Evaluation of growth dynamics of Lithuanian cultivars of *Hordeum sativum* ssp. *distichum* L. after treatment with aluminium

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Aluminium (Al) toxicity is a predominant growth-limiting factor in acid soils. Over 18% of agricultural areas of Lithuania belong to acidic soils. The use of aluminium-tolerant genotypes together with liming is a very promising strategy for agriculture. Knowledge of inheritance mechanisms of aluminium tolerance contributes to obtaining tolerant and productive genotypes. Aluminium toxicity was investigated by analysing the reaction of two Lithuanian spring barley (*Hordeum sativum* ssp. *distichum* L.) cultivars 'Aura ' and 'Auksiniai-3'. The aim of the present work was to evaluate residual effects of aluminium within two weeks followed after one week growth of seedlings of spring barley in the solution containing Al ions (148 μM, 296 μM, 592 μM $AlKSO_4$, pH 4.5). The length of roots and overground parts, also dry weight and leaf areas were recorded during seedling growth (7th, 14th and 21st day). The following growth parameters were calculated: relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and shoot/root ratio. Significant differences ($p < 0.05$) in root/shoot length ratio were found between control and Al treatments (296, 592 μM). Significant differences ($p < 0.05$) in the root/shoot weight ratio were found between control and all Al treatments (148, 296, 592 μM). After 21 days of growth in 296 μM treatment, specific leaf area was lower (93%; $p < 0.05$) than in control for cultivar 'Aura' and in the same treatment higher (106%) for cultivar 'Auksiniai-3'. Under 592 μM treatment the reaction of cultivars was opposite: for 'Aura' SLA was higher ($p < 0.05$; 108%) while for 'Auksiniai-3' lower (96% compared to control).

Our study has revealed that for both cultivars the shoot/root dry weight ratio was more affected than the shoot/root length ratio under Al treatment of all concentrations. The overground part was less depressed than the underground part of plants grown in metal solutions. Two weeks after removal of metal, its strong effect was still left in the roots, while in the overground part decreased significantly. The effect of aluminium on the spring barley (*Hordeum sativum* ssp. *distichum* L.) cultivars 'Aura' and 'Auksiniai-3' was not significant according to relative growth rate, net assimilation rate and leaf area ratio.

Key words: spring barley, cultivars, aluminium toxicity, metal tolerance, plant growth

INTRODUCTION

Soil acidity-causing ions might have strong effects on plants [10]. Aluminium toxicity is among the main factors limiting the growth of crops. Unfortunately, liming of soils is not effective in cases when repeated acidification is taking part [1]. One of the ways to overcome metal toxicity in plants is creation of Al-tolerant cultivars by using a variety of existing crop genotypes [6]. World-wide research has shown different acidity tolerance in various cultivars, some of them being more or less resistant to that harmful factor [5].

During the previous decade acidification of soils in Lithuania took place due to a shortage of money required to cover expences for liming. Increase of soil acidification is the most probable forecast for the nearest future in our country. Each year in the limed soils acidification occurs in the magnitude of 0.1–0.2 pH [9]. At the same time a potential hazard of Al toxicity is increasing. In the neutral or low acidity soils the solubility of Al is negligible. In the soils with pH 5 the concentration of Al^{3+} does not exceed 10 mg kg^{-1}, while at pH 4 it causes accumulation of Al^{3+} up to 100 mg kg^{-1}. The concentration of solubile aluminium ranges in the interval of 3–200 mg kg^{-1} for the soils never treated against acidity [9]. The epiblem and meristems are the first target tissues in the roots effected by aluminium, and cell division and elongation are primarily inhibited processes [6]. As a consequence, tips of the roots turn reddish and die, deformation and abnormal branching of the roots takes place [6, 12]. Al^{3+} competes with Ca^{2+} in stoping absorbtion and transportation of phosphorus [14]. Usually higher contents of aluminium are found in the roots than in the green organs of the plant. In the overground part aluminium concentrates mainly in the cells of photosynthetic parenchyma. The crucial organelles attacked are the nucleus, mithochondrias and chloroplasts, disturbance of recovery of chloroplasts, especially photosynthetic phosphorylation take place [13].

Spring barley is among the crops most sensitive to acidic soils and aluminium. It is grown widely in Lithuania. In our previous papers the sensitivity of some barley cultivars to heavy metals and aluminum are described. On the basis of the survey done, genotypes with the most contrasting sensitivity to metals were selected for a deeper evaluation of the processes taking place after aluminium treatment [11].

The present work was aimed at the evaluation of the distribution of the products of photosynthesis and growth dynamics followed after a short-term aluminium treatment of seedlings of spring barley cultivars 'Aura' and 'Auksiniai-3' of Lithuanian origin.

