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INVESTIGATION OF THE EFFECTS OF ALUMINUM STRESS ON SOME MACRO AND MICRO-NUTRIENT CONTENTS OF THE SEEDLINGS OF *LYCOPERSICON ESCULENTUM* MILL. BY USING SCANNING ELECTRON MICROSCOPE

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Abstract

This study was planned to see the affect of aluminum stress on plant nutrition and metabolism. The effects of aluminum stress on uptake level of some macro- and micro-nutrients from the nutrition solution into the seedlings of *Lycopersicon esculentum* Mill. and on mobilization of some nutrient elements in the seedlings were examined at the level of epidermal cells. The elemental structure of root, hypocotyl and cotyledon epidermal cells were determined by Energy Dispersive X-ray Microanalysis (EDX) performed in a local area ~50 nm in diameter at the level of a single epidermal cell cytoplasm by using low vacuum (~24 pascal) Scanning Electron Microscope. EDX analysis revealed that aluminum content of the cells was increasing with the increased concentrations of aluminum in the nutrient solution and that aluminum largely accumulated in the roots. Aluminum concentration was much higher in the root epidermal cells of the seedlings incubated in aluminum containing media for 17 days without adding any nutrient solution; it was also true for the local EDX analysis of radicle epidermal cells from the same series.

Aluminum stress was found to tend to modify the plant nutritional element content of the cells and this was particularly of critical importance in terms of some macro- and micro-nutrients. The assessments performed at the level of epidermal cells of young seedlings of *Lycopersicon esculentum* suggest that aluminum stress leads to an absolute change in the plant nutritional element composition of the cells and in the mobilization of some nutritional elements in the seedlings.

Introduction

Plants growing on acid-reactive soils with a pH value of <5, the aluminum toxicity is identified as an important abiotic stress factor that often inhibits the water and nutrient uptake, thus causing decreased plant growth and productivity (Hoekenga *et al*., 2003; Doncheva *et al*., 2005; Postma *et al*., 2005; Rana & Aery, 2005; Wang *et al*., 2006). The literature reveals a large number of studies dealing with the various aspects of interaction of aluminum and plant nutritional elements.

According to an opinion: "the prolonged exposure of the plants to the aluminum can prevent the growth of the shoots due to nutritional deficiencies (magnesium, calcium, phosphorus), drought stress and phytohormone imbalances" (Horst, 1995). According to another view: "aluminum interacts with the phosphorus, calcium, magnesium, potassium and nitrogen uptake of the plants; aluminum toxicity is often associated with increased manganese and iron contents and decreased calcium and magnesium contents in the plants; and aluminum-induced chlorosis can be seen in some plants due to impaired iron metabolism" (Aller *et al*., 1990).

In a study of four different culture varieties of *Lolium multiflorum,* aluminum has considerably reduced the amount of divalent cations (Ca^{+2}, Mg^{+2}) desorbed from Donnan free space, while it has increased the amount of desorbed K^+ and Na^+ (Rengel & Robinson, 1989). In *Triticum aestivum*, both uptake and transport of the potassium, phosphorus and calcium have been influenced by administration of aluminum (Pettersson & Strid, 1989). For *Lolium multiflorum,* the high sulfate concentration and phosphate in mineral nutrient solutions has been shown to have a positive effect on the detoxification of aluminum (Mora *et al*., 2005). Aluminum has reduced the nutrient concentrations except manganese and zinc in the above-ground organs of *Oryza sativa,* whereas almost all of nutrient concentrations in *Phaseolus vulgaris* crop have tended to increase with increasing concentrations of aluminum (Fageria & Santos, 1998). Increasing concentrations of aluminum administered to *Zea mays* has been found to reduce nitrogen, phosphorus and iron concentrations in the shoots (Lidon *et al*., 1999). The researchers have also reported that the aluminum concentrations higher than 9 mgl^{-1} led to reduced magnesium concentrations in contrast to increased manganese content (Lidon *et al*., 1999). In the study of Baligar *et al*., (1993), a greater amount of phosphorus, potassium, calcium, magnesium, zinc and iron has been up-taken and transported by aluminum-tolerant genotypes than aluminum-sensitive genotypes of *Sorghum*.

In the study of Goransson & Eldhuset (1991), aluminum added to the nutrient solution in the form of $AlCI_3$ or $AI(NO_3)_3$ and at a concentration of 0.2-30 mM has reduced the rate of calcium and magnesium uptake into *Picea abies* and *Pinus sylvestris* at 0.2 mM and 1 mM of aluminum concentrations respectively, without affecting the relative growth rates. In the another study of *P. sylvestris* seedlings incubated in nutrient solutions containing 0.5-4 mM $AI(NO_3)_3$, the presence of aluminum in the solution has reduced the concentrations of phosphorus, potassium, calcium, magnesium, manganese, iron, copper, zinc and boron in the root and needle (Oleksyn *et al*., 1996). The researchers have reported that the nutritional status of the roots was generally much more affected than that of shoots (Oleksyn *et al*., 1996). In *Leucaena leucacephala,* the increasing aluminum concentrations in the nutrient solutions has resulted in decreased phosphorus, potassium, calcium and

magnesium contents in the leaves and roots, whereas the nitrogen content has remained relatively unchanged (Koffa & Mori, 1987). In a study of *Betula pendula,* the potassium, magnesium and iron concentrations in the root were significantly reduced with the administration of aluminum (Kidd & Proctor, 2000). In *Nicotiana tabacum,* aluminum has been found to have a synergistic interaction with iron, an antagonistic interaction with copper, and no effect on cadmium element (Yamamoto *et al*., 1994).

On the other hand, recent studies mainly emphasize the aluminum-silicon interactions (Jansen *et al*., 2003; Ryder *et al*., 2003; Wang *et al*., 2004) and aluminum toxicity has been suggested to be diminished by silicon administration to the plants in a similar manner that was observed in animal systems (Hodson & Evans, 1995). The studies of magnesium-aluminum interaction conducted by Silva *et al*., (2001) on *Glycine max,* Watanabe & Okada (2005) on *Oryza sativa* and Yang *et al*., (2007) on *Vigna umbellata* have also contributed greatly to the knowledge in the field of physiology of plant nutrition through understanding the mechanisms of adaptation of the plant to the aluminum stress. However, despite numerous studies examining the underlying physiological mechanisms of aluminum toxicity and tolerance (Matsumoto, 2000; Silva *et al*., 2004), more research is warranted in this field because ‘’the vast majority of these studies have been focused on monocot crop plants, detailed analysis of potential mechanisms of inhibition of dicot plant growth caused by aluminum toxicity is lacking’’ (Ahn *et al*., 2001). The cellular distribution of aluminum is still a subject of debate’’ (Eticha *et al*., 2005) and it is possible to observe very important genotypic differences in different genotypes of the same species in terms of aluminum sensitivity.

In the present study, we aimed to investigate the effects of aluminum on the uptake of some macro- and micro-nutrients from the nutrient solutions into 17-day old *L. esculentum* Mill. seedlings and on the mobilization of some macro- and micro-nutrients in these seedlings by EDX analysis of the single cells obtained from root, hypocotyl and cotyledon epidermal cells. Although aluminum is known to affect the uptake of some nutritional elements into the plant, as we stated above, the vast majority of research in this field is conducted at the level of a complete plant or organ. On the other hand, present study was conducted at the level of epidermal cells and examined effects of aluminum administration on cellular change of 17 different elements using a single epidermal cell. The present study may have a small contribution to explain the physiological mechanisms underlying the decreased growth caused by aluminum and the interaction between aluminum and nutritional elements in the plants.

Materials and Methods

A culture variety of *Lycopersicon esculentum* Mill. (*Solanaceae*) was used in hte present stuty. The seeds of *Lycopersicon esculentum* Mill. cv. H-2274 constituting our research material were obtained from Anatolia Agricultural Research Institute.

As is known, in the today’s agriculture, cultivated plants are usually exposed to various biotic and abiotic stresses mostly as a result of agricultural applications (Anwar *et al*., 2011; Shakeel & Mansoor, 2012). The fertility of one of the world's most widely cultivated plant, *Lycopersicon esculentum,* is determined by a large number of genetic and environmental factors and has been reported that this plant, particularly the young seedlings, is also sensitive to various abiotic stresses (Ali *et al*., 2011; Zdravkovic *et al*., 2011).

The seeds were subjected to serial superficial sterilization procedures. For sterilization, the modified techniques recommended for standard tissue culture were used (Başaran, 1990; Babaoğlu *et al*., 2001). Following the sterilization procedures, 100 seeds were sown in sterile petri dishes containing sterile filter paper inside.

