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Physiological Role of Root Surface Phosphatases in Adaptation Strategies of *Alyssum bertolonii* Desv. to Serpentine Edaphic Conditions

By

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With 3 Figures

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Summary

Gabbrielli R., Pandolfini T. & Pucci B. 1995. Physiological role of root surface phosphatases in adaptation strategies of *Alyssum bertolonii* Desv. to serpentine edaphic conditions. – Phyton (Horn, Austria) 35 (2): 189–197, 3 figures. – English with German summary.

Root surface phosphatase activity and P nutrition in the presence of Ni^{2+} was studied in *Alyssum bertolonii* Desv., a Ni-accumulating species of Tuscan serpentine soils. Increasing external concentrations of inorganic phosphate reduced phosphatase activity. In the presence of an inhibiting concentration of inorganic phosphate, Ni^{2+} stimulated phosphatase activity of seedlings treated for seven days but did not affect root P content. Root surface phosphatase showed a higher activity towards phytate in comparison with other P organic substrates. Increased P levels were detected in the roots of Ni-treated seedlings supplied with phytate. We discuss the possible physiological role of root cell wall phosphatases, which in this study was shown to be tolerant to Ni^{2+}.

Zusammenfassung

Gabbrielli R., Pandolfini T. & Pucci B. 1995. Die physiologische Rolle der Wurzeloberflächen-Phosphatasen in den Anpassungsstrategien von *Alysssum*

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bertolonii DESV. auf Serpentinböden. – Phyton (Horn, Austria) 35 (2): 189–197, mit 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die Aktivität der Wurzeloberflächen-Phosphatase und P-Ernährung in Anwesenheit von Ni^{2+} wurde am *Alyssum bertolonii* DESV. untersucht, einer Ni-akkumulierenden Art, die auf den Serpentinböden der Toskana wächst. Steigende äußere Konzentrationen anorganischen Phosphats verminderten die Aktivität der Wurzeloberflächen-Phosphatase. Bei einer hemmenden Pi Konzentration stimulierte Ni^{2+} die Phosphatase-Aktivität von Sämlingen, die sieben Tage behandelt worden waren, aber beeinflußte nicht den Gehalt an Wurzel-P. Die Wurzeloberflächen-Phosphatase zeigte dem Phytat gegenüber eine höhere Aktivität im Vergleich zu anderen P-haltigen organischen Substraten. Es ergaben sich erhöhte P-Werte in den Wurzeln Ni-behandelter Sämlinge , denen Phytat zugeführt worden war. Wir diskutieren die mögliche physiologische Rolle der Phosphatasen der Wurzel-Zellwand, die, wie diese Studie zeigte, Ni^{2+} tolerierten.

Introduction

Phosphate deficiency has often been indicated as one of the main factors involved in soil infertility (BIELESKI 1973). A low P concentration has frequently been observed in serpentine soils (for instance in Tuscan ophiolitic outcrops total P is about 90 $\mu g\ g^{-1}$) as well as a high content of heavy metals, (as Ni^{2+}, Cr^{2+},Co^{2+}), and Mg^{2+} and a low level of Ca^{2+} and nitrates (BROOKS 1987).

Root extracellular phosphatases may be important to P nutrition efficiency, as they may enhance the availability and utilization of soil organic P (BIELESKI & JOHNSON 1972, DRACUP & al. 1984, HELAL 1990). In plants subjected to phosphate deprivation, an increased phosphatase activity together with a higher capacity of P uptake has been reported (CLARKSON & SCATTERGOOD 1982, LEE 1982, 1988).

It had been observed in two serpentine plants, *Festuca rubra* L. and *Alyssum bertolonii* DESV. that phosphatase activity is increased by high Ni^{2+} concentrations, which is one of the factors responsible for the phytotoxicity of serpentine soils (JOHNSTON & PROCTOR 1984, GABBRIELLI & al. 1989). In these researches it has not been investigated if the increase in phosphatase activity is a direct effect of Ni^{2+} or it is a consequence of a reduced P uptake due to Ni^{2+} toxicity. Therefore it is also unclear whether this response could represent an adaptation to the P deficiency of serpentine soils.

The aim of this study has been to verify in *A. bertolonii*, a Ni-accumulating species of Tuscan serpentine soils, if the Ni-induced stimulation of root surface phosphatase activity was associated to an alteration of P tissue content. Moreover, in order to elucidate the physiological role of root surface phosphatases, the affinity of these enzymes towards different P organic substrates was evaluated, and the isoenzymatic patterns and Ni^{2+} resistance of wall-bound and soluble phosphatases were compared.

