Effect of aluminium on peroxidase activity in roots of Al-sensitive and Al-resistant barley cultivars

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ABSTRACT

The effect of Al stress on peroxidase activity and peroxidase isozymes was studied in roots of two barley cultivars with contrasting sensitivity to Al. Al treatment induced a tremendous enhancement of guaiacol peroxidase activity especially in roots of Al-sensitive barley cv. Alfor. After 48 h of Al treatment activity of peroxidase in roots of cv. Alfor was up to 5.5 times higher than the control roots. In contrast, activity of peroxidase in the roots of Al-resistant cv. Bavaria was about one half than that in roots of Al-sensitive Alfor. SDS-PAGE analysis revealed that at least five peroxidase isozymes are activated by Al treatment. Using IEF we determined that three of Al-induced peroxidase isozymes are cationic with pI about 8.2, 8.4 and 8.6, while two other are anionic isoperoxidases with pI about 4.0 and 4.5. Al induced increase in the activity of root peroxidases correlated with the extent of Al induced root growth inhibition. The inhibition of root growth in Al-sensitive Alfor represented 44% but in Al-resistant Bavaria only 21% in comparison with control plants. Higher peroxidase activity, as well, as higher inhibition of root growth in Al-sensitive Alfor suggest that enhanced oxidative stress generated by Al treatment is significantly more stressful in Alfor than in the Al-resistant Bavaria.

Keywords: spring barley (*Hordeum vulgare* L.); roots; growth; isozymes; peroxidase; aluminium-stress

Aluminium at pH values below 5.0 is toxic for many plants; therefore, Al ions are major crop production limiting factor in acid soils. The inhibition of root elongation is a general and very sensitive response of plants to micromolar concentrations of aluminium. The primary site of Al toxicity is the root meristem. Within this apical root zone, the distal part of the transition zone is the most sensitive site of Al induced injury of plants (Sivaguru and Horst 1998). Large numbers of Al toxicity have been described, including apoplastic injury such as Al induced disjunction of cell wall, plasma membrane permeability and ion fluxes or symplastic targets of Al, such as Ca homeostasis, cytoskeleton and nuclei (for review see Kochian 1995, Matsumoto 2000).

Aluminium ions show strong affinity for the negatively charged cell wall pectin and for the negative outer surface of plasma membrane. It has been suggested that Al-membrane interaction causes structural changes of phospholipide bilayer and accelerates their peroxidation, which affects the function of membranes, such as permeability and membrane protein activities (Cakmak and Horst 1991). The relationship between plasma membrane surface negativity and sensitivity to Al also supports the idea about the membranes as a target site of Al toxicity (Yermiyahu et al. 1997). Recently, it has been reported that Al facilitates Fe-mediated free radical production and thus lipid peroxidation, which is a direct cause of plasma membrane disruption and cell death (Yamamoto et al. 1997). On the other hand, antioxidant molecules and antioxidant enzymes prevent Al-induced lipid peroxidation (Ezaki et al. 1996). Direct evidence for link between Al and oxidative stress is that Al induces expression of the oxidative stress genes in *Arabidopsis thaliana* and their expression in transgenic plants ameliorate Al or oxidative stress caused damages (Richards et al. 1998, Ezaki et al. 2000). One of the genes expressed in Al treated tobacco cells is *pAL142*, which is highly homologous to the *parB* gene (Takahashi and Nagata 1992). Since the *parB* protein is reported to have a glutathione S-transferase activity, it is suggested that the function of glutathione, as an antioxidant is to prevent the peroxidation process caused by Al ions. Another gene *pAL201* expression of which is induced by Al treatment encoded a moderately anionic peroxidase suggest that peroxidase enzymes have some function in Al stress (Ezaki et al. 1996).

The aim of the present study was to investigate Al induced changes in the activity of guaiacol peroxidase in roots of two barley cultivars with contrasting sensitivity to Al. Activity of root peroxidases as well as induction of individual peroxidase isozymes were analysed 24 and 48 h after exposure of plants to 10 mM $AlCl_3$ to characterise the ability of Al-sensitive and Al-resistant barley cultivars to cope with Al and oxidative stress.

