ARBORETUM KÓRNICKIE
ROCZNIK 40, 1995

GABRIELA LORENC-PLUCIŃSKA

Effects of aluminium on free inorganic phosphate levels in Scots pine roots

Abstract

Lorenc-Plucińska G. 1995. Effects of aluminium on free inorganic phosphate levels in Scots pine roots. Arbor. Kórnickie 40: 135-141.

Scots pine seedlings were grown for different periods (3, 6, or 9 weeks) in hydroponic culture with various concentrations of aluminium (0.0, 0.5, 1.0, 2.0, 4.0, mM of $Al(NO_3)_3$, Al) at pH 4.2. The free inorganic phosphate level (P_i) and phosphorylation potential (PP) were determined. In roots of seedlings treated with 0.5 and 1.0 mM of Al, the P_i levels and PP values did not change over a period of 3 weeks. In contrast, the roots treated with 2.0 and 4.0 mM od Al showed higher P_i levels and PP values. Six-weeks incubation of seedlings in the nutrient solution increased P_i at 0.5 and 1.0 mM Al but decreased it at 4.0 mM Al. At this duration of incubation, PP did not undergo any significant changes. After 9 weeks, P_i levels and PP values were both greatly reduced indicating Al stress conditions. The results were discussed with respect to the effect of Al on energy metabolism in roots.

Additional key words: phosporylation potential.

Address: G. Lorenc-Plucińska, Polish Academy of Sciences, Institute of Dendrology, 62-035 Kórnik, Poland.

Accepted for publication, February 1995.

INTRODUCTION

Aluminium (Al) toxicity to plants, especially in acid soils, is well established (Lüttge and Clarkson 1992). One of the first symptoms of Al toxicity involves disturbances in root physiological functions.

Aluminium causes inhibition of meristematic cell division by interfering with DNA replication and related mitotic activity (Matsumoto and Morimura 1980), inducing alterations of root-cell plasma membrane and membrane transport proteins (Horst et al. 1992, Kinraide et al. 1992, Basu et al. 1994),

impairment of enzyme activity (Haug 1984, Pettersson et al. 1988, Copeland and De Lima 1992), injury of the respiratory metabolic pathway (Collier et al. 1993, De Lima and Copeland 1994) and disturbances in the uptake, transport and use of minerals, in particular Ca, Mg, K and P (Cumming et al. 1986, Rengel 1992, Nichol et al. 1993, Kinraide et al. 1994).

Phosphate represents one of the structural constituents of many biomolecules and plays a metabolic role in energy transfer and regulation of activities of different enzymes. Low Al concentrations ($\leqslant 0.1$ mM) may enhance phosphate uptake (Macklon and Sim 1992). Higher Al concentrations ($\geqslant 1.0$ mM) reduce phosphorus uptake and the intracellular fraction of phosphorus in the roots (Bengtsson et al. 1994). Aluminium phosphate precipitation inside the cells makes P immobile and affects phosphorus metabolism and P related processes (Lüttge and Clarkson 1992).

In studying the influence of long-term exposure of Scots pine seedlings to various Al concentrations we have found that Al ions exhibit pronounced effects on adenylate levels in tissue roots (Lorenc-Plucińska and Ziegler 1995). We have suggested that this may result from restricted availability of inorganic phosphate for metabolic processes. Therefore, the purpose of the present study has been to examine the long-term effect of various Al ion concentrations on free inorganic phosphate content in roots of Scots pine seedlings.

MATERIAL AND METHODS

One-year-old nursery-grown seedlings of Scots pine (*Pinus sylvestris* L.) were used. In early May the seedlings were washed free of soil and grown hydroponically in a glass house under conditions previously described (Karolewski and Giertych 1994). The seedlings were treated with aluminium ions (Al, $Al(NO_3)_3$) at concentrations of 0.5, 1.0, 2.0 or 4.0 mM (pH 4.2) according to the method described by Lorenc-Plucińska and Ziegler (1995). Seedlings growing in Al free nutrient solution served as controls. Following 3, 6 or 9 weeks of Al treatment, randomly selected seedlings were harvested, the roots were rinsed with distilled water, blotted between filter paper, then separated from shoots, weighed and frozen in degassed liquid N_2. Each treatment combination in the experiment was studied on 4-6 seedlings (replicates).

