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Author: Barbara Wojciechowska, Halina Kocik

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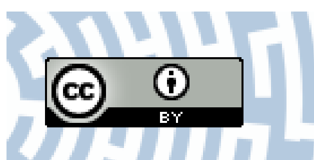

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Influence of aluminium chloride and sulphate on the root meristem of *Vicia faba* L.

BARBARA WOJCIECHOWSKA, HALINA KOCIK

Department of Plant Anatomy and Cytology, Silesian University,
Jagiellońska 28, 40-032 Katowice, Poland

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Abstract

The influence of various (0.1, 0.05, 0.01, 0.001 and 0.0001 M) aluminium chloride and aluminium sulphate concentrations on the mitotic activity of the root meristem of the bean *Vicia faba* L. was investigated after 24 h of incubation. A mito-depressive action of the tested compounds, irreversible at higher concentrations was observed. The tested substances induced chromosome aberrations (fragmentation and bridges in anaphase or telophase, micronuclei, binuclear cells) and inhibited elongation of roots. The results of topochemical analysis are described.

Key words: Vicia faba L., root, $AlCl_3$, $Al_2(SO_4)_3$.

INTRODUCTION

Aluminium has so far not been considered as an element indispensable for the life of plants, it is present, however, in all their tissues and organs (Grzesiuk 1967, Nowaczyk and Borys 1974, Nowotny-Mieczyńska 1976) on the average, according to the data of Chenery (1955, quoted after Nowotny-Mieczyńska 1976) in the amount of 200 ppm per 1 kg dry weight. According to Nowosielski (1974), aluminium in a 0.1-0.2 ppm concentration in the medium has a stimulating effect on the development of many plant species. Higher concentrations, however, have a toxic influence, causing dwarfing of roots (Keeridge et al. 1971) and dying back of the root tips (Fleming and Foy 1968).

Aluminium take up and its cumulation in the root tissues of particular plants varies in intensity and depends on the specific properties of the plants and pH of the medium (Sójkowski 1971).

Among the numerous investigations on the toxicity of aluminium (McLean and Gilbert 1927, Hewitt 1947, Kirkpatrick et al. 1975, Clark 1977) the experiments of Clarkson (1965) on the root meristem of *Allium cepa* L. were of model character. In continuation of these studies a trial was undertaken to establish the degree of cytotoxicity of aluminium chloride and sulphate to the root meristem of *Vicia faba* L. It also seemed useful to study the accumulation and distribution of this element in the root cells by the histochemical method.

MATERIAL

The experiments were performed with *Vicia faba* L. seeds of the variety Hangdown White obtained from the District Garden Plant and Seed Breeders in Cracow from the 1979 harvest.

METHODS

DETERMINATION OF THE MITOTIC INDEX

Rooted broad bean seeds were incubated in Petri dishes in aqueous aluminium chloride and sulphate solutions of the following concentrations: 0.1, 0.05, 0.01, 0.001 and 0.0001 M. Two batches of seeds were first incubated for 24 h in a thermostat at 24 ± 1°C. Thereupon one batch was transferred to water and postincubated for a further 24 h under the same conditions. In the first batch 8 seeds were placed on each dish, in the second — 5. At the same time control samples were set in water. The root length was measured with millimetre paper. Cut off roots were fixed in Carnoy's solution (absolute ethanol:glacial acetic acid, 3:1) for 24 h and stained with acetoorcein (Zeilinga 1956) for a dozen hours or so at room temperature. Squashes were prepared from the root apexes and embedded in Canada balsam. At least 1000 cells were counted in each preparation and note was taken of the particular stage of mitosis and any disturbances. For the particular roots mitotic indexes were calculated and further mean mitotic indexes and mitosis inhibition. Phase indexes and the per cent of mitotic disturbances were also recorded. The mean length increment of the roots and its inhibition were also determined.

ALUMINIUM LOCATION

For topochemical analysis of aluminium in the tested material three aluminium chloride and three sulphate solutions were prepared of 0.1, 0.01 and 0.0001 M concentrations. After 24 h of incubation the tested

and control roots were cut off and from each root zone cross and longitudinal sections were prepared separately by means of a freezing microtome.

Aluminium was detected by the method of Aimi and Murakami (1964, quoted after Morimura et al. 1978), with the use of aluminon which forms a chelate complex of red colour with aluminium salts. The sections were placed in a mixture of 0.1 per cent aluminon and 3 M ammonium acetate (1:1) for 1 h. The material was washed in distilled water and subsequently in 3.2 M ammonium carbonate solution.

