Aluminium induced acid phosphatase activity in roots of Al-sensitive and Al-tolerant barley varieties

J. Huttová, L. Tamás, I. Mistrík

Institute of Botany, Slovak Academy of Science, Bratislava, Slovak Republic

ABSTRACT

In barley roots significant increase of acid phosphatase activity was observed during Al treatment. Especially steep increase was found in the roots of Al-sensitive cv. Alfor treated with Al in the range of 1–10mM which was followed by sudden decline when higher concentration (10–100mM) was applied. Continual, but significantly lower increase in phosphatase activity was also demonstrated in the roots of Al-tolerant cv. Bavaria in the range of 1–50mM Al. In both cases, Al-induced increase of acid phosphatase activity was accompanied by the increase in the amount of one phosphatase isoforme. Contrary to cv. Alfor where Al-induced changes reached their maximum in the first day of Al treatment in the Al-tolerant cv. Bavaria slight increase continued also on the second day of Al treatment. Our results indicate that different behaviour of acid phosphatase enzyme in barley cultivars during Al stress may play an important function in coping by the plants with Al induced phosphate deficiency syndrome.

Keywords: aluminium; stress; acid phosphatase activity; isoenzymes; root growth inhibition; spring barley (*Hordeum vulgare* L.)

Acid soils represent up to 30% of the ice-free land and due to the natural, agricultural and industrial processes, area of acid soils increases from year to year (Uexküll and Mutert 1995). Acid soils are naturally low in available phosphorus (P) and have high P retention capacity. In addition, acid soils contain several toxic forms of metals. Aluminium is the most toxic metal in acid soils limiting crop productivity all over the world. Many plants are sensitive to micromolar concentration of soluble forms of Al appearing in soils with pH below than 5.0. Several forms of Al toxicity and tolerance have been described in various plant species (Kochian 1995, Matsumoto 2000, Hocking 2001, Matsumoto et al. 2001). In spite of considerable research efforts the biochemical and molecular background of Al-induced symptoms is still poorly understood.

Al toxicity is frequently associated with reduced water and nutrient uptake. In many plants Al toxicity resembles phosphorus, calcium or iron deficiency syndrome (Rout et al. 2001). Al forms insoluble and stable complexes with inorganic and organic phosphates, therefore their solubilisation is prerequisite for P uptake by plants. The role of secreted organic acids in solubilisation of Al complexes and in Al detoxification is well known in several plant species (Hocking 2001, Ma et al. 2001). Relation between Al and P-deficiency stress is also supported by the observation that P-starved cells are more tolerant to Al toxicity than normally growing cell (Yamamoto et al. 1996) and that the Al-induced genes are activated also in P-deficient plants (Ezaki et al. 1995).

One of the initial responses of plants to P-deficiency stress is an increase of root acid phosphatase activity (Goldstein et al. 1988, Duff et al. 1994). Its function is the hydrolysis of Pi from orthophosphate-monoesters used for plant nutrition in the soil. Similarly to P-deficiency, also salt stress induces enhancement of acid phosphatases (Pan 1987) and considerable stimulation of phosphatase activity was reported by nickel in tolerant plants while in sensitive plants slight decrease of phosphatase activity was detected (Gabbrielli et al. 1989).

In this paper, we characterise Al-induced changes in acid phosphatase activity in two barley cultivars differing in their sensitivity to Al. Total changes in the root acid phosphatase activity are amended by the changes in isoenzyme pattern.

MATERIAL AND METHODS

Barley seeds (*Hordeum vulgare* L. cv. Alfor – Al-sensitive, and cv. Bavaria – Al-tolerant were selected and provided by the Research Institute of Plant Production, Piešťany) were surface sterilised with 12% H_2O_2 for 10 min and then rinsed five times with distilled water (Tamás and Huttová 2000). Seeds were germinated on filter paper for 36 h in dark at 24°C. Germinated seeds were transferred onto the new filter paper moistened by distilled H_2O (control) or with various concentrations (ranging from 1 to 100mM) of $AlCl_3.6\,H_2O$, pH 4.5 (Al-treated). The transferred plants (60 seedlings were included in each treatment) were incubated 24 or 48 h under the same condition as for germination. The collected whole roots were stored at –70°C until analysed. All experiments were repeated at least three times.

