

Pea roots affect immobilisation and solubilisation of phosphorus depending on genotype, stage and phosphorous source

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To assess the efficiency of pea roots to mobilize available phosphorus (P) from P compounds we subjected various pea genotypes to a post-treatment method. Axenic seedlings were raised on P-deficient semisolid synthetic medium using control blanks without a plant otherwise treated in the same way. AlPO_4 , CaHPO_4 , FePO_4 , apatite and meat-bone-meal (MBM) were tested. A genotype was tested from 1-day through 15-days of growth. There were differences between the compounds ($p < 0.001$). P was dissolved from CaHPO_4 with apparent maxima at 72-h intervals and to a significantly lesser extent from MBM. With AlPO_4 , FePO_4 and apatite, the roots did not show a dissolving effect, but, on the contrary, significantly immobilised P.

In each case a correlation with an increase in acidity, H^+ ($p < 0.001$) was observed. The correlation was negative in the AlPO_4 , FePO_4 and apatite series. A CaHPO_4 treatment combined with apatite or MBM significantly decreased solubility of P from that of CaHPO_4 singly. Tests with six additional genotypes showed that all solubilised P from CaHPO_4 , some to a significant extent from apatite, MBM or slightly from FePO_4 , but none from AlPO_4 . The accumulation of nearly water-insoluble aluminium and iron phosphates in field and virgin soils is partly explainable by the immobilisation through the root action on P, which we have found also with other plant species. The root responses must also have ecophysiological functions distinct from P acquisition.

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The resources of fertilizer phosphates are declining globally (VANCE et al. 2003). It is, therefore, imperative to understand the P (phosphorus) acquisition of crops. Of all the nutrient elements, soil P is the most immobile, inaccessible and unavailable one (HOLFORD 1997).

What little native P there is in germinating pea (*Pisum sativum*) grains is a minor and shortly exhausted source. The mean P content of dry pea grains was found to be only 0.37 or 0.38% (SCHERZ and KLOOS 1981; RASTAS et al. 1989). The main P reservoirs in the pea grains are nucleic acids and inositol phosphates. All of the grain P is probably not in the form susceptible to be mobilized at germination. About 80% of the inositol phosphates, the apparent storage P in pea, were degraded in 8 days after germination (HONKE et al. 1998). Active means of P acquisition are supposed to begin already in germination in association with relatively young sections of roots. We (AHOKAS and MANNINEN 2001) have found that such activities start on the 4th day of germination in barley (*Hordeum vulgare*).

Case studies in Finland (SAARELA et al. 2003, 2004) show that insoluble phosphates derived from fertilizers seem to have accumulated in soils. Fertilizers contain phosphate in the form of CaHPO_4 . Aluminium phosphate or iron phosphate, or both, are known to accumulate in

Finland in peat soils (VALMARI 1970; NIEMINEN and JARVA 1996), virgin mineral soils (KAILA 1963) and field soils (KAILA 1963, 1964; HARTIKAINEN 1989; SAARELA et al. 2003) creating a soil sediment of P. The mobilisation of this resource is of interest here. It seems that the accumulated sediments of P in the field soil are not used in plant production. In the roots of barley, aluminium is absorbed by cell walls and root surfaces and precipitate phosphate there (CLARKSON 1967), a process which may contribute to the insoluble phosphate in post-harvest soils grown with barley.

This study is an extension of the previous one on barley (AHOKAS et al. 2007). The aim of the study was to assess genetic variation using different putative phosphorus sources with pea.

Because P availability in the form of annually added fertilizer free from polluting elements is becoming more expensive, it may be prudent to start breeding crops with properties which utilize the soil-bound resources of P or bone meal, a recyclable P source. The meat bone meal product studied here was accepted by EU to be used as a fertilizer. It was thought to have about 40% of its P available to crops (KALLAMA and UUSIHONKO 2006). The apatite type studied here has been tried as a P source in organic culture (SEURI et al. 2001).