RESULTS

A macroscopically noticeable effect of the toxic action both of the chloride and the sulphate was a considerable reduction of elongation growth of the roots, correlated with the concentration of the solution used for incubation. Growth inhibition at higher concentrations of these salts seemed irreversible (Fig. 1). After transfer of the broad bean seeds from the incubation solutions to water the morphological changes in the roots were exacerbated. Browning of the roots, particularly strong in the elongation zone (0.1 and 0.5 M solutions) was associated with cracking and desquamation of the surface layers (0.01 and 0.001 M solutions, Fig. 4) and further disintegration of the rhizodermis and primary

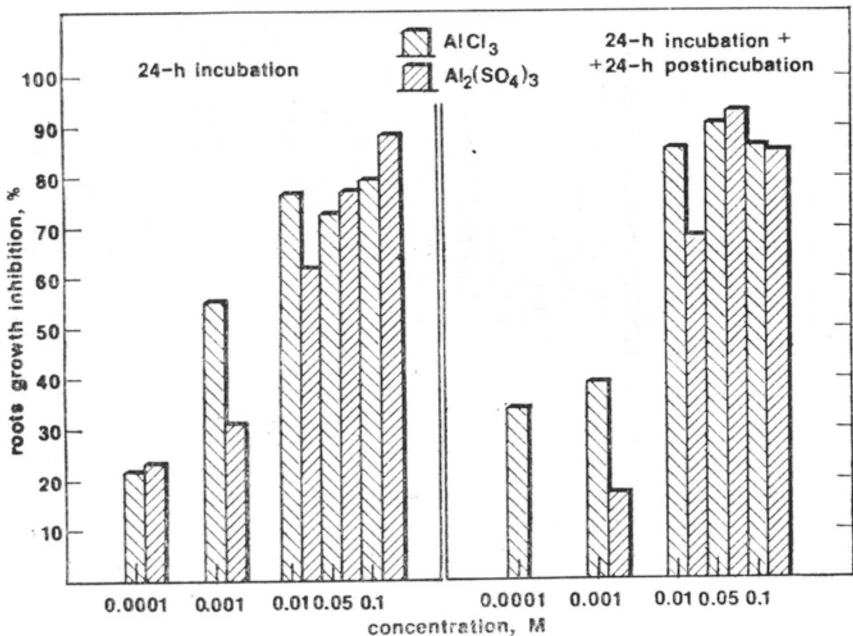


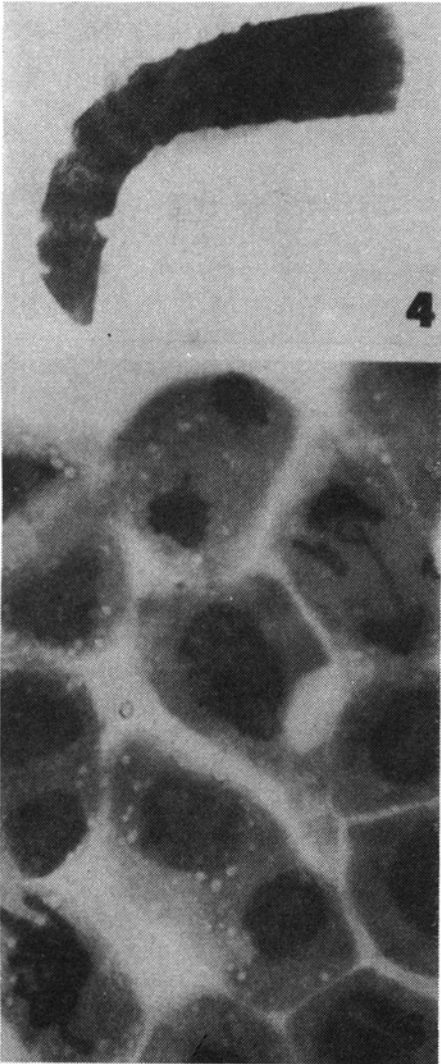
Fig. 1. Inhibition of root elongation growth in *Vicia faba* L. under the influence of aluminium chloride and sulphate solutions