Root tissues were ground to a fine powder in a cold mortar in liquid nitrogen and the resulting powder was rehomogenised in 40mM succinic acid/NaOH, buffer, pH 4.0 with homogenisator (Heidolph DIAX 900). After filtration the homogenate was centrifuged at 1 500 *g* for 5 min, then

Table 1. Effect of 24 h Al-treatment on root length increments (final root length minus initial root length) of Al-sensitive cv. Alfor and Al-tolerant cv. Bavaria grown at different Al concentrations (0–100mM); values are the mean of at least three independent experiments (± *SD*)

Al (mM)	Root length increments (mm ± *SD*)	
	Alfor	Bavaria
0	2.67 ± 0.11	2.85 ± 0.08
1	2.57 ± 0.04	2.75 ± 0.06
5	2.19 ± 0.11	2.66 ± 0.06
10	1.47 ± 0.07*	2.20 ± 0.10
25	0.68 ± 0.08**	1.01 ± 0.12*
50	0.49 ± 0.02**	0.53 ± 0.15**
100	0.11 ± 0.06**	0.31 ± 0.07**

* significant at 95% confidence
** significant at 99% confidence

at 12 000 *g* for 15 min and finally at 150 000 *g* for 30 min (Beckman L8-M). The resulting supernatant (soluble fraction) after concentration and passing through Sephadex G-25 was used for analysis. Proteins were quantified with bovine serum albumin as a standard by the method of Bradford (1976).

Acid phosphatase (EC 3.1.3.2) activity was determined photometrically using microplate reader (SLT-Laborinstruments, Austria). The reaction mixture contained 100 µl of 0.1 mol.l^{-1} Na-acetate buffer, pH 5.2, 50 µl of 4-nitrophenylphosphate (2 mg.ml^{-1}) and 50 µl of sample. The reaction was stopped after 30 min incubation at 37°C by adding of 50 µl of 0.4 mol.l^{-1} Na-phosphate buffer and activity was measured at 555 nm against the control reaction without sample. Specific enzyme activities were expressed as OD.µg^{-1} protein (OD – optical density). Changes in enzyme activities were expressed as a percentage of control.

Acid phosphatase isoenzymes were separated under non-denaturating conditions on 7% slab polyacrylamide gels using the discontinuous buffer system (Laemmli 1970) and acid phosphatase activity was visualised by incubating the gel in a solution consisting of 0.1 mol.l^{-1} Na-acetate buffer (pH 5.2), 0.1% α-naphthyl phosphate and 0.1% Fast Garnet GBC for 1 h at 30°C (Panara et al. 1990).

RESULTS AND DISCUSSION

The inhibition of root growth revealed significant difference between these cultivars. Al-caused inhibition of root growth was higher in Al-sensitive Alfor than in Al-tolerant Bavaria (Table 1). In Alfor inhibition of root growth started at 5mM concentration of Al and significant decrease of root increment was observed at 10mM Al concentration. In more Al-tolerant cv. Bavaria significant reduction occurred at higher – 25mM Al concentration. At higher Al concentrations (50 and 100mM), the root growth inhibition was similar in both cultivars. The inhibition of root growth is general syndrome of Al-treated plants. However, as demonstrated Quartin et al. (2001) sub-lethal concentration of Al caused stronger inhibition of dry matter production in the shoot than in the root of *Triticale*. By the view of these authors, the biomass reduction of *Triticale* seedlings grown in nutrient solution with Al was caused by phosphorus deficiency.

Many plant species are able to induce increase in the acid phosphatase activity under P-deficiency stress condition. Similarly, in our experiments exposure of barley plants to phytotoxic concentration of Al resulted in considerable increase of phosphatase activity especially at the lower concentrations of Al (Figure 1A). Exposure of the Al-sensitive cv. Alfor to 24 h Al treatment significantly increased the activity of acid phosphatase already at 1mM concentration of Al. This increased rapidly up to

