ACTA AGROBOTANICA
Vol. 48, z. 2 – 1995
83-93

The cytotoxic influence of aluminium on *Cucumis sativus* L. seedling roots

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(Received: September 13, 1995)

Abstract

The roots of 5-days old cucumber seedling cv. Wisconsin were incubated in the Al solutions ($AlCl_3$) of pH 4.2. Al was applied in the following concentrations: 20, 30 and 40 mg/dm^3. All the Al concentrations caused both the inhibition of root elongation and reduction of mitotic activity of apical meristem. The complete inhibition of mitoses and elongation growth was determined in the presence of 30 and 40 mg Al/dm^3 after 4 and 3 days of incubation. After 5 incubation days the changes in the morphology of seedling roots, resulting mainly from the inhibition of the main root elongation and formation of a greater number of lateral roots, were noted. The reduction of root cap dimensions was accompanied by the apical meristem shortening and in short distance, differentiation of numerous lateral root primordia. The meristematic cell got elongated while their nuclei and nucleoli enlarged (particularly at 20 and 30 mg Al/dm^3) and cytoplasm vacuolized. In the region of elongation and lateral roots differentiation, the cells of epidermis and primary cortex got shrunk, degenerationed and after falling off the tissue they made bigger (40 mg Al/dm^3) or smaller (20 and 30 mg Al/dm^3) hollows and cracks on the outer side of the root.

INTRODUCTION

The symptoms of Al toxicity appear firstly on the roots of young plants. Under the conditions of aluminium stress, the morphology of root system changes and its elongation is inhibited (Andersson, 1988; Berzonsky and Kimber, 1986; Borkowska, 1988; Borkowska et al., 1995; Foy et al., 1978; Foy, 1983; Lee and Pritchard, 1984; Ślaski, 1992). According to stress intensity, that is Al concentration and its operation time, a root meristems and even already differentiated tissues gets desintegrated (Bennet et al., 1985 a; Wagatsuma et al., 1987; Wallace and Andersson, 1984). It is widely known that the inhibition

of root elongation results from the reduction of mitotic division in its apical meristem (C l a r k s o n, 1965; C l a r k s o n, 1969]. A S phase of cell growth cycle is blocked due of Al influence. Al ions bonds with phosphate rests are causing disturbance in DNA replication (C l a r k s o n, 1965; C l a r k s o n, 1969; W a l l a c e and A n d e r s o n, 1984).

There is great variability of plants response to toxical activity of Al. This variability can be conditioned by various factors from genetic to environmental ones (Ś l a s k i, 1992). The nature of root damage, beside some symptoms common for all vascular plants, shows a species and even cultivar specificity, which is closely connected with plants readiness to mobilize the defense mechanism against a stressogene. Mechanism of Al toxicity has been an object of numerous studies from agriculture, biology, to the medical sciences, and is still open for further investigations. So, the aim of presesnt work were the investigations on the influence of high Al concentrations on mitotic activity of apical meristem, root elongation and specificity of cells and tissues damage of cucumber plants cv. Wisconsin. According to other studies (S z y m a ń s k a and M o l a s, 1995 a, b) this cultivar demonstrates different responses to high and low concentrations of Al.

MATERIAL AND METHODS

The investigations were carried out on the cucumber seedlings (*Cucumis sativus* L. cultivar Wisconsin). Young seedlings (a few days after the seed germination) were treated for 5 days with aluminium solution. Al was used as $AlCl_3$ in the concentrations of 20, 30 and 40 mg/dm^3. The pH of Al solutions was adjusted to 4.2. Distilled water was used as the control.

The following experiments and observations were carried out:

1. Rate of root elongation – the mesaures of root length taken after succesive (1-5) days of incubation in Al solutions.

2. Mitotic activity of root apical meristem – determination of mitotic index (MI in %). To obtain that, the decapitated 3-4 mm in length apices of root were fixed in Cornoy fixer and than stained in acetoorcein (G e r l a c h, 1972). On so-called pressed preparations (smears), a number of cells in the mitotic stage per 1000 cells was determined. The mitotic index was established after 1, 2, 3 and 4 days of roots incubation in the Al solutions.