MATERIAL AND METHODS

Plants and growing medium

Breeding lines and old cultivars of the pea were selected without previous information of their P uptake properties. The pea line L1703 was obtained from Dr. Stig Blixt, Weibullshom Plant Breeding Institute, Sweden. It is a selection of cv. 'Vincio' (MURFET 1967). The origin of the line V84-77 was described previously (AHOKAS 1987); it is in principle the genotype of L200 in BLIXT's (1972) collection. The line 93-893 is of hybrid origin (*Pisum elatius* × Ville) × (V84-77 × Ville) and of F₈ or later generation crossed and bred by one of us (H. Ahokas). The old Finnish pea entries, Linie von Nord (K-2402), the landrace lines, Linie von Landerbse (K-2406), and South-West Finnish (K-3668) and cv. 'Kellervä' (K-2403) were obtained from the N. I. Vavilov All-Russian Scientific Research Institute of Plant Genetic Resources, St. Petersburg, Russia. The seeds were produced in greenhouse. To reduce possible genetic variation within the accessions, single plant origins were used. The seeds were usually picked from mature pods stored unopened with sterile forceps, pencil-marked, weighed and surface sterilized and washed in sterile water series as described (AHOKAS and MANNINEN 2001; AHOKAS et al. 2007), however, using the vacuum treatment only in the Linie von Nord series, because the germination in some of the pea lines appeared to be impaired by the vacuum step during the sterilisation in 0.01% HgCl₂. The plants, single per a 14-ml polypropylene tube (Falcon 2059, USA), were grown for 1 to 15 days on about 7 ml of medium made semisolid with 0.6% agar or about 42 mg per tube (Plant Cell Culture Reagents, A-1296, Sigma, USA) having the inorganic constituents of Barley Medium II (NORSTOG 1973) but lacking phosphate the plants being subjected to P starvation but supplied with other macro and micro minerals of Barley Medium II. The salts were of analytical (Merck, Germany) or Plant Cell Culture Reagents grade (Sigma, USA). The masses of both the empty tube before the experiment and the medium were recorded. In calculations, 1 g of the medium was taken as 1 ml. At least two control tubes without any plant were included to each sample series of the different P compounds to determine the zero level. The daily series comprised of 12 to 24 tubes. The natural pH (in mean 5.74) of each control medium (without a plant) at the sampling day was used as the reference for the calculations of the acidification by the plant. For the pH measurements with pH-Meter CG840 (Schott, Germany), the plantless blank medium was mixed with a sterile glass rod and the electrode (Schott N 6180) was inserted midway in the medium in the tube. The pH measuring apparatus was calibrated before each series of measurements. The electrode solution was changed once a month. The pH was expressed as

micro-equivalent l⁻¹ of H⁺. The experiments were done in an air-conditioned greenhouse between late autumn and early summer with night minima of 9–13°C and day maxima of 20–25°C at the plant level. Additional light was supplied with high-pressure sodium lamps (Philips SON-T AGRO 400, Belgium) to guarantee a 16-h day-length, being turned on and off by a photosensor outside the greenhouse during the illumination period. At different periods of the year, the series were subjected to the northern latitude of about 61° with varying natural light intensities and spectral distributions.

Testing the phosphorous compounds

CaHPO₄ (= CaP) (BDH 27598, UK), FePO₄ (= FeP) (Merck 103923, Germany), AlPO₄ (= AIP) (Aldrich 34,145-2, Germany) and Siilinjärvi Apatite (Kemira GrowHow, Finland) were ground in a mortar, washed at least five times during a day with sterile Milli-Q water using 10 volumes or more, collected by centrifugation and dried at 62–65°C for three days on Whatman 1 (UK) filter paper and stored exsiccated. The ground meat bone meal (= MBM) (Honkajoki Oy, Finland) was used as such. The MBM has been subjected to autoclaving at 133°C for 20 min in the process of the factory and was stored at –20°C during the experiment. The P content of MBM is indicated to be 5.5%. Siilinjärvi Apatite represents fluoroapatite (NEUVONEN 1960) with a reported total P content of 14% and with water soluble P of 0% and with original particle size fractions of < 0.06 mm 40%, < 0.1 mm 60% and < 0.3 mm 95% (ANONYMOUS 2003). Each compound containing phosphorus was weighed (10 mg) on partly cut 1-ml pipettor tips in advance used to insert the compound on the medium in the growth tube.