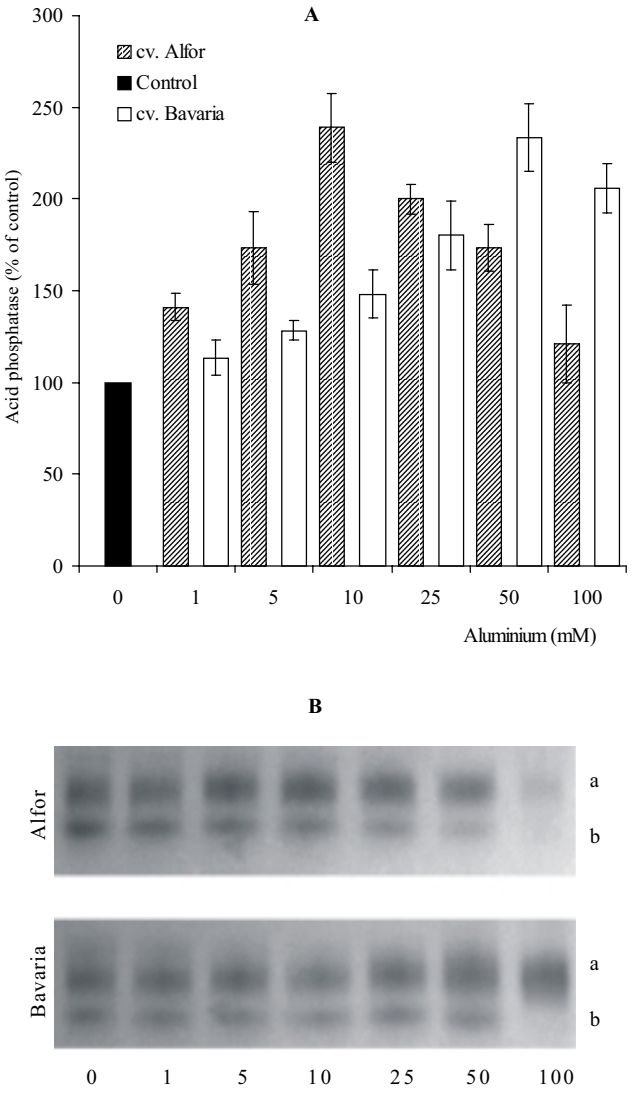

Figure 1. Aluminium dose dependency of acid phosphatase activity (A) and accumulation of acid phosphatase isozymes (B) after 24 h of Al treatment

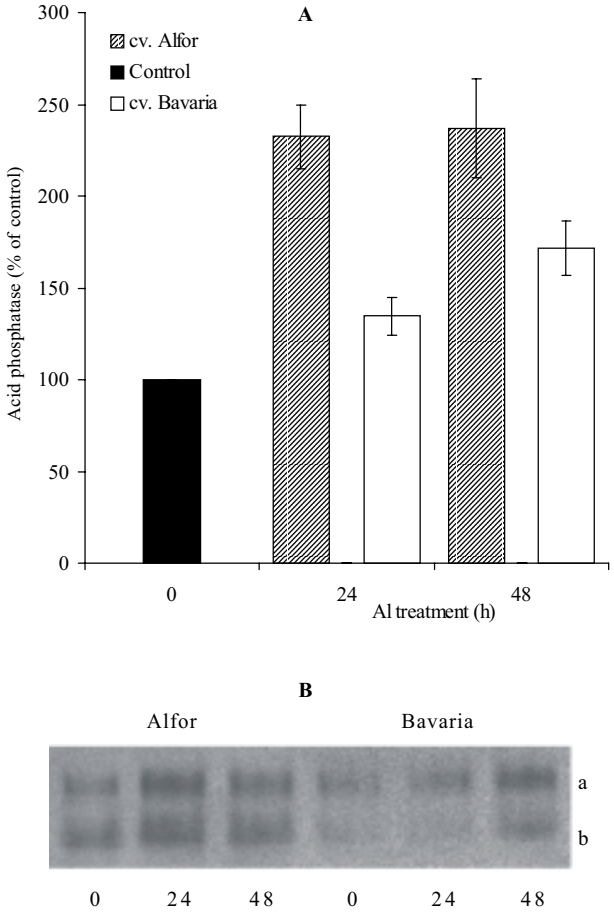

Figure 2. Time course analysis of acid phosphatase activity (A) and accumulation of acid phosphatase isozymes (B) induced by 10mM Al treatment

10mM Al. However, at Al concentrations above 10mM activity of acid phosphatase slowly declined and at 100mM Al was only slightly higher than in control plants. Probably in Al-sensitive plants at higher Al concentrations destructive processes caused by Al toxicity dominated and the decline of acid phosphatase activity was only the consequence of this process. In Al-tolerant plants, an increase of acid phosphatase activity was more moderate than in sensitive one and raised linearly up to 50mM Al concentration. At this high Al concentration the activity of acid phosphatase was similar to that in the case of 10mM Al treatment in Al-sensitive Alfor. These data clearly demonstrate the significant difference between Al-tolerant and Al-sensitive barley cultivars. Similar results were described by Patra et al. (1994) where stronger activation of acid phosphatase was observed in tolerant grass than in non-tolerant during mercury and cadmium treatment.