3. Symptoms of damages of root cells and tissues by Al – examined in light microscope, on the semithin sections prepared out from particular regions of root. The prepared sections of seedling roots (incubated in the Al solutions for 5 days) were fixed in 2 % glutare aldehyde for 8 hours in the temperature of 4°C, rinsed in phosphate buffer (pH 7.4), dehydrated in ethanol and submerged in epoxide resin – Araldit. The semithin sections were prepared on the ultramikrotome LKB stained with 0.1% solution of azure II.

RESULTS

Rate of root elongation

Al in concentrations of 30 and 40 mg/dm^3 inhibited the rate of cucumber seedling roots as early as after 24 hours of incubation. With prolongation of Al ions operation time, the increase in a growth reduction degree was observed (Table 1). After 3 days of incubation in the Al solutions (30 and 40 mg/dm^3), the elongation of roots was completely inhibited. On the other hand in the presence of lower Al concentration (20 mg/dm^3), the slight stimulation of root growth was noted after the 1st day of incubation, the inhibition only after 2nd day, but even after 5 days of incubation the growth of roots was not completely blocked (Table1).

Mitotic activity of root apical meristem

In the presence of Al ions at 30 and 40 mg/dm^3 concentrations the mitotic activity (MI) of root apical was already reduced after 24 hours of incubation and then after 72 hours the mitotic divisions stopped completely (Table2). In the case of lower Al concentration (20 mg/dm^3) the mitotic activity was twice smaller as in control series after 5 days (Table 2).

Symptoms of root injury caused by Al

The morphological changes of cucumber seedlins roots caused by Al (20-40 mg/dm^3), were accompanied by some anatomical changes. The symptoms of Al toxicity were expressed by the inhibition of main and lateral roots elongation, reduction of length and density of root hairs and changes of root thickness, particularly of root tip. The thickness of root apex decreased in the presence of Al concentration at 20 and 30 mg/dm3 (Fig. 1, 2, 3), while it increased at Al 40 mg/dm^3 (Fig. 1, 4). There was increase in the number of differentiated lateral roots and even the secondary roots on hypocotyl were formed.

Table 1

Inhibition of roots elongation of cucumber seedlings caused by aluminium

Days of incubation in AL solution	Aluminium concentration (mg/dm^3)						
	0	20		30		40	
	mean length increment of roots (cm)*	mean length increment of roots (cm)*	growth inhibition (%)	mean length increment of roots (cm)*	growth inhibition (%)	mean length increment of roots (cm)*	growth inhibition (%)
1	1.10	1.19	0.00	0.60	45.5	0.55	50.0
2	1.05	0.90	14.3	0.43	50.5	0.25	76.2
3	1.22	0.80	33.4	0.20	83.4	0.00	100
4	1.05	0.60	42.9	0.00	100	0.00	100
5	1.20	0.55	54.2	0.00	100	0.00	100

*mean length increment of 20 roots incubated in water (control) and Al solution

Table 2

Mitoses inhibition in root meristem of cucumber seedlings caused by aluminium

Days of incubation in AL solution	Aluminium concentration (mg/dm^3)						
	0	20		30		40	
	mitotic index (%)	mitotic index (%)	mitosis inhibition (%)	mitotic index (%)	mitosis inhibition (%)	mitotic index (%)	mitotic index (%)
1	8.04 ± 0.18	6.69 ± 0.15	26.8	4.37 ± 0.17	55.6	3.28 ± 0.23	52.5
2	8.37 ± 0.26	5.48 ± 0.21	34.5	3.06 ± 0.28	63.4	0.88 ± 0.07	89.5
3	7.97 ± 0.31	3.68 ± 0.27	53.8	1.20 ± 0.28	83.8	0.00	100
4	8.20 ± 0.14	3.43 ± 0.12	58.2	0.00	100	0.00	100

± standard deviation

After 5 days of root incubation in the solutions containing high concentrations of Al (20, 30 and 40 mg/dm^3), the damage of root tissues both in the meristematic region and the other examined root zones (elongation, proliferous and young zone of lateral roots differentiation) was observed.