MATERIALS AND METHODS

After sterilization and one-day germination of seeds in the dark, the plants were placed into water solutions of $AlKSO_4$ (148 μM, 296 μM, 592 μM, adjusted to pH 4) and grown for 7 days. For the same period the control plants were kept in the water solutions (pH 5.8). Later the roots were thoroughly washed and the seedlings transferred into a nutritient-containing solution (0.4 mM $CaCl_2$, 0.65 mM KNO_3, 0.25 mM $MgCl_2$ $6H_2O$, 0.01 mM $(NH_4)_2SO_4$, 0.04 mM NH_4NO_3 as recommended by Aniolas A. [2]). The observation took place within 2 weeks following the exposure to aluminium. The gravimetric (weight of roots and shoots) and morphological (length of the mentioned organs and leaf areas) parameters were recorded for the 7-, 14- and 21-day-old seedlings (respectively at 0, 7th and 14th day of the growth after exposure to aluminium). From the weight and length data, calculations of various growth indices [7] were performed using Excel and SPSS statistical packages. Biomass and leaf area data of the individual harvests were used to calculate changes in plant relative growth rate (RGR) and its components, net assimilation rate (NAR), leaf area ratio (LAR), using the standard formulas: $RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$; $NAR = (W_2 - W_1) (\ln L_2 - \ln L_1) / (L_2 - L_1) (t_2 - t_1)$; $LAR = (L_2 - L_1) (\ln W_2 - \ln W_1) / (W_2 - W_1) (\ln L_2 - \ln L_1)$; where W_1 and L_1 were the total dry weight and leaf areas of the plants at time t_1, and W_2 and L_2 at time t_2. Specific leaf areas (SLA) were calculated by dividing the total leaf area by the dry weight of the leaves fraction of each plant.

RESULTS AND DISCUSSION

After 7 days-long growth of 'Aura' cultivar seedlings, significant differences ($p < 0.05$) were found between control and those treated with 296 μM and 592 μM solutions of $AlK(SO_4)_2$ according to the root–shoot length ratios (100%, 81%, 75% respectively; Table 1). The same tendency was registered after 14 days of growth following exposure to aluminium treatment, though differences were significant ($p < 0.05$) only between control and 296 μM $AlK(SO_4)_2$ treatment (100%, 86%). Twenty one-day-old seedlings showed significant differences ($p < < 0.05$) between the most contrasting cases of the experiment: 592 μM $AlK(SO_4)_2$ concentration and control plants (81% compared to 100% in control). As for the root–shoot dry weight ratios, the effect of metal was increasing with each subsequent harvest: for the 7-d seedlings significant differences were found between control and the highest concentration treatment (592 μM $AlK(SO_4)_2$; 77% and 100%) respectively, for the 14-d seedlings significant differences were found between control and the intermediate as well as the highest concentration treatments (296 μM and 592 μM $AlK(SO_4)_2$) and control; 88%, 91% and 100% respectively; $p < 0.05$); for the 21-d seedlings treatment with all concentrations showed significant differences when compared to control (77%, 81%, 83% and 100%, Table 1).

For the 'Auksiniai-3' cultivar seedlings the root–shoot length ratios showed significant differences between 592 μM, 296 μM $AlK(SO_4)_2$ treatments and control after one week of growth following exposure to metal effect (89%, 89%, 100%, $p < 0.05$;

Table 1. **Root-shoot length and root-shoot weight ratios of seedlings of spring barley cultivars 'Auksiniai-3' ir 'Aura' grown for 7 (14-days old plants) and 14 (21-day-old plants) days after a 7-day treatment with 148, 296 and 592 µM solutions of $AlKSO_4$**

Age of seedlings (days)		'Aura'				'Auksiniai-3'			
		Concentration of $AlK(SO_4)_2$ (µM)							
		0	148	296	592	0	148	296	592
Root-shoot length ratios	7	0.789	0.746	0.640*	0.592*	0.699	0.7293	0.696	0.672
	14	0.539	0.510	0.465*	0.505	0.693	0.676	0.598*	0.619*
	21	0.472	0.425	0.411	0.382*	0.979	0.948	0.844	0.714*
Root-shoot dry weight ratios	7	0.679	0.709	0.636	0.523*	0.775	0.615*	0.594*	0.640*
	14	0.396	0.392	0.359*	0.348*	0.413	0.422	0.412	0.369*
	21	0.475	0.393*	0.387*	0.365*	0.5204	0.494	0.402*	0.405*

* Significant differences ($p < 0.05$) compared to control.

