# Physiological responses of Lithuanian cultivars of *Hordeum sativum* ssp. *distichum* L. to Al exposure

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The aim of the present work was to evaluate the residual effects of aluminium within a two-week period followed after one-week growth of seedlings of spring barley (*Hordeum sativum* ssp. *distichum* L.) cultivars 'Aura' and 'Auksiniai' in the solution containing Al ions. Under the influence of metal (148–592 mM $AlKSO_4$, pH 4.5) significant ($p < 0.05$) changes were detected in the length of overground part (85–95%), leaf area (83%) and dry weight (84–91%). The effect was similar in both cultivars at the final time. According to the dry weight of roots, cultivar 'Aura' was more sensitive than 'Auksiniai-3' (85–87% and 84–91% respectively, cases when differences between treatments and control were significant), and the opposite tendency held for the length of roots: 'Auksiniai-3' was more sensitive than 'Aura' (70–91% and 75–89%, respectively). After two weeks, the recovery-depressing effect of aluminium was still present in the roots and very week in the stems. Elevated contents of the free proline was observed in Al-treated 7-day-old seedlings, the effect being higher for 'Auksiniai-3' than for 'Aura'.

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

## INTRODUCTION

Aluminium (Al) ions are regarded as a main toxic factor affecting plant growth in mineral soils at a pH below 5.5 [1]. In neutral and lightly acid soils the amount of Al is lower than 10 mg $kg^{-1}$, while at pH 4 the content of Al increases and may reach 100 mg $kg^{-1}$ [2]. Roots are the first target for aluminium in most of plant species [3–5]. Especially sensitive are roots of seedlings and juvenile plants. Under the effect of aluminium shorter, thicker and twisted roots, also brown and dead tips of overground parts of wheat were detected [6–8]. Aluminium may disturb the uptake of nutrients, especially cations. In addition, Al toxicity is related to an increased uptake of Fe, Mn, also to accumulation of heavy metals [9]. In the previous decade, acidification in Lithuania increased due to higher costs of liming and shortage of this type activities. It is estimated that without liming the pH decreases by 0.1–0.2 each year [1]. It is realistic to forecast an increase of acidic soils. Aluminium tolerance is under genetic control, and different sensitivity of species and cultivars is a well documented fact in the literature [9]. Selection of Al-tolerant cultivars is one of the ways to overcome the problems of acidity and aluminium toxicity. In our previous studies, several cultivars of spring barley grown in Lithuania were examined for aluminium and heavy metal sensitivity and the most contrasting varieties were defined [10].

The aim of the present work was to evaluate the effect of Al on two selected Lithuanian cultivars of spring barley under the influence of a range of Al concentrations and to follow the processes of recovery after the treatment.

## MATERIALS AND METHODS

'Aura' and 'Auksiniai-3' cultivars were selected. After one day germination in the dark seeds were placed into solutions of $AlKSO_4$ (148 μM, 296 μM and 592 μM) ajusted to pH 4.5. Control plants were kept in water solutions (pH 5.8). After 7 days of Al treatment, roots were washed and seedlings were transferred into a solution containing 0.4 mM $CaCl_2$, 0.65 mM $KNO_3$, 0.25 mM $MgCl_2 \cdot 6H_2O$, 0.01 mM $(NH_4)_2SO_4$, 0.04 mM $NH_4NO_3$ [11] and grown for 14 days. Gravimetric (weight of roots and shoots) and morphological (length of the longest root and overgroud part) parameters were recorded straight after aluminium treatment ($7^{th}$ day in Results) and one-two weeks later (days $14^{th}$ and $21^{st}$). Leaf areas of the leaves were defined only at days 14 and 21. The content of free proline in 7-d seedlings was

determined by the method described in [12]. Twenty five seedlings were grown in each replicate and four replicates were run over the time of the experiment.

## RESULTS AND DISCUSSIONS

*Dynamics of changes in root growth.* All concentrations of Al significantly ($p < 0.05$) depressed root elongation in 7- and 14-day-old 'Aura' seedlings (length of roots in treatments was 72–85% and 80–89%, respectively), and only the highest concentration had the same significant inhibitory effect (77%) on 21-day-old seedlings (Table 1). No significant differences of the lowest concentration were detected for 'Auksiniai-3' at any time of observation, and the opposite tendency held for the highest concentration (91–70%). In addition, the effect was increasing each week. 296–592 μM $AlKSO_4$ caused a significant ($p < 0.05$) inhibition of root weight of 'Aura' within all period of investigation (Table 2.). The effect was increasing with time (83–65%), and at the final harvest a significant depression was also observed at the lowest concentration. Only 7- and 21-d seedlings of 'Auksiniai-3' treated with 296–592 μM $AIKSO_4$ had significantly ($p < 0.05$) lower dry weights of the roots (81–86%).