Aluminium brought about injury of cap cells of cucumber seedling roots on the longitudinal cross-sections showed heavily shrunk and deformed, numerous incrustations were identified on their area (Fig. 1-4). At Al 20 mg/dm^3, cap cells elongated quite clearly (Fig. 2).

The apical meristem was considerably shortened due to Al ions presence, extremely at Al 40 mg/dm^3. After 5 days of root incubation in the solution containing 20 mg Al/dm^3, the meristematic cells as well as their nuclei and nucleoli, mainly in the zone of intensive divisions, were enlarged in relation to control root meristematic cells (Fig. 5, 6). In the region of cap meristem and quiscent centre, the cells were strongly vacuolized and nuclei and nucleoli showed the size like in the control (Fig. 6). The dimension of meristematic cells, their nuclei and nucleoli enlarged also in the presence of 30 mg Al/dm^3, whereas cytoplasm got vacuolized in the whole area of meristem (Fig. 7). At Al 40 mg/dm^3 the root meristem was strongly shortened and far thicker in comparison to the meristem of roots not treated with Al (Fig. 1, 8). Its cells were slightly enlarged, much less than at 20 and 30 mg Al/dm^3, and no changes in the size of nuclei and nucleoli were observed (Fig. 8). Alike the lower Al concentrations, the cells were vacuolized (Fig. 8). Smaller or bigger vacuoles were also observed in the region of cell nucleoli treated with Al (20-40 mg/dm^3).

In meristematic region of root treated with 20 and 30 mg/dm^3 aluminium numerous smaller and bigger intercellular breaks were observed (Fig. 2, 3). In the region of the meristem only in roots incubated at 20 mg Al/dm^3 small numerous cells in the mitosis phase were found (Fig. 6). Mitoses were not observed at higher aluminium concentrations (30 and 40 mg/dm^3).

