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Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms

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Summary. The efficiency of phosphatases produced by clover, barley, oats and wheat was investigated in soils treated with sodium glycerophosphate, lecithin and phytin. Root exudates of aseptically grown clover were also examined for the breakdown of different organic P compounds in order to test the efficiency of plant-produced phosphatases. In general, the plants were able to use P from all the organic sources used in the study almost as efficiently as inorganic sources. Dry-matter yield, P uptake, acid and alkaline phosphatase activity and microbial population were increased in all the P treatments. Organic P enhanced alkaline phosphatase activity. Lecithin increased fungal, and phytin bacterial growth. There was no alkaline phosphatase activity in the asepticallly grown clover root exudates. Phosphatase released in aseptic culture after 4 weeks of clover growth was able to efficiently hydrolyse sodium glycerophosphate, lecithin and phytin. The amount of organic P hydrolysed in this and in the soil experiment surpassed plant uptake by a factor of 20. This suggests that the limiting factor on plant utilization of organic P is the availability of hydrolysable organic P sources.

Key words: Organic soil P – Phosphatase – P uptake – *Trifolium alexandrinum* – *Hordeum vulgare* L. – *Avena sativa* L. – *Triticum aestivum* L.

In most agricultural soils, organic P comprises 30%–80% of total P. The largest fraction of organic P – approximately 50% – appears to be in the form of phytin and its derivatives (Pederson 1953; Dalal 1978). The nucleic-acid content is not known, although some authors suggest that it comprises not more than 5% of the organic P (Adams et al. 1954). The amount of lecithin in the soil is small, possibly about 1% of the total soil P. The remainder of the soil organic P occurs in unknown forms.

The significance of these organic P compounds for the P nutrition of plants is not clear. In order to become available these P compounds must be hydrolysed by phosphatases, which may be of plant or microbial origin. The production and distribution of phosphatases in soils have been extensively studied in recent years (Tarafdar and Chhonkar 1978; Boero and Thien 1979).

Helal and Sauerbeck (1984) found increased phosphatase activity in the vicinity of maize roots but no significant change in organic P in this region. Tarafdar and Jungk (1987), using a technique that divides the soil next to the root into layers about 0.2 mm thick, showed that phosphatase activity at the root surface was up to eight times greater than in soil further away. They also found an appreciable decrease in organic P very close to the root surface. It has not yet been established, however, whether soil phosphatases are derived from the root or from an increased microbial rhizosphere population. The present results indicate that organic P might be of significance for the P nutrition of plants.

The aim of the present research was to study the capacity of plant roots to secrete phosphatase and to hydrolyse organic P compounds similar to those found in soil, and to investigate the extent to which plants can use organic P compounds for growth.

Materials and methods

Solution culture experiment. In order to study the capacity of plants to produce phosphatases, clover (*Trifolium alexandrinum* L.)

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was grown under aseptic conditions. Healthy, viable seeds were surface-sterilized by treatment with a 1:1 (v/v) mixture of H_2O_2 and absolute alcohol for 2 min, followed by treatment with 0.05% $HgCl_2$ with traces of HCl for 2 min. The sterilizing agents were drained off aseptically, and the seeds were washed 10–12 times in sterile distilled water to remove traces of the sterilizing chemicals.

Fifty sterilized seeds were transferred aseptically into each sterilized agar plate for germination (Junga 1984). The agar plates were then transferred to the aseptic room under a 16-h day with a 2500–3000 lux light and 65% ± 5% relative humidity. The day temperature was 25 ± 1 °C and the night temperature 20 °C.

After 7 days 30 aseptic plants from each plate were placed in a sterilized flask containing 1200 ml of nutrient solution with the following composition: NH_4^+ 225 μ*M*; NO_3^- 675 μ*M*; K 250 μ*M*; Ca 450 μ*M*; Mg 315 μ*M*; SO_4 315 μ*M*; Cl 15 μ*M*; Fe 5 μ*M*; Mn 1 μ*M*; B 5 μ*M*; Zn 0.1 μ*M*; Cu 0.1 μ*M* and Mo 0.12 μ*M*. Sterilized air was bubbled through each flask to provide aeration and mixing of the nutrient solution. The treatments consisted of four doses (2, 5, 10 and 20 mg P/100 ml) of sodium glycerophosphate ($C_3H_5(OH)_2PO_4Na_2 \cdot H_2O$), lecithin ($C_{36}H_{74}NO_9P$) or phytin ($C_6H_{18}O_{24}P_6$). The treatments were replicated three times and the plants were grown in an aseptic room for 4 weeks. An aliquot sample was drawn from each flask and streaked on yeast-extract mannitol agar prior to the harvest of plants to detect possible contamination, and only those that were free of contaminants were used for analysis. The nutrient solution was analysed for phosphatase activity and inorganic P immediately after harvest. Linear regression was used to test the relationship between enzyme activity and inorganic P present in the solution.