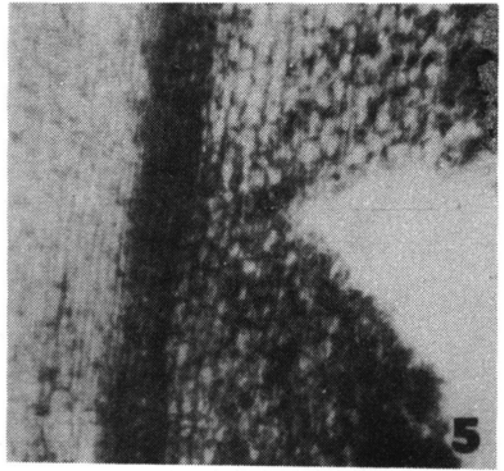
Table 1

Mitosis inhibition in root meristem of *Vicia faba* L. caused by various aluminium salt concentrations

Concentration, M	$AlCl_3$				$Al_2(SO_4)_3$			
	incubation		postincubation		incubation		postincubation	
	mean mitotic index	mitosis inhibition, %	mean mitotic index	mitosis inhibition, %	mean mitotic index	mitosis inhibition, %	mean mitotic index	mitosis inhibition, %
0.1	0	100.0	0	100.0	6.15± 0.87	93.5	0	100.0
0.05	0.87± 0.48*	99.4	12.56± 6.59	92.1	7.87± 1.92	91.7	0	100.0
0.01	2.50± 1.84	98.3	28.41± 6.66	82.2	23.73± 5.76	74.9	54.01± 5.31	34.6
0.001	33.40±13.17	77.1	71.60± 8.39	55.1	58.90±12.12	37.1	75.50±12.12	8.6
0.0001	99.38±18.83	31.9	130.70±29.18	17.9	74.19± 7.58	21.4	83.55± 4.44	0
Control	145.93±16.47		159.31±16.82		94.38±14.07		82.62± 8.43	

* mean±SEM





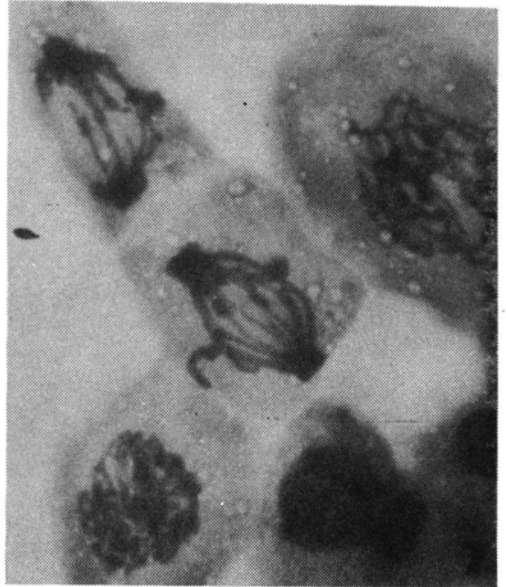
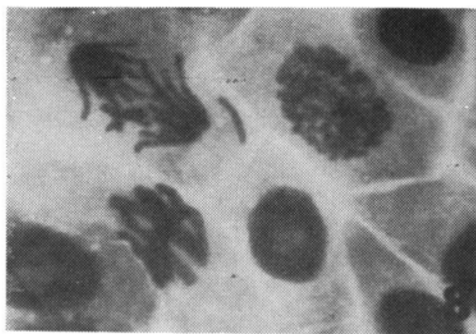
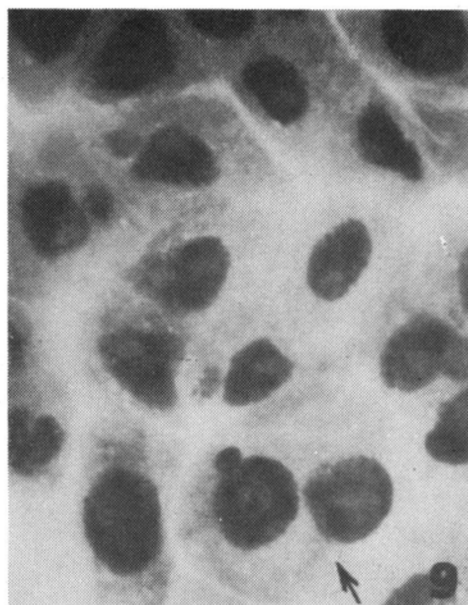
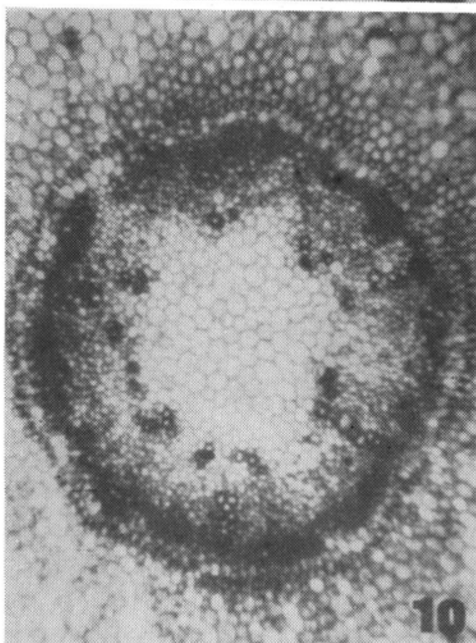
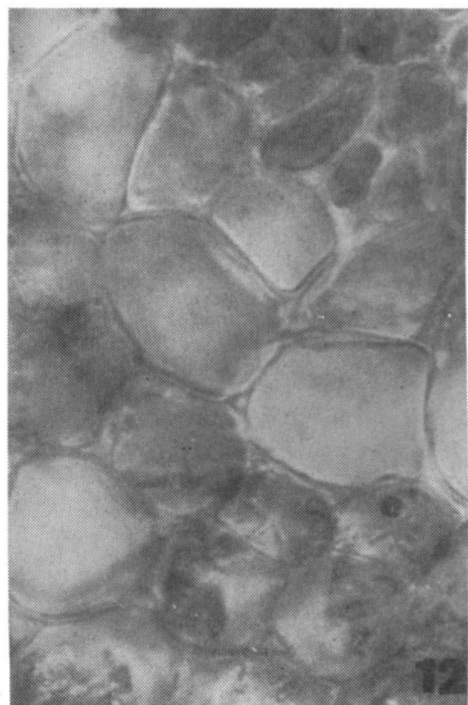