Table 1). The effects were less significant after two weeks of recovery: pronounced effects were found only between the most contrasting groups of the experiment – 592 µM $AlK(SO_4)_2$ treatment and control (73% and 100% respectively). Root-shoot dry weight data have demonstrated residual effects of the highest concentration of aluminium treatment at all time intervals studied (7-d, 14-d and 21-day old seedlings, 79%, 89% and 81%, respectively). The lowest aluminium concentration exerted a significant effect only on the 7-d seedlings (83%), and the influence of the intermediate concentration was significant on the 7-d and 21-day-old seedlings (77% and 78%, respectively, $p < 0.05$).

According to specific leaf area, significant differences between 296 µM, 592 µM treatments and control were found for 21-d seedlings (for 'Aura' cultivar 93% and 108% and for 'Auksiniai-3' cultivar 106% and 96%; Table 2). For the both cultivars no reliable differences between control and treatments were found according to the parameters of the RGR and NAR growth dynamics.

In the present experiment, the calculated differences between control and aluminium-treated plants

Table 2. **Specific leaf area (SLA, cm^2 g^{-1}), relative growth rate (RGR, day^{-1}), net assimilation rate (NAR, g cm^{-2} day^{-1}) of seedlings of spring barley cultivars 'Auksiniai-3' and 'Aura' grown for 7 and 14 days after a 7-day treatment with 148, 296 and 592 µM solutions of $AlKSO_4$**

Parameter of the growth	'Aura'				'Auksiniai-3'			
	Concentration of $AlK(SO_4)_2$ (µM)							
	0	148	296	592	0	148	296	592
SLA 14th day	0.042	0.042	0.041	0.040	0.044	0.038	0.042	0.036
SLA 21st day	0.044	0.045	0.040*	0.047*	0.041	0.042	0.044*	0.040*
RGR (d^{-1})	0.041	0.046	0.052	0.036	0.063	0.054	0.062	0.065
NAR (g cm^{-2}d^{-1})	62.37	67.94	81.35	54.74	131.157	111.055	131.68	151.55

* Significant differences ($p < 0.05$) compared to control.

were caused by the effect of metal on roots rather than on shoots. It is in support of our data obtained in earlier experiments and in the studies of other laboratories, which showed the strongest influence of metal on the underground parts of plants [3, 8, 10]. A comparison of the root–shoot length and the root-shoot weight data has revealed a higher inhibition of biomass formation than of elongation process. The reaction of both cultivars was quite similar. The low effect of aluminium on the overground part of seedlings could be explained by a low mobility and suspension of the biggest part of the absorbed metal in the roots. These facts were confirmed by element analyses performed in other laboratories of the world [4]. In our study, after 1 and 2 weeks a stronger toxical effect of Al was still obserwed in the roots, while the residual influence on the overground part of the seedlings became very weak. Green peas [13] showed a different reaction of Al-tolerant and Al-sensitive cultivars according to a decreased synthesis of nucleic acids after 3 days of exposure to metal (the effect on the tolerant cultivar was 48% and on the sensitive cultivar 36%, and the subsequent 3-day-long recovery diminished the inhibitory effect by 90% and 47%, respectively, in the tolerant and sensitive genotypes). It shows the existance of strong and different reparation processes ongoing in plants after aluminium treatment [14]. Low effects of metal obtained in our experiment are in agreement with the facts described in the literature. In addition, it has been demonstrated that spring barley as well as wheat, soybean and bean cultivars belong to the group of plants that exhibit no significant differences in the content of metal in the overground part of the tolerant and sensitive cultivars, while tolerant cultivars accumulate much lower contents of aluminium in the roots as compared to the sensitive genotypes [6]. To explain the results obtained in our experiment, a deeper examination of the processes ongoing in the roots of the seedlings and mature plants are needed.

CONCLUSIONS

1. Under the influence of aluminium significant ($p <$ < 0.05) changes occur in the distribution of biomass: root–shoot weight ratio and root–shoot length ratio decreases by 77–91% and 75–89%, respectively.

2. Analyses of growth dynamics (RGR and NAR) after aluminium treatment did not reveal any notable residual effects of aluminium on the spring barley genotypes studied.

3. Reaction to short-term aluminium treatment of the selected spring barley cultivars 'Auksiniai-3' and 'Aura' of Lithuanian origin was very similar.