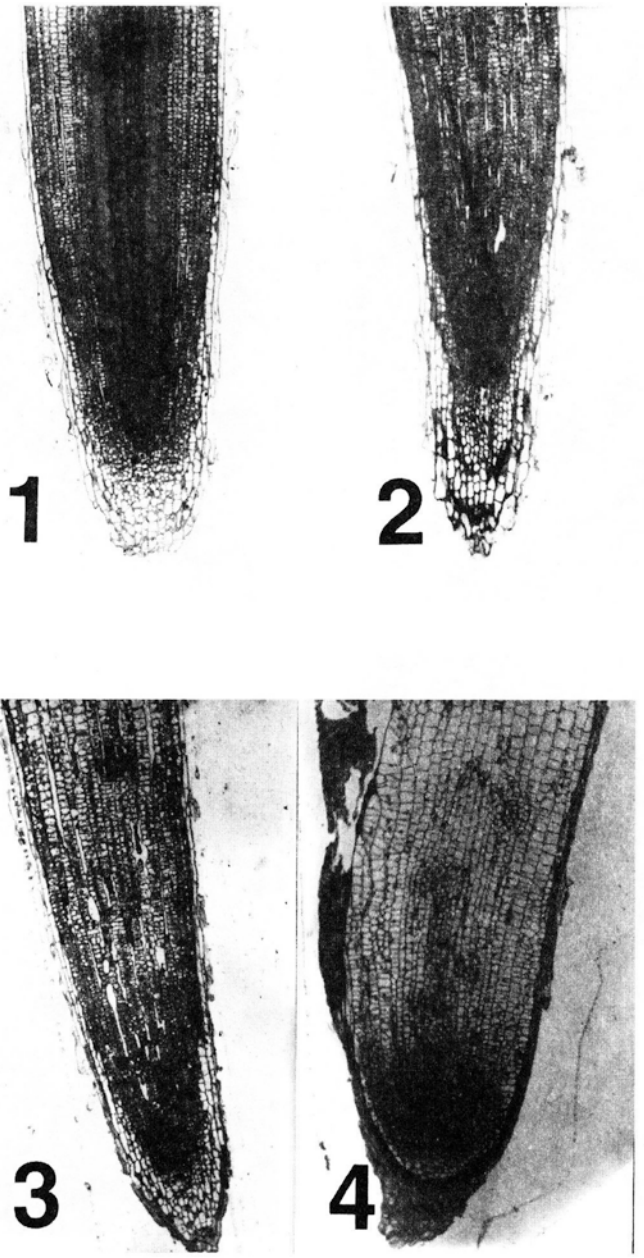


Fig. 1-4. The longitudinal sections of root apices of cucumber seedlings not treated with Al (1), and treated with Al at the following concentrations: 20 mg/dm^3 (2), 30 mg/dm^3 (3), 40 mg/dm^3 (4)
The Figures illustrate the changes in thickness of root apex treated with Al in comparison to control. Wats more, they show the specificity of cap injury which dimensions decrease together with Al concentration increase while the cells got elongated (2) or damaged on the peripherial side. The intersections manifest so called "intercellular cracks" in the meristem region (2, 3).

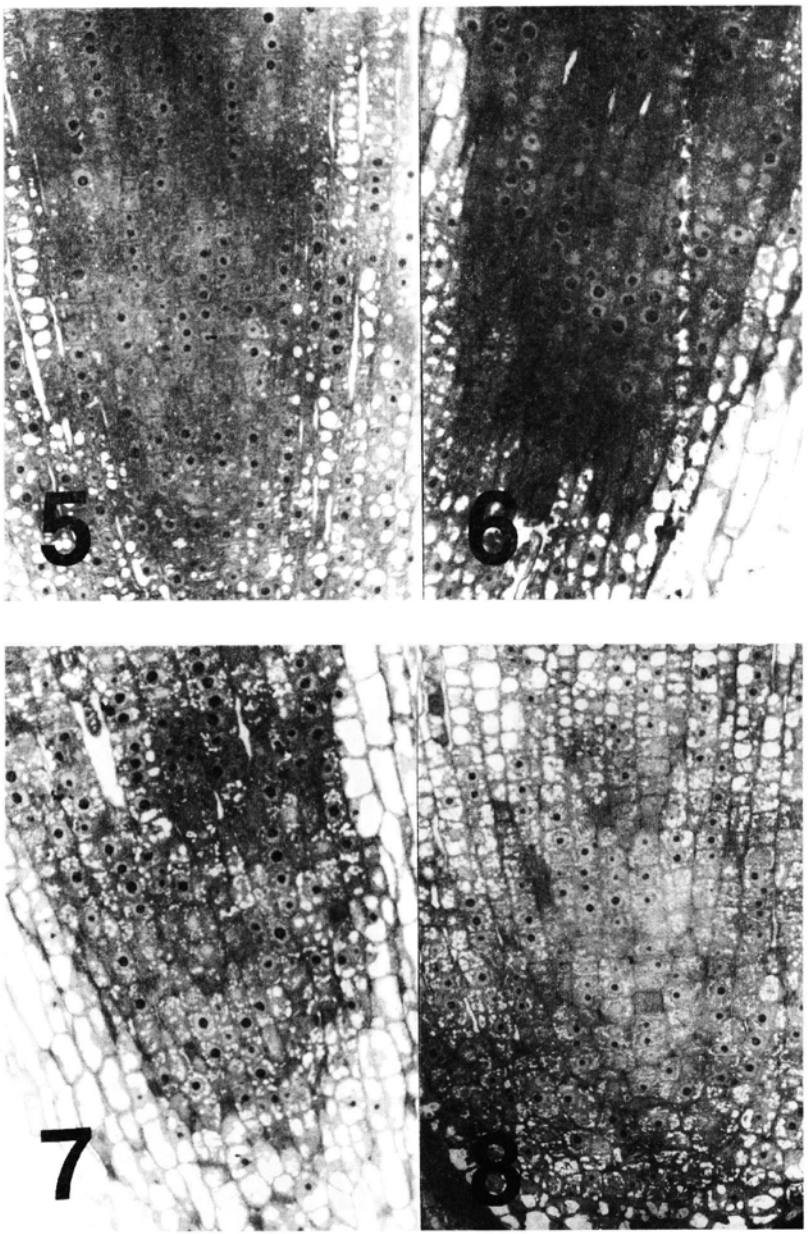
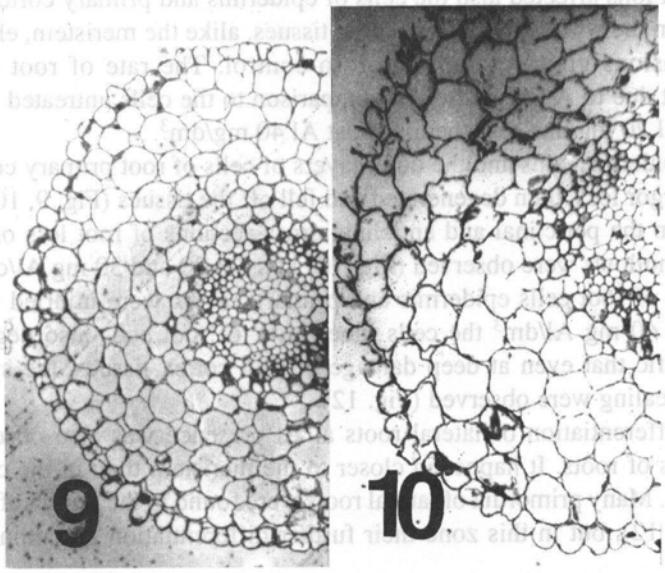