The macro- and micro-nutrients of Murashige and Skoog medium were used as the nutrient solution (Murashige & Skoog, 1962). The aluminum administrations were performed by using the solutions containing the aluminum in the form of $AlCI_3.6H_2O$ and prepared in 4 different concentrations (10, 50, 500 and 1000 ppm Al^{+3}).

The first series of 100 seeds was used as control and only Murashige and Skoog medium (Murashige & Skoog, 1962) was administered to this group of seeds during the study period. Thus, the genotypic analysis of 17-day-old *L. esculentum* cv. H-2274 seedlings in terms of the potential of uptake of relevant nutritional elements was performed and the nutritional element contents of the cytoplasms of root, hypocotyl and cotyledon upper and lower epidermal cells were identified. In addition to macro- and micro-nutrients of Murashige-Skoog medium (Murashige & Skoog, 1962), $AlCI_3.6H_2O$ solutions were administered to 4 distinct series of seeds in 4 different concentrations (10, 50, 500 and 1000 ppm Al^{+3}). Other series were treated only with $AlCI_3.6H_2O$ solutions containing 50, 500 and 1000 ppm Al^{+3} during the incubation period. The macro- and micro-nutrients of Murashige-Skoog medium (Murashige & Skoog, 1962) were not administered to these series. Thus, the possibility of co-existence of aluminum and some macro-and micro-nutrients was also evaluated in epidermal cells of the embryos and young seedlings. After completing the sterilization and sowing processes, the seeds were incubated in a culture room under 16/8 h light/dark photoperiod and at 25 +/- 2^{o}C for 17 days.

At the end of the incubation period, the root, hypocotyl and cotyledon upper and lower epidermal cells of seedlings isolated from the nutrient solutions were analyzed for their carbon, nitrogen, oxygen, sulfur, phosphorus, sodium, magnesium, potassium, calcium, manganese, iron, cobalt, copper, zinc, chlorine and aluminum contents using low vacuum (~24 pascal) Scanning Electron Microscopy and EDX analysis (Energy Dispersive X-ray Microanalysis) performed on a local region (~50 nm in diameter) at the level of cytoplasm of a single epidermal cell. During these analyses, we took care of selecting the same type of cells settling in similar local regions of the studied organs.

Results

In the present study, administration of increasing concentrations of aluminum to the nutrient solutions used for the incubation of *L. esculentum* cv. H-2274 seeds resulted in increased aluminum content in the cells and accumulation of aluminum largely in the roots. Indeed, after an incubation period of 17 days, the aluminum content (percent elemental weight) of the root epidermal cells were 0.07, 0.48, 0.33 and 0.40 with the administration of Al^{+3} at a concentration of 10, 50, 500 and 1000 ppm, respectively (Figs. 1, 2).

Aluminum content of hypocotyl epidermal cells was 0.03, 0.01 and 0.07 with the administration of Al^{+3} at a concentration of 10, 50 and 500 ppm, respectively; while the aluminum content of cotyledon upper and lower epidermal cells was at the ranges of 0.02-0.01 and 0.01-0.04 with the administration of Al^{+3} at a concentration of 10 and 50 ppm, respectively (Figs. 3-5).

Administration of Al^{+3} at a concentration of 1000 ppm resulted in an aluminum content reaching to 0.65 in some of the radicle epidermal cells. However, the radicles differentiated under such high levels of aluminum accumulation could not grow into full-fledged seedlings that exhibited the development of root, hypocotyl and cotyledons. The germination process was limited to only appearance of the radicle through testa.

The aluminum content in the root epidermal cells of the seedlings which were incubated in medium containing only aluminum for 17 days and without adding any nutrient solution was much higher. Indeed, while the aluminum content of the root epidermal cells was at the level of 0.47 and 0.94 with the administration of Al^{+3} at a concentration of 500 and 1000 ppm respectively, Al^{+3} administered at a concentration of 1000 ppm resulted in aluminum accumulation in the radicle epidermal cells reaching to the level of 1.22%. However, these radicles also exhibited a similar pattern of development with the preceding ones. While 500 ppm Al^{+3} resulted in aluminum accumulation at a level of 0.03 in the hypocotyl epidermal cells, administration of 50 ppm Al^{+3} led to aluminum accumulation at a level of 0.03 and 0.01 in cotyledon upper and lower epidermal cells, respectively (Figs. 6, 7).

The EDX analysis of a single root epidermal cell of *L. esculentum* cv. H-2274 seedlings revealed that administration of 10 and 50 ppm of aluminum resulted in no significant change in the carbon content of the cells compared to control cells, while the carbon content of the epidermal cells was decreased with the administration of 500 and 1000 ppm of aluminum. In the EDX analyses of the series treated with up to 1000 ppm of aluminum concentration, no significant difference was found in nitrogen content of the cells compared to that of control group. The nitrogen content of the cells was slightly increased with the administration of aluminum at a concentration of 1000 ppm. The sulfur content of the root epidermal cells was regularly decreased with increasing concentration of aluminum in the nutrient solution (Table 1).

Administration of 10 ppm of aluminum resulted in significantly higher sodium content in the root epidermal cells compared to the control group, while the decline in sodium content that began at the aluminum concentration of 50 ppm was observed to persist in regular manner up to the concentration of 1000 ppm. An obvious increase was recorded in potassium content of the root epidermal cells with administration of 10 ppm of aluminum. The values obtained with the administration of 50 ppm of aluminum were similar to those obtained with 10 ppm of aluminum. When the aluminum was administered at a concentration of 500 ppm or higher, a progressive decline was observed in the potassium content of the epidermal cells. The decrease was more remarkable at the aluminum concentration of 1000 ppm (Table 1).

In the comparison of EDX analysis results of the root epidermal cells in the control group and percent elemental weights in radicle epidermal cells, the carbon content was found to be higher and oxygen and nitrogen contents were found to be lower in radicle epidermal cells compared to controls. The sulfur, potassium and calcium content of the radicles were also significantly lower than that of controls. However, these reductions were much more significant for potassium, and particularly for calcium. The zinc and copper contents of radicles were significantly higher than that of control group and the iron, which was undetectable in the control group, could be detected in the EDX analysis of radicle epidermal cells. Although cobalt, magnesium and sodium concentrations were quite high in the root epidermal cells constituting the control group, all three elements were undetectable in the EDX analysis of radicles. Manganese was also undetected in the radicle epidermal cells. Chlorine content of the cells was found to be significantly higher than that of control group and one of the most striking finding found in the radicle epidermal cells was the high levels of phosphorus accumulation in these cells (Table 1).

In present study, the EDX analysis from a single cell of hypocotyl epidermal cells of the seedlings revealed regular increases in the carbon content of the cells parallel to the increases in aluminum concentration in the nutrient solution. At a concentration of 10 ppm of aluminum, the nitrogen content of the hypocotyl epidermal cells was not very different from the control values, while it was decreased at the 50 and 500 ppm of aluminum concentration. While the sodium content of hypocotyl epidermal cells was significantly lower than that of control values after the administration of 10 and 50 ppm of aluminum, the values obtained with these two aluminum concentrations were similar. On the other hand, sodium element was not detected in the hypocotyl epidermal cells with the administration of 500 ppm of aluminum. The potassium content of all hypocotyl epidermal cells treated with aluminum was lower than control values. At an aluminum concentration of 10 ppm, the percent elemental weight of oxygen in the cells was very close to the control values. A slight and a significant decline were observed with the administration of 50 and 500 ppm of aluminum, respectively (Table 2).

In the EDX analysis performed on a single cotyledon lower epidermal cell, regular increase in the carbon, sulfur, phosphorus and calcium contents and decrease in the oxygen and magnesium contents of the cells were recorded with the administration of increasing concentration of aluminum. The nitrogen content of the cells was also lower than that of control group with the administration of 10 and 50 ppm of aluminum (Table 3).

The results of the EDX analysis showing the endogen macro- and micro-nutrient contents of the root, hypocotyl and cotyledon epidermal cells of the seedlings incubated in the medium containing only 50, 500 or 1000 ppm of Al^{+3} for 17 days and without adding MS nutrient solutions are given in Tables 2 and 4.