References

1. Alva A. K., Asher C. J., Edvards D. G. The role of calcium in alleviating aluminium toxicity. *Aust. J. Agric. Rest*. 1986. No. 37. P. 375–382.
2. Aniol A. Plant breeding: theories, achievements and problems. Ed. V. Ruzgas et al. *Proceedings of International Conference*. Dotnuva-Akademija, 1997. P. 14–22.
3. Aniol A. *Plant and Soil*. 1990. Vol. 123. P. 223–227.
4. Benet R. J., Breen C. M. The aluminium signal: New dimension to mechanisms of aluminium tolerance. *Plant and Soil*. 1991. Vol. 134. P. 153–166.
5. Duke J. A. Plant germplasm resources for beeding crops adapted to marginal environments. In: N. M. N. Chistiansen, C. F. Levis (Eds.). *Breeding Plants for Less Favorable Environments*. Chapter 12. John Wiley & Sons, New York, 1982.
6. Foy C. D. The physiology of plant adaptations to mineral stress. *Iowa state jornal*. 1983. Vol. 57. P. 355–391.
7. Kupčinskienė E., Ashenden T. Effects of low concentration mixtures of acidic atmosphere pollutants on some plants in controlled condition experiment. *Aplinkos inžinerija. Environmental Engineering*. 1997. No. 1. Vol. 7. P. 16–21.
8. Kupčinskienė E., Ramaškeviciene A., Sliesaravičius A., Jankauskaite R. Comparison of growth of *Lepidium sativum* L in the media containing air pollutants of various chemical origin. In: Ed. D. A. Lovejoy. *Heavy metals in the environment: an integrated approach*. Vilnius, 1999. P. 179–185.
9. *Lietuvos dirvožemių agrocheminės savybės ir jų kaita*. Red. J. Mažvila Kaunas, 1998. P. 14–50.
10. Marschner H. *Mineral Nutrition of Higher Plants*. 2nd ed., Academic Press, London, 1995.
11. Ramaškevičienė A., Kupčinskienė E., Sliesaravičius A. *Sodininkystė ir daržininkystė*. 2000. No. 19, Vol. 3. P. 419–428.
12. Slanski J. J. Aniol A. Effect of camodulin inhibitors on aluminium toxicity in cereals. *Acta Phys. Plant*. 1987. Vol. 9, No. 1. P. 13–23.
13. Климашевский Э. Л., Бернатская М. Л. Активностъ АТ Фазы и кислой фосфатазы в зонах роста корней гороха, неодинаково чувствительных к Al^{3+}. *Физиол. раст*. 1973. Т. 20, No. 2.
14. Удовенко Г. В. и др. *Физиологические основы селекции растений*. Санкт-Петербург, 1995. Т. II(1). С. 119–123.

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LIETUVIŠKŲ VEISLIŲ MIEŽIŲ (*Hordeum sativum* ssp. *distichum* L.) DAIGŲ AUGIMO DINAMIKOS ĮVERTINIMAS PAVEIKUS JUOS AL JONAIS

Santrauka

Per 2 savaites ištirti liekamojo aliuminio poveikio po vienos savaitės vasarinių miežių (*Hordeum sativum* ssp. *distichum* L.) 'Aura' ir 'Auksiniai-3' veislių daigų auginimo aliuminio druskų tirpaluose ypatumai. Augimo metu (7, 14 ir 21-ą daigų augimo dieną) buvo pamatuotas daigų šaknų bei antžeminės dalies ilgis, sausa masė ir lapų plotas. Apskaičiuoti šie augimo dinamiką įvertinantys rodikliai: augimo greitis (SAG), asimiliacinis greitis (AG), lapų ploto santykis (LPS), specifinis lapų plotas (SLP), šaknų ir antžeminės dalies ilgių bei sausosios masės santykiai. Dėl metalo (148 µM – 592 µM $AlKSO_4$, pH 4,5) įtakos (p < 0,05) sumažėjo šaknų ir antžeminės dalies svorių santykiai. Šaknų ir antžeminės dalies ilgių santykiai patikimai, palyginnit su kontrole, sumažėjo tirpaluse, kuriuose Al koncentracija buvo 296 µM ir 592 µM $AlKSO_4$. Specifinis lapų plotas 21-ą dieną variante (296 µM $AlKSO_4$, pH 4,5) patikimai, palyginti su kontrole, veislės 'Aura' (93%) buvo mažesnis, o veislės 'Auksiniai-3' – didesnis (106%). O 592 mM variante buvo gauti priešingi rezultatai: 'Aura' SLP – 108%, o 'Auksiniai-3' – 96%, palyginti su kontrole. Mūsų darbe nustatyta, kad visos aliuminio koncentracijos labiau keitė abiejų veislių sausos masės santykį negu ilgių santykį. Aliuminis labiau slopino šaknų negu antžeminės dalies augimą. Praėjus dviems savaitėms po metalo pašalinimo išliko stiprus poveikis šaknyse, tuo tarpu antžeminėje dalyje labai sumažėjo.