After the plants were pulled out, the semisolid medium mixed and the pH measured, the ability of the medium in each tube to dissolve phosphorus from CaP, FeP, AIP, apatite or MBM was determined. Two persons worked together without any delays until the final sample was obtained for the P determination. A tube with the medium was mixed with the 10 mg compound with a glass rod for 30 s, the tubes were capped, struck by hand to lower the medium in the tube and incubated in a 25°C water bath for 1 h. After 15 s vigorous vortexing, the tubes were centrifuged at about 9500 g in an angle rotor (Beckman JA-20.1, USA) for 10 min at +8°C setting. The supernatant was collected with wide-pore 1-ml tips (Geno-DNA-tip, Finnpiipette, Finland) on a pipettor in two chilled 1.5-ml Eppendorf (Germany) tubes. The tubes were centrifuged at about 15 000 g in a Sorvall FA-MICRO rotor (USA) for 5 min at 8°C setting, and the supernatants pooled usually to make > 1.5 ml by pouring to a pre-chilled 2-ml tube. The samples were stored in ice and determined within a few hours or frozen (–20°C) for determination later. In a

joined test, both 10 mg aliquots of AIP, apatite, CaP, FeP or MBM were mixed together with 10 mg of CaP simultaneously and the sample processed otherwise as those with individual phosphorous compounds.

Phosphate determination

Phosphate was determined in 1.5-ml samples of the supernatant in 14-ml Falcon 2059 tubes in the method of DICK and TABATABAI (1977) in a final volume of 10 ml. Both the samples and control blanks without plants were read with a spectrophotometer (HP 8452A, Germany) at 700 nm against water. The readings were corrected for volumes of the original samples. In case that less than 1.5 ml of the supernatant was recovered, the volume was corrected by calculation. For a standard plot of phosphate, dilute K_2HPO_4 solution was added to the tube with the same agar medium, Barley Medium II (see above), mixed for 30 s with a glass rod, incubated in a 25°C water bath for 1 h, centrifuged as the samples, and then determined and calculated on the mass basis as with the sample tubes.

Statistics

The observed values were calculated on medium bases without seed mass correction or corrected to a standard grain mass of 200 mg, which was approximately the mean single pea mass in Linie von Nord. To circumvent any errors caused by nonnormality, the nonparametric statistics for probability (p), Kruskal-Wallis one-way analysis of variance (H), Spearman rank correlation (r_s) and Friedman two-way analysis of variance (χ_r^2) were used and the significances determined according to SIEGEL (1956). SEM means the standard error of the mean, and n , the number of samples.

RESULTS

The values shown are differences between the samples and the control blanks obtained with media without a plant (Barley Medium II). The values can vary from positive (solubilising) to negative (immobilising). In many cases this makes the standard error of the mean (SEM) values numerically apparently high with regard to the numeral of the means. The values are presented on the μmol phosphate per ml of the medium often both without and with a correction for the seed mass. Within a genotype such correction for seed mass has a marginal effect, but between genotypes the correction is meaningful especially because L1703 has nearly double the single-seed mass of the others.

Time series from 1-day germinants to 15-days seedlings

The responses of seedlings in the time series were studied with the pea line Linie von Nord. The line was chosen as a

representative of the oldest pea cultivar (Nord) released in Finland in 1904 (AHOKAS 2000). Linie von Nord was selected from it in about 1910 when very little artificial fertilizers were used. Linie von Nord also germinated uniformly under the experimental conditions. The mean pH level of the control medium was found to be 5.74, but the root-induced acidification expressed as the increase of equivalents of H^+ ml^{-1} was strongest at root branching in between 8- and 10-days samples declining later under the test conditions (Fig. 1). The acidification and primary root elongation showed a rhythm of 72 h during the 9-days period (Fig. 1), after which also the secondary roots start to play a role.