Pot experiment. A low-P loess subsoil taken from a depth of about 1.5 m was air dried, mixed and ground to pass through a 2-mm sieve. The soil had a pH of 7.7 and 0.64 mg 4 *N* HCl extractable P/100 g. Each pot was filled with 600 g of dry soil. All treatments received a basic fertilizer per 100 g soil of: NH_4NO_3 120 mg; K_2SO_4 51.6 mg; $MgSO_4 \cdot H_2O$ 24 mg; H_3BO_3 0.12 mg; $CuSO_4 \cdot 5\ H_2O$ 2.4 mg; $MnSO_4 \cdot H_2O$ 2.4 mg; $(NH_4)_6\ MO_7O_{24} \cdot 4\ H_2O$ 0.06 mg; $Co(NO_3)_2 \cdot 6\ H_2O$ 0.06 mg; $ZnSO_4 \cdot 7\ H_2O$ 0.12 mg; Fetrilon 3 mg. The inorganic P-treated pots received in addition 20 mg P/100 g soil as $Ca(H_2PO_4)_2 \cdot H_2O$ and the organic P-treated pots received 20 mg P as sodium glycerophosphate or lecithin or phytin. There were three replications in each treatment.

The following four plant species were used: clover (*Trifolium alexandrinum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) and wheat (*Triticum aestivum* L.). Ten plants were grown in each pot. The experiment was conducted in a controlled-climate room under a 14-h day with light at 56 W/m², a temperature of 27 ± 1 °C and 65% ± 5% relative humidity, and a 10-h dark period at 18 ± 1 °C. The pots were watered daily to field capacity with deionized water.

The plants were harvested after 40 days of growth and the dry-matter yield was recorded. Plant materials were analysed for P. The soil of each pot was mixed and the roots separated carefully from the soil. A portion of the mixed sample was placed immediately in a cold room at 4 °C and analysed within 7 days for phosphatases. The remaining samples were analysed for inorganic P and the microbial population.

Analytical procedures. Soil inorganic P was extracted by shaking 1 g of soil in 50 ml 4 *N* HCl for 2 h (Wrenshall and Dyer 1939). Plant P was measured colorimetrically after wet ashing and complexing as molybdovanadophosphoric acid (Kitson and Mellon 1944). The microbial popuiation was determined by the standard dilution plate technique, using selective media. Fungi were counted using Martin's Rose Bengal agar with streptomycin (Martin 1950), and bacteria on Thorton's medium (Thorton 1922). Acid phosphatase activity was assayed by the method of Tabatabai and Bremner (1969), using acetate buffer (0.1 *M*; pH 5.4) and *p*-nitrophenyl phosphate. Alkaline phosphatase activity was assayed using disodium phenyl phosphate, by the method of Kramer and Yerdei (1960). All phosphatase activity was expressed in terms of enzyme units (EU). One unit of acid phosphatase is that amount of enzyme per ml solution or g soil which hydrolyses 1.0 μmol of *p*-nitrophenyl phosphate per min at pH 5.4 and 35 °C, and one unit of alkaline phosphatase is that amount of enzyme which hydrolyses 1.0 μmol of nitrophenyl phosphate per min at pH 9.4 and 35 °C.

Results

In the solution experiment the plants only produced acid phosphatase (Table 1). There was no alkaline phosphatase present in the solution. The P uptake by

Table 1. Production of acid phosphatase and hydrolysis of three different organic P compounds by clover (4 weeks old) in aseptic solution culture

P compound	Dose (mg P/100 ml)	Acid phosphatase activity (EU × 10^{-3})	Inorganic P in solution (mg/flask)	P uptake by plants (mg/flask)	Applied organic P recovered in inorganic form (%)
Without P	–	0.108	–	–	–
Sodium glycerophosphate	2	0.255	14.4	0.85	60.0
	5	0.376	21.2	0.91	35.3
	10	0.374	21.1	0.92	17.6
	20	0.334	19.7	0.91	8.2
Lecithin	2	0.238	13.4	0.87	55.8
	5	0.319	18.0	0.90	30.0
	10	0.376	21.1	0.92	17.6
	20	0.356	20.4	0.94	8.5
Phytin	2	0.249	14.0	0.88	58.3
	5	0.359	20.0	0.91	33.3
	10	0.378	22.6	0.94	18.8
	20	0.094	1.1	– [a]	0.5