Fig. 4. Deep root lesions due to 0.1 M aluminium sulphate treatment. × 5. Fig. 5. Longitudinal section through differentiation zone, torn rhizodermis and primary cortex cells (0.01 M $Al_2(SO_4)_3$). × 70. Fig. 6. Chromosome agglutination in metaphase, telophase bridge and free chromosomes (0.1 M $AlCl_3$). × 720. Fig. 7. Anaphase bridges and nonuniform distribution of chromosomes (0.01 M $AlCl_3$). × 720









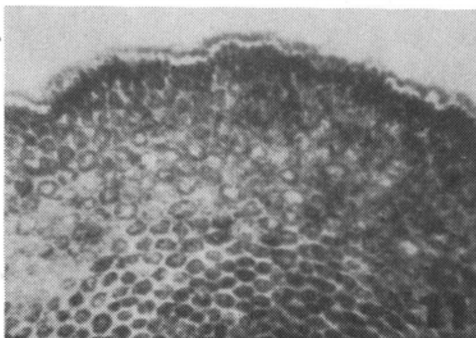


Fig. 8. Anaphase with free chromosome (0.01 M $AlCl_3$). × 720. Fig. 9. Nucleus with micronucleus and binuclear cells with micronucleus (0.01 M $AlCl_3$). × 530. Fig. 10. Fragment of differentiation zone. Positive reaction for aluminium with aluminon in the endodermis and vascular tissues (0.01 M $AlCl_3$). × 70. Fig. 11. Positive reaction of aluminium with aluminon in primary cortex cells (0.01 M $AlCl_3$). × 70. Fig. 12. Segment of differentiation zone — no reaction for aluminium in periocycle cells. × 720

cortex cells (Fig. 5). Aluminium sulphate was found to inhibit root elongation more than does the chloride (Fig. 1).

Both aluminium salts inhibited the meristem mitotic activity in the roots. As seen from Table 1, aluminium chloride proved a stronger karyokinesis inhibitor. It was mitostatic in the highest concentration used, and in lower concentrations had a strong mitodepressive action, even in a concentration as low as 0.0001 M (31.9% inhibition).

The cytotoxic effects of both the tested compounds were irreversible within the period of the experiment. Applied in the maximal concentration (0.1 M) they durable reduced mitotic activity, whereas in lower concentrations (0.01-0.0001 M) their mitodepressive influence partly receded after a period of postincubation (Table 1). The cytotoxic effects caused by aluminium chloride seemingly regressed slower than those after sulphate treatment.

