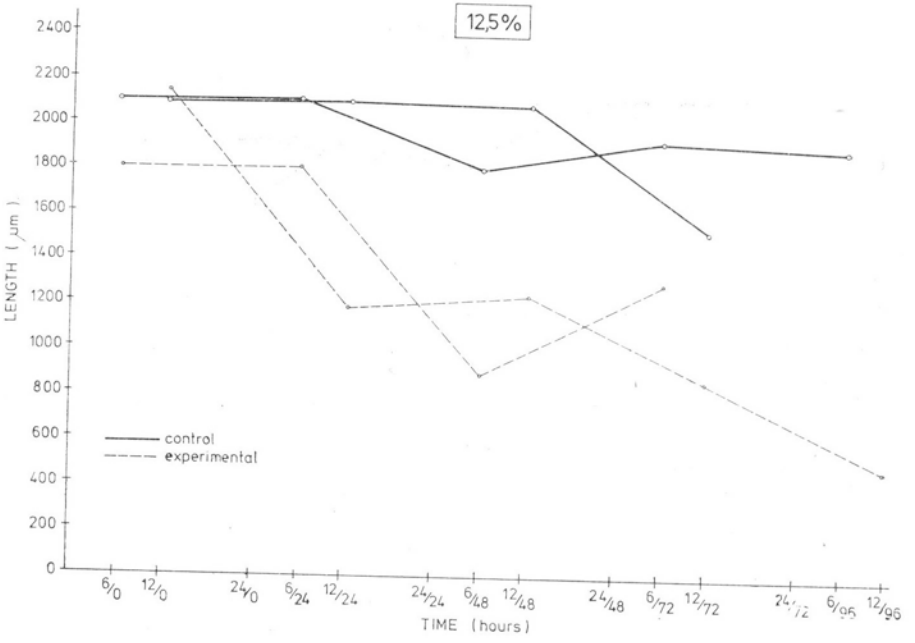

Fig. 2. Length (in µm) of periblem in root meristematic zone during incubation in 12.5 per cent extract and postincubation in water. Particular curves denote data for 6 and 12 h of incubation and following postincubation

Incubation in extract shortens in each combination the zone of dividing cells. Figs 3 and 4 show the changes in frequency and distribution of mitoses for two chosen representative combinations: 24-h incubation in 5 per cent extract and 12-h incubation in 12.5 per cent extract. In both cases the zone of mitotic activity is reduced in the incubation period to 1/2; furthest reaching mitoses in basal direction were observed in sector V at the level of 1050 µm. The apical shift of the division zone occurs similarly in each of the histogens. Slight differences in mitotic activity between the histogens suggest that periblem is relatively least susceptible to the extract.

During postincubation after exposure to 5 per cent extract the mitotically active zone extends in basal direction by 2 sectors (reaching sector VIII, to the 1680 µm level), however, the regions of highest frequency of mitoses shift in apical direction (Fig. 3, data for 48 and 72 h of postincubation). These data indicate that the main region of the root where mitotic activity is resumed is the apical region extending over sectors I, II and III. Special significance is ascribed to the higher than control frequency of mitosis in sector I (data for 48 and 72 h of postincubation, Fig. 3).

The present data are in agreement with the results of K a s z y ń s k a (1970, unpublished) concerning the influence of 5-aminouracil on apical

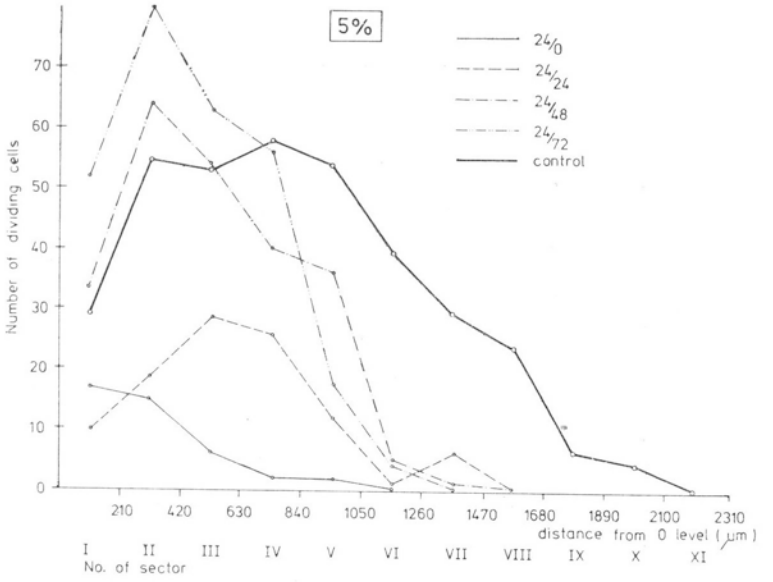

Fig. 3. Number of mitoses in successive 210-µm meristem sectors during 24-h incubation in 5 per cent extract and various stages of postincubation