Fig. 5-8. The longitudinal sections of root apical meristem of cucumber seedlings not treated with Al (5), those treated with Al in concentrations of 20 mg/dm^3 (6), 30 mg/dm^3 (7), 40 mg/dm^3 (8)
The Figures show the changes in meristematic cells dimensions and their injures owing to Al influence. Particularly in the presence of Al at 20 and 30 mg/dm^3 the dimensions of cells increased as well as their nuclei and nucleoli grew (6, 7). The cell cytoplasm vacuolized, some single smaller or bigger vacuoles were also identified in the nucleoli. At 20 mg Al/dm^3 only fewer cells are visible in mitosis stage.



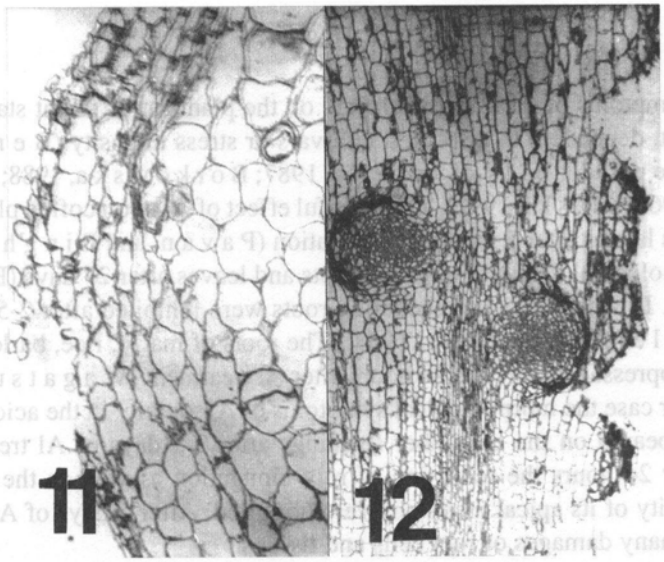


Fig. 9-12. The cross-sections (9-11) and a longitudinal sections (12) of cucumber seedling roots in the elongation and lateral roots differentiation