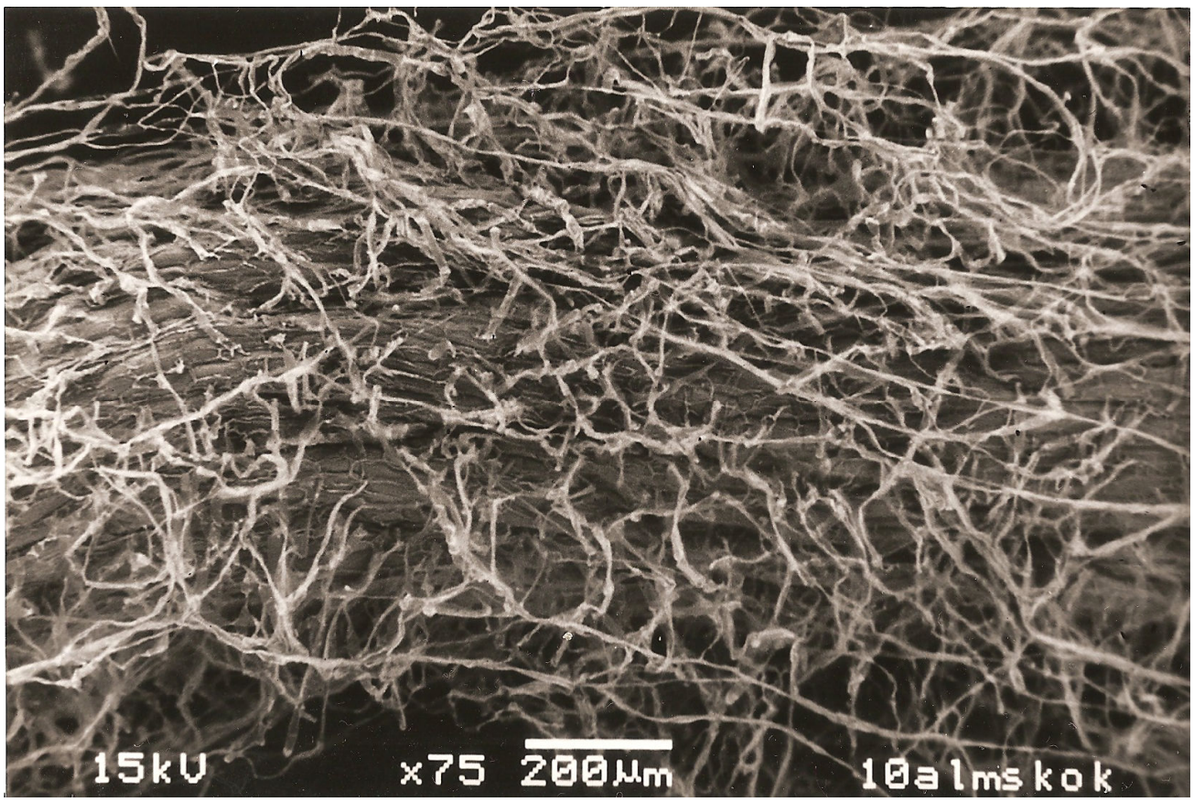

Fig. 1. SEM images of the root of *L. esculentum* seedlings incubated in the nutrient solutions containing 10 ppm of aluminum.

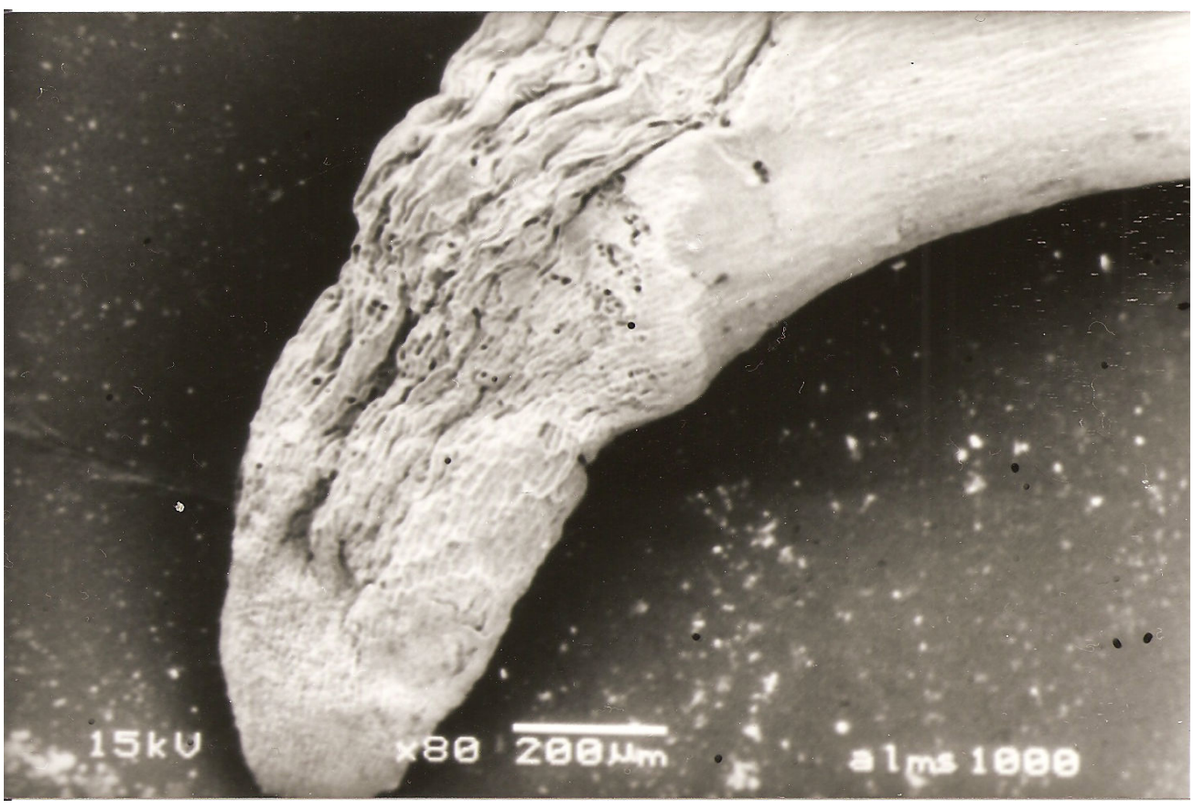

Fig. 2. SEM images of the roots and the deformation observed in the root tips of some *L. esculentum* seedlings incubated in the nutrient solutions containing 1000 ppm of aluminum.

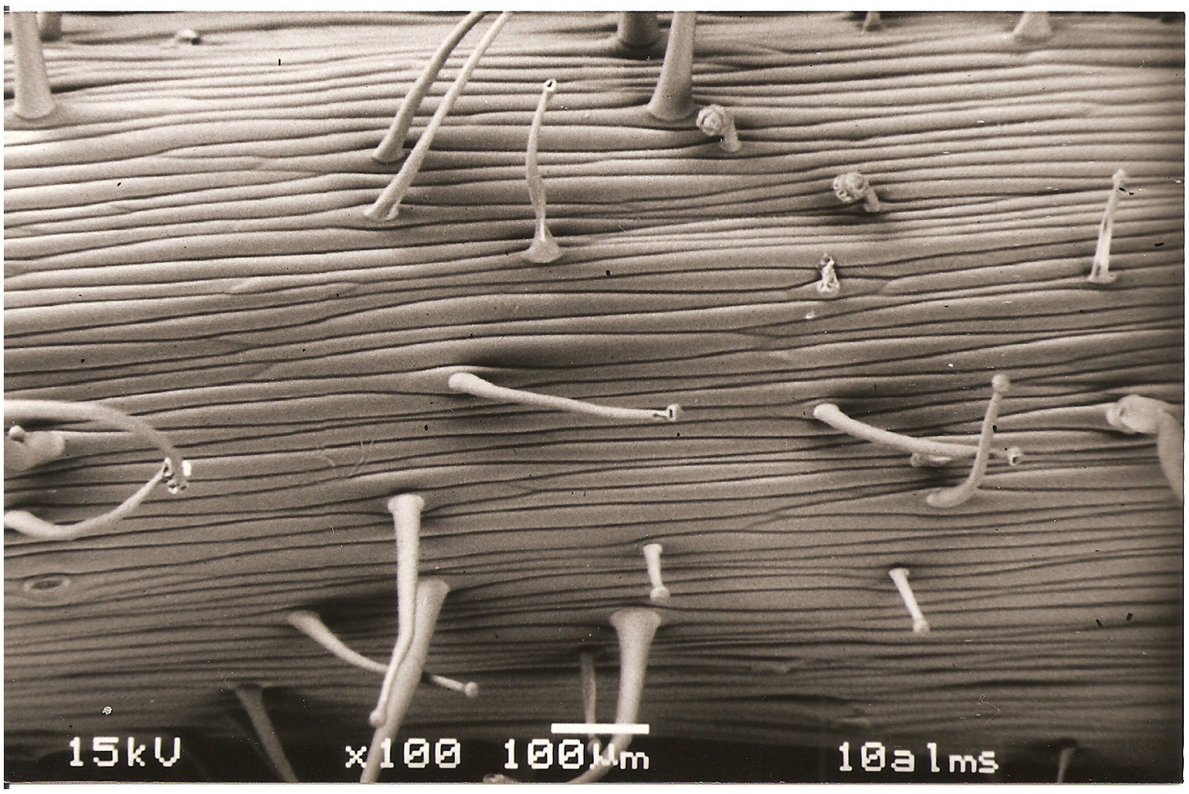

Fig. 3. SEM images of the hypocotyl epidermal cells of *L. esculentum* seedlings incubated in the nutrient solutions containing 10 ppm of aluminum.