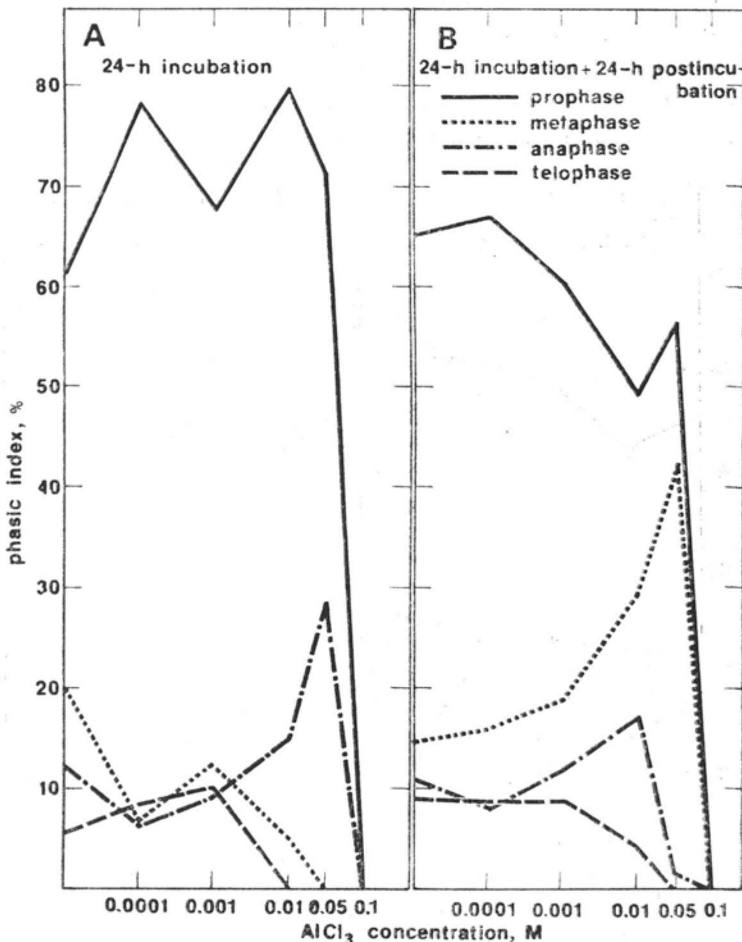


Fig. 2. Mean phase indexes for *V. faba* root meristems incubated in solutions of aluminium chloride (A) and then postincubated in water (B)

Both salts (Figs. 2 and 3), and especially aluminium chloride produced a considerable rise of the prophase index in dependence on the solution concentration. The highest index was noted under the action of 0.01 and 0.0001 M aluminium chloride (Fig. 2). In 0.05 M aluminium chloride the value of the prophase index decreased and in the 0.1 M solution prophases disappeared. Aluminium sulphate in a 0.0001 M concentration depressed the number of prophases, but in higher concentrations it increased them (Fig. 3). The increase in the number of prophases under the influence of 0.0001 M aluminium chloride was connected with an increased number of telophases as compared with the control. Disappearance of meta- and telephases was observed in meristems incubated in 0.01-0.1 M solutions. In meristems treated with 0.05 and 0.1 M aluminium sulphate solutions the greatest number of prophases was, however, associated with an important depression of the telophase number, while the metaphases remained at the same level as in the control.

Both aluminium compounds induced various mitotic disturbances more numerous in the case of the chloride than the sulphate (Table 2). Aluminium chloride mainly induced chromosome fragmentation in meta- and anaphase, pseudometaphases and formation of micronuclei (Fig. 9),