*Aluminium effect on shoots*. The length of the overground part of both cultivars was much less effected by aluminium. Significantly ($p < 0.05$) lower seedlings were observed only straight after metal treatment with all concentrations for both cultivars. The effect for 'Auksiniai-

Table 1. **Length of roots and shoots (cm) of 7-, 14- and 21-d seedlings of 'Aura', 'Auksiniai-3' cultivars of spring barley grown for the first week in 148–592 μM $AlK(SO_4)_2$ solutions**

| Cultivars | | | | 'Aura' | | | | 'Auksiniai-3' | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Al concentration (μM) | | | | 0 | 148 | 296 | 592 | 0 | 148 | 296 | 592 |
| Length (cm) | Roots | 7th day | M | 7.12 | 6.04* | 5.12* | 5.34* | 5.82 | 5.77 | 5.35 | 5.28* |
| | | | *SD* | *0.31* | *0.16* | *0.53* | *0.26* | *0.39* | *0.42* | *0.80* | *0.32* |
| | | 14th day | M | 9.30 | 8.04* | 7.45* | 8.25* | 11.26 | 10.63 | 9.61* | 9.97* |
| | | | *SD* | *2.23* | *2.04* | *2.11* | *2.58* | *1.96* | *2.05* | *2.17* | *2.08* |
| | | 21st day | M | 11.31 | 9.96 | 9.86 | 8.70* | 20.42 | 19.56 | 17.29 | 14.19* |
| | | | *SD* | *4.38* | *2.74* | *3.39* | *2.73* | *6.41* | *7.71* | *8.53* | *8.19* |
| | Shoots | 7th day | M | 9.05 | 9.00* | 7.70* | 7.70* | 8.36 | 7.92* | 7.65* | 7.90* |
| | | | *SD* | *0.09* | *0.38* | *0.18* | *0.15* | *0.23* | *0.24* | *0.22* | *0.30* |
| | | 14th day | M | 17.46 | 16.40 | 16.32 | 16.43 | 16.58 | 16.11 | 16.24 | 6.24 |
| | | | *SD* | *2.34* | *2.84* | *2.95* | *2.91* | *2.44* | *2.90* | *2.31* | *2.48* |
| | | 21st day | M | 24.29 | 23.77 | 24.25 | 23.03 | 20.81 | 20.40 | 20.06 | 19.69 |
| | | | *SD* | *3.45* | *3.35* | *3.45* | *3.49* | *2.54* | *2.70* | *3.43* | *2.69* |

* Significant difference ($p < 0.05$) as compared to control

Table 2. **Weight of shoots and roots (g) of 7-, 14- and 21-d seedlings of 'Aura', 'Auksiniai-3' cultivars of spring barley grown for the first week in 148–592 μM $AlK(SO_4)_2$ solutions**

| Cultivars | | | | 'Aura' | | | | 'Auksiniai-3' | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Al concentration (μM) | | | | 0 | 148 | 296 | 592 | 0 | 148 | 296 | 592 |
| Weight (g) | Roots | 7th day | M | 0.115 | 0.108 | 0.093* | 0.094* | 0.116 | 0.103 | 0.094* | 0.094* |
| | | | *SD* | *0.004* | *0.003* | *0.010* | *0.004* | *0.011* | *0.006* | *0.013* | *0.01* |
| | | 14th day | M | 0.161 | 0.145 | 0.131* | 0.133* | 0.153 | 0.154 | 0.153 | 0.137 |
| | | | *SD* | *0.012* | *0.004* | *0.003* | *0.12* | *0.012* | *0.017* | *0.010* | *0.021* |
| | | 21st day | M | 0.263 | 0.213* | 0.211* | 0.172* | 0.253 | 0.240 | 0.161* | 0.169* |
| | | | *SD* | *0.014* | *0.011* | *0.035* | *0.013* | *0.009* | *0.038* | *0.015* | *0.012* |
| | Shoots | 7th day | M | 0.180 | 0.162 | 0.156* | 0.155* | 0.150 | 0.143 | 0.137* | 0.126* |
| | | | *SD* | *0.008* | *0.004* | *0.005* | *0.008* | *0.002* | *0.005* | *0.003* | *0.009* |
| | | 14th day | M | 0.407 | 0.37 | 0.363 | 0.382 | 0.370 | 0.365 | 0.373 | 0.370 |
| | | | *SD* | *0.041* | *0.22* | *0.011* | *0.032* | *0.014* | *0.025* | *0.016* | *0.034* |
| | | 21st day | M | 0.555 | 0.541 | 0.547 | 0.472* | 0.487 | 0.484 | 0.401 | 0.417 |
| | | | *SD* | *0.017* | *0.144* | *0.070* | *0.020* | *0.018* | *0.037* | *0.024* | *0.017* |

* Significant difference ($p < 0.05$) as compared to control

3' ranged between 91–95% (Table 1) and for 'Aura' 91–85%. The weight of shoots was depressed ($p < 0.05$) only for 7-d seedlings of 'Auksiniai-3' treated with 296–592 μM $AlKSO_4$ (91–84%) and for the same age seedlings of 'Aura' (87–86%; Table 2). Inhibition of shoot weight increase was also found for 21-d 'Aura' seedlings under the influence of the highest metal concentration (85%). The only significant effect for leaf areas was documented after 21-day growth of 'Auksiniai-3' seedlings treated with 592 μM $AlKSO_4$ (83%, Table 3).