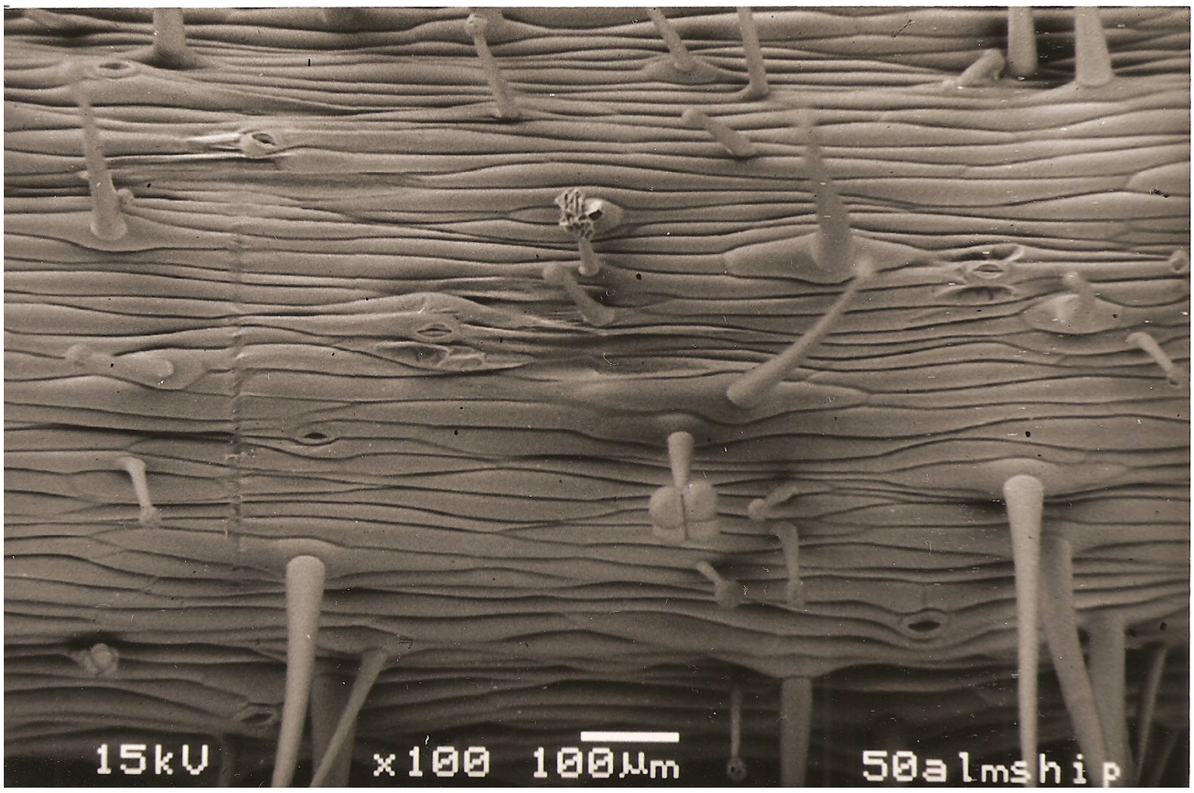

Fig. 4. SEM images of the hypocotyl epidermal cells of *L. esculentum* seedlings incubated in the nutrient solutions containing 50 ppm of aluminum.

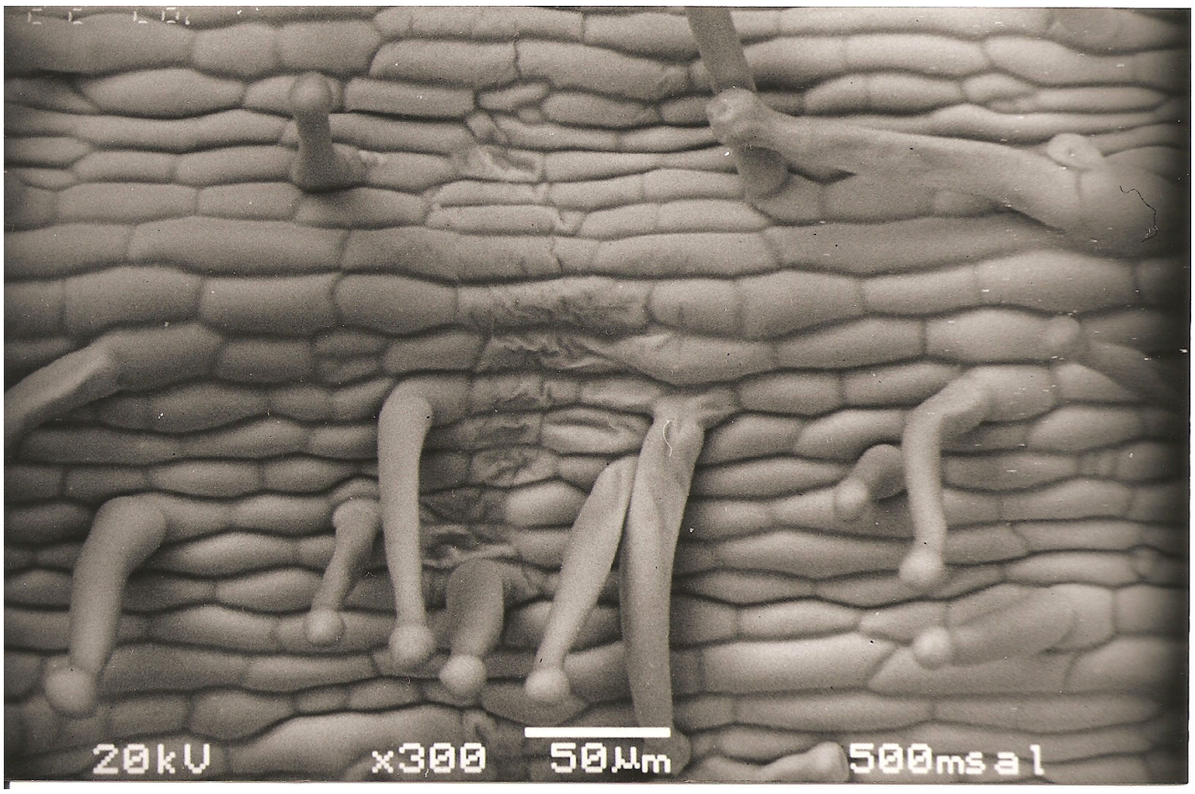

Fig. 5. SEM images of the hypocotyl epidermal cells of *L. esculentum* seedlings incubated in the nutrient solutions containing 500 ppm of aluminum.