Statistically, the five tested compounds showed highly significant differences over the 15-days periods: $\chi_r^2 = 305.9$, $p < 0.001$. After a 6-days period all the tested compounds seem to diverge from the previous solubilisation values. CaP, however, showed an earlier response, with its first peak in 3-days, the second in 6-days and the third in 9-days samples. After that, the activities of the secondary roots may have become overwhelming and the conditions for development may also be stressful.

CaP and MBM showed highly significant ($p < 0.001$) positive correlation between acidifying of the medium and ability to solubilise phosphorus, while AIP, apatite and FeP showed highly significant ($p < 0.001$) negative correlation (Table 1).

Phosphorous compounds in combination with CaP

For further studies, the 8-days sample age was selected to represent a stage not much yet affected by the secondary roots or by the putative stresses caused by the minute space for the plant. The effect of the individual phosphorous compound together with a 10 mg aliquot of CaP and singly was tested in a series at the 8-day samples. The results are given in Table 2. FeP, AIP and CaP, 10 mg each with an additional 10 mg of CaP did not change the amount of P solubilised from that of the single 10 mg dose of CaP, while apatite significantly at $p < 0.01$ and MBM at $p < 0.05$ lowered the amount of P solubilised compared with the single (10 mg) samples of CaP.

Genotypic effects

The results with six pea genotypes at their efficiency to make P soluble from the five tested compounds were determined with 8-days seedlings both on single plant basis and corrected for the differences in single-seed masses (Table 3, 4). The results of the uncorrected and corrected values were, however, coherent. With AIP all the genotypes consistently precipitated P compared with the controls without a plant, even though L1703, 93–893 and Kellervä precipitated somewhat less. Significant differences were found, in particular, if corrected for the seed mass. This mostly moves L1703 with its large seeds