Table 3. **Leaf area ($cm^2$) of 14- and 21 d seedlings of *Hordeum sativum* ssp. *distichum* L. after one week exposure ($1^{st}$–$7^{th}$ day) to $AlK(SO_4)_2$ solutions (148–592 μM)**

| Cultivar | Age (days) | Concentration of aluminium (μM) | | | |
|---|---|---|---|---|---|
| | | 0 | 148 | 296 | 592 |
| 'Aura' | 14 | 170 | 155 | 150 | 153 |
| | 21 | 242 | 243 | 218 | 223 |
| 'Auksiniai-3' | 14 | 140 | 123 | 131 | 128 |
| | 21 | 201 | 201 | 176 | 166* |

* Significant difference ($p < 0.05$) compared to control

In 7-d seedlings of 'Aura', the concentration of free proline ranged between 0.0115 and 0.0122 μM and all Al concentrations had a significant ($p < 0.05$) effect (103–112%). The amount of proline in 'Auksiniai-3' was 0.0115–0.0139 μM, and a significant effect of Al was observed ($p < 0.05$) only at its highest concentration (121%) and was stronger than in the 'Aura' cultivar.

Stronger Al effects on roots than on overground parts observed in the present study are in agreement with results obtained at other laboratories [8]. They may be due to a lower amount of metal transported to shoots [13]. The amount of free proline is used as an indicator of stress caused by drought and other factors [14]. Though, elevated proline content in the aluminium-treated seedlings of our cultivars may reflect the state of stress and a higher scope of it for the 'Auksiniai-3' cultivar. In the present study, a higher sensitivity of 'Aura' was reflected by the data on root and shoot length, while the weight statistics showed a similar reaction of both cultivars. Tolerance to Al is genetically controlled, and various genotypes of cultivated plants show various degrees of Al tolerance at various stages of development [8]. Further investigations including other parameters and extension of observation time are needed to evaluate the sensitivity of selected crop genotypes.

## CONCLUSIONS

1. Under the influence of aluminium: (2) significant ($p < 0.05$) changes were detected in the length of the overground part (85–95%), leaf area (83%) and dry weight (84–91%). The effect was similar in both cultivars; (2) according to the dry weight of roots, the cultivar 'Aura' was more sensitive than 'Auksiniai-3' (85–87% and 84–91%, respectively); (3) the opposite dendencyw obserwed for the length of roots: 'Auksiniai-3' was more sensitive than 'Aura' (70–91% and 75–89%, respectively); (4) 'Auksiniai-3' was more sensitive according to the content of free proline.

2. After two weeks the recovery-depressing effect of aluminium was obvious for the roots and very week for the stems.

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**LIETUVIŠKŲ VEISLIŲ *HORDEUM SATIVUM SSP. DISTICHUM* L. ATSAKAS Į ALIUMINIO POVEIKĮ**


Santrauka

Buvo ištirtas 2 savaites liekamasis aliuminio poveikis po vienos savaitės vasarinių miežių (*Hordeum sativum ssp. distichum* L.) 'Aura' ir 'Auksiniai-3' veislių daigų auginimo aliuminio druskų tirpaluose. Dėl metalo (148–592 mM $AlKSO_4$, pH 4,5) ($p < 0,05$) sumažėjo antžeminės dalies ilgis (85–95%), lapų plotas (83%) ir sausoji masė (84–91%). Poveikis buvo panašus abiejose veislėse. Pagal šaknų sausąją masę (atitinkamai 85–87% ir 84–91%, kai reikšmingi skirtumai, palyginti su kontrole) veislė 'Aura' buvo jautresnė už 'Auksiniai-3', ir atvirkščiai: 'Auksiniai-3' buvo jautresni už 'Aura' pagal šaknų ilgį (70–91% ir 75–89% atitinkamai). Praėjus dviem savaitėms po metalo pašalinimo, išliko stiprus poveikis šaknyse, tuo tarpu antžeminėje dalyje labai sumažėjo. Rastas reikšmingai ($p < 0,05$) didesnis laisvojo prolino kiekis Al augusiuose 7-dieniuose daiguose, ypač 'Auksiniai-3' veislėje.