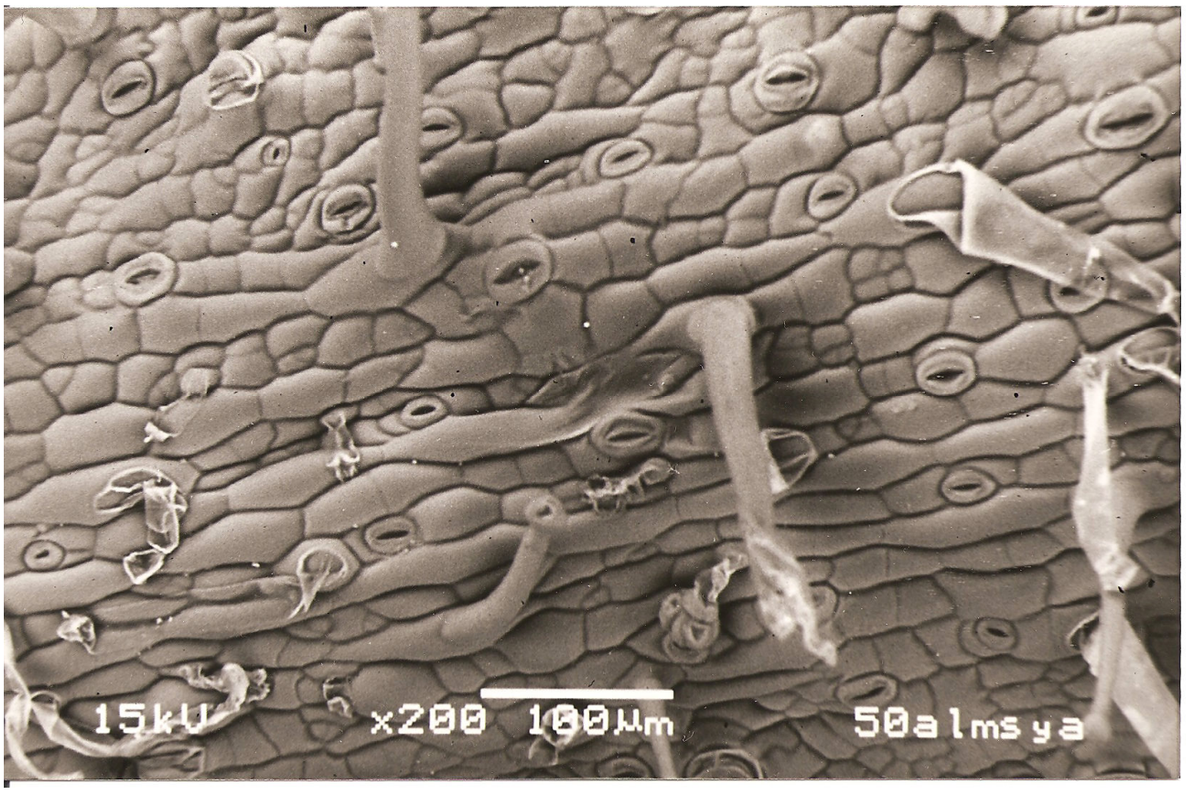

Fig. 6. SEM images of the cotyledon lower epidermal cells of *L. esculentum* seedlings incubated in the nutrient solutions containing 50 ppm of aluminum.

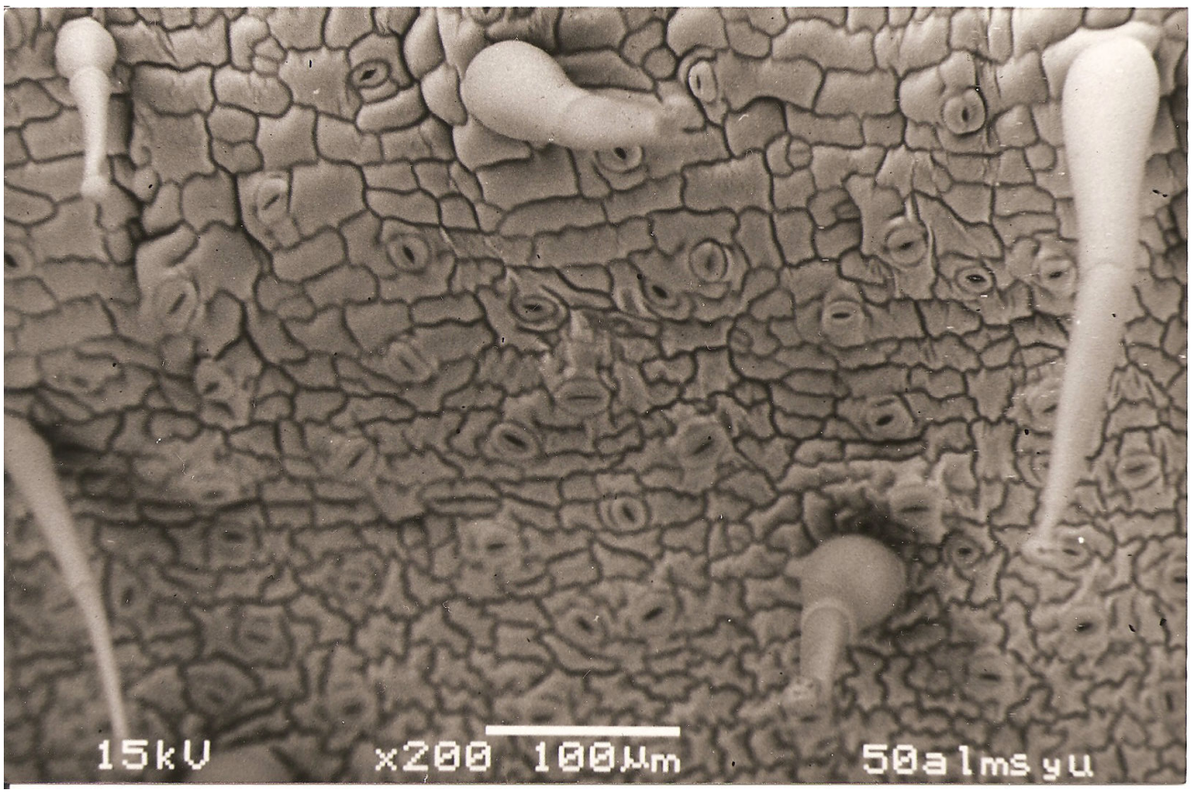

Fig. 7. SEM images of the cotyledon upper epidermal cells of *L. esculentum* seedlings incubated in the nutrient solutions containing 50 ppm of aluminum.

Table 1. The results of EDX analysis performed on a single cell from the root and embryonic radicle epidermal cells of *L. esculentum* cv. H-2274 seedlings incubated in nutrient solutions containing aluminum.

Element	Control		10 ppm Al^{+3}		50 ppm Al^{+3}		500 ppm Al^{+3}		1000 ppm Al^{+3}		1000 ppm Al^{+3} r*	
	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom
C	54.79	60.94	54.17	60.32	55.03	61.61	50.76	57.06	45.96	52.25	75.25	80.30
N	18.43	17.58	19.84	18.94	17.40	16.71	18.79	18.11	20.67	20.15	7.34	6.72
O	24.70	20.62	23.73	19.84	24.52	20.61	28.54	24.08	31.48	26.86	15.10	12.10
Se	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S	0.27	0.11	0.23	0.09	0.17	0.07	0.11	0.05	0.08	0.04	0.11	0.04
P	0.20	0.09	0.18	0.08	0.16	0.07	0.32	0.14	0.18	0.08	0.46	0.19
Na	0.22	0.13	0.51	0.29	0.11	0.06	0.09	0.05	0.07	0.04	0.00	0.00
Mg	0.07	0.04	0.05	0.03	0.08	0.04	0.06	0.03	0.03	0.02	0.00	0.00
K	0.23	0.08	0.43	0.15	0.43	0.15	0.26	0.09	0.08	0.03	0.04	0.01
Ca	0.44	0.15	0.17	0.06	0.11	0.04	0.01	0.00	0.02	0.01	0.01	0.00
Mn	0.02	0.00	0.03	0.01	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.00
Fe	0.00	0.00	0.01	0.00	0.08	0.02	0.01	0.00	0.03	0.01	0.04	0.01
Co	0.04	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cu	0.08	0.02	0.38	0.08	0.65	0.14	0.24	0.05	0.12	0.03	0.21	0.04
Zn	0.01	0.00	0.00	0.00	0.35	0.07	0.14	0.03	0.20	0.04	0.08	0.02
CI	0.18	0.07	0.20	0.08	0.43	0.16	0.33	0.12	0.65	0.25	0.72	0.26

The results of the EDX analysis of radicle epidermal cells (column with r*) and the root epidermal cells (the remaining columns)

Table 2. The results of EDX analysis performed on a single cell from the hypocotyl epidermal cells of *L. esculentum* cv. H-2274 seedlings incubated in nutrient solutions containing aluminum.

