

CHANGES IN THE DEVELOPMENT AND STRUCTURE OF *Raphanus sativus* L. var. *radicula* Pers. ROOT UNDER ALUMINUM STRESS CONDITIONS

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Abstract. Roots of radish (*Raphanus sativus* L. var. *radicula* Pers.) of Rowa cv. seedlings grown in water cultures (pH 4.3) have been tested. Aluminum as $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ has been applied at 0, 10, 20 and 40 $\text{mg} \cdot \text{dm}^{-3}$ concentrations. Inhibition of the growth of root elongation and the following changes of root morphology have been observed after aluminum treatment: browning and thickening, root cap elongation or falling off, bending of the apex root and cracks on its surface. Roots have been characterized with the change of hair length and lateral zones. First lateral roots occurred closer to the basal root apex than first hair. Reduction of hair length was noticed too. Atrophy of outer root tissues has been accompanied by great enlargement of cortex cells size as a result of their hypertrophy.

Key words: aluminum toxicity, radish, root, growth, morphology, anatomy

INTRODUCTION

Symptoms of mobile aluminum toxicity occurring at large quantities in acidic soils, are mainly observed on roots of young plants. It results from easy and quick intake and accumulation of toxicant ions in an organ's tissues [Lazof et al 1996, Clune and Copeland 1999]. Reduction of roots elongation growth, their browning, cracks on surface and thickening of apical parts are the most characteristic symptoms of aluminum toxicity [Budiková 1999, Weryszko-Chmielewska et al. 1999, Čiamporová 2000, Li et al. 2000, Tabuchi and Matsumoto 2001]. The injured root system is characterized by less "efficiency", reduced resistance to diseases and soil pests as well as insufficient supply with waters and minerals [Pintro et al. 1996, Bennet and Steward 1999].

There is only partial information about reaction of crops to aluminum toxicity and great differentiation of Al-tolerance within the species (variety). Therefore, the aim of this study was to analyze the influence of different levels of Al on radish roots (Rowa cv.) and to determine the effects of Al on the morphology and anatomy of radish root.

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MATERIALS AND METHODS

Seeds of radish (*Raphanus sativus* L. var. *radicula* Pers.) of Rowa cv. were sterilized in 70% ethanol and HgCl_2 and then germinated on filter paper in Petri dishes moistened with deionized water. Three days after germination, the seedlings were transferred to water cultures. Plants were grown in plastic pots of 4 dm³ capacity filled with modified Knop's medium [Brauner and Bunkatsh 1987], to which aluminum in a form of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added at the following rates: 0 (control), 10, 20 and 40 mg·dm⁻³. pH was adjusted to 4.3 using 0.1M HCl or 0.1M NaOH. The solution was aerated and completed for the whole experiment, and after a week, it was replaced with a new one. The experiment was carried out in three replications and completed after 14 days.

Morphological observations of the root system were made during seedling growth and the main root length was measured in 30 plants after 1, 3, 5, 7 and 14 days of aluminum treatment. The root tolerance index (IT) was calculated according to Wilkins [1978]. Measurements (n = 10) of root cap height and main root diameter at 0.5 cm distance from the apex were made after 4 days. Moreover, the beginning and length of hair and lateral root zones were estimated along with the percentage of these zones in total root length. Length of the longest hair formed in the hair zone was measured as well. On the 14th day, the main root length, mean fresh matter of roots for a single seedling, as well as length of the longest hair were evaluated. Apical parts of the main root (about 10 mm) were taken for anatomical tests. Slides were prepared by paraffin method. The root samples, after dehydration in ethanol and Poly Clear Solvent (Polysciences, 1-methyl-4-isopropenylcyclohexane) series, were embedded in paraffin (Poly-Fin, 40°). The material was cut along and across into slices of 10-15 μm thickness using MPS-2U 4.2. microtome. Paraffin was removed applying Poly Clear Solvent. The slices were stained in 0.05% toluidine blue or 1% safranin and 0.1% durable green and closed in Euparal. Observations were made and photos taken of anatomical changes using microscope of Eclipse 400 (Nikon) type.