Phosphatase isoenzyme pattern always contain different numbers of isoenzymes. In aleurone layer of barley, rapid increases of several phosphatase isoenzymes were detected during imbibition by Gabard and Jones (1986). From mature barley roots Panara et al. (1990) separated two soluble acid phosphatase activities one of which represented the most abundant acid phosphatase form in barley root extract. In our experiments, analysis of isoenzymes similarly revealed two isoforms of soluble (cytoplasmic) acid isophosphatase activity on native PAGE (Figure 1B). One of them, isoform-*b* showed only a small changes during Al treatment except in the case when 100mM Al concentration was used. At this concentration, we did not measure any activity of this phosphatase isoenzyme. Accumulation of isoform-*a* followed the same pattern as we found during phosphatase activity determination in solution (Figure 1A). From these results it is clear that changes in accumulation of acid phosphatase isoenzyme-*a* is responsible for Al-induced acid phosphatase activity in Al-treated roots.

Time course analysis revealed that acid phosphatase activity did not change significantly during the second day of Al (10mM) treatment (Figures 2A, B). Only moderate increase of the acid phosphatase activity was detected in Al-tolerant Bavaria. This suggests that activation of acid phosphatase occurs at the beginning of Al stress and therefore plays crucial role in Al-toxicity reaction in plants. The secreted forms of acid phosphatases are non-specific enzymes probably with function of mobilisation of P in soils, while intracellular isoforms may play role in mobilisation of P reserves in the cell. Apoplastic P may play a role in tolerance mechanism by precipitation of Al in cell wall interior and thus immobilise Al in apoplast and protect the cytoplasm of root cells from Al toxicity (Marienfeld and Stelzer 1993). Transport of Al-P deposits from apoplast into the vacuole was reported by Vázquez et al. (1999) in maize Al-tolerant variety. In these cases intracellular phosphatases may play an important function in mobilisation of such P reserves in the cell during Al stress and help the plants to reduce the Al induced P starvation stress.

Acknowledgement

Mrs Anna Grycová for the excellent technical assistance.

This work was partially supported by the Slovak Grant Agency VEGA, project No. 2/1066/21.

REFERENCES

Bradford M. (1976): A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principle of protein-dye binding. Anal. Biochem., *72*: 248–254.

Duff S.M.G., Sarath, G., Plaxton W.C. (1994): The role of acid phosphatase in plant phosphorus metabolism. Physiol. Plant., *90*: 791–800.

Ezaki B., Yamamoto Y., Matsumoto H. (1995): Cloning and sequencing of the cDNAs induced by aluminium treatment

and Pi starvation in cultured tobacco cells. Physiol. Plant., *93*: 11–18.

Gabard K.A., Jones R.J. (1986): Localization of phytase and acid phosphatase isoenzymes in aleurone layers of barley. Physiol Plant., *67*: 182–192.

Gabbrielli R., Grossy L., Vergnano O. (1989): The effects of nickel, calcium and magnesium on the acid phosphatase activity of two *Alyssum* species. New Phytol., *111*: 631–636.

Goldstein A.H., Baertlein D.A., Mcdaniel R.G. (1988): Phosphate starvation inducible metabolism in *Lycopersicon esculentum*. I. Excretion of acid phosphatase by tomato plants and suspension-cultured cells. Plant Physiol., *87*: 711–715.

Hocking P.J. (2001): Organic acids exuded from in phosphorus uptake and aluminum tolerance of plants in acid soils. Adv. Agron., *74*: 63–97.

Kochian L.V. (1995): Cellular mechanisms of aluminium toxicity and resistance in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol., *46*: 237–260.

Laemmli U.K. (1970): Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature, *277*: 680–685.

Ma J.F., Ryan P.R., Delhaize E. (2001): Aluminium tolerance in plants and the complexing role of organic acids. Tr. Plant Sci., *6*: 273–278.