Element	Control		10 ppm Al^{+3}		50 ppm Al^{+3}		500 ppm Al^{+3}		**500 ppm Al^{+3}	
	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom
C	18.82	22.93	20.54	24.73	25.61	30.49	31.50	36.89	28.92	34.06
N	30.60	31.97	30.55	31.53	26.25	26.79	25.63	25.73	27.08	27.35
O	48.28	44.16	48.02	43.39	47.52	42.46	42.25	37.14	43.38	38.36
Se	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
S	0.02	0.01	0.01	0.00	0.01	0.00	0.05	0.02	0.03	0.01
P	0.67	0.32	0.23	0.11	0.16	0.07	0.04	0.02	0.05	0.02
Na	0.08	0.05	0.01	0.01	0.01	0.01	0.00	0.00	0.04	0.02
Mg	0.20	0.12	0.10	0.06	0.10	0.06	0.02	0.01	0.01	0.01
K	0.15	0.05	0.05	0.02	0.10	0.04	0.11	0.04	0.04	0.01
Ca	0.76	0.28	0.26	0.09	0.12	0.04	0.04	0.01	0.04	0.01
Mn	0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Fe	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Co	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cu	0.27	0.06	0.17	0.04	0.01	0.00	0.06	0.01	0.16	0.03
Zn	0.05	0.01	0.00	0.00	0.08	0.02	0.09	0.02	0.00	0.00
CI	0.04	0.02	0.02	0.01	0.02	0.01	0.15	0.06	0.21	0.09

**: The EDX analysis showing some endogen macro- and micro-nutrient contents of hypocotyl epidermal cells of seedlings incubated without MS nutrient solutions and left to develop in a medium only containing 500 ppm of Al^{+3} for 17 days.

Table 3. The results of EDX analysis performed on a single cell from the cotyledon upper and lower epidermal cells of *L. esculentum* cv. H-2274 seedlings incubated in nutrient solutions containing aluminum.

Element	Cotyledon upper epidermal cells						Cotyledon lower epidermal cells					
	Control		10 ppm Al^{+3}		50 ppm Al^{+3}		Control		10 ppm Al^{+3}		50 ppm Al^{+3}	
	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom
C	22.10	26.58	31.72	37.47	28.59	33.74	25.32	30.18	32.60	38.37	35.94	41.74
N	27.73	28.60	21.54	21.82	26.89	27.22	27.98	28.61	22.69	22.90	24.97	24.87
O	49.09	44.31	45.03	39.94	43.60	38.63	45.56	40.78	43.19	38.16	37.69	32.86
Se	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
S	0.03	0.01	0.07	0.03	0.03	0.01	0.03	0.01	0.05	0.02	0.07	0.03
P	0.37	0.17	0.54	0.25	0.28	0.13	0.24	0.11	0.41	0.19	0.43	0.20
Na	0.01	0.00	0.02	0.01	0.11	0.07	0.00	0.00	0.06	0.03	0.02	0.01
Mg	0.38	0.23	0.53	0.31	0.16	0.09	0.18	0.11	0.15	0.09	0.06	0.03
K	0.10	0.04	0.13	0.05	0.07	0.03	0.09	0.03	0.11	0.04	0.10	0.03
Ca	0.05	0.02	0.10	0.03	0.09	0.03	0.12	0.04	0.18	0.06	0.24	0.08
Mn	0.03	0.01	0.00	0.00	0.02	0.00	0.08	0.02	0.02	0.01	0.10	0.03
Fe	0.02	0.01	0.00	0.00	0.01	0.00	0.02	0.00	0.07	0.02	0.00	0.00
Co	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.01
Cu	0.06	0.01	0.12	0.03	0.09	0.02	0.14	0.03	0.25	0.05	0.25	0.05
Zn	0.00	0.00	0.10	0.02	0.03	0.01	0.14	0.03	0.17	0.04	0.00	0.00
CI	0.04	0.02	0.04	0.02	0.02	0.01	0.07	0.03	0.03	0.01	0.07	0.03

Table 4. The results of the EDX analysis showing the endogen macro- and micro-nutrient contents of the radicle, root and cotyledon epidermal cells of *L. esculentum* cv. H-2274 incubated with aluminum without adding MS nutrient solutions.

Element	50 ppm Al^{+3}		50 ppm Al^{+3}		500 ppm Al^{+3}		1000 ppm Al^{+3}		1000 ppm Al^{+3}	
	Cotyledon upper epidermal cells		Cotyledon lower epidermal cells		Root epidermal cells		Root epidermal cells		Radicle epidermal cells	
	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom
C	20.84	25.06	33.61	39.44	53.95	59.88	66.39	72.71	68.69	76.10
N	29.45	30.37	26.79	26.96	19.98	19.02	12.27	11.52	9.65	9.16
O	49.13	44.36	36.79	32.41	24.64	20.53	17.12	14.08	14.08	11.71
Se	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S	0.01	0.01	0.05	0.02	0.14	0.06	0.14	0.06	0.27	0.11
P	0.13	0.06	1.19	0.54	0.21	0.09	0.57	0.24	1.35	0.58
Na	0.00	0.00	0.00	0.00	0.02	0.01	0.13	0.07	0.03	0.02
Mg	0.07	0.04	0.47	0.27	0.00	0.00	0.00	0.00	0.00	0.00
K	0.02	0.01	0.09	0.03	0.00	0.00	0.01	0.00	0.02	0.01
Ca	0.03	0.01	0.62	0.22	0.04	0.01	0.03	0.01	0.09	0.03
Mn	0.01	0.00	0.10	0.03	0.01	0.00	0.00	0.00	0.05	0.01
Fe	0.01	0.00	0.03	0.01	0.00	0.00	0.09	0.02	0.04	0.01
Co	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.07	0.02
Cu	0.10	0.02	0.11	0.02	0.17	0.03	0.20	0.04	0.14	0.03
Zn	0.13	0.03	0.11	0.02	0.05	0.01	0.00	0.00	0.00	0.00
CI	0.03	0.01	0.03	0.01	0.31	0.12	2.10	0.78	4.29	1.61

Discussion

The main target of the phytotoxic aluminum, which is defined as the major growth-limiting factor for the plants growing on the acid-reactive soils with a pH value of <5 (Fageria & Santos, 1998; Neogy *et al*., 2002; Yamamoto *et al*., 2003), has been reported to be the roots of the plant (Doncheva *et al*., 2005). One study has stated that aluminum mainly accumulated in the root tips which are at the cell division and cell elongation sites and that the inhibition of the rate of cell division was directly related to the interaction of aluminum with the nuclear chromatin (Horst, 1995). In another study the kinetics of aluminum uptake and distribution in roots of *Triticum aestivum* were determined (Archambault *et al*., 1996). In an aluminum-sensitive *Triticum aestivum* genotype, exposure to 10µM aluminum has resulted in immediate inhibition of the root elongation and this has been attributed to the time-dependent accumulation of aluminum in the root tissues (Ma *et al*., 2004). In all the studies conducted by Lidon & Barreiro (1998) on *Zea mays*, by Ahn *et al*., (2001) on *Cucurbita pepo,* by Shen & Ma (2001) on *Fagopyrum esculentum*, by Collet *et al*., (2002) on *Zea mays*, by Meriga *et al*., (2004) on *Oryza sativa,* by Nian *et al*., (2005) on *Glycine max,* by Silva *et al*., (2004) on *Eucalyptus globulus*, *E. urophylla*, *E. dunnii*, *E. saligna*, *E. cloeziana* and *E. grandis*, and by Gallon *et al*., (2004) on *Typha latifolia* and *Sparganium multipedunculatum*, the aluminum up-take into the plant has been suggested to accumulate mainly in the root, whereas the aluminum concentration in the young leaves, mature leaves, old leaves and root of the *Melastoma malabathricum* has been reported to be 8.0, 9.2, 14.4 and 10.1 mgg^{-1}, respectively (Watanabe *et al*., 1998).

In the studies conducted to determine the cellular location pattern of aluminum, for example when the seedlings of *Glycine max* was exposed to 1.45 µM Al^{+3} activity, the aluminum has been found to enter the cells of the aluminum-sensitive genotype within 30 minutes and to locate at nuclei in the meristematic region of the root tips (Silva *et al*., 2000). A significant level of aluminum has been also found in the cell periphery (Silva *et al*., 2000). On the other hand, aluminum has been reported to accumulate mainly in the epidermal and outer cortical cell layers, especially in the 1-2 mm of apical root zone in an aluminum-sensitive genotype of *Zea mays* (Sivaguru *et al*., 2006). In the study of Eticha *et al*, the aluminum has been found to be located in the epidermal, cortical and endodermal cells in the root apex of *Zea mays*, but ‘’the aluminum could not be detected in neither living nor non-living cells in the central cylinder because of the restricted radial aluminum transport caused by the endodermis’’ and the nucleus has been reported to be the region with most abundant aluminum accumulation in the cytosol (Eticha *et al*., 2005). In another study of *Zea mays,* aluminum has been reported to reach to the stele in the root tips within 60 minutes, whereas it has been reported to accumulate only in the rhizodermis and outer cortex cells in the roots

of *Vicia faba* and to have limited radial movement ability (Marienfeld *et al*., 2000). During this time, intracellular aluminum could be detected in both species, while the highest aluminum concentrations have been always reported to be present in the cell walls (Marienfeld *et al*., 2000). In the study of Kollmeier *et al*. ‘’aluminum has been reported to mainly bind strongly to the cell walls in the epidermal and cortical root cells (Delhaize *et al*., 1993)’’ and it has been suggested that ‘’the apoplast plays the main role in the perception of aluminum in the cell (Horst, 1995)’’ (Kollmeier *et al*., 2000).