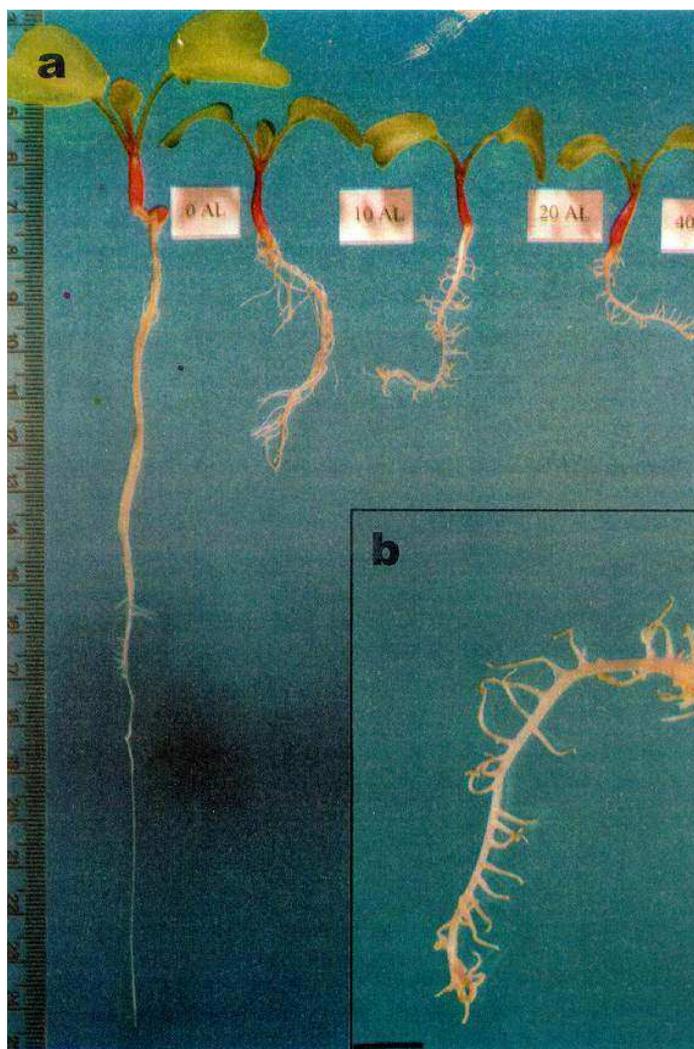
The results were statistically worked out. They were subjected to variance analysis for complete randomization system (single classification) and the least significant difference for pairs of mean values (LSD) was calculated.

RESULTS

Inhibition of root elongation growth was the earliest change observed that lasted for the whole experiment as a reaction to toxic aluminum ions concentrations (photo 1a, fig. 1). Sensibility of roots on increasing aluminum rates in medium at the first vegetation period was estimated on the basis of tolerance index (IT), whose values much decreased along with the aluminum concentration increase in medium on subsequent days of the experiment (tab. 1). Roots were characterized with slow length gains in the presence of 10 mg·dm⁻³ of aluminum chloride. Doses of 20 and 40 mg·dm⁻³ caused complete inhibition of root elongation on the 5th day of seedling growth on the medium (fig. 1).

After 14 days of vegetation, the mean length of root system in the presence of aluminum was reduced over 2.5 times at the lowest rate, about 3 times at 20 mg·dm⁻³ AlCl_3

and 5 times at the maximum rate of the toxicant (tab. 2, fig. 1, phot. 2a). Moreover, reduction of lateral roots length (phot. 3b, 3c) and decrease of mean root fresh matter as compared to the control after aluminum treatment was recorded (tab. 2).



Phot. 1. Root development of radish seedlings after one week of the Al-treatment (0, 10, 20 and 40 $\text{mg}\cdot\text{dm}^{-3}$ $\text{AlCl}_3\cdot 6 \text{H}_2\text{O}$): a – complete view of plants; b – 40 $\text{mg}\cdot\text{dm}^{-3}$ $\text{AlCl}_3\cdot 6 \text{H}_2\text{O}$, Al-treated lateral roots turned curved and shorter, bar = 5 mm

Fot. 1. Korzenie siedmiodniowych siewek rzodkiewki hodowanej w kulturach wodnych w obecności różnych stężeń glinu w pożywce (0, 10, 20 i 40 $\text{mg}\cdot\text{dm}^{-3}$ $\text{AlCl}_3\cdot 6 \text{H}_2\text{O}$): a – widok całych roślin; b – 40 $\text{mg}\cdot\text{dm}^{-3}$ $\text{AlCl}_3\cdot 6 \text{H}_2\text{O}$, widoczne skrócone korzenie boczne o zakrzywionych odcinkach szczytowych, kreska = 5 mm