MATERIAL AND METHODS

Barley seeds (*Hordeum vulgare* L., cv. Alfor – Al-sensitive, and cv. Bavaria – Al-resistant) were sterilised with 12% H_2O_2 for 10 min and then rinsed five times with distilled H_2O. The seeds were germinated on filter paper for 1.5 days in the dark at 24°C. Germinated seeds were transferred to the fresh filter paper moistened in distilled H_2O (control) or in 10 mM $AlCl_3.6\ H_2O$, pH 4.5 (Al-treated). After 24 or 48 h of exposure of roots to distilled water (control) or Al treatment root tissues were (the terminal

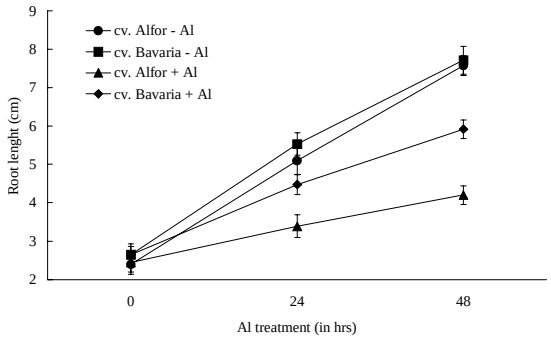

Figure 1. Root length of Al-sensitive (Alfor) and Al-resistant (Bavaria) barley cultivars exposed to Al for 0, 24 or 48 h

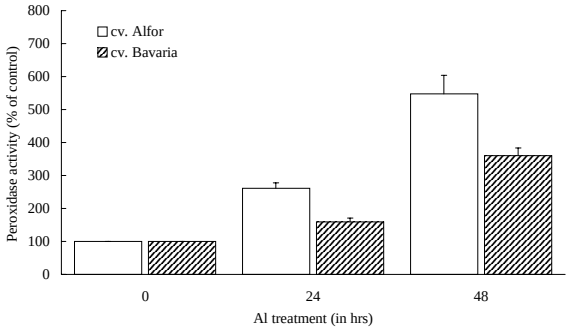

Figure 2. Activity of guaiacol peroxidase of Al-sensitive (Alfor) and Al-resistant (Bavaria) barley cultivars exposed to Al for 24 or 48 h

1 cm long root segment) harvested and homogenised in a cold mortar using extraction buffer (0.05 M Na-phosphate pH 7.2 and 2 mM EDTA). The homogenate was centrifuged at 12 000 *g* for 15 min.

Peroxidase (EC 1.11.1.7) activity was determined photometrically using guaiacol as substrate at 405 nm (EASY READER SLT-Laborinstruments Austria). Specific enzyme activities were expressed as OD.μg^{-1} protein (OD = optical density). Changes in enzyme activities are expressed as a percentage of control. Isozymes were separated on 8% SDS-PAGE or on native IEF and stained with benzidine (Tamás and Frič 1995). For pH gradient determination the gel after IEF was cut into strips and incubated 1 h in 1 ml of 10 mM KCl. Lipid peroxidation was determined by TBA method (Cakmak and Horst 1991).

RESULTS

To characterise the sensitivity of two barley cultivars to Al, root elongation was measured 24 and 48 after Al application (Figure 1). Results obtained showed significant differences in the ability of two cultivars to grow in the presence of Al. Inhibition of root growth was found in both cultivars, but in Al-sensitive cultivar Alfor was two times stronger than in the Al-resistant cultivar Bavaria. The overall root growth in Al-sensitive Alfor cultivated under Al stress represented 44% that of control plants, while Al treatment induced only 21% inhibition of root growth in Al-resistant Bavaria.

Activity of peroxidase in root tissues dramatically increased after Al treatment. Especially in Al sensitive Alfor activity of peroxidase after 24 h Al treatment was almost three times higher than in control roots. In roots of Al-resistant cultivar Bavaria this increase represented 180% compared with the control, but was about one half of roots of Al-sensitive Alfor (Figure 2). Peroxidase activity after 48 h of Al treatment increased approximately two times in both cultivars in comparison with the peroxidase activity in roots after 24 h Al treatment. Lipid peroxidation in root tissues of both barley cultivars was not significantly affected by Al treatment.

SDS-PAGE analysis showed that at least five peroxidase isozymes are activated by Al treatment (Figure 3). Two of them reached the highest activity in cultivar Alfor after 24 hr of Al treatment. Using IEF we observed that three of Al-induced peroxidase isozymes are cationic with pI about 8.2, 8.4 and 8.6, while two others are anionic isoperoxidases with pI about 4.0 and 4.5 (Figure 4).

DISCUSSION

Peroxidases are ubiquitous enzymes in plant kingdom and their specific isozymes are expressed in response to development, senescence, biotic and abiotic stresses (Siegel 1993). In the study presented here, we demonstrate induction of guaiacol peroxidase activity in barley roots by Al treatment. Increase in peroxidase activity correlated with the extent of root growth reduction. Activity of peroxidase was higher in the roots of Al-sensitive cultivar Alfor than in Al-resistant Bavaria and was accompanied by higher root growth inhibition than in Al-resistant Bavaria. Similarly, Cakmak and Horst (1991) described tremendous increase in peroxidase activity in root tips of soybean after 24 h Al treatment. Recently, Richards et al. (1998) have suggested, that Al induces expression of genes encoding peroxidase within 1 h of exposure of *Arabidopsis thaliana* roots to Al stress and this expression increases over 48 h of Al treatment.