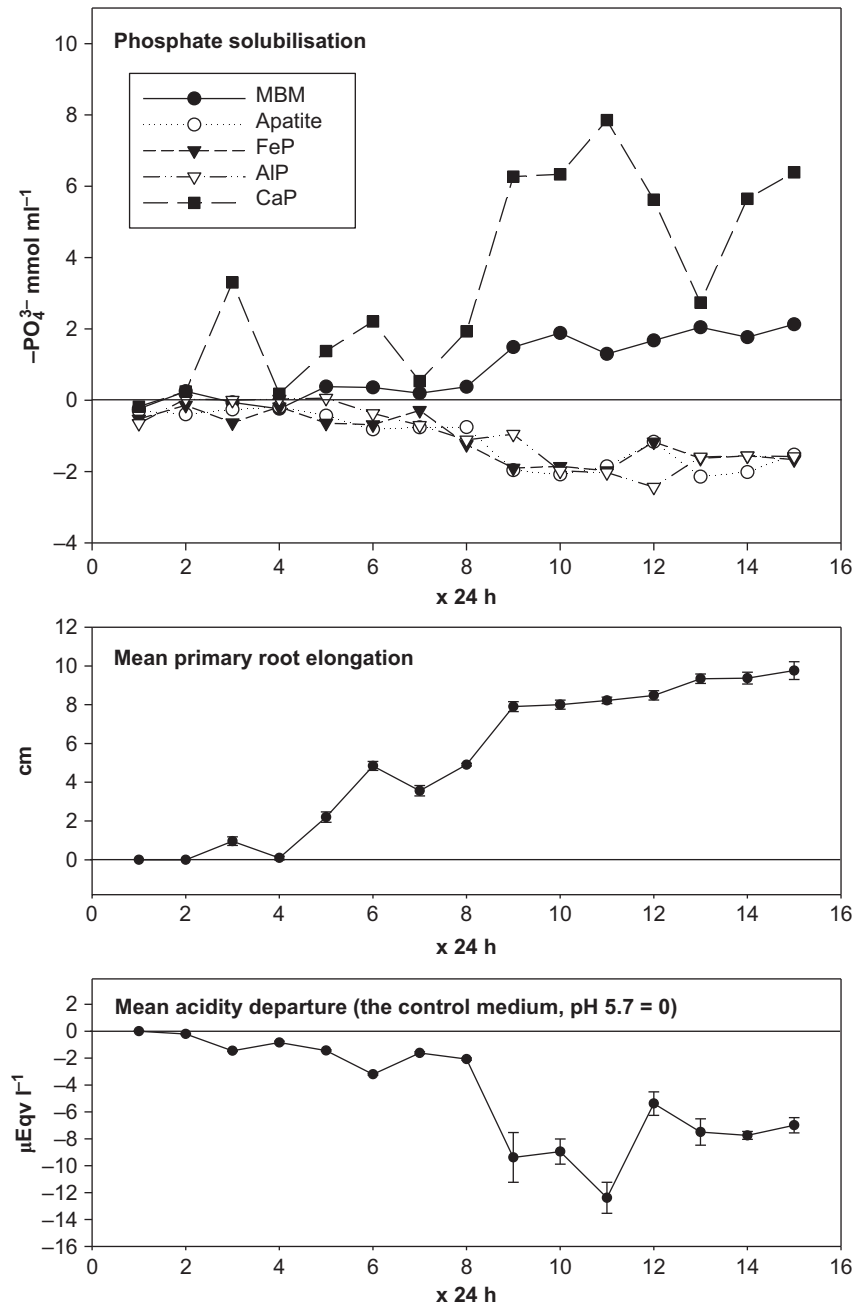


Figure 1. Results on soluble phosphate quantities from different phosphorous substances in axenic media grown for 1 to 15 days *in vitro* with pea, Linie von Nord. Values were corrected for the true grain weight to correspond results with a 200 mg grain. – Top plot: phosphate solubilisation or immobilisation (negative values) relative to control blanks without a plant sampled for each MBM, apatite, FeP, AlP and CaP. – Central plot: the elongation of the primary root (mean \pm SEM) of all samples. – Bottom plot: acidification in micro-equivalents per litre of the media relative to control blanks without a plant (mean \pm SEM) of all samples. The change from 0 to -10 means an acidification of one pH unit from pH 5.7.

towards the zero value. Highly significant differences ($p < 0.001$) were observed with apatite as the source, where L1703 and V84–77 showed solubilising properties, the other genotypes showing consistently precipitation of P. From CaP all the genotypes could make soluble P, there being no significant differences between the genotypes.

With FeP, L1703 and 93–893 showed positive values, the other genotypes precipitating P and with significant differences ($0.02 > p > 0.01$). The genotypes L1703 and 93–893 also showed positive values with MBM, the other genotypes being negative though nearly zero, giving no statistical significances.

Table 1. Coefficients of Spearman rank correlations between soluble phosphate and acidification with the *Linie von Nord* pea from 1 day through 15 days without correction for seed weight differences (cf. Fig. 1).

Substance	Spearman rank correlation, r_s	Significance, p (df = 13)
AIP	-0.767	<0.001
Apatite	-0.889	<0.001
CaP	0.932	<0.001
FeP	-0.925	<0.001
MBM	0.818	<0.001

DISCUSSION

The method of P determination used is insensitive to organic phosphates (DICK and TABATABAI 1977). It is therefore suitable to accurately determine any inorganic P that has previously been in contact with the seedling of the test plant and allows also using the organic MBM as a test source of P. The little amount, about 42 mg per sample of agar of plant cell culture purity also appeared in the control blank tubes and the tubes for the phosphate standard and its possible effects are, therefore, fully controlled.

It is apparent that only the relatively new roots are effective at dissolving phosphate containing compounds in the soil. Under the present test conditions the ability persisted up to 11 or 12 days, after which new branches appear and largely take over the activities of the primary root in pea. *Linie von Nord* studied for 1 day through 15 days, showed a 3-day (72-h) periodicity at solubilising phosphate from CaP somewhat associated with the primary root elongation rate and secretion of acidity into the root environment. This lasted at least for 3×3 days on the primary roots. The periodic appearance of the solubilisation capabilities at about 72 h intervals towards CaP

mimics the cluster-root initiation detected in many species under low P in the soil.