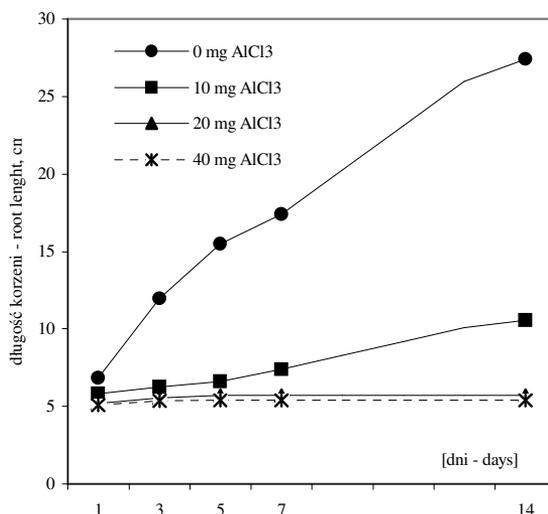


Fig. 1. Root elongation rate of radish seedlings treated with different levels of Al
Rys. 1. Tempo wzrostu systemu korzeniowego siewek rzodkiewki po wprowadzeniu różnych dawek Al

Table 1. The mean values of the root system length and aluminum tolerance index (IT) of radish seedlings in the first week of the experiment

Tabela 1. Średnia długość korzeni siewek rzodkiewki w pierwszym tygodniu wzrostu w kulturach wodnych oraz wartości korzeniowego indeksu tolerancji (IT)

Days Dni	Level of AlCl ₃ – Poziom AlCl ₃ , mg·dm ⁻³						
	The mean value of basal root length – Średnia długość korzenia głównego, cm						
	0	10	IT (%)	20	IT (%)	40	IT (%)
1	6.86	5.81	84.7	5.19	75.7	5.10	74.3
3	11.97	6.24	52.1	5.55	46.4	5.37	44.9
5	15.50	6.62	42.7	5.70	36.8	5.38	34.7
7	17.40	7.38	42.4	5.70	32.8	5.38	30.9

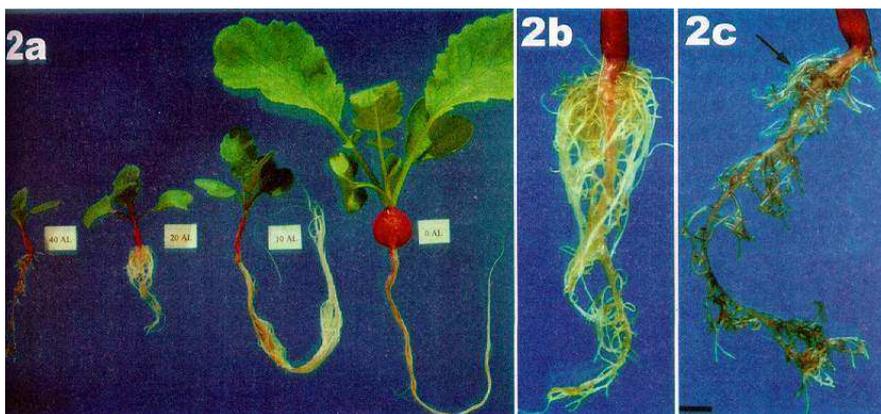
The root system formed by control radish seedlings of Rowa cv. was characterized by long, uniform and narrow – in apical part – main root as well as numerous lateral roots of the first and subsequent orders. Roots had creamy-white color. After seven days of exposure to aluminum ions, roots changed the color of apical fragments of main and lateral roots at the distance of several millimeters from creamy-white to yellow-brown or grey-blue. At the presence of 20 and 40 mg·dm⁻³ AlCl₃, numerous reduced-length lateral roots were curled and grew at 90° angle in relation to the main root in the neighborhood of apical point (phot. 1).