[a] Plants died after 7 days

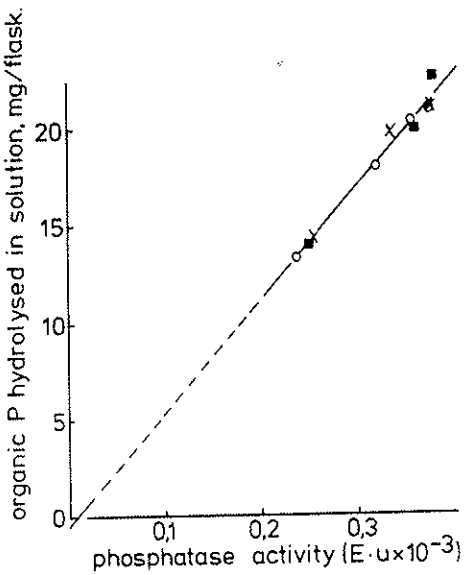

Fig. 1. Relationship between acid phosphatase activity and amount of P hydrolysed from different organic P compounds in aseptic solution-culture of clover. ×, sodium glycerophosphate; ○, lecithin; ■, phytin; regression equation: $y = 0.54 + 58.6x$, $r = 0.99$

clover was almost constant, whatever the organic P compound and rate used. Only the plants grown in the solution treated with 2 mg P/100 ml showed a slight decrease in P uptake while 20 mg P/100 ml phytin was toxic to plants. Phosphatase activity, inorganic P in solution after plant growth and P uptake were the same for all P compounds. Increasing the P application rate from 2 to 5 mg P/100 ml mainly increased phosphatase activity and inorganic P in solution but only a small effect on P uptake. Increasing the P in solution beyond 5 mg/100 ml had no further effect. A comparison of the inorganic P in solution with P uptake shows that in all cases the amount of organic P hydrolysed exceeded plant needs by a factor of about 20. The amount of P hydrolysed was closely related to the phosphatase activity (Fig. 1), the relationship being the same for all three compounds used.

The pot experiment was set up to determine whether plants can grow by using mainly organic sources of P. Table 2 shows that adding 20 mg inorganic P/100 g soil increased the yield of clover by a factor of 9 and that of small grains by a factor of 2.

Table 2. Dry-matter (DM) production and P uptake of four plant species grown on low-P subsoil treated with inorganic P and three different organic phosphates

Treatments[a]	Clover[b]		Barley[b]		Oats[b]		Wheat[b]	
	DM yield (g/pot)	P uptake (mg/pot)	DM yield (g/pot)	P uptake (mg/pot)	DM yield (g/pot)	P uptake (mg/pot)	DM yield (g/pot)	P uptake (mg/pot)
Control (no P)	0.22a	0.25a	1.62a	0.42a	1.31a	0.28a	1.17a	0.50a
Inorganic P	1.85c	0.95e	2.51c	1.77c	2.35c	1.26c	2.25bc	2.28c
Sodium glycero-phosphate	1.68c	0.79c	2.56c	1.77c	2.45c	1.29c	2.09c	2.19c
Lecithin	1.31b	0.73b	2.03b	1.48b	1.83b	1.10b	1.85b	2.07b
Phytin	1.21b	0.85d	2.49c	1.76c	2.02b	1.23c	2.50c	2.42d

[a] P applied at 20 mg/100 g soil
[b] Results followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple-range test

Table 3. Phosphatase activity in rhizosphere of several plant species treated with inorganic P and three different organic phosphates

Treatments[c]	Phosphatase activity[a,b] (EU $\times 10^{-3}$)							
	Clover		Barley		Oats		Wheat	
	Acid	Alkaline	Acid	Alkaline	Acid	Alkaline	Acid	Alkaline
Control (no P)	0.27a	0.28a	0.32a	0.36a	0.25a	0.37a	0.27a	0.47a
Inorganic P	0.38b	0.38b	0.40ab	0.44b	0.33c	0.53b	0.34c	0.61b
Sodium glycero-phosphate	0.36b	0.47c	0.36a	0.65d	0.33c	0.71c	0.34c	0.65b
Lecithin	0.43c	0.44bc	0.35a	0.57c	0.40d	0.62bc	0.32b	0.63b
Phytin	0.35b	0.42b	0.37ab	0.46b	0.30b	0.56b	0.32b	0.59b

[a] Phosphatase activity of the initial soil was 0.20×10^{-3} (acid) and 0.14×10^{-3} (alkaline) enzyme units (EU)
[b] Results followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple-range test
[c] P applied at 20 mg/100 g soil

Table 4. Microbial population in soil treated with inorganic P and several organic phosphates after growth of different plant species