Determination of free inorganic phosphate (P_i). The frozen roots were ground in 10% (v/v) $HClO_4$ and 10% (w/v) insoluble polyvinylpyrrolidone (Polyclar AT, Serva) and centrifuged for 15 min at 30000 g. Aliquots of the supernatants were brought to pH 7.8 by adding 1 M bicine in 5 M KOH. After 40 min on ice, the precipitated $KClO_4$ was pelleted (20000 g, 10 min) and the supernatant was used to assay P_i. Free inorganic phosphate was measured spectrophotometrically by a two-step method, which increased sensitivity and

decreased nonextract blanks deriving from biochemicals and enzymes, as described by Wirtz et al. (1980).

Statistical analyses. Statistical data analyses were performed using analysis of variance with the statistical package STATGRAPHICS (INTERSOFT-LAND, USA).

RESULTS AND DISCUSSION

The content of free inorganic phosphate (P_i, Fig. 1) and the values of phosphorylation potential (Fig. 2) in the roots of seedlings not exposed to Al ions increased throughout the 9 weeks incubation of plants in the nutrient solution. The increases fully corresponded to the earlier demonstrated augmented redox status, phosphorylation capacity, ATP/ADP ratios and to the maintained high values (0.85) of adenylate energy charge in Scots pine seedlings growing in the same conditions (Lorenc-Plucińska and Ziegler 1995).

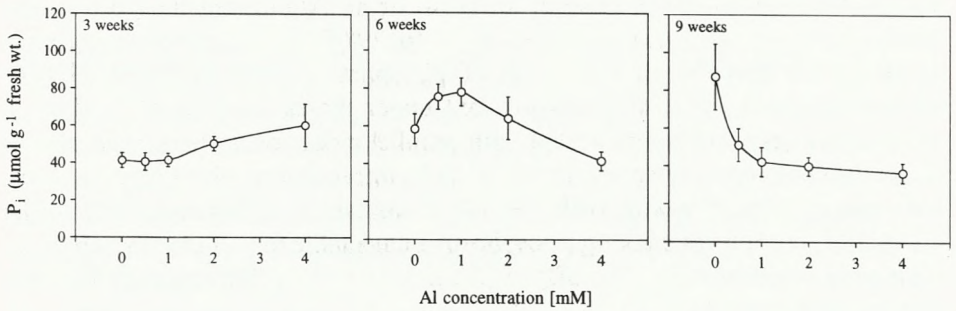

Fig. 1. Effect of Al on free inorganic phosphate (P_i) contents of Scots pine roots. Concentration of Al ($Al(NO_3)_3$) used: 0.0 (control)-4.0 mM; duration of Al treatment: 3, 6 and 9 weeks. Data represent means ±SD.

Growth of Scots pine in a medium with Al induced changes in the P_i content in the roots, depending on the Al concentration used and on the time of exposure. Treatment with Al at concentrations of 0.5 and 1.0 mM did not significantly change P_i levels in the roots over 3 weeks (Fig. 1). On the other hand, P_i levels were enhanced after incubation in the presence of 2.0 or 4.0 mM Al. Increased P_i levels in the roots could also be induced by 6 weeks of seedling incubation in the presence of 0.5 or 1.0 mM Al (Fig. 1). The increase could reflect stimulated uptake of phosphates subsequent to the reduction of negative surface potential by Al^{3+} (Bengtsson et al. 1988), formation of an aluminium phosphate complex on the surface and/or in the free space of the roots

(Pettersson and Strid 1989), absorption of the Al/P complex into root cell vacuoles (Pfeffer et al. 1986, Jensen et al. 1989, Macklon and Sim 1992) and also a decrease of phosphate leakage (Jensen et al. 1989, Asp and Berggren 1990).

The stimulated uptake of phosphates and higher accumulation of phosphorus in Al-treated roots could have been observed when low Al levels ($\leqslant 0.1$ mM) and short-term exposure have been applied (Cumming et al. 1986, Asp et al. 1991, Jan 1991, Macklon and Sim 1992). The increase in P_i, noted in this study under effects of higher Al levels (>0.1 mM) and longer exposure (3 or 6 weeks), could have been obtained probably due to differences in procedure and due to different susceptibility of plant material to Al. In contrast to many tree species and most agricultural crops, Scots pine has been classified as a species which is resistant to soluble inorganic aluminium (Schaedle et al. 1989, Boudot et al. 1994).