14-day-old radish roots after the highest toxicant concentration treatment became beige-brown (phot. 2a, 2c). Lateral roots occurred at the whole length of the main root

Table 2. The mean value of the root system length and root fresh matter of radish root on the last day of the experiment (14 day)

Tabela 2. Średnia długość systemu korzeniowego i średnia świeżej masy korzeni siewek rzodkiewki w dniu zakończenia doświadczenia (po 14 dniach)

Investigated feature Badana cecha	Level of AlCl ₃ Poziom AlCl ₃ mg·dm ⁻³				LSD NIR 0.05
	0	10	20	40	
The root system length Długość systemu korzeniowego, cm	27.40	10.55*	5.70*	5.38*	2.70
The root system fresh matter Świeża masa korzeni, g	0.46	0.27	0.17*	0.17*	0.27

* indicates a statistically significant difference from the control (0 mg·dm⁻³ AlCl₃)* oznacza wyniki istotnie różniące się z kontrolą (0 mg·dm⁻³ AlCl₃).Phot. 2. Root development of radish seedlings after two weeks of the Al-treatment (0, 10, 20 and 40 mg·dm⁻³ AlCl₃·6 H₂O): a – complete view of plants; b – 20 mg·dm⁻³ AlCl₃·6 H₂O; c – 40 mg·dm⁻³ AlCl₃·6 H₂O, Note Al-treated root turned brown, bars = 5 cmFot. 2. Korzenie czternastodniowych siewek rzodkiewki hodowanej w kulturach wodnych w obecności różnych stężeń glinu w pożywce (0, 10, 20 i 40 mg·dm⁻³ AlCl₃·6 H₂O): a – widok całych roślin; b – 20 mg·dm⁻³ AlCl₃·6 H₂O; c – 40 mg·dm⁻³ AlCl₃·6 H₂O, korzenie przyjmują brązowe zabarwienie, kreska = 5 mm

and made the root system conformation look like a brush (phot. 2c). Formation of white root gains with a good condition was observed at lower aluminum levels (phot. 2b).

Morphological observations of *Raphanus sativus* roots carried out after 4 days of seedling growth on the medium revealed that apparently formed root cap was an apical part of the control root, whose length at the following toxicant rates increased from 30% to 60%. However, numerous main roots were characterized by peeling and falling off of the root cap or its complete lack at the highest dose of aluminum (tab. 3). Furthermore, roots with no cap had a hooked bend of their apical fragment. The root's reaction towards aluminum ions was also manifested in the increase of the main root diameter at 0.5 cm distance from its apex, proportional to increasing toxicant rates (tab. 3). Differentiation of root level where the first short hair were formed was observed as well. Hair occurred on radish seedling roots treated with aluminum at a larger distance from its apex than on control ones (1.1, 2.3 and 1.3 times higher, respectively) (tab. 3). At 40 mg·dm⁻³ AlCl₃ rate, the length of hair zone was about 40% of that in control plants (tab. 3). However, the share of the zone in the total main root length was much larger in Al-treated objects than in controls (tab. 3). The length of the root hair zone was 90% and 72% of the total main root length for two highest aluminum rates. This value increased even by 54.5% and 36.5% in relation to the control (tab. 3).

Table 3. The morphological features of basal radish root after 4 and 14 days of aluminum treatment

Tabela 3. Cechy morfologiczne korzenia głównego rzodkiewki po 4 i 14 dniach traktowania glinem

