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SHORT COMMUNICATION

# Aluminium-induced drought and oxidative stress in barley roots

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Al uptake;
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## Summary

The aim of the present study was to examine the relation between Al accumulation in root tissues, root growth inhibition, root water content, cell viability and expression of oxidative and drought stress-related genes in barley roots growing on the filter paper. Al-induced root growth inhibition correlated with Al uptake and cell death. Water content of Al-treated root represented only half of the control one. The expression of the dehydrin gene dhn4, which is a marker for drought stress in plant tissues, was strongly induced during Al stress. Al treatment also induced expression of oxidative stress-related genes such as glutathione peroxidase (gpx), pathogen-related peroxidase (prx8), glutathione reductase (gr) and dehydroascorbate reductase (dhar). The present results suggest correlation between Al uptake, Al-induced drought stress, oxidative stress, cell death and root growth inhibition.
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## Introduction

Dehydrins or late embryogenesis abundant (LEA) proteins are induced during dehydration by several environmental stresses (drought, low temperature and salinity) but also during developmental stages such as seed development or pollen maturation (Scott and Close, 1997). They act as stabilizers of macromolecules by interacting with proteins or membranes during low water conditions. In drought stressed cereals dehydrins accumulate to levels representing more than 1% of total soluble proteins extractable with law salt buffers (Scott and Close, 1997), therefore the expression of dehydrin genes is a suitable marker for diminished water activity in plant tissues.

The first target site of Al action is probably the apoplast. Distribution of absorbed Al differs among plant species but the significant part (30–90%) was found in the apoplast (Rengel, 1996). Horst (1995) supposed that binding of Al to sensitive binding sites of the apoplast and competition for these binding sites with other ions determines Al-induced inhibition of root elongation. These rapid Al-induced changes in cell wall (CW) can lead to the inhibition of water and mineral uptake imitating drought stress. In addition,

*Abbreviations:* AOS, active oxygen species; CW, cell wall; PM, plasma membrane

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the rigid structure of the plasma membrane (PM) caused by metal toxicity can also affect the uptake of water and ions (Fodor et al., 1995).

A common feature of several stresses including Al toxicity and drought is perturbation of cell redox homeostasis and as a consequence the enhanced production of active oxygen species (AOS) (Elstner and Osswald, 1994). Studies of Al toxicity in roots suggest that production of AOS may significantly contribute to Al-induced inhibition of root elongation (Yamamoto et al., 2003; Tamás et al., 2004b). During drought stress mainly the impairment of the electron transport chains on injured membranes leads to the formation of AOS and to subsequent activation of antioxidative defense system (Hoekstra et al., 2001). Induction of oxidative stress-related genes by Al and drought stress confirmed the important role of AOS during both stresses (Richards et al., 1998; Jiang and Zhang, 2002; Tamás et al., 2003).

The aim of the present study was to examine the relation between Al accumulation in root tissues, root growth inhibition, root water content, cell viability and expression of oxidative and drought stress-related genes in barley roots growing on the filter paper.

## Materials and methods

Barley seeds (*Hordeum vulgare* L. cv. Jubilant) were germinated on filter paper (Whatman No. 1) for 35 h in the dark at 24 °C. Uniformly germinated seeds were selected and transferred to the fresh filter paper (Whatman No. 1) moistened with 0.2 mM $CaCl_2$ (control) or with 10 mM $AlCl_3$, pH 4.0 (Al-treated). The transferred plants were incubated for the relevant time period under the same conditions as for germination. Root length was measured by a ruler and excised root tips (1 cm) were used immediately for analysis or stored at −70 °C until analyzed. Water content was expressed as a difference between fresh weight (FW) and dry weight (DW). DW was recorded after drying the roots for 24 h at 60 °C. Evans blue and hematoxylin staining was used for evaluation of the loss of PM integrity and for determination of Al accumulation, respectively, as described in our previous work (Šimonovičová et al., 2004). The loosely bound Al was removed from apoplast by soaking the root tips in 0.5 mM citric acid at 4 °C for 30 min before hematoxylin staining. Each experiment was repeated at least five times with 60 seedlings per Petri dish.

Total RNA was isolated from root tips using Rneasy Plant Kit (Qiagen) and cDNA was synthesized from Dnase-treated total RNA with Omniscript RT Kit (Qiagen) using oligo-dT-primer. Primers were prepared according to sequences published by Finkemeier et al. (2003) and Choi et al. (2002). Cycle numbers were optimized to assure that the amplification reaction was tested in the exponential phase and the gene product was detectable also in control roots. The expression of actin was used as a positive control. The PCR products were applied to 5% PAGE and silver stained.

## Results and discussion

In our previous work we demonstrated that due to binding of Al to filter paper and root-induced alkalization of the root milieu the concentration of free Al is about 25 times lower than originally applied to filter paper (Šimonovičová et al., 2004). During the first 3 h root growth increment was observed neither in control nor in Al-treated seedlings, probably as a consequence of adaptation lag phase after transferring the seedlings to new filter paper (Fig. 1). Root growth inhibition was evident 6 h after 10 mM Al treatment and after 12 h root elongation represented only about 20% of control roots. However, during the following hours some increase in root elongation was observed in Al-treated plants. It is possible that in roots growing on filter paper some Al detoxifying mechanisms (e.g. intact root cap with root border cells, pH change around root tip, organic acid secretion) are manifested to larger extent compared to hydroponics. In our previous work (Tamás et al., 2003) we found out that 10 mM Al applied on filter paper did not cause lethal root injuries after 24 h and root growth was restored after transferring the plants into Al-free conditions.

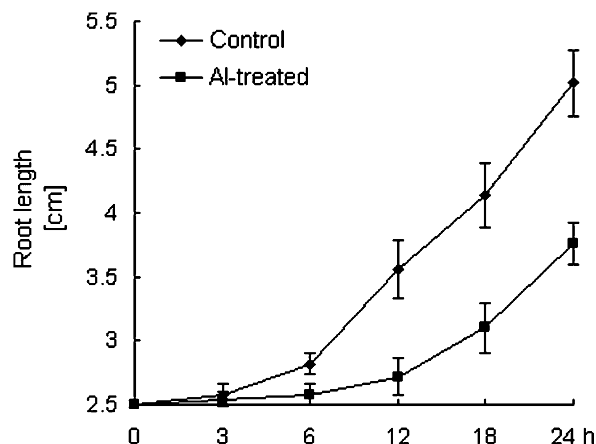


**Figure 1.** Root length of barley seedlings exposed to Al for 0, 3, 6, 12, 18 and 24 h (0 – root length at the start). Mean values ± SD ($n = 5$).

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