Exposure of plants to 4.0 mM Al for 6 weeks or to 0.5-4.0 mM Al for 9 weeks significantly reduced the amount of P_i in the roots (Fig. 1). The inhibitory effect of Al on P_i content may be due to precipitation with P (Asp et al. 1991, Nichol et al. 1993), a modification of the permeability of membranes by Al (Horst et al. 1992, Basu et al. 1994) or an Al-induced decrease in the ATP-dependent H^+ transport (Matsumoto 1988). The drastically reduced levels of P_i may be also a result of phosphate uptake inhibition by Al (Bengtsson et al. 1994). P_i levels lowered under the effect of Al stress (Fig. 1) need not necessarily be associated with parallel reduction in total pool-size of P or phosphates. However, even at high intracellular phosphate concentrations, Al interacts with orthophosphate-containing substances and forms Al-H_2PO_4 complexes, making phosphorus unavailable for cellular metabolism. This may result in symptoms of phosphorus starvation (Pettersson et al. 1988, Jensen et al. 1989).

One of the important metabolic roles played by cellular phosphates involves participation in the synthesis and utilization of ATP and thus in maintenance of appropriate cytoplasmic phosphorylation potential ($PP=[ATP]/[ADP][P_i]$), which controls respiration (Lehninger 1985). Depending upon the level of energy metabolism, ATP/ADP ratio and P_i levels, the values of the PP have varied between 200 and 800 M^{-1}. The higher are PP values, the greater is the energetic potential of the cell (Lehninger 1985).

After 3 and 9 weeks of incubation with Al, PP (Fig. 2) and P_i (Fig. 1) have changed in the same direction while after 6 weeks of the Al stress PP has remained relatively unaltered (Fig. 2) despite changes in P_i level (Fig. 1). On the other hand, changes in PP values have corresponded with the earlier described disturbed ATP/ADP ratios (Lorenc-Plucińska and Ziegler 1995). The increased ATP/ADP ratios after 3 weeks of exposure to Al concentrations of 2.0 or

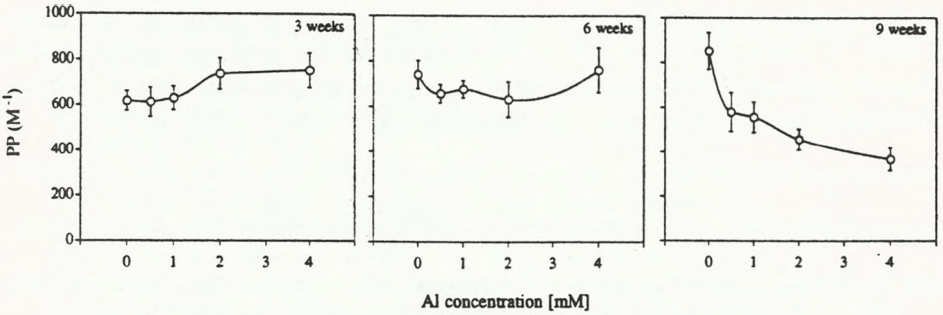

Fig. 2. Effect of Al on the phosphorylation potential (PP = [ATP]/[ADP][P_i]) levels in Scots pine roots. Concentration of Al ($Al(NO_3)_3$) used: 0.0 (control)-4.0 mM; duration of Al treatment: 3, 6 and 9 weeks. The values of ATP and ADP originate from Lorenc-Plucińska and Ziegler 1995, Fig. 1. Data represent means ±SD.

4.0 mM, absence of changes in ATP/ADP ratios after 6 weeks of Al influence at 0.5 to 4.0 mM and decreased ATP/ADP ratios seedlings treated with the same range of Al concentrations for 9 weeks appear to indicate, respectively, stimulated anabolic activity, reduced demand for energy and drastically inhibited metabolic activity (Lorenc-Plucińska and Ziegler 1995). Results of the present study confirm this suggestion. The increased PP values after 3 weeks of incubation with 2.0 or 4.0 mM Al have pointed to high energetic potential in the cells (Fig. 2) with the resulting stimulation of ATP-consuming processes and induction of the metabolic pathway for the repair mechanism. The mechanism however has ceased to function after 6 weeks of Al action, either due to Al stress-reduced requirement for additional pool of energy or because the additional energy source has been exhausted, resulting in unchanged PP values (Fig. 2) and ATP/ADP ratios (Lorenc-Plucińska and Ziegler 1995) despite disturbed P_i content (Fig. 1). One the other hand, the drastic decrease in PP level (Fig. 2) after 9 weeks of Al action indicates deranged energy metabolism, probably due to decreased P_i level (Fig. 1), inhibited respiratory activity of mitochondria (Collier et al. 1993, De Lima and Copeland 1994), stimulated fermentation metabolism (Copeland and De Lima 1993) and interference of Al with cellular compartmentation, i.e. damage of membranes (Horst et al. 1992, Basu et al. 1994).