Treatments[c]	Clover[a,b]		Barley[a,b]		Oats[a,b]		Wheat[a,b]	
	Fungi $\times 10^3\ g^{-1}$	Bacteria $\times 10^4\ g^{-1}$	Fungi $\times 10^3\ g^{-1}$	Bacteria $\times 10^4\ g^{-1}$	Fungi $\times 10^3\ g^{-1}$	Bacteria $\times 10^4\ g^{-1}$	Fungi $\times 10^3\ g^{-1}$	Bacteria $\times 10^4\ g^{-1}$
Control (no P)	3.8a	5.9a	4.3a	7.3a	4.6a	8.5a	7.4a	10.1a
Inorganic P	10.2b	18.2b	11.2b	24.4b	17.2b	41.6b	20.3b	42.5b
Sodium glycero-phosphate	10.4b	29.5c	11.3b	39.2d	17.4b	118.5c	20.4b	77.8d
Lecithin	36.5c	35.4d	39.5c	32.8c	58.9d	45.3b	59.5c	56.7c
Phytin	11.2b	77.5e	11.6b	76.8e	19.1c	115.3c	21.1b	105.9e

[a] Microbial population of the initial soil was 1.0×10^2 (fungi) and 1.5×10^3 (bacteria) per gram soil
[b] Results followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple-range test
[c] P added at 20 mg/100 g soil

Table 5. Amount of P released in inorganic form from three different organic phosphates which were added to the soil after 40 days of growth of four plant species

Organic P compound (120 mg/pot)	Added P released in inorganic form (%)			
	Clover	Barley	Oats	Wheat
Sodium glycero-phosphate	41.3	43.0	44.5	44.9
Lecithin	44.3	49.1	48.6	50.2
Phytin	57.0	55.6	55.9	58.7

P uptake showed a similar trend. Fertilizing the soil with organic P compounds showed a similar increase in yield and P uptake. Thus the plants used organic P sources as efficiently as inorganic sources. Only P uptake from lecithin was somewhat lower than that from the other sources.

Table 3 shows that there was a strong increase in phosphatase activity in the presence of plants. Acid phosphatase activity increased from 0.20×10^{-3} EU by about 50% in the control and another 50% when P, inorganic or organic, was added. There were no large differences in phosphatase activity with different P compounds added or with different plant species. Alkaline phosphatase activity was 0.14×10^{-3} EU in the unplanted soil, and increased by a factor of 2 to 3 in the planted non-fertilized soil, and further in the planted fertilized soil. The addition of organic P compounds caused a larger effect than inorganic P. Among the organic P compounds sodium glycerophosphate had the largest effect.

The microbial population also increased when plants were grown in the soil (Table 4). The largest increase in fungi and bacteria occurred under oats and wheat. Among the organic P compounds, lecithin treatment gave the largest number of fungi and phytin the largest bacterial population.

Table 5 shows the proportion of inorganic P released from the three organic P sources used. The amount of inorganic P released was calculated from the increase in inorganic P in the soil after plant growth and the P uptake by the plants. The greatest release of inorganic P occurred after phytin treatment, about 57% of the P added. There was no consistent plant species effect on the amount of organic P hydrolysed.

Discussion

The results of the solution experiment show that clover only produced acid phosphatases. These phosphatases readily hydrolyse organic P in the form of phytin, lecithin and sodium glycerophosphate. Szember (1960) reported that microorganisms are also capable of breaking down lecithin or phytin. The amount of P hydrolysed (Table 1) surpassed, under aseptic conditions, the amount of P taken up by a factor of about 20. This shows the significance that plant-produced phosphatases may have for the P nutrition of plants. According to Ridge and Rovira (1971) they are probably more important than phosphatases produced by rhizosphere organisms. The ability of plant roots to utilize organic P compounds was also reported by Rogers et al. (1940).

In the pot experiment, four plant species grew on a very P-deficient soil and were therefore dependent on added P for their growth. Table 2 shows that all types of P fertilization increased growth and P uptake. The increase was similar for both inorganic and organic P sources, indicating that plants can use organic P sources under septic conditions, also. The presence of plants in the soil increased both alkaline and acid phosphatase activity (Table 3) and the microbial population (Table 4). The bacterial population was further increased after the addition of organic P sources especially phytin, whereas fungi increased most after the lecithin treatment. This in-

dicates that the two compounds are preferred substrates for bacteria and fungi, respectively. These results agree with those of Hoffmann and Elias-Azar (1965), who observed higher phosphatase activity in soils with a higher microbial population. The increase in alkaline phosphatase activity can only be attributed to the increased microbial population since these enzymes were not produced by plants, as shown in the solution culture experiment and by Chhonkar and Tarafdar (1981). Ramkov and Dimitrov (1971) also observed a marked increase in phosphatase activity in soils treated with organic manure and inorganic N, P and K fertilizers. The measured increases in enzyme activity and the microbial population are average values for the bulk soil, and the increase at the root surface, where P uptake takes place, was probably much higher, as shown by Tarafdar and Jungk (1987). Both experiments show that if hydrolysable organic P is present, the activity of phosphatases in plant-soil systems is able to hydrolyse sufficient P to meet and even surpass the P demand of the plant (Tables 1 and 5). This result suggests that phosphatase activity is not the limiting factor in the use of organic P but rather the availability of phosphatase hydrolysable P compounds.

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