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Materials and Methods

Seeds of *A. bertolonii* were collected on the ultramafic outcrop of Pieve S. Stefano (Arezzo, Tuscany). The seedlings were grown for 10 d hydroponically as reported in GABBRIELLI & al. 1989. In the nutrient solution P was present as $NH_4H_2PO_4$, while in the treatments NaH_2PO_4 was used to avoid the effects of different concentrations of NH_4^+ on phosphatase activity (O'CONNELL & GROVE 1985); Ni^{2+} was added as sulphate. The presence of Ni^{2+} inhibited bacterial growth.

All experiments were repeated three times. The significance of differences between control and treatments was evaluated by analysis of variance.

Experiments on seedlings

Experiment 1. The seedlings were kept in diluted Arnon solution (1:10 v/v) containing increasing concentrations of P. Control seedlings were grown in a P-deprived Arnon solution. After 10 d biomass, root and shoot P concentration and root surface phosphatase activity were measured.

Experiment 2. The seedlings were first grown for 3 d in diluted Arnon solution and then kept for 7 d in solutions containing increasing concentrations of Ni^{2+} (0, 5, 10 µM) and inorganic (0.1 mM NaH_2PO_4) or organic (0.05 mM Na-phytate) P. Root surface phosphatase activity and root P and Ni^{2+} concentrations were measured.

Experiments on excised roots.

Experiment 3. Roots excised from 10 d old seedlings were treated for 1 h with each of the following phosphoric substrates (2.5 mM): AMP, ADP, ATP, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-phosphate, p-nitrophenyl phosphate, Na-phytate). The extent of hydrolysis of these substrates was calculated from the quantity of P released into the external solution. These values were corrected by substracting the quantity of P released by each substrate solution in the absence of roots. P hydrolyzed by root surface phosphatase was expressed as mmol P g^{-1} DW h^{-1}.

Experiment 4. Root surface phosphatase activity was measured in excised roots kept for 1 h in a medium containing 0.05 mM p-nitrophenyl phosphate (pNPP) and increasing concentrations of Ni^{2+} (5, 50, 500 µM). P concentration was determined in excised roots treated for 24 h with 3 mM pNPP and the same Ni^{2+} concentrations.

P and Ni^{2+} analysis

The samples, rinsed in distilled water, were dried at 80° C for 24 h and wet-ashed in a nitric and perchloric acid mixture (5:2 v/v). Ni^{2+} was determined by atomic absorption spectrophotometry and P concentration measured according to MURPHY & RILEY 1962.

Phosphatase assay

Phosphatase activity was assayed by spectrophotometric measurement of the rate of formation of p-nitrophenol (pNP) produced by enzyme catalysis of pNPP hydrolysis (SHINSHI & al. 1976). For the determination of root surface phosphatase activity, freshly cut 1 cm root tips were incubated in Na-acetate buffer (pH 4.8) with 3 mM pNPP (GABBRIELLI & al. 1989). Enzyme activity was expressed as µmol pNP g^{-1} root DW min^{-1}.

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Preparation of enzymic fractions

Enzymic fractions were separated from roots of control seedlings following the method of HASEGAWA & al. 1976. Root homogenates, obtained as described by HASEGAWA & al. 1976, were filtered with Whatmann 1 and the filtrate was centrifuged at 15000 xg for 10 min at 4° C . The resulting supernatant was designated as "soluble fraction". The residue remaining on the filter paper was used for the extraction of cell wall phosphatases. It was washed several times with cold 0.2 % Triton X-100 and distilled water and strained through four layers of cheesecloth. Afterwards the residue was incubated in 1M NaCl at 4° C overnight and centrifuged at 1500 xg at 4° C. The supernatant, designated as "NaCl-soluble wall fraction" was dialyzed against distilled water and applied to a Sephadex G-25 column. The pellet was treated overnight at room temperature with 0.5 % (w/v) cellulase and 2.5 % (v/v) pectinase in 0.1 M Na-acetate buffer (pH 5.0) giving the "enzyme-extracted wall fraction". Phosphatase activity was assayed in all the three fractions in the presence (10, 100 μM) and absence of Ni^{2+}.

Isoelectric focusing

Multiple forms of acid phosphatase were separated by isoelectric focusing using polyacrylamide gels (LKB T = 5 %) containing 2.4 % ampholytes (pH 3.5–9.5).

Isoenzymes were stained with Na-naphtyl-(1)-phosphate and Fast blue BB salt (MAIER 1978) and fixed in 0.2 M Na-acetate buffer (pH 5.0) for 24 h.

Results

The effect of Pi external concentration on seedling P content and on the activity of root surface phosphatases of *A. bertolonii* seedlings is shown in figure 1 (Exp. 1). The progressive increase of root P concentration and translocation to the aerial part is evident.

In the same conditions (Fig. 1), the activity of root extracellular phosphatases decreased as Pi concentration in the culture medium increased. The plants grown in a P-deprived medium exhibited the highest phosphatase activity.