In conclusion, aluminium ions affect the free inorganic phosphate levels and the values of phosphorylation potential in cells of Scots pine roots and as a consequence the bioenergetic state of the roots is disturbed Aluminium toxicity increases with increase in concentration of aluminium ions and with duration of exposure.

Acknowledgements – This work was partly supported by the Polish-German Collaboration Fund from resources of the Federal Republic of Germany. I wish to thank Ass. Prof. Dr. P. Karolewski for his generous gift of plants, Ass. Prof. Dr. J. Oleksyn for assistance in graph preparation as well as Mrs. K. Grewling and A. Niemir for their technical assistance.

LITERATURE

ASP H., BERGGREN D.,1990. Phosphate and calcium uptake in beech (*Fagus sylvatica*) in the presence of aluminium and natural fulvic acids. Physiol. Plant. 80: 307-314.

ASP H., BENGTSSON B., JENSEN P., 1991. Influence of aluminium on phosphorus and calcium localization in roots of beech (*Fagus sylvatica*). Physiol. Plant. 83: 41-61.

BASU A., BASU U., TAYLOR G.J., 1994. Induction of microsomal membrane proteins in roots of an aluminium-resistant cultivar of *Triticum aestivum* L. under conditions of aluminium stress. Plant Physiol. 104: 1007-1013.

BENGTSSON B., ASP H., JENSEN P., BERGGREN D., 1988. Influence of aluminium on phosphate and calcium uptake in beech (*Fagus sylvatica*) grown in nutrient solution and soil solution. Physiol. Plant. 74: 299-305.

BENGTSSON B., ASP H., JENSEN P., 1994. Uptake and distribution of calcium and phosphorus in beech (*Fagus sylvatica*) as influenced by aluminium and nitrogen. Tree Physiol. 14: 63-73.

BOUDOT J.P., BECQUER T., MERLET D., ROUILLER J., 1994. Aluminium toxicity in declining forests: a general overview with a seasonal assessment in a silver fir forest in the Vosges mountains (France). Ann. Sci. For. 51: 27-51.

COLLIER D.E., ACKERMANN F., SOMERS D.J., CUMMINS W.R., ATKIN O.K., 1993. The effect of aluminium exposure on root respiration in an aluminium-sensitive and an aluminium-tolerant cultivar of *Triticum aestivum*. Physiol. Plant. 87: 447-452.

COPELAND L., DE LIMA M.L., 1992. The effect of aluminium on enzyme activities in wheat roots. J. Plant Physiol. 140: 641-645.

CUMMING J.R., EVANS L.S., ECKERT T.R., 1986. Effect of aluminium on ^{32}P uptake and translocation by red spruce seedlings. Can. J. For. Res. 16: 864-867.

DE LIMA M.L., COPELAND L., 1994. The effect of aluminium on respiration of wheat roots. Physiol. Plant. 90: 51-58.

HAUG A.R., 1984. Molecular aspects of aluminium toxicity. CRC Crit. Rev. Plant Sci. 1: 345-373.

HORST W.J., ASHER C.J., CAKMAK I., SZULKIEWICZ P., WISSEMEIER A.H., 1992. Short-term responses of soybean roots to aluminium J. Plant Physiol. 140: 174-178.

JAN F., 1991. Aluminium effects on growth, nutrient net uptake and transport in 3 rice (*Oryza sativa*) cultivars with different sensitivity to aluminium. Physiol. Plant. 83: 441-448.

JENSEN P., PETTERSSON S., DRAKENBERG T., ASP H., 1989. Aluminium effects on vacuolar phosphorus in roots of beech (*Fragus sylvatica* L.). J. Plant Physiol. 134: 37-42.

KAROLEWSKI P., GIERTYCH M.J., 1994. Influence of aluminium, cadmium, manganese and lead ions on the level of phenols in needles and roots and on root respiration of Scots pine (*Pinus sylvestris* L.) seedlings grown in laboratory conditions. Acta Soc. Bot. Pol. 63: 29-35.

KINRAIDE T.B., RYAN P.R., KOCHIAN L.V., 1992. Interactive effects of Al^{3+}, H^+, and other cations on root elongation considered in terms of cell-surface electrical potential. Plant Physiol. 99: 1461-1468.