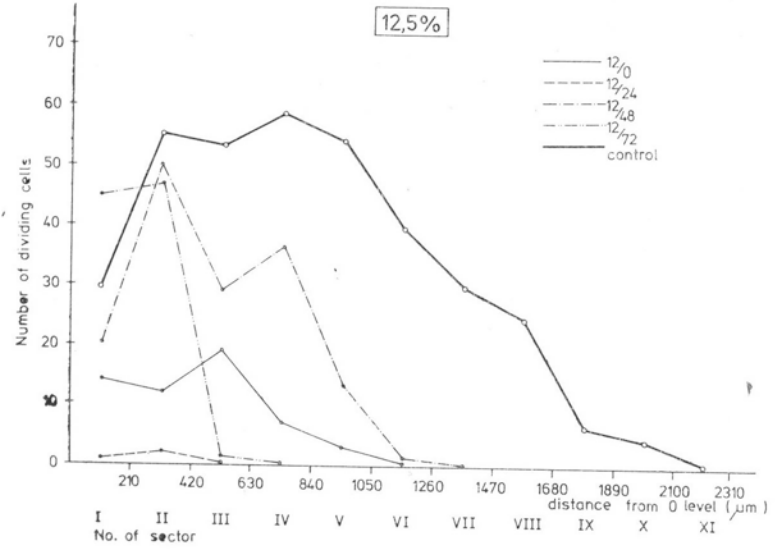

Fig. 4. Number of mitoses in successive 210-µm root meristem sectors during 12-h incubation in 12.5 per cent extract and at various stages of postincubation

meristem of onion roots. This author noted shortening of the meristematic zone during incubation at the first hours after incubation as well as a shift of the mitosis high frequency region into apical direction.

The shift of the regions of maximum mitosis frequency to the apical sectors suggests a renoval of meristem from the cells constituting the

Table 1

Number of mitoses in dermatogen, periblem and plerome after incubation in 5% and 12.5% extract and after different periods of the postincubation (Averages from 3 median sections each from two roots)

Extract concentration	Incubation (hours)	Post incubation (hours)	Absolute number of mitoses in meristem				Relative frequency of mitoses (%)		
			dermatogen	periblem	plerome	total	dermatogen	periblem	plerome
Control			117	126	98	341	34	37	29
5%	24	0	1.5	8	33	42.5	3.5	19	77
5%	24	24	44	124	65	23	18.4	53.2	28.3
5%	24	48	29	21.5	47.5	98	29	21	49
5%	24	72	51	130.5	93.5	275	19	47	34
12.5%	12	0	10.5	19.5	25	55	19	36	45
12.5%	12	24	0	2	1	3	—	—	—
12.5%	12	48	18	77	53.5	148.5	12	52	36
12.5%	12	72	12	48	33	93	13	51	36

quiscent centre. Apical meristem reconstruction in roots was investigated among others by Clowes (1961, 1963) in roots of *Allium cepa*, Benbadis (1965) in roots of *Allium sativum*, Webster and Langenauer (1973) in roots of *Zea mays*. Meristem reconstruction occurred after the action of strong X-ray doses (Clowes, 1963), β-radiation from ^{3}H-thymidine of 20 μCi/ml concentration (Clowes, 1961) and triethylmelanine (Benbadis, 1965).

Meristem reconstruction from its most apical part is well illustrated by the postincubation data after exposure to sublethal conditions (12.5% extract for 12 h, Fig. 4). Mitotic activity after 72 h of postincubation is limited almost exclusively to sectors I and II, with mitosis level higher than in control in sector I. The wide zone of mitoses formed during the earlier postincubation period (48 h) shows that part of the cells then underwent the last mitotic cycle and passed to the elongation zone.

Mitotic activation of the particular histogens during postincubation is not simultaneous. It may be concluded from the data in table 1 that the highest mitotic activity is first resumed by periblem (53.2% of all mitoses after 24 h of postincubation after exposure to 5 per cent extract and 22% of all mitoses in meristem after 48 h of postincubation after treatment with 12.5% extract).

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Wpływ rozpuszczalnej w wodzie onkostatycznej frakcji z kłączy Rheum officinale Baill. na merystem wierzchołkowy korzeni Allium cepa L.

II. *Zmiany zasięgu strefy merystematycznej oraz rozmieszczenia w niej mitoz*

Streszczenie

Inkubacja w 5% i 12,5% ekstrakcie z kłączy *Rheum officinale* powoduje zakłócenie stanu dynamicznego równowagi między liczbą dzielących się komórek a liczbą komórek przechodzących do strefy wydłużania. 24-h inkubacja w 5% ekstrakcie powoduje skrócenie strefy komórek merystematycznych do 2/3, a strefy występowania mitoz do 1/2, co sugeruje, że w warunkach znacznej depresji mitotycznej może odbywać się wydłużeniowy wzrost komórek. Inkubacja w warunkach subletalnych (12-h w 12,5% ekstrakcie) nie wywołuje skrócenia strefy komórek merystematycznych, mimo skrócenia strefy mitoz do 1/5. Sugeruje to, że w tych warunkach nastąpiło zahamowanie wzrostu elongacyjnego.

Aktywizacja mitotyczna merystemu w początkowym okresie postinkubacji (24-h w 5%, 48-h w 12,5%) odbywa się w rozległym obszarze. W bazalnej części skróconego uprzednio merystemu przebiega wtedy ostatni przed elongacją cykl mitotyczny. Podczas dalszej postinkubacji (48-h i 72-h po 5% i 72-h po 12,5%) — strefa merystematyczna zostaje bowiem silnie skrócona, a jednocześnie strefa najwyższej częstotliwości mitoz zostaje przesunięta w kierunku apikalnym. Głównym obszarem merystemu, w którym odbywa się wznowienie aktywności mitotycznej jest zatem obszar apikalny, położony w I i II sektorze, obejmujący centrum spoczynkowe. Znacznie wyższa niż w kontroli aktywność mitotyczna apikalnego sektora sugeruje, że w rekonstrukcji merystemu bierze udział centrum spoczynkowe.