Several studies have shown that Al enhances generation of reactive oxygen species and lipid peroxidation (Cakmak and Horst 1991, Yamamoto et al. 1997). Catalase, but not superoxide dismutase, blocked such generation suggesting hydrogen peroxide as an intermediate in reaction of lipid peroxidation (Bondy et al. 1998). The cationic peroxidases may catalyse the synthesis of H_2O_2 as a consequence of several oxidase reactions (Vianello and Marci 1991). Most recently, Kawano and Muto (2000) have proposed model for the mechanism of generation of reactive oxygen species in which the crucial role have extracellularly secreted peroxidases. Similarly to our results, Ezaki et al. (1996) reported that Al treatment of cultured tobacco cells activated two cationic peroxidases with higher pI (9.2 and 9.7) than we found in barley (pI 8.2, 8.4 and 8.6). However, in spite of high peroxidase activity during Al stress, we did not detect any changes in lipid peroxidation. These results suggest that peroxidase

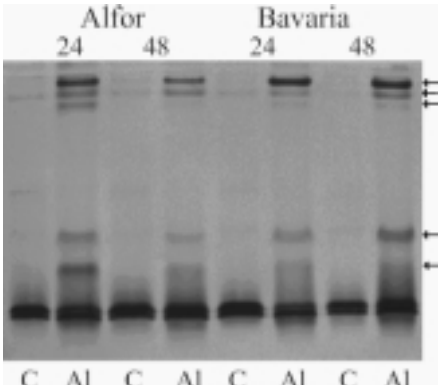

Figure 3. SDS-PAGE of peroxidase isozymes in root tips of Al-sensitive (Alfor) and Al-resistant (Bavaria) barley cultivars exposed to Al for 24 or 48 hours; an arrow indicates the activated isoperoxidases

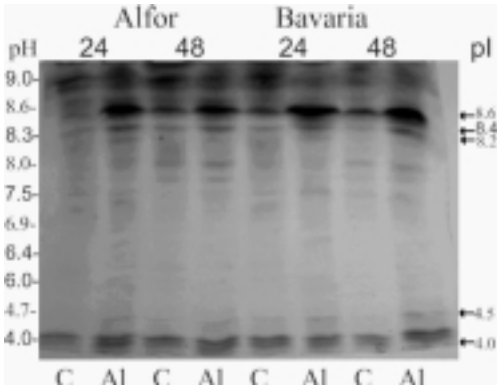

Figure 4. IEF of peroxidase isozymes in root tips of Al-sensitive (Alfor) and Al-resistant (Bavaria) barley cultivars exposed to Al for 24 or 48 hours; an arrow indicates the activated isoperoxidases

isozymes may play some other role in stress response of roots to Al in addition to generate oxygen radicals to lipid peroxidation. Recently, Yamamoto et al. (2001) have reported that butylated hydroxyanisole and lipophilic antioxidant completely prevented lipid peroxidation during Al stress, but did not prevent inhibition of root elongation. On the other hand, lipid peroxidation can occur in very early stage of Al stress and its product after releasing from membrane to cytosol can be detoxified by cytoplasmic peroxidases.

Anionic isoperoxidases are involved in lignification and their activity increases upon wounding. Anionic peroxidase isoforms are induced by wounding and their activity increased up to 72 h in tobacco plants (Lagrimini and Rothhstein 1987). Several Al induced morphological changes are characterised, such as swelling of root tip, sloughing of epidermis and cracks on the root surface. Therefore, it is not surprising that similarly to wounding Al also induce deposition of lignin, and the lignification is positively correlated with Al-sensitivity of plants (Sasaki et al. 1996). In our experiments Al induced in barley roots two anionic peroxidases (pI 4.0 and 4.5), similarly to Ezaki et al. (1996) who reported induction of two anionic isozymes in tobacco cells after Al treatment or Pi starvation. Chang et al. (1984) reported that salt stress tends to shunt growth presumably through lignification, where lignin can serve also as a mechanism to rid the cells of excess oxygen via peroxidation.

Based on our results, we can conclude that Al-induced dramatic enhancement of peroxidase activity in roots of both barley cultivars belongs to the group of nonspecific responses of plants to stress conditions. The fact that the highest activity of peroxidases in roots of Al-sensitive barley cultivar was connected with larger inhibition of root growth should indicate that in Al-sensitive cultivar the range of oxidative stress is more severe than in roots of Al-resistant cultivar. On the other hand, the fact that peroxidation of lipids was not affected by Al suggests that enhanced peroxidase activity can act in similar way as it behaves in salicyl acid induced generation of active oxygen species which, in turn, triggers an increase in cytosolic Ca^{2+} concentration (Kawano and Muto 2000).