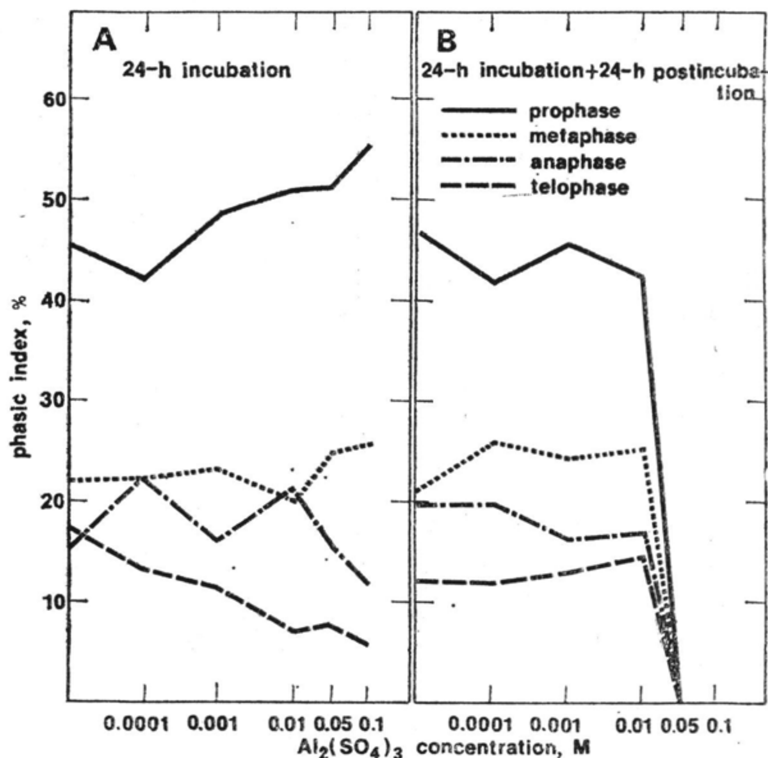


Fig. 3. Mean phase indexes for *V. faba* root meristems incubated in aluminium sulphate solutions (A) and then postincubated in water (B)

Table 2

Mitotic disturbances (%) caused by various aluminium salt concentrations

	Kind of disturbance	$AlCl_3$, M								$Al_2(SO_4)_3$, M							
		incubation				postincubation				incubation					postincubation		
		0.05	0.01	0.001	0.0001	0.05	0.01	0.001	0.0001	0.1	0.05	0.01	0.001	0.0001	0.01	0.001	0.0001
In metaphase	chromosome agglutination						3.8	9.3	5.3								
	shortening and thickening of chromosomes			17.1	23.1		10.1	10.3	9.9								
	pseudometaphase		12.5	36.6	23.1	87.1	39.2	22.4	25.7								
In anaphase	chromosome bridges						10.1	3.7				63.0	33.3	60.0	25.0		100.0
	chromosome fragmentation	40.0	12.5	17.1	23.1		11.4	22.4	17.1								
	tropokinesis			12.2	23.1	3.2	7.6	1.9	13.2		16.7						
	pseudoanaphase			12.2			2.5	3.7	1.3								
In telophase	chromosome bridges							3.7				37.0		20.0			
	chromosome fragments			2.4	3.8			9.3	19.7					20.0			
	nonuniform chromosome distribution			2.4	3.8		1.3	5.6	5.9								
Other disturbance	micronucleus	40.0	50.0			9.7	11.4	7.5	2.0								
	binuclear cells	20.0	12.5				1.3			77.7	83.3		50.0				
	trinuclear cells		12.5				1.3										
	pycnotic nuclei									22.3					75.0		

whereas under the action of the sulphate chromosome bridges in ana- and telophase were noted as well as cytokinesis inhibition leading to the formation of binuclear cells. At higher aluminium sulphate concentrations (0.1 and 0.05 M) pycnotic nuclei appeared. After a period of postincubation the chromosome anomalies in the meristems incubated in the aluminium chloride solution were more frequent, while their number decreased in meristems treated with the sulphate solution (Table 2). It results from quantitative analysis of the mitotic disturbances that certain mitotic anomalies, although not numerous, appear also in the controls, probably owing to contamination of the tap water.

Location of aluminium in the root tissue was done by means of aluminon giving a red colour. According to the intensity of the reaction, the maximum concentration of aluminium in the meristematic zone of the root was evaluated, mainly in the protoplasts of cells, and minimal absorption in the cells of the differentiation zone. In the elongation zone aluminium was located in the rhizodermis wall cells, in the primary cortex around the nuclei and at the cell walls and in the intercellular spaces. It was also detected in the cytoplasm and walls (mainly Caspary strands) of endodermal cells, in the protoplasts of phloem cells and xylem elements (Fig. 10). Aluminium deposits were absent in the pericycle and heart cells (Figs. 10 and 12).

It is noteworthy that similar accumulation zones were found in all the incubated roots in all aluminium chloride and sulphate solutions, differing only in colour intensity resulting from concentration of the compound. At the lowest concentration (0.0001 M) the main site of aluminium accumulation were meristematic cells.