Doubling the amount of CaP from 10 to 20 mg did not affect the amount of phosphate solubilised (Table 2) suggesting that the solubility is dependent on the quantity of one or more specific substances secreted from the roots and that the amount of the tested P substances was not rate limiting. The inability of some plant species to use P from calcium phosphate was shown to render them calcifuge (TYLER 1994; ZOHLEN and TYLER 2004). The best availability of P from CaP found in this study suggests that pea is rather a calcicole than calcifuge. There were significant lowering interferences by apatite and MBM with the availability of phosphate from CaP in the joined experiments (Table 2). If such a phenomenon would occur in the soil environment too, mixing of different types of P fertilizers, at least CaP with apatite might be wasteful for P in the pea root environment. In the case of apatite as fertilizer, including Siilinjärvi Apatite, the field trials with annual barley in organic farming (SEURI et al. 2001) corroborate the finding that P of apatite is unavailable or poor available to barley. This was confirmed with 7 other barley genotypes with an *in vitro* method (AHOKAS et al. 2007). In soil, the tested compounds would be subjected to biological leaching. Then it is, however, more likely that the organisms making P available preferentially take up the soluble P by their own cells rather than supply it to other species.

Solubilisation of P from FeP, AIP and apatite by pea showed a negative correlation with the acidification of the medium (Table 1). SUBBARAO et al. (1997) found that the pH of the root exudates of pigeon pea (*Cajanus cajan*) did not correlate with the P solubilisation, but they ascribed the property to the chelating compound released by roots. Evidently the secretion of organic acids that is attributed to participate in the mobilisation of P in the rhizosphere have, or mainly have other functions for the

Table 2. Interference of phosphorous compounds with the solubilisation of P from CaP in media after eight days growth of the *Linie von Nord* pea.

Compound, 10 mg each tested singly or joined with 10 mg CaP	Phosphate solubilised $\mu\text{mol ml}^{-1}$ (Mean \pm SEM)		Phosphate solubilisation from CaP-joined compound vs 10 mg CaP singly	
	Compound singly $n = 7$	Compound joined with additional 10 mg CaP $n = 7$	H	Significance, p
AIP	-1.19 \pm 0.16	1.93 \pm 0.32	0.494	>0.30
Apatite	-1.07 \pm 0.10	0.51 \pm 0.11	8.265	<0.01
CaP	2.13 \pm 0.30	2.08 \pm 0.44	0.510	>0.30
FeP	-0.80 \pm 0.28	1.72 \pm 0.53	0.690	>0.30
MBM	-0.50 \pm 0.34	1.74 \pm 0.55	4.592	<0.05
H	20.038	15.395		
Significance,	$p < 0.001$	$p < 0.01$		

Table 3. Actions of the root environment of six pea genotypes on five phosphorous compounds relative to the controls without correction for the seed weight differences.

Pea	Phosphate, $\mu\text{mol ml}^{-1}$ Mean \pm SEM [n]				
	AIP	Apatite	CaP	FeP	MBM
Kellervä	-0.48 ± 0.23 [7]	-0.59 ± 0.42 [7]	1.90 ± 0.33 [7]	-0.90 ± 0.31 [7]	-0.16 ± 0.37 [8]
L1703	-0.37 ± 0.17 [5]	2.11 ± 1.24 [7]	2.74 ± 0.59 [9]	0.78 ± 0.71 [8]	0.73 ± 0.50 [8]
Linie von Landerbse	-1.14 ± 0.19 [6]	-1.08 ± 0.05 [7]	2.20 ± 0.18 [8]	-1.06 ± 0.15 [7]	-0.32 ± 0.13 [7]
South–West Finnish	-1.34 ± 0.21 [6]	-0.98 ± 0.15 [7]	2.30 ± 0.39 [8]	-1.13 ± 0.22 [7]	-0.25 ± 0.25 [7]
V84–77	-1.08 ± 0.21 [7]	0.15 ± 0.51 [7]	1.50 ± 0.29 [7]	-1.11 ± 0.08 [7]	-0.22 ± 0.32 [7]
93–893	-0.30 ± 0.54 [7]	-0.75 ± 0.33 [6]	2.40 ± 0.48 [7]	0.50 ± 0.59 [9]	0.60 ± 0.64 [8]
H	9.697	21.758	5.087	14.874	5.870
Significance	$0.10 > p > 0.05$	$p < 0.001$	ns	$0.02 > p > 0.01$	ns