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REFERENCES

Bondy S.C., Guo-Ross S.X., Pien J. (1998): Mechanisms underlying the aluminum-induced potentiation of the pro-oxidant properties of transition metals. Neurotoxicologu, *19*: 65–72.

Cakmak I., Horst W.J. (1991): Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiol. Plant., *83*: 463–468.

Chang H., Siegel B.Z., Siegel S.M. (1984): Salinity-induced changes in isoperoxidases in Taro, *Colocasia esculenta*. Phytochemistry, *23*: 233–235.

Ezaki B., Gardner R.C., Ezaki Y., Matsumoto H. (2000): Expression of aluminum-induced genes in transgenic *Arabidopsis* plants can ameliorate aluminum stress and/or oxidative stress. Plant Physiol., *122*: 657–665.

Ezaki B., Tsugita S., Matsumoto H. (1996): Expression of moderately anionic peroxidase is induced by aluminum treatment in tobacco cells: Possible involvement of peroxidase isozymes in aluminum ion stress. Physiol. Plant., *96*: 21–28.

Kawano T., Muto S. (2000): Mechanism of peroxidase actions for salicylic acid-induced generation of active oxygen species and an increase in tobacco cell suspension culture. J. Exp. Bot., *51*: 685–693.

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

Lagrimini L.M., Rothstein S. (1987): Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. Plant Physiol., *84*: 438–442.

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

Richards K.E., Schott E.J., Sharma Y.K., Davis K.R., Gardner R.C. (1998): Aluminum induces oxidative stresss genes in *Arabidopsis thaliana*. Plant Physiol., *116*: 409–418.

Sasaki M., Yamamoto Y., Matsumoto H. (1996): Lignin deposition induced by aluminum in wheat (*Triticum aestivum*) roots. Physiol. Plant., *96*: 193–198

Siegel B.Z. (1993): Plant peroxidases – an organismic perspective. Plant Growth Regul., *12*: 303–312.

Sivaguru M., Horst W.J. (1998): The distal part of the transition zone is the most aluminum sensitive apical root zone of maize. Plant Physiol., *116*: 155–163.

Takahashi Y., Nagata T. (1992): ParB: An auxin-regulated gene encoding gluthatione S-transferase. Proc. Nat. Acad. Sci., USA, *89*: 56–59.

Tamás L., Frič F. (1995): Pathogenesis-related proteins in barley induced by powdery mildew infection. Biologia, Bratislava, *50*: 79–83.

Vianello A., Marci F. (1991): Generation of superoxide anion and hydrogen peroxide at the surface of plant cells. J. Bioenerg. Biomembr., *23*: 409–423.

Yamamoto Y., Hachiya A., Matsumoto H. (1997): Oxidative damage to membranes by a combination of aluminum and iron in suspension-cultured tobacco cells. Plant Cell Physiol., *38*: 1333–1339.

Yamamoto Y., Kobayashi Y., Matsumoto H. (2001): Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. Plant Physiol., *125*: 199–208.

Yermiyahu U., Brauer D.K., Kinriade T.B. (1997): Sorption of aluminum to plasma membrane vesicles isolated from roots of Scout 66 and Atlas 66 cultivars of wheat. Plant Physiol., *115*: 1119–1126.

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ABSTRAKT

Účinek hliníku na aktivitu peroxidázy v kořenech citlivé a tolerantní odrůdy ječmene

Aktivita a izozymové složení peroxidáz izolovaných z kořenů dvou odrůd jarního ječmene s různou citlivostí vůči hliníku byly sledovány během hliníkem indukovaného stresu. Aplikace hliníku do kořenového prostředí vyvolala výrazné zvýšení aktivity peroxidáz zejména v kořenech citlivé odrůdy Alfor, kde dosáhla po 48 h působení hliníku až 5,5násobku hodnoty kontrolních rostlin. Na rozdíl od této odrůdy aktivita peroxidázy v kořenech tolerantní odrůdy Bavaria se zvýšila jen na poloviční hodnotu aktivity odrůdy Alfor. Analýza izozymového složení peroxidázy (SDS-PAGE) ukázala, že v průběhu stresu dochází k aktivaci syntézy nejméně pěti izozymů peroxidázy, z nichž tři jsou kationické (pI 8,2; 8,4 a 8,6) dvě anionické (pI 4,0 a 4,5). Zvýšení aktivity peroxidázy v kořenech ječmene korelovalo s rozsahem inhibice délkového růstu kořenů, což svědčí o tom, že hliníkem indukovaný oxidační stres je v případě citlivé odrůdy Alfor mnohem výraznější než u tolerantní odrůdy Bavaria.

Klíčová slova: jarní ječmen (*Hordeum vulgare* L.); kořeny; růst; izozymy; peroxidáza; hliníkový stres

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