Fig. 9 not treated with Al; Fig. 10-12 treated with Al. The illustration of outer injures of root tissues (epidermis and primary cortex) due to Al effect. The outer layer of epidermis and cortex cells shrunk, degenerate and falling off the tissue they make smaller or deeper losses called "hollows". It is characteristic that even at deep damage of the there were recorded the symptoms of wounding cicatrization (Fig. 12) after 5-days incubation in the solution with 40 mg Al/dm^3.

The Al ions affected also the cells of epidermis and primary cortex of cucumber roots. On the whole, the cells of these tissues, alike the meristem, elongated and their dimensions grew in comparison to control. The rate of root cortex cells enlargement due to Al incubation in comparison to the cells untreated with Al was highest at Al 20 mg/dm^3 and the lowest at Al 40 mg/dm^3.

The epidermis cells and the outer layers of cells of root primary cortex shrunk owing to turgor loss, then degenerated and fell off the tissues (Fig. 9, 10). As a consequence, on the periclinal and anticlinal cross-sections of root less or more deep breaks and hollows were observed (Fig. 10, 11). At 20 and 30 mg Al/dm^3 only the outer layers of root cells epidermis and primary cortex were injuried while in the presence of 40 mg Al/dm^3 the cells inner layer of root cells also degenerated. It seems specific that even at deep damage of root cortex tissues, the symptoms of wounding healing were observed (Fig. 12).

The differentiation of lateral roots at Al presence was also observed in the bottom parts of roots. It happened closer to the meristem than in the control roots (Fig. 1, 3, 4). Many primordia of lateral roots were found in the region of the primary cortex (Fig. 12), but in this zone their further differentiation and elongation were suppressed.

DISCUSSION

The symptoms of Al toxicity appears on the plants at different stages of their development, dependent to a species, cultivars or stress intensity (B e r z o n s k y and K i m b e r, 1986; B i l s k i and F o y, 1987; B o r k o w s k a, 1988; F l e m i n g and F o y, 1968; Ś l a s k i, 1992). The harmful effect of Al upon coffee plants growth was noted as late as after 5 month of vegetation (P a v a n and B i n g h a m, 1982), while morphological damages of peach roots and leaves after 27 days (E d w a r d s et al., 1976). In wheat varieties seeddling roots were inhibited after 2-5 days of Al treatment (F l e m i n g and F o y, 1968). The roots of maize, rice, barley, oats and pea were suppressed as early as 24 hours after Al treatment (W a g a t s u m a et al., 1987). In our case the morphological symptoms of Al toxicity in the acid conditions (pH 4.2) appeared on the cucumber seedlings after 2-5 days of Al treatment, but already after 24 hours the reduction of root elongation as well as the changes in mitotic activity of its apical meristem were observed. After 5 days of Al treatment, there were many damages of root cells and tissues.

In the presence of different Al concentrations the root apical meristem as well as epidermis and primary cortex at the elongation, proliferous and young zone of lateral roots differentiation were damaged. The nature of damage observed in the cells and tissues of epidermis and primary cortex resembled the damages of these tissues at elongation zone of maize, barley and pea roots as reported by W a g a t s u m a et al. (1987). A characteristic symptoms of Al ions effects upon cucumber seedling roots was elongation of meristematic cells and primary cortex as well as

enlargement of their nuclei and nucleoli dimension and finally the vacuolization of cytoplasm and nucleoli advancing from the quiscent centre of the meristem.