plant and the interaction of plant and root environment. Malate was found to be the major carboxylate found to be exuded from pea in soil (NURUZZAMAN et al. 2005). Al-induced citrate secretion by tap and basal roots of bean (*Phaseolus vulgaris*) showed genotypic differences (SHEN et al. 2004). The secreted compounds of the Linie von Nord pea under P-deficiency probably have Al- and Fe-detoxification as one of their functions for the adaptation to the soil environment.

Our post-treatment method uses axenic conditions devoid of interactions with organisms in the rhizosphere, which are known to interfere in P acquisition of plants in soil (RENGEL and MARSCHNER 2005). SUBBARAO et al. (1997) ran tests lasting for weeks with the perennial pigeon pea on non-sterile soil. They also found negative P solubilisation from iron phosphate at some stages. Phosphates may be made available to plants with the aid of a mycorrhiza (BUCHER 2007). Under iron deficiency, alfalfa (*Medicago sativa*) root exudates contain a furan derivative capable of dissolving ferric phosphate (NOGUCHI et al. 1994). Elephant grass (*Pennisetum* sp.) was found to benefit from iron and aluminium phosphate in acid soil in a fashion not explainable with rhizosphere acidification.

Here the underlying cause was related to exuded pentanedioic acid (SHEN et al. 2001). In a pot experiment using neutral soils, synthetic iron phosphate was found to serve as P source to rye-grass (*Lolium perenne*) (JOHNSTON and RICHARDS 2003). Broad bean (*Vicia faba*) grown in non-sterile soil was thought to be capable to use P from iron and aluminium phosphate, even though calcium phosphate was the most favourable (LI et al. 2007).

The observation that pea lines L1703 and 93-893 made P soluble from FeP, while the other ones precipitated P (Table 2, 3, 4) in the slightly acidic pH suggests that genotypic differences exist in pea in this characteristic. In literature, FePO_4 is, in a careless way, listed among the P sources available to plants in soil. However, closer studies have shown that FePO_4 is really an unavailable P-source to many plants, including the Poaceae species maize, sorghum and pearl millet (AE et al. 1990, 1993; AE and SHEN 2002). Genotypic variation for P uptake from iron phosphate was found in pigeon pea (SUBBARAO et al. 1997) and groundnut (WISSUWA and AE 1999). A positive correlation was found between P solubilising of iron phosphate and of aluminium phosphate in pigeon pea in tests, where microbial participation was interfered

Table 4. Actions of the root environment of six pea genotypes on five phosphorous compounds relative to the controls with seed weights standardized to 200 mg from the results in Table 3.

Pea	Phosphate, $\mu\text{mol ml}^{-1}$ Mean \pm SEM [n]				
	AIP	Apatite	CaP	FeP	MBM
Kellervä	-0.37 ± 0.18 [7]	-0.60 ± 0.35 [7]	1.52 ± 0.22 [7]	-0.87 ± 0.28 [7]	-0.21 ± 0.31 [8]
L1703	-0.17 ± 0.08 [5]	1.00 ± 0.58 [7]	1.39 ± 0.32 [9]	0.38 ± 0.37 [8]	0.39 ± 0.28 [8]
Linie von Landerbse	-1.12 ± 0.20 [6]	-1.02 ± 0.06 [7]	2.07 ± 0.17 [8]	-0.97 ± 0.15 [7]	-0.