A positive result of reaction in the control sections appeared in some rhizodermis cells, intercellular spaces of the primary cortex, endodermal and vascular walls. This reaction as compared with the material subjected to the action of aluminium salts was markedly weaker. The presence of aluminium traces in the control sections may be explained by accumulation of this element in seeds and contamination of the tap water.

DISCUSSION

The present results allow the classification of *Vicia faba* L. to species susceptible to aluminium. Since Clarkson (1965) observed at similar concentrations of aluminium sulphate complete elongation growth inhibition of *Allium cepa* L. roots and disappearance of mitoses as early as after 6-8 h of exposure, it may be assumed that the sensitivity of *V. faba* is lower than that of onion. In spite, namely, of a four times longer incubation time the toxic effect of aluminium was weaker. Under the influence of higher concentrations of both salts local necroses appeared,

however, penetrating deeper into the root primary cortex. Clarkson (1965) does not mention reactions of this type, perhaps because post-incubation was omitted.

Chloride proved to be a stronger inhibitor of mitosis than aluminium sulphate. In the highest concentration (0.1 M) it acted mitostatically and in the lowest (0.0001 M) the per cent of mitosis inhibition was still relatively high amounting to 31.9, whereas in the case of the sulphate it was 21.4.

The differences in the action of both salts appeared also in the number and kind of chromosome aberrations. Aluminium chloride induced numerous durable chromosome anomalies in the form of fragmentation and micronuclei, whereas sulphate application resulted in the appearance of chromosome bridges which according to Levan (1945) are considered to indicate a change in chromosome stickiness.

The observed cytotoxic symptoms may be the result of acidity of the solution (pH=3.2 of the 0.1 M $AlCl_3$ solution and pH=3.6 of the 0.1 M $Al_2(SO_4)_3$ solution). It is, namely, known that in solutions with lower pH aluminium in the form of the Al^{3+} ion is taken up faster (Sójkowski 1971).

It results from literature data (Fleming and Foy 1968, Kerridge et al. 1971) that in susceptible plants aluminium is accumulated in the protoplasm, especially in the nucleus and mitochondria. In more tolerant plants accumulation of this element is not noted. According to the investigations of Stiles (1958) the main site of aluminium concentration in root is supposed to be the cortex. Foy et al. (1972) consider that aluminium taken up from the soil is deposited above all in the growth apexes of the main root and lateral ones, the cells of the epidermis and primary cortex, however, and markedly poorer in this element.

Klimashevskij et al. (1976) found in two pea varieties, a tolerant and a susceptible one to aluminium, very rapid uptake of this substances after 1 h of exposure, but after its penetration into the cells the rate of absorption increased only in the sensitive variety. These differences may be explained by the change in stickiness and permeability of the cytoplasm and irreversible damage to the membranes.

Topochemical analysis of *V. faba* roots confirmed in the present study that, independently of the salt applied, meristematic tissue is particularly susceptible. In the higher part of the root zonality is observed in the localisation of aluminium. Particular accumulation of aluminium deposits was noted in the nucleus and the cytoplasm zone near the wall in the meristematic cells. In the zone of determination and differentiation positive reaction results were most pronounced in the cells of the cortex and the endodermis and the phloem and xylem

strands. Against the background of these intensively coloured tissues the cells of the pericycle and heart and the central part of the rhizodermis seemed optically empty. Accumulation of aluminium deposits proved proportional to its concentration in the solution applied.

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Wpływ chlorku i siarczanu glinu na merystem korzeniowy Vicia faba L.

Streszczenie

Określono cytotoksyczne oddziaływanie chlorku i siarczanu glinu na korzenie bobu. Stwierdzono różnice w inhibującym działaniu obydwu badanych soli na kariokinezę. Silniejszy i trwalszy efekt mitodepresyjny wywoływał chlorek glinu. Pod wpływem tego związku ujawniono również liczniejsze aberracje chromosomowe (fragmentacja chromosomów w anafazie i w telofazie, mikrojądra). Siarczan glinu indukował głównie mostki chromosomowe w anafazie i w telofazie. Obydwa związki hamowały wydłużanie korzeni, a w wyższych stężeniach (0,1-0,01 M) działały destrukcyjnie na tkanki kory pierwotnej powodując głębokie pęknięcia i ubytki. Metodą histochemiczną, przy użyciu aluminonu, zlokalizowano maksymalne nagromadzenia strątów glinu w tkankach merystematycznych, natomiast różne w strefie dyferencjacji.