KINRAIDE T.B., RYAN P.R., KOCHIAN L.V., 1994. $Al^{3+}-Ca^{2+}$ interactions in aluminium rhizotoxicity. Planta 192: 104-109.

LEHNINGER A.L., 1985. Biochemie. Verlag Chemie, Weinheim, Deerfield Beach, Florida, Basel, pp. 443.

LORENC-PLUCIŃSKA G., ZIEGLER H., 1995. The effect of aluminium on adenylate levels in Scots pine roots. Acta Physiol. Plant. 17, 225-232.

LÜTTGE U., CLARKSON D.T., 1992. Mineral nutrition: aluminium. In: Behnke H.D., Esser K., Kubitzki K., Runge M., Ziegler H. (eds). Progress in Botany, vol. 53. Springer Verlag Berlin, Heidelberg, New York, 63-77.

MACKLON A.E.S., SIM A., 1992. Modifying effects of non-toxic levels of aluminium on the uptake and transport of phosphate in ryegrass. J. Exp. Bot. 43: 915-923.

MATSUMOTO H., 1988. Inhibition of proton transport activity of microsomal membrane vesicles of barley roots by aluminium. Soil Sci. Plant Nutr. 34: 499-506.

MATSUMOTO H., MORIMURA S., 1980. Repressed template activity of chromatin of pea roots treated by aluminium. Plant and Cell Physiol. 21: 951-959.

NICHOL B.E., OLIVEIRA L.A., GLASS A.D.M., SIDDIGI M.Y., 1993. The effects of aluminium on the influx of calcium, potassium, ammonium, nitrate, and phosphate in an aluminum-sensitive cultivar of barley (*Hordeum vulgare* L.). Plant Physiol. 110: 1263-1266.

PETTERSSON A., HÄLLBOM L., BERGMAN B., 1988. Aluminium effects on uptake and metabolism of phosphorus by the cyanobacterium *Anabaena cylindrica*. Plant Physiol. 86: 112-116.

PETTERSSON S., STRID H., 1989. Initial uptake of aluminium in relation temperature and phosphorus status of wheat (*Triticum aestivum* L.) roots. J. Plant Physiol. 134: 672-677.

PFEFFER P.E., TU S.I., GERASIMOWICZ W.V., CAVANAUGH J.R., 1986. *In vivo* ^{31}P NMR studies of corn root tissue and its uptake of toxic metals. Plant Physiol. 80: 77-84.

SCHAEDLE M., THORNTON F.C., RAYNAL D.J., TEPPER H.B., 1989. Response of tree seedlings to aluminium. Tree Physiol. 5: 337-356.

RENGEL Z., 1992. Role of calcium in aluminium toxicity. New Phytol. 121: 499-513.

WIRTZ W., STITT M., HELDT H., 1980. Enzymic determination of metabolites in the subcellular compartments of spinach protoplasts. Plant Physiol. 66: 187-193.

Wpływ glinu na zawartość wolnego, nieorganicznego fosforanu w korzeniach sosny zwyczajnej

Streszczenie

Badano wpływ jonów glinowych (Al) na zawartość wolnego, nieorganicznego fosforu (P_i) oraz poziom potencjału fosforylacyjnego (PP) korzeni sosny zwyczajnej. Doświadczenia wykonano na jednorocznych siewkach traktowanych roztworami azotanu glinowego w stężeniach: 0.0-kontrola, 0.5, 1.0, 2.0 i 4.0 mM (pH 4.2) przez 3, 6 i 9 tygodni.

Stwierdzono, że toksyczność Al wzrasta wraz ze wzrostem stężenia Al i czasu ekspozycji. Zawartość P_i w korzeniach była stymulowana pod wpływem 3-tygodniowego działania Al w stężeniu 2.0 i 4.0 mM oraz 0.5 i 1.0 mM Al przez 6 tygodni. Natomiast 6-tygodniowa inkubacja siewek w pożywce z Al w stężeniu 4.0 mM oraz 9-tygodniowa w stężeniu od 0.5 do 4.0 mM drastycznie obniżała P_i.

Poziom PP wzrastał pod wpływem 3-tygodniowej ekspozycji roślin na działanie Al w stężeniu 2.0 i 4.0 mM. Z drugiej strony, 6- i 9-tygodniowe traktowanie siewek jonami glinu w stężeniu od 0.5 do 4.0 mM prowadziło, odpowiednio, do nieznacznych zmian i istotnego poziomu PP.

W pracy przedyskutowano wpływ powyższych zaburzeń na regulację metabolizmu energetycznego komórek korzeni.