In the plant organs other than the root, in the x-ray microanalysis of needles of *Picea glauca,* the aluminum has been reported to be often removed from the cell walls and to be almost confined to the epidermis of the needle (Hodson & Sangster, 1998). In another study conducted by the same method, the aluminum has been found to accumulate in the needle tips of *Pinus strobus* and the transfusion tissues have been shown to be the major sites of aluminum accumulation (Hodson & Sangster, 2002). In the *Melastoma malabathricum,* aluminum has been located mainly in the upper epidermal cells in the leaf sections, while it has been also found to spread into the mesophyll cells (Watanabe *et al*., 1998). On the other hand, aluminum has been reported to be present in all root tissues, particularly in the epidermis and endodermis in the root sections (Watanabe *et al*., 1998). Ma *et al*., (1998) also reported the accumalation of about 90% of almuium in the cell sap of the *Fagopyrum esculentum*, a high aluminum tolerant species.

Accordingly, in our study, the administration of increasing concentrations of aluminum to the nutrient solution resulted in increased aluminum content of the cells and it was found that the aluminum up-taken into the plant was mainly accumulated in the roots. However, this accumulation pattern was not linear for the root epidermal cells with the increasing concentrations of aluminum from 50 ppm. On the other hand, the increase observed in the aluminum content of the radicle epidermal cells was much more striking. The aluminum concentration in the root epidermal cells of the seedlings incubated in medium containing only aluminum for 17 days and without adding any nutrient solution was found to be much higher. This was particularly more pronounced in the radicle that was grown in the incubation medium containing 1000 ppm of aluminum. In the case of administration of 500 ppm of aluminum to the nutrient solution, a significant increase was observed in the aluminum content of hypocotyl epidermal cells, however, the aluminum content of the root epidermal cells were found to be much higher after administration of the same concentration of aluminum. In the assessments performed on hypocotyl and cotyledon upper and lower epidermal cells with the presence of 50 ppm of aluminum, a slight increase was observed in aluminum content of cotyledon lower epidermal cells.

Studies of interactions between aluminum and plant nutrients have put emphasis on some nutrient elements including calcium. It is well-known that aluminum stress can cause calcium deficiency in the plant, particularly in genotypes with high sensitivity to aluminum. Negative correlations have been reported between the Al/Ca ratio and root mass, mean relative root growth rate, mean relative total plant growth rate, and proportional allocation of biomass in the roots of *Pinus sylvestris* seedlings (Oleksyn *et al*., 1996). One study has reported that Al^{+3} displaced Ca^{+2} from pectic binding sites of cell wall up to a critical size in isolated cell wall material of *Lycopersicon esculentum* roots (Postma *et al*., 2005). Aluminum has been found to cause growth inhibition and decreased concentration of cytoplasmic free Ca^{+2} in *Nicotiana tabacum* cell cultures (Jones *et al*., 1998). Administration of toxic concentrations of aluminum to an aluminum-sensitive genotype of *Triticum aestivum* has inhibited Ca^{+2} uptake dramatically immediately after the administration of aluminum (Huang *et al*., 1992). In a study, aluminum has been found to result in rapid changes in the concentrations of free calcium and potassium and in cytosolic pH in the root protoplasts of *Triticum aestivum* (Lindberg & Strid, 1997). In another study of *Triticum aestivum*, exposure to aluminum has relatively decreased concentrations of calcium and magnesium in the shoots, but this effect was much more pronounced for the calcium than magnesium (Strid, 1996). The researcher has also reported that the calcium reached critical concentrations that can cause the growth inhibition in the shoots, particularly at a root zone temperature of 10°C (Strid, 1996). In a similar study conducted on *Lolium multiflorum,* the increased aluminum concentrations in the nutrient solution has been found to increase K/Ca+Mg ratios in the shoots (Rengel & Robinson, 1990). Active potassium uptake of the *Picea abies* has been inhibited by the administration of aluminum, while all tested concentrations of aluminum have been reported to also severely inhibit the passive calcium uptake (Widell *et al*., 1994).

In present study, the EDX analysis of a single cell from root and hypocotyl epidermal cells of the seedlings of *L. esculentum* cv. H-2274 incubated with increased aluminum concentrations in the nutrient solutions revealed regular decrease in the calcium content of the cells. This was confirmed by the EDX analysis revealing the endogenous calcium content of the root and hypocotyl epidermal cells of the seedlings which were incubated in medium containing only aluminum for 17 days and without adding any nutrient solution. Although 10 and 50 ppm of aluminum led to higher calcium contents in cotyledon upper and lower epidermal cells compared to the controls, this increased concentration was not as high as to explain the decreased calcium content in the root and hypocotyl epidermal cells by only increased ability of mobilization of the element. The calcium content of radicle epidermal cells was also extremely low. In present study, administration of aluminum at a concentration of 1000 ppm led to a significant decrease in the magnesium content of the root epidermal cells compared to the controls. On the other hand, magnesium element was undetectable in the EDX analysis of the radicles. The magnesium contents of the root epidermal cells in the other aluminum-treated series were not significantly different from that of controls. The decreased magnesium content in the cells was more pronounced particularly for the hypocotyl epidermal cells. Indeed, although administration of 10 and 50 ppm of aluminum resulted in decreased magnesium content in the hypocotyl epidermal cells by 50%, this decrease was much more striking in the case of administration of 500 ppm of aluminum. While increased aluminum concentrations led regular decreases

in the magnesium content of cotyledon lower epidermal cells, the magnesium content of the cotyledon upper epidermal cells tended to increase with the administration of 10 ppm of aluminum. On the other hand, 50 ppm of aluminum also resulted in a significant decrease in magnesium content of the cells. Aller *et al.,* (1990) and Horst (1995) have also reported that aluminum toxicity may lead to magnesium deficiency in the plants. In the studies conducted by Koffa & Mori (1987) on *Leucaena leucacephala*, by Goransson & Eldhuset (1991) on *Picea abies* and *Pinus sylvestris,* by Jan (1991) on *Oryza sativa*, by Simon & colleagues (1994) on *Lycopersicon esculentum*, by MacDiarmid & Gardner (1996) on *Saccharomyces cerevisiae*, by Oleksyn *et al*., (1996) on *Pinus sylvestris*, by Strid (1996) on *Triticum aestivum*, by Lidon *et al*., (1999) on *Zea mays,* and by Kidd & Proctor (2000) on *Betula pendula* have also draw attention to aluminum-related magnesium deficiencies.

It has been reported that aluminum toxicity may often lead to phosphorus deficiency in the plants in addition to the calcium deficiency (Aller *et al*., 1990). Likewise, the aluminum did not inhibit the growth of the shoots of *Oryza sativa* pre-cultured with phosphates, whereas the addition of aluminum has delayed the growth of shoots pre-cultured without phosphate and it has also inhibited the root elongation in any case regardless of the existence of phosphates in the pre-culture solution (Nakagawa *et al*., 2003). In the homogeneous solution experiments in which a uniform distribution of the aluminum and phosphorus has been provided, administration of phosphorus has significantly increased the aluminum tolerance of 4 *Glycine max* genotypes differentiating in the phosphorus efficiency, while two phosphorus-efficient genotypes has been reported to be more resistant to aluminum under these high-phosphorous conditions (Liao *et al*., 2006). The researchers have suggested that ‘’phosphorus-efficient genotypes may have increased tolerance to aluminum not only through direct Al-P interactions but also through indirect interactions related to the stimulation of exudation of various aluminum chelating organic acids in the specific roots and root regions’’ (Liao *et al*., 2006). In a study examining the effects of aluminum on nutrient uptake and transport in *Oryza sativa*, high levels of phosphorus accumulation has been found in the shoots and roots of the aluminum-resistant genotypes and decreased rates of total uptake of the phosphorus and calcium were recorded in aluminum-sensitive genotypes (Jan, 1991). In *Lycopersicon esculentum,* aluminum has been reported to increase phosphate accumulation in the roots and to inhibit the transport of this element into the leaves and stem of the plant (Simon *et al*., 1994). In two *Vigna unguiculata* genotypes exhibiting differences in aluminum tolerance, the administration of aluminum has significantly decreased the phosphorus accumulation in both aluminum-resistant (28%) and aluminum-sensitive (95%) genotypes (Jemo *et al*., 2007). However, although the aluminum has not affected the phosphorus concentration in the roots of *Betula pendula* (with the exception of an aluminum-tolerant genotype treated with 5 and 35 mgl^{-1} of aluminum), high aluminum concentrations have not also affected the phosphorus concentration in the shoots with the exception of the most aluminum-resistant genotype (Kidd & Proctor, 2000).