Marienfeld S., Stelzer R. (1993): X-ray microanalyses in roots of Al-treated *Avena sativa* plants. J. Plant Physiol., *141*:569–573.

Matsumoto H. (2000): Cell biology of aluminum toxicity and tolerance in higher plants. Int. Rev. Cytol., *200*: 1–46.

Matsumoto H., Yamamoto Y., Devi S.R. (2001): Aluminum toxicity in acid soils. Plant responses to aluminum. In: Prassad M.N.V. (ed.): Metals in the environment. Okayama Univ., Kurashiki, Japan: 289–320.

Pan S. (1987): Characterisation of multiple acid phosphatases in salt-stressed spinach leaves. Austral. J. Plant Physiol., *14*: 117–124.

Panara F., Pasqualini S., Antonielli M. (1990): Multiple forms of barley root acid phosphatase: purification and some characteristics of the major cytoplasmic isoenzyme. Biochem. Biophys. Acta, *1037*: 73–80.

Patra J., Lenka M., Panda B.B. (1994): Tolerance and co-tolerance of the grass *Chloris barbarata* Sw. to mercury, cadmium and zinc. New Phytol., *128*: 165–171.

Quartin V.I., Azinheira H.G., Nunes M.A. (2001): Phosphorus deficiency is responsible for biomass reduction of *Triticale* in nutrient solution with aluminum. J. Plant Nutr., *24*: 1901–1911.

Rout G.R., Samantaray S., Das P. (2001): Aluminium toxicity in plants: a review. Agronomie, *21*: 3–21.

Tamás L., Huttová J. (2000) Effect of pH and some metals (Al, Cu, Cd and Co) on root growth of barley. Poľnohospodárstvo, *46*: 725–732.

Uexküll H.R. von, Mutert E. (1995): Global extent, development and economic impact of acid soils. Plant Soil, *171*: 1–15.

Vázquez M.D., Poschenrieder C., Corrales I., Barcelo J. (1999): Change in apoplastic aluminum during the initial growth response to aluminum by roots of a tolerant maize variety. Plant Physiol., *119*: 435–444.

Yamamoto Y., Masamoto K., Rikiishi S., Hachiya A., Yamaguchi Y., Matsumoto H. (1996): Aluminum tolerance acquired during phosphate starvation in cultured tobacco cells. Plant Physiol., *112*: 217–227.

Received on May 20, 2002

ABSTRAKT

Působení hliníku na aktivitu kyselé fosfatázy v kořenech senzitivní a tolerantní odrůdy ječmene

Aktivita kyselé fosfatázy byla sledována u dvou odrůd ječmene, lišících se citlivostí vůči hliníku. Hliník u obou odrůd indukoval signifikantní zvýšení aktivity kyselé fosfatázy. Obzvlášť výrazné zvýšení bylo detekované u senzitivní odrůdy Alfor při koncentracích hliníku od 1 do 10 mM. Vyšší koncentrace hliníku však indukovala pokles aktivity kyselé fosfatázy. Na rozdíl od odrůdy Alfor u rezistentní odrůdy Bavaria hliník indukoval mírnější, avšak plynulejší zvyšování aktivity kyselé fosfatázy v koncentračním rozmezí od 1 do 50 mM Al. V obou případech zvýšená aktivita kyselé fosfatázy korelovala s akumulací jedné izoformy kyselé fosfatázy. Časová analýza ukázala, že v případě senzitivní odrůdy se aktivita kyselé fosfatázy po prvním dni působení hliníku již nezvyšovala, u rezistentní odrůdy se její aktivita zvyšovala ještě i druhý den. Získané výsledky naznačují možnou účast různých fyziologických mechanismů v příjmu a metabolismu fosfátu v odrůdách ječmene s různou citlivostí vůči hliníku.

Klíčová slova: hliník; stres; kyselá fosfatáza; izoenzymy; inhibice růstu kořene; jarní ječmen (*Hordeum vulgare* L.)

Corresponding author:

RNDr. Ladislav Tamás, Ph.D., Botanický ústav, Slovenská akadémia vied, Dúbravská cesta 14, 842 23 Bratislava, Slovenská republika, tel.: + 421 259 426 116, fax: + 421 254 771 948, e-mail: botutama@savba.sk