The changes in elongation growth rate of plant roots due to Al operation are, generally, related to a changes in mitotic activity of apical meristems (B e n n e t and B r e e n, 1991; C l a r k s o n, 1965, 1969; S z k o l n i k, 1980). The obtained results seems to suggest that inhibition of cucumber root elongation resulted mainly from reduction of the number of mitoses in apical meristem. However, it doesn't exclude other mechanisms mobilized earlier than mitoses reduction as a primary response to Al toxicity. According to B e n n e t and others (B e n n e t and B r e e n, 1991; B e n n e t et al., 1985 b; B e n n e t et al., 1987) the primary response to Al is the electrophysiological and ultrastructural chnges occuring in the cap cells what, among others, results in cap volume decrease. Al-induced changes in root elongation rates are paralleled by coresponding changes in the volume of the root cap, and it seems to suggest that root growth may depend on minimum levels of activity being maintained in the cap (B e n n e t et al., 1987; B e n n e t and B r e e n, 1991). The obtained results seems to confirm this suggestion. The reduction of the cap volume of cucumber seedling roots treated with Al was noticed. The changes of cap volume were coincided with a decrease in root elongation rates.

The observed effect of Al on the meristematic cells and primary cortex suggests that endogenous phytohormones could take part in this phenomena. Al may induce the changes in the level of auxins, cytokinins and ABA which could modified the course of physiological processes in Al-treated plants (B a r l o w and P i l e t, 1984; B e n n e t and B r e e n, 1989; B e n n e t and B r e e n, 1991; F o y, 1988; H a s e n s t e i n and E v a n s, 1988; P a n et al., 1989; R i v e r et al., 1977). The obtained results suggest, and in some way confirm B e n n e t and B r e e n' s hypothesis (1991) that under the conditions of Al stress an auxin level in root changes which is denoted by the root cells elongation from its apical part. Presumably an ABA level may also change that influences a DNA synthesis (H a s e n s t e i n and E v a n s, 1988) and in turn, mitotic activity of cells as well as root elongation (B e n n e t and B r e e n, 1991). The induction of differentiation of far greater number of lateral root primordia and even differentiation of the secondary roots on hypocotyl in shorter time considerably closer the meristem than in the roots of seedlings not treated with Al, seems to confirm a suggestion concerning a primary hormonal role in the mechanisms of Al influence on the growth and development of plants.

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Cytotoksyczny wpływ jonów glinu na korzenie *Cucumis sativus* L.

Streszczenie

Korzenie 5-dniowych siewek ogórka odmiany Wisconsin inkubowano w roztworach glinu ($AlCl_3$) o pH 4.2. Glin zastosowano w koncentracjach 20, 30 i 40 mg/dm^3. W obecności Al we wszystkich badanych stężeniach hamowany był wzrost elongacyjny korzenia oraz redukowana była aktywność mitotyczna jego merystemu wierzchołkowego. Zupełne zahamowanie mitoz i wzrostu elongacyjnego w obecności 30 i 40 mg Al/dm^3 notowno po 4 i 3 dobach inkubacji. Po 5 dobach inkubacji korzeni w roztworach Al obserwowano zmiany w morfologii systemu korzeniowego siewek, wynikające głównie z zahamowania elongacji korzenia głównego, uformowania większej liczby korzeni bocznych, a nawet przybyszowych na hypokotylu. Redukcji rozmiarów czapeczki korzenia towarzyszyło skracanie merystemu wierzchołkowego i w bliskiej od niego odległości różnicowane były liczne zawiązki korzeni bocznych. Komórki merystematyczne ulegały wydłużeniu, a ich jądra i jąderka powiększeniu (szczególnie w obecności 20 i 30 mg Al/dm^3), cytoplazma zaś wakuolizacji. W strefie elongacji, włośnikowej i różnicowania korzeni bocznych komórki epidermy i kory pierwotnej ulegały kurczeniu, degeneracji i odpadając od tkanki tworzyły większe (40 mg Al/dm^3) lub mniejsze (20 i 30 mg Al/dm^3) zagłębienia i pęknięcia od zewnętrznej strony korzenia.