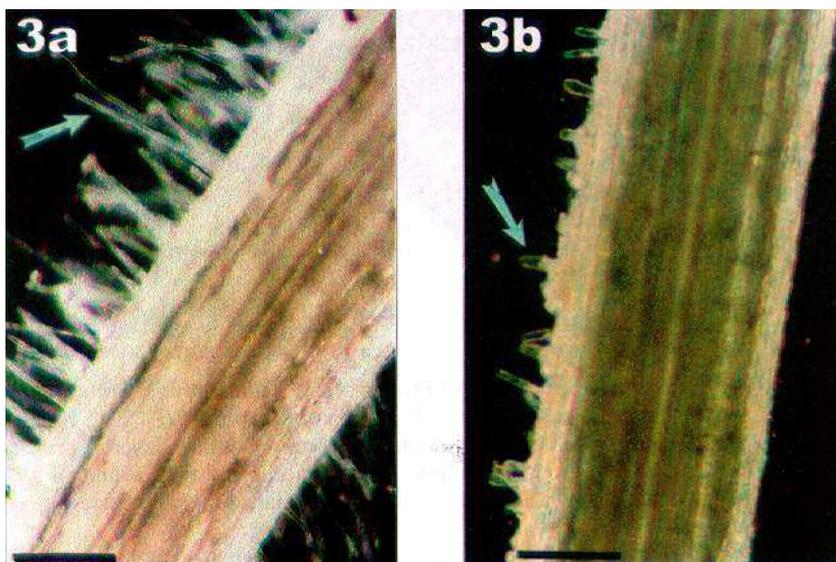
Investigated feature Badana cecha	Level of AlCl ₃ – Poziom AlCl ₃ mg·dm ⁻³				LSD NIR P = 0.05
	0	10	20	40	
The cap length Długość czapeczki (μm)	191.30	215.90	309.67*	250.48 or no	93.99
The root diameter (0.5 cm from the top) Średnica korzenia 0,5 cm od wierzchołka (μm)	341.6	328.3	350.0	400.0*	55.40
The hair zone beginning Początek strefy włosnikowej (mm)	3.40	3.81	7.83*	4.30	2.85
The hair zone length Długość strefy włosnikowej (mm)	97.4	60.5	50.7*	38.6*	41.80
The length of basal root zone with hair Udział strefy włosnikowej w całkowitej długości korzenia głównego (%)	35.5	57.3	88.9	71.7	-
The hair length after 4 days Długość włosników 4 dnia (μm)	1764.6	1764.6	1764.6	1764.6	-
The hair length after 14 days Długość włosników 14 dnia (μm)	210.33	217.25	60.42*	14.76*	77.02
The lateral roots zone beginning Początek strefy korzeni bocznych (mm)	47.00	33.80	5.14*	2.66*	15.87
The lateral roots zone length Długość strefy korzeni bocznych (mm)	90.4	30.5*	51.2	51.1	50.00
The length of basal root zone with lateral roots Udział strefy korzeni bocznych w całkowitej długości korzenia głównego (%)	65.8	47.4	90.9	95.0	-

* indicates a statistically significant difference from the control (0 mg·dm⁻³ AlCl₃)

* oznacza wyniki istotnie różniące się z kontrolą (0 mg·dm⁻³ AlCl₃).

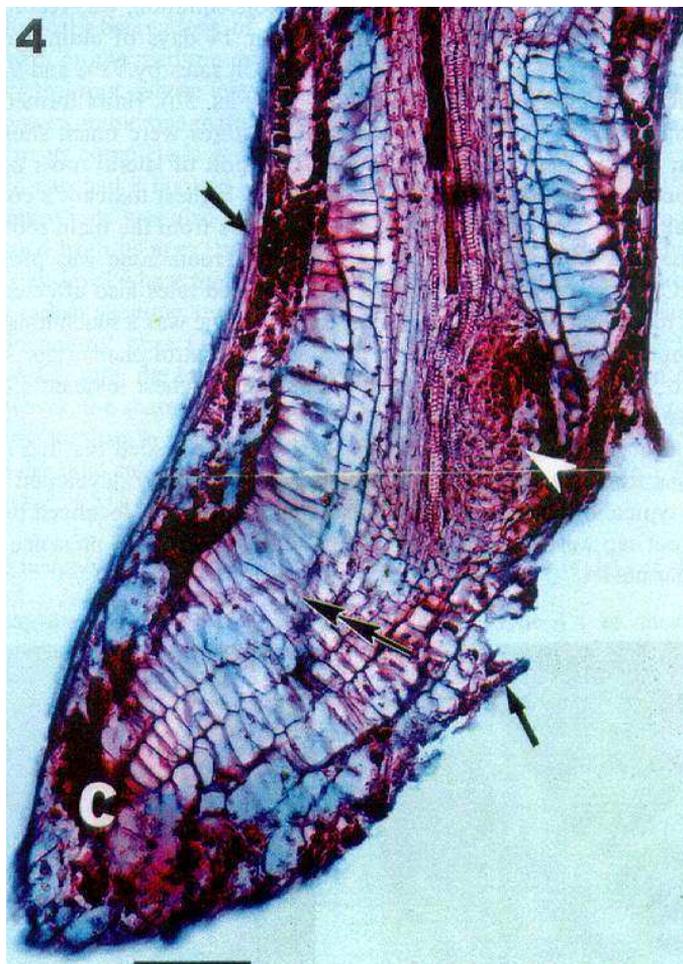
At the beginning of the experiment (after 4 days), no aluminum influence on the length of formed hair, regardless of the toxicant's concentration, was recorded (tab. 3). Measurements of radish root hair length made after 14 days of aluminum treatment indicated a reducing effect of 20 and 40 mg·dm⁻³ AlCl₃ rates by 71% and 93%, respectively, as compared to control seedlings (tab. 3, phot. 3a, 3b). Hairs formed by control radish seedlings after 14 days of growth in water cultures were much shortened comparing to their length after 4-day culture (tab. 3). A shift of lateral roots zone towards apical point occurred due to aluminum action. At the highest toxicant's concentration, the first lateral roots were formed just a few millimeters from the main root apex, even closer than the first hair (tab. 3). Lowering of lateral roots zone was proportional to increasing AlCl₃ concentration. Aluminum at the applied rates also affected the reduction of lateral roots zone length (tab. 3). However, the zone was a much longer fragment of the main root at two highest aluminum rates than in control plants (tab. 3). The zone included up to 95% of the total main root length at the highest toxicant's dose. It was a 29% increase in relation to control (tab. 3).

