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**The threshold values of colchicine action in some
cultivated plants**

**Wartości graniczne działania kolchicyny u niektórych
roślin uprawnych**

This investigation continues the previous work of Levan published in *Hereditas* (1946). The same technique is applied in the present study. Seeds of flax, rape, turnip, clover, soy beans and corresponding tetraploids (except in the case of soy bean where only diploids were studied) were placed on canvas, which covered jars filled with water. After the roots of the germinating plants reached the length of about 3 cm., the water was poured out and the jars were filled with colchicine solution. Following solutions were applied: 0.1, 2.5, 5.25, 100, 250, 500, 750, 1000, 1500×10^{-6} mol per liter. The Merck colchicine puriss. cryst. used in this investigation contains about 10% chloroform*). All experiments were carried on at room temperature of about 20°C. The fixation of roots took place after 6 and 24 hours. To determine the degree of the colchicine effect a scale of 5 grades was used. Grade 5 signified full effect, grade 1 but slight spindle disturbance and few c-mitoses. The results of our observations are summarized in Table I. This comparison makes evident, that flax and rape showed the highest threshold value, clover the lowest, the thresholds being distributed between 25 and 500×10^{-6} mol/l. The tetraploids showed no certain difference in relation to the corresponding diploids. Certain indications were found however, that, the tetraploids were somewhat less resistant to colchicine than the diploids. The

*) Steinegger and Levan (1947 b) have shown that at the low concentrations (at which the threshold values are situated in such a plant as *Allium cepa*), the chloroform content is so diluted as to be of no c-mitotic influence.



full effect of colchicine action was often reached in the tetraploids after 6 hours, whilst the diploids showed the same effect after 24 hours.

To determine the behaviour of plants cultivated in different media, the threshold value in distilled water and in Knop's solution was compared. *Allium cepa* and *Linum usitatissimum* were used as experimental material. The results are represent in Table II.

From this table is seen that the thresholds are lower in distilled water than in Knop. In *Allium* it was found that the threshold value in Knop after 10 days has been lowered and almost coincided with that of distilled water.

The experiment indicates that plants cultivated in distilled water are more rapidly influenced by colchicine than those grown in Knop's nutrition; but after the regeneration of roots the colchicine effect is quite

Table II

Colchicine concentration in 10^{-6} mol/liter	O n i o n								F l a x							
	Distilled H ₂ O				Knop-solution				Distilled H ₂ O				Knop-solution			
	4h	24h	48h	240h	4h	24h	48h	240h	6h	24h	6h	24h	6h	24h	6h	24h
	D i p l o i d								2x	2x	4x	4x	2x	2x	4x	4x
1500									5	5	5	5	5	2	2	2
1000		5	5		5		5		5	5	5	5	1	2	2	1
750		5	5		5		5		3	3	3	3				
500		4	5		5	4	5		2	3	3	3	—	—	—	—
250		3	3		1	3	3		—	—	—	—	—	—	—	—
200	—	3	3	5	—	1	1	3								
150	—	2	2	<4	—	—	—	2								
125		2	2	1	—	—	—	1								
100	—	1	1	—	—	—	—	—								
50	—	—	—	—	—	—	—	—								
25	—	—	—	—	—	—	—	—								
10	—	—	—	—	—	—	—	—								
5	—	—	—	—	—	—	—	—								
2.5	—	—	—	—	—	—	—	—								
0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

the same. The regeneration begins after 2 days of treatment and goes on more quickly by plants kept in Knop's solution, than in distilled water. The roots of the former grew more rapidly than those of latter. The cultivation in distilled water evidently makes the plants more sensitive to colchicine, which may be due to a weakening influence of the pure water medium.

Cytological observations on the investigated material showed, that colchicine causes different effects on plants according to the length of chromosomes. Among the plants investigated by me only *Allium* has large chromosomes, the others having smaller. After the colchicine treatment small chromosomes are more easily pushed together, forming lumps of chromatine (Figure 1 b, c). Only in rare cases typical c-mitosis with scattered c-pairs were seen (Figure 1 a). This formation of irregular chroma-