On the other hand, 500 ppm of aluminum led to a significant phosphorus accumulation in the root epidermal cells in our study. The percent elemental weights in other aluminum-treated series were similar to that found in the controls. However, quite high levels of phosphorus accumulation were observed in radicle epidermal cells. While the phosphorus content was quite high in the hypocotyl epidermal cells constituting the control group, the phosphorus content of the cells began to decrease significantly and regularly from the aluminum concentration of 10 ppm. The root epidermal cells of the seedlings left to grew in solutions containing 500 and 1000 ppm of aluminum without adding any nutrient solutions for 17 days exhibited significant phosphorus accumulation. The increase in phosphorus content of the cells was much more striking in the EDX analysis performed on radicle epidermal cells. At the concentration range of 50-500 ppm of aluminum, phosphorus content of the hypocotyl epidermal cells tended to decrease. Although the phosphorus content was increased in cotyledon upper epidermal cells after the administration of 10 ppm of aluminum and in cotyledon lower epidermal cells after the administration of 10 and 50 ppm of aluminum, it was not expected to explain or tolerate the decreased phosphorus content in the hypocotyl epidermal cells.

Studies in the literature examining the interactions between aluminum and micro-elements have reported very distinct results at the level of species and even genotypes. While it has been suggested that aluminum toxicity can be associated with increased manganese and iron contents in the plants (Aller *et al*., 1990). Other researchers have reported significantly decreased iron concentrations in the shoots of *Zea mays* with the administration of increased concentrations of aluminum (Lidon *et al*., 1999). Exposure to aluminum has led to significant decreases in iron content of the roots of *Betula pendula*, as well as the iron transport to the shoots has also often decreased significantly with the administration of high aluminum concentrations (Kidd & Proctor, 2000). For *Lycopersicon esculentum,* exposure to increasing concentrations of aluminum has been found to result in decreased uptake of copper, manganese, molybdenum, zinc, boron and iron from the nutrient solutions, to decrease the manganese, iron and zinc contents of the root, stem and leaves, and, in contrary, to increase the molybdenum and copper accumulation in the roots and inhibited the transport of these two elements into the stem and leaves (Simon *et al*., 1994). In three genotypes of *Oryza sativa* differing in the sensitivity to aluminum, administration of aluminum has increased the iron, copper and zinc accumulation in the roots of aluminum-sensitive genotype as a result of high zinc and copper uptake rates; the overall net absorption rate of manganese has been found to decrease regardless of the sensitivity of genotypes to aluminum (Jan, 1991). For the seedlings of *P. sylvestris* incubated in nutrient solutions containing 0.5-4 mM of $AI(NO_3)_3$, although the presence of aluminum in the solution has resulted in decreased Mn, Fe, Cu and Zn contents in the roots and needles, these decreases did not constitute a critical situation in terms of foliar concentrations (Oleksyn *et al*., 1996). In

another study analyzing the aluminum, silicon, iron, manganese and copper concentrations in the leaves of ten different species of *Rubiaceae,* all studied species of *Rubiaceae* have been found to have relatively high concentrations of silicon, whereas no significant correlations have been found between aluminum and other metals tested (Jansen *et al*., 2003). In our study, aluminum administered at concentrations of 10 and 50 ppm led to significant and regular copper accumulations in the root epidermal cells of the seedlings. Although the copper content of the cells began to decrease from the aluminum concentrations of 500 ppm, the percent elemental weights were higher than that of controls. For example, at an aluminum concentration of 500 ppm, it was 3 times higher than that of controls. The copper content of the radicles was also found to be quite high. Copper content of the hypocotyl tended to decrease with the increasing aluminum concentration in the nutrient solution, whereas the copper contents of cotyledon upper and lower epidermal cells were higher than that of controls. Therefore, we can suggest that 10 and 50 ppm of aluminum concentrations in the nutrient solution lead to high levels of copper accumulation in the epidermal cells with the copper up-take by the plant accumulating mainly in the root and cotyledon epidermal cells and that copper content of the hypocotyl tends to decrease with the increasing aluminum concentration in the nutrient solution. On the other hand, after the administration of 500 and 1000 ppm of aluminum, the copper content of the roots was found to be higher than that of controls. In our study, 50 ppm of aluminum resulted in significantly higher zinc content in the root epidermal cells than the control group. Although zinc content of the root epidermal cells began to decrease from the aluminum concentration of 500 ppm, the percent elemental weights found to be higher than that of controls. The same was applied to radicles grown in the nutrient solutions containing 1000 ppm of aluminum. Although zinc content of the hypocotyl epidermal cells of the seedlings was also found to increase at the aluminum concentrations of 50 ppm and above, this increase was not as striking as that observed in the root epidermal cells. In the cotyledons, zinc content of the cells was increased at an aluminum concentration of 10 ppm and an overall decline was observed at an aluminum concentration of 50 ppm, whereas the zinc element was undetectable in the root and hypocotyl after the administration of 10 ppm of aluminum. Manganese content of the root epidermal cells did not significantly changed with the administration of aluminum. After the addition of increased concentrations of aluminum to the nutrient solution, manganese element was undetectable in the hypocotyl. However, although no manganese element was found in the cotyledon upper epidermal cells at an aluminum concentration of 10 ppm, 50 ppm of aluminum led to a slight decrease in the manganese content of upper epidermal cells and a slight increase in that of lower epidermal cells compared to the controls.

In conclusion, our assessments performed at the level of epidermal cells of young seedlings of *Lycopersicon esculentum* cv. H-2274 suggested that aluminum stress led to an absolute change in the plant nutritional element composition of the cells and in mobilization of some plant nutritional elements in the seedlings. The antagonistic interactions between aluminum and some plant nutritional elements present in the nutrient solutions have possibly resulted in much higher concentrations of aluminum found in the root epidermal cells and radicles of the seedlings incubated in medium containing only aluminum for 17 days without adding any nutrient solution.

The appearance of radicle from the testa, i.e. germination was observed in some of the seeds used in our study, but these radicles did not differentiate into full-fledged young seedlings displaying development of complete root, hypocotyl and cotyledon. Because the radicles are the root draft or the region that differentiate into the root, we compared the nutritional elemental composition of the root epidermal cells of the control group with that of radicle epidermal cells in an attempt to explain the possible physiological mechanisms of the inhibition of germination and found that particularly the oxygen, nitrogen and calcium contents of the radicle epidermal cells was quite lower than that of control group. The sulfur and particularly the potassium contents of the radicles were also significantly lower than that of the control group. The nutritional elements such as magnesium, sodium and manganese were undetectable in the EDX analysis performed on radicle epidermal cells. However, some micro-nutritional elements such as iron, zinc, and copper accumulated in higher levels compared to the controls. As previously reported in the study of Simon *et al* (1994) on the roots of *Lycopersicon esculentum*, very high levels of phosphorus accumulation found in these cells is possibly attributable to the inhibition of phosphate mobilization caused by aluminum in our root drafts. Together with all of these results, the higher levels of aluminum and chlorine accumulation found in these as a result of experimental design and due to the higher potential of genotypic uptake probably inhibited the differentiation of these germinating embryos into full-fledged seedlings that displayed more advanced stages of the development, i.e., development of root, hypocotyl and cotyledon.

Conclusion

The assessments performed at the level of epidermal cells of young seedlings of *Lycopersicon esculentum* suggest that aluminum stress leads to an absolute change in the plant nutritional element composition of the cells and in the mobilization of some nutritional elements in the seedlings. However, we suggest that our observations in this study alone is not enough to explain the phytotoxic effects of aluminum stress on the young seedlings and embryos of *L. esculentum* cv. H-2274.

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