Anatomical observations made in a light microscope revealed that tissues of longitudinal sections from the roots of control plants were properly developed and formed regular lines, typical of root meristem. Meristematic tissue cells localized over properly a developed root cap were characterized by a regular shape and the presence of centrally situated cellular nuclei.



Phot. 3. Differences between the root hair (arrow) length in two weeks' radish seedlings grown in water culture; a – control, b – 40 mg·dm⁻³ AlCl₃·6 H₂O, bars = 150 μm

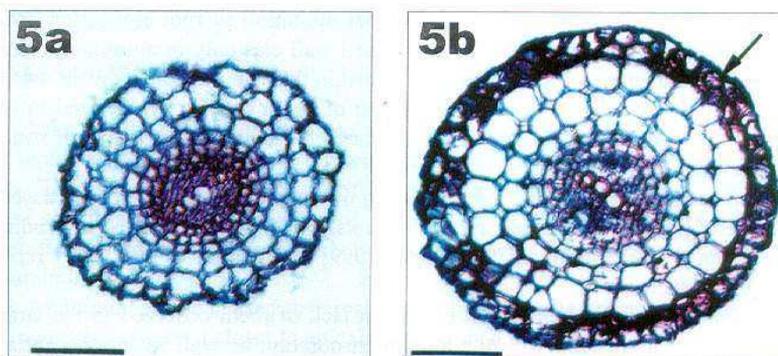
Fot. 3. Zróżnicowana długość włosników (strzałka) na korzeniach czternastodniowych siewek rzodkiewki uprawianej w kulturach wodnych; a – kontrola, b – 40 mg·dm⁻³ AlCl₃·6 H₂O, kreska = 150 μm



Phot. 4. The longitudinal section of root apices of radish seedling after 14 days of the $40 \text{ mg} \cdot \text{dm}^{-3}$ $\text{AlCl}_3 \cdot 6 \text{ H}_2\text{O}$ treatment. Disorganization of root cap cells is visible (C). Thick layer of disintegrated and disorganized cells with transverse lesion in surface layer of primary root are visible (arrows) Note irregular pattern of cortical cells and their abnormal size with hypertrophy (double arrow). Emergence of the first lateral root appeared near apex (arrowhead), bar = $100 \mu\text{m}$

Fot. 4. Przekrój podłużny wierzchołkowej strefy korzenia głównego 14-dniowych siewek rzodkiewki w obecności $40 \text{ mg} \cdot \text{dm}^{-3}$ $\text{AlCl}_3 \cdot 6 \text{ H}_2\text{O}$. Widoczne degenerujące komórki czapeczki (C), szeroka warstwa obwodowych komórek kory z objawami nekrozy lub spękaniem oraz pustymi przestrzeniami (strzałki), a także wzrost rozmiarów różnicujących się komórek i ich nieregularny układ (podwójna strzałka). W bliskiej odległości za zagiętym wierzchołkiem korzenia głównego widoczny początek pierwszego korzenia bocznego (grot strzałki); kreska = $100 \mu\text{m}$

Changes of topography and structure of the main root tissues in radish seedlings were observed at the highest concentration of aluminum (phot. 4). Atrophy or the falling off root cap as well as destruction or necrosis of cortex outer cell layers caused the root deformation and bending of its apical part from the vertical axis. Also cracks and tissue losses as well as coming off of the cortex outer layers were found (phot. 4). It was difficult to distinguish the zone of cellular divisions in roots of seedlings treated with aluminum. Irregular arrangement of particular cell layers was observed in cortex and cylinder parenchyma. Cells of that root sector were characterized by variable shapes and sizes, and also showed hypertrophy (phot. 4).