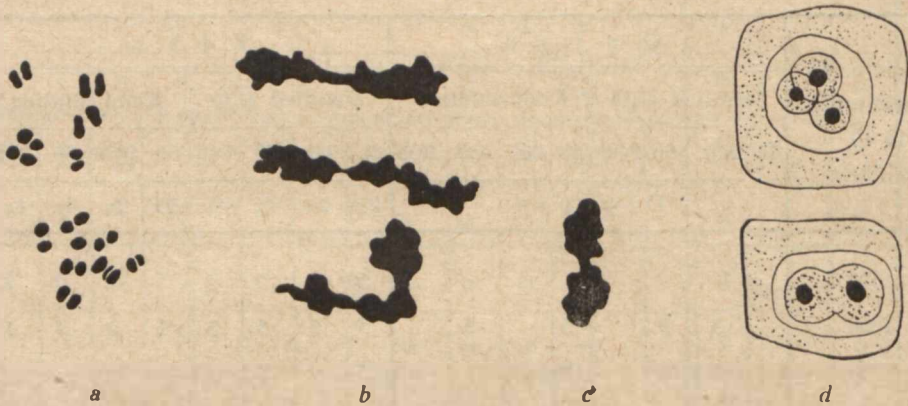


Fig. 1. a) — Rare type of c-mitosis in clover with scattered chromosomes.
 b) — Agglutination of chromosomes after colchicine treatment in clover.
 c) — The same in flax.
 d) — 2 abnormal nuclei formed after colchicine treatment in flax (notice 2 and 3 nucleoli).

Microscopic magnification: 15×40 .

tine lumps in which single chromosomes are hardly or not at all distinguishable is probably caused by the „stickiness“. The formation of bridges between chromosomes groups is the best proof of this. Steinegger and Leván (1947 a) stated that iso-colchicine can produce similar sticky effect on *Allium*, in consequence of which the division of chromosomes is retarded. Stickiness in connection with c-mitosis has been found in many other cases.

The lumps of chromosomes were often seen to form two groups joined together with chromatine bridges or completely separated. This may be

the results of the spindle not being quite inactivated. On the other hand, the large chromosomes of *Allium* and also those of *Hordeum* (observed by other authors) more seldom form lumps and are usually scattered over the whole cell. As a result of the elongated or separated chromosome groups formed in the plants with small chromosomes nuclei with abnormal and irregular shape arise (fig. 1 d, e). The determination of the c-mitotic action in plants with small chromosomes is easy enough, because the characteristic groups may be observed without difficulty even under low magnification, and the threshold value is very distinct. As shown in the Table I, the transition from full effect to complete absence of c-mitosis is distinct and there is almost only one intermediate grade. On the contrary, plants with large chromosomes such as *Allium* and *Hordeum* often show more than one intermediate grade and the transition to full effect is less rapid.

The cytological results may be supported with macroscopic observations on morphological qualities. In order to study the variation caused by different colchicine concentrations, measurement were performed on

Table IIIa. Length of roots

Colchicine concentration in 10^{-6} mol/liter	Flax dist. H ₂ O		Rape dist. H ₂ O		Turnip dist. H ₂ O		Clover dist. H ₂ O		Soy beans dist. H ₂ O	Flax Knop-solution	
	2x	4x	2x	4x	2x	4x	2x	4x	2x	2x	4x
1500	20,2	6,3	11,9	10,6						18,0	22,7
1000	23,1	19,7	15,4	14,9	7,8	10,1	13,0	18,0	27,2	21,1	16,5
750	23,2	21,5	24,0	18,4	14,8	17,5	16,9	17,2			
500	29,2	25,3	26,1	20,3	11,3	7,6	15,3	13,5	45,1	36,7	36,4
250	55,8	44,5	30,6	28,1	16,8	11,3	14,0	14,8	50,1	63,4	56,0
100	42,3	43,0	22,2	27,4	24,9	24,3	19,0	15,0	43,2	65,2	56,6
50	39,2	32,4	21,9	19,9	24,5	24,0	18,5	20,4	70,4	69,6	57,2
25	37,1	48,1							63,1	63,6	55,9
10	38,0	27,7	23,4	29,0			17,1	25,7	77,8	66,9	59,7
5	48,6	37,7	19,5	26,4			27,3	24,3	82,5	65,3	59,2
2.5	47,8	44,0							61,0	71,7	60,7
1	41,9	47,6	26,6	22,3			24,8	20,8	90,4		
0	55,9	64,0	25,9	20,0	48,7	75,0	28,7	27,9	102,8	70,1	65,2