In seedlings of *A. bertolonii* treated with Ni^{2+} for 7 days (Exp. 2), phosphatase activity was stimulated despite the inhibitory effect of 0.1 mM NaH_2PO_4 in the medium (Tab. 1, Fig. 1).

Ni^{2+} did not directly affect P absorption from inorganic substrates, however an enhanced root P concentration was observed when P was supplied as organic substrate (Na-phytate) (Tab. 1).

Root surface phosphatase of *A. bertolonii* hydrolyzed the phosphoric esters of various organic substrates and showed the highest affinity towards Na-phytate (Fig. 2, Exp. 3). On the other hand, there was a low affinity towards organic anhydrides, especially ATP.

In experiments carried out using excised roots (Exp. 4), 1 h treatments with different Ni^{2+} concentrations and pNPP, did not have any stimulatory effect either on root phosphatase activity or on P root concentration (Tab. 2).

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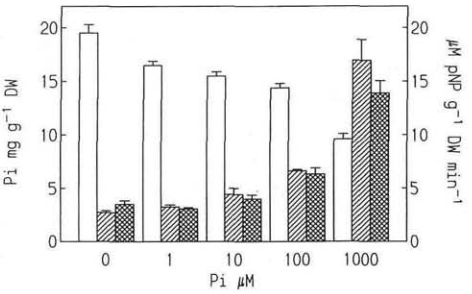

Fig. 1. Effect of external Pi (as NaH_2PO_4) concentration on root surface phosphatase activity (□) and on root (▨) and shoot (▩) P concentration. Values are means ± SE (n = 3).

Table 1

Phosphatase activity (µmol pNP g^{-1} DW min^{-1}), phosphorus (mg g^{-1} DW) and Ni^{2+} concentration (µg g^{-1} DW) in roots of seedlings treated with Ni^{2+} and with (A) inorganic (0.1 mM NaH_2PO_4) or (B) organic (0.05 mM Na-phytate) phosphorus for 7 d. Values are means ± SE (n = 3).

Treatment Ni^{2+} µM	Phoshatase activity (µmol pNP g^{-1} DW min^{-1})	P (mg g^{-1} DW)		Ni^{2+} (µg g^{-1} DW)
		A	B	
0	13.3 ± 0.8	14.2 ± 0.8	23.0 ± 0.3	369 ± 40
5	25.9 ± 10.6	15.2 ± 1.0	27.0 ± 0.5*	509 ± 23
10	29.4 ± 4.7**	16.0 ± 0.6	29.7 ± 1.1*	676 ± 48*

* $P < 0.05$, ** $P < 0.01$.

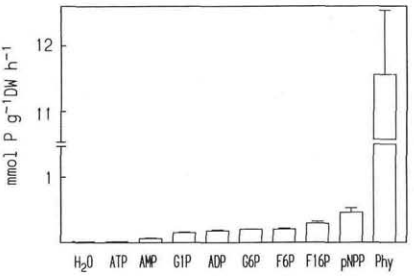

Fig. 2. Specificity of root surface phosphatase for various organic P compounds. The extent of hydrolysis of the substrates was calculated from the quantity of P released into the external solution. Treatment solutions contained the following substrates (2.5 mM): ADP, AMP, ATP, G1P (glucose-1-phosphate), G6P (glucose-6-phosphate), F6P (fructose-6-phosphate), F16P (fructose-1,6-phosphate), pNPP (p-nitrophenyl phosphate), PHY (Na-phytate). Values are means ± SE (n=3).

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Table 2

Phosphatase activity (μmol pNP g^{-1} DW min^{-1}) in excised roots treated for 1 h with increasing concentrations of Ni^{2+} and 0.05 mM pNPP. Pi uptake measured after a 24 h treatment with the same Ni^{2+} concentrations and 3 mM pNPP. Values are means ± SE (n = 3).

Treatments	Phosphatase activity (μmol pNP g^{-1} DW min^{-1})	root P (mg g^{-1}DW)
pNPP	5.2 ± 1.5	5.41 ± 0.19
pNPP + 5μM Ni^{2+}	7.1 ± 0.9	4.63 ± 0.30
pNPP + 50μM Ni^{2+}	5.4 ± 1.3	4.62 ± 0.23
pNPP + 500μM Ni^{2+}	6.4 ± 1.3	5.67 ± 0.23

Similar results were obtained studying the *"in vitro"* effects of Ni^{2+} on "NaCl-soluble wall fraction" and "enzyme-extracted wall fraction" (Tab. 3). Both Ni^{2+} concentrations had no effect on phosphatase activity of both fractions. In the same conditions, phosphatase activity of the "soluble fraction" was reduced (Tab. 3). Differences among the phosphatases of the three fractions are also shown by isoelectrophoretic analysis (Fig. 3). The isoenzymatic patterns of soluble and wall phosphatases varied. Particularly relevant were the differences between the "soluble fraction" and the "NaCl-soluble wall fraction". The phosphatase of the "NaCl-soluble wall fraction" showed a cathodic band which is absent in those of the "soluble fraction" and of the "enzyme-extracted wall fraction".