Phot. 5. Cross-sections of two weeks' radish seedlings root treated with: a – $0 \text{ mg}\cdot\text{dm}^{-3} \text{ AlCl}_3\cdot 6 \text{ H}_2\text{O}$ (control), b – $40 \text{ mg}\cdot\text{dm}^{-3} \text{ AlCl}_3\cdot 6 \text{ H}_2\text{O}$; note: Al-treated root become thicker; hypertrophy of cortical cells is visible, cell walls of epidermis and outer layer of cortex are thickening (arrow), bars = $100 \mu\text{m}$

Fot. 5. Przekroje poprzeczne przez korzeń główny czternastodniowych siewek słonecznika: a – kontrola, b – $40 \text{ mg}\cdot\text{dm}^{-3} \text{ AlCl}_3\cdot 6 \text{ H}_2\text{O}$; w obecności glinu widoczny wzrost średnicy korzenia w wyniku hipertrofii komórek kory oraz szeroka warstwa obwodowych komórek o zgrubiałych ścianach komórkowych (strzałka), kreska = $100 \mu\text{m}$

Analysis of tissues from cross-sections of Rowa cv. radish roots cultivated in the aluminum presence indicated a significant increase of root diameter as a result of size enlargement, in particular primary cortex cells and enlargement of thickness of cell wall in outer root layers (phot. 5a, 5b).

DISCUSSION

It was found on the basis of results achieved in the present study that *Raphanus sativus* var. *radicula* of Rowa cv. is a plant that shows considerable sensitivity to higher aluminum concentrations in a medium (20 and $40 \text{ mg}\cdot\text{dm}^{-3} \text{ AlCl}_3$), and it is fairly tolerant to a rate of the lower toxicant ($10 \text{ mg}\cdot\text{dm}^{-3} \text{ AlCl}_3$).

Diversity of defense mechanisms towards aluminum ions that may occur in plants determines the great variability of aluminum-tolerance not only among species, but even

within the same species [Foy et al. 1974, Llugany et al. 1995, Weryszko-Chmielewska et al. 1999]. Opinions of many authors on plants resistant and sensitive to aluminum much differ, which depends on many factors, including the tested variety, the applied form of aluminum and the experimental conditions [Wheeler 1994, Kobayashi et al. 2004]. However, there is no information on the level of radish sensitivity to aluminum ions in up-to-date numerous studies. Only studies performed by Michałek [1997] revealed that radish of Sopol Lodu cv. reacted towards aluminum stress in a form of significant change of carbohydrates and ascorbic acid.

Inhibition of root elongation growth and their browning observed in the present study corresponds with the results of other authors [Weryszko-Chmielewska *et al.* 1999, Čiamporová 2000]. Some scientists suggest that inhibition of root elongation growth, and thus cell elongation, is due to the loss of cell wall elasticity in aluminum presence [Tabuchi and Matsumoto 2001]. It is also probable that the phenomenon in the presence of the toxicant resulted from the inhibition of auxin activity synthesized in root's apical point responsible for cell elongation through loosening of the cell wall structure [Kopcewicz and Lewak 1998].

In the presence of aluminum, a deep peeling or complete lack of root cap caused the disturbance of positive geotropism typical of roots, which was manifested in bending of the main root's apical fragment. Budikova [1999] and Comin et al. [1999] reported similar dependencies.

Reduction of the root hair length or even a lack of them observed in the author's own studies was associated with the aluminum duration as well as its concentration. Forming of a lower number of shorter and deformed hair or their complete lack in the presence of aluminum ions was also described by some other authors [Care 1995, Jones and Kochian 1995, Weryszko-Chmielewska et al. 1999]. Perhaps the observed necrosis of cover tissue cells and root growth in water culture caused that seedlings did not form such long root hair as in the soil. Reduction of hair length even in the control object after 14 days of plant's growth in the medium would this confirm. Furthermore, disturbance of microtubular activity and the decrease of free calcium ions level, which usually accompanies aluminum toxicity, may also reduce the root hair growth [Blancaflor et al. 1998, Kopcewicz and Lewak 1998].

Forming of lateral roots closer to the apical part of the main root than the first hair was observed in the present study, which might have resulted from the shortening of particular root fragments, meristematic and elongation in particular. Similar results were also described by Clune and Copeland [1999] as well as Čiamporová [2000]. Moreover, the observed accelerated tissue differentiation and maturation, as well as necrosis or lignification of root apex outer layers, may also contribute to earlier formation of lateral roots, which was observed by Lenoble et al. [1996] and Mangabeira et al. [1999].

Cracks and tissue losses reaching through the cover tissue up to cortex parenchyma and coming off of degraded primary cortex cell layer were observed on the surface of roots treated with aluminum, which corresponds with the results of Budiková and Mistrík [1999]. In the opinion of McQuattie and Schier [1990], necrosis of root circumferential tissues might have been a result of cell destruction, disorganization and amorphization of their cytoplasm due to plasmatic membrane cracking or breaking of cytoplasm continuity.

In the presence of the toxicant, parenchyma cell hypertrophy causing great thickening of root's apical part, was a result of isotropic growth of these cells, which was found by Sasaki et al. [1996] and Čiamporová 2000]. Microtubules, whose structure and action becomes disturbed in the presence of aluminum and which determine the direction of cellulose fibers deposition in cell walls, are responsible for that process [Blancaflor et al. 1998, Horst et. al. 1999]. Furthermore, tissues of root circumferential sectors observed in a light microscopes were sometimes characterized by thickened cell walls, which Sasaki et al. [1996] and Votrubová et al. [1997] accounted for by deposited aluminum precipitates or deposition of larger amounts of components that take part in building the cell walls (e.g. hemicelluloses and lignin).

CONCLUSIONS

1. Root system of *Raphanus sativus* var. *radicula* of Rowa cv. was characterized by great sensitivity to aluminum stress (growth inhibition, necrosis, cracks, and deformations).
2. Changes of color and bending of apical fragment of the main root, strong inhibition of elongation growth and enlarged diameter of roots, reduction of root hair length and shift of lateral roots zone towards the root apex were the most important symptoms of aluminum toxicity.
3. Aluminum action caused disturbances in the root zone arrangement: the first lateral roots were observed closer to apical root part than the first hair.
4. The highest aluminum concentration ($40 \text{ mg}\cdot\text{dm}^{-3} \text{ AlCl}_3$) influenced the occurrence of numerous disturbances of radish root anatomy such as: reduction of root cap height or its falling off, thickening of walls and atrophy of circumferential cell layers, cracks and gaps in the outer tissues of the main root as well as hypertrophy of many cortex parenchyma cells.

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ZMIANY W ROZWOJU I BUDOWIE KORZENI RZODKIEWKI (*Raphanus sativus* L. var. *radicula* Pers.) w warunkach stresu glinowego

Streszczenie. Badano korzenie siewek rzodkiewki 'Rowa' (*Raphanus sativus* L. var. *radicula* Pers.) hodowanych w kulturach wodnych o pH 4,3. Glin zastosowano w stężeniach: 0, 10, 20 i 40 mg·dm⁻³ w formie AlCl₃·6 H₂O. W obecności glinu obserwowano inhibicję wzrostu elongacyjnego korzeni oraz zmiany w ich morfologii: brązowienie, wydłużanie się lub odpadanie czapeczki, zaginanie się szczytowego odcinka korzenia oraz spękania na jego powierzchni. Korzenie o znacznie powiększonej średnicy odznaczały się zmianą długości i układu stref korzeniowych: włośnikowej i korzeni bocznych oraz redukcją długości włośników. Obumieraniu tkanek obwodowej strefy korzenia towarzyszyło znaczne powiększenie rozmiarów komórek kory w wyniku ich hipertrofii.

Słowa kluczowe: toksyczność glinu, rzodkiewka, korzeń, wzrost, morfologia i anatomia

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