Table III b. Length of stems

Colchicine concentration in 10^{-6} mol/liter	Flax dist. H ₂ O		Rape dist. H ₂ O		Turnip dist. H ₂ O		Clover dist. H ₂ O		Soy beans dist. H ₂ O	Flax Knop-solution		
	2x	4x	2x	4x	2x	4x	2x	4x	2x	2x	4x	
1500	12,2	11,8	21,2	15,6							6,7	10,3
1000	16,1	23,3	17,4	14,6	7,4	9,3	16,1	26,6			12,0	7,1
750	14,5	18,3	33,4	21,6	11,0	6,4	24,3	22,4				
500	17,5	21,9	41,1	28,1	9,2	5,9	29,6	28,6			19,0	21,8
250	44,6	45,2	52,8	53,1	13,1	7,3	24,2	23,8	D O F F W I D		22,3	22,5
100	47,4	47,0	59,3	52,6	27,4	23,4	29,5	25,3			27,3	26,7
50	52,5	48,4	46,1	54,0	27,8	37,1	27,7	33,1			27,8	26,4
25	61,1	48,2									31,3	29,9
10	53,2	51,6	55,7	51,7			30,1	34,0			27,4	35,4
5	60,3	45,2	51,8	49,2			29,2	34,9			29,1	31,0
2.5	49,0	50,9									32,6	30,8
1	43,4	49,8	40,0	44,5			30,0	29,7				
0	64,2	62,0	46,7	50,1			34,7	36,1			29,2	33,0

roots and stems in all plants investigated. From each concentration at least 10 measurements were made. The average values are represented in Table III. The variation in the roots length was very great and it often happened, that in one concentration only some plants were affected, whereas the rest were normally developed. This may be due to the influence of genetical or modifying factors.

In spite of the great irregularity of the average root length, it is quite obvious that the high concentrations of colchicine stopped the growth much more than the low ones. On the base of these observations, threshold values may be traced, which correspond quite well to the threshold values determined by means of cytological studies. The results obtained from the stem measurements are more distinct than those from roots, except in clover, which shows a gradual diminuation of stem length with increasing colchicine concentration. In all other plants threshold values may be traced without difficulties. The study of the tumours on roots gave similar results. The swelling of the roots is regarded as a parallel reaction accompanying the c-mitosis. In flax and in soy beans the threshold values of

c-tumour were somewhat lower than those of c-mitosis. The tumours on the stems are often more pronounced than on the roots. This was the case in rape, turnip and tetraploid clover. On the contrary in flax the morphological response of the influence of colchicine is stronger on roots than on stems. In both cases, however, the threshold value obtained from observations on the c-tumours differs from that determined by cytological investigation no more than one grade of colchicine concentration. The comparison of diploids with tetraploids shows that in many cases the tetraploids are more strongly influenced by the alkaloid, than the corresponding diploids. The length of roots and stems is often smaller, and the root tumours of tetraploid flax, turnip and clover are more pronounced. In consequence the determined threshold values support the above-mentioned results obtained at the cytological study, that the tetraploids may be somewhat more sensitive than corresponding diploids.

As mentioned above, the cultivation in nutrition solution makes the plants more resistant to colchicine treatment. This is also evidenced by observations on root and stem length as well as by tumour reaction. If we compare the same plants (*Allium cepa*, *Linum usitatissimum*) cultivated in distilled water with those growing on Knop's solution, we see, that in all cases the threshold values of colchicine action is lower in distilled water than in nutrition solution.

Summarising the results we come to the following conclusions:

- 1) Different plant species show different threshold values for colchicine action. Plants belonging to related species exhibit similar but not identical colchicine reaction (rape, turnip).
 - 2) Tetraploids are in some cases more sensitive to colchicine than corresponding diploids.
 - 3) Plants with small chromosomes show a somewhat different reaction to colchicine than those with large ones. During c-mitosis small chromosomes are pushed together and form lumps, large chromosomes are scattered all over the cell.
 - 4) The threshold values of c-mitotic action are higher in plants cultivated in nutrition solution, than those in distilled water.
 - 5) The observations on root and stem length support the results obtained from cytological studies, though the corresponding values may differ in one grade of colchicine concentration.
 - 6) The same is true concerning the c-tumour reaction on roots and stems.
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The experimental part of this study was carried out during a stay at the Cyto-genetic department of the Swedish Seed Association, Svalöf. I am deeply indebted to Professor A. Akerman for his help in carrying out my work and to Docent A. Levan for his valuable suggestions and advices.

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STRESZCZENIE

Badania nad wpływem kolchicyny na różne rośliny uprawne wykazały, że wartości graniczne działania alkaloidu są różne w zależności od gatunku rośliny. Rośliny tetraploidalne są nieco wrażliwsze na działanie kolchicyny, niż odpowiednie diploidy. Wartości graniczne c-mitotycznego działania są wyższe dla roślin hodowanych na pożywcze Knop'a niż w wodzie destylowanej. Pomiarzy długości korzeni i łodyg oraz obserwacje nad obrzękami korzeni potwierdziły w zupełności rezultaty otrzymane przy pomocy badań cytologicznych.

A-11525

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W WARSZAWIE

Nakł. 1500 61 × 86 V kl. 80 g