Table 3

Effects of Ni^{2+} on the activity of soluble and cell-wall phosphatases. (A) wall phosphatases solubilized with 1 mM NaCl. (B) wall phosphatases solubilized with 0.5 % pectinase- 2.5 % cellulase. Values are means ± SE (n = 3).

Treatments Ni μM	Phosphatase activity ($\triangle A_{400}$ h^{-1})		
	soluble	cell-wall	
		A	B
0	3.10 ± 0.036	2.41 ± 0.018	1.88 ± 0.042
10	2.83 ± 0.048*	2.39 ± 0.024	1.87 ± 0.054
100	2.51 ± 0.024**	2.52 ± 0.078	1.85 ± 0.024

* $P < 0.05$, ** $P < 0.01$

Conclusions

Plants growing on serpentine soils seem to possess an adaptation mechanism which is the result of tolerance strategies developed in response to the various factors determining serpentine infertility (KRUCKEBERG 1992). In *A. bertolonii* the tolerance strategy to high concentrations of Ni^{2+} may be related to the adaptation mechanism to low P.

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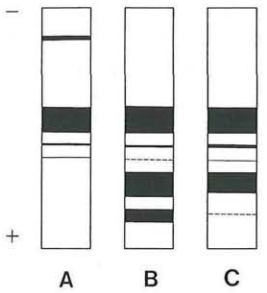

Fig. 3. Isoelectric focusing in polyacrylamide gel of phosphatases from different enzymic fractions. (A) "NaCl-soluble wall fraction", (B) "enzyme-extracted wall fraction" and (C) "soluble fraction".

P deficiency causes an increase in phosphatase activity (BIELESKI & JOHNSON 1972, DRACUP & al. 1984). In *A. bertolonii* this enzyme is also stimulated by Ni^{2+} (GABBRIELLI & al. 1989). In this species, increasing the concentration of Ni^{2+} does not depress or increase root total P concentration when plants are supplied with inorganic P. Therefore, the effect of Ni^{2+} seems unlikely to be the result of an alteration of P uptake. However, Ni^{2+} does not affect phosphatase activity directly, since a stimulatory effect occurs only in seedlings grown in Ni^{2+} for several days and not in excised roots treated for 1 h nor in crude phosphatase extracts obtained from control seedlings to which Ni^{2+} was added. The Ni-induced increase in phosphatase activity might be the consequence of an enhanced release of the enzymes to the root extracellular spaces or a *"de novo"* synthesis.

In *A. bertolonii*, an increasing Ni^{2+} concentration stimulates root extracellular phosphatase activity and consequently the hydrolysis of organic phosphate. This leads to a greater availability of Pi, which is easily absorbed. Therefore, the most plausible role of root surface phosphatases of *A. bertolonii* might be to make possible the utilization of organic P compounds derived from the decomposition of organic matter from other organisms or from the plant itself. This is consistent with the high affinity of these enzymes towards organic substrates such as Na-phytate, a common form of organic P storage in the soil.

The differences in the isoenzymatic patterns of soluble and cell wall phophatases, particularly evident in the case of "NaCl-soluble fraction", might be related to distinct physiological functions. Moreover it is interesting to note that *"in vitro"* cell wall phosphatases of both fractions are more resistant to Ni^{2+} than soluble enzymes, the majority of which are presumably of intracellular origin. In metal tolerant plants intracellular enzymes can be protected by cytoplasmic mechanisms of defence (ERNST 1976).

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Further research is necessary to verify how and to what extent, in natural conditions, the effect of Ni^{2+} on phosphatase activity and on P nutrition could contribute to the adaptation process to serpentine soils.

Acknowledgements

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Recensiones

Schierwater, B., Streit, B., Wagner, G. P., Desalle R. (editors) 1994. Molecular Ecology and Evolution: Approaches and Applications. 640 pages, Hardcover. – sfr 228,–/ DM 269,–/ öS 2090,40/ US $ 165.00/ 98,–/ FF 998,–. – Birkhäuser Verlag.

The last 25 years have witnessed a revolution in the way that ecologists and geneticists approach their disciplines. This perplexing fast change has been fueled by the ability to use modern molecular techniques that are now reshaping the spectrum of questions in ecology and evolution. This molecular revolution has appeared in waves. First isozyme electrophoresis was the technique around which much of genetic work in ecology and evolution was based. Next recombinant DNA technology and finally the ability to amplify DNA sequences via polymerase chain reaction (PCR) are extensively used in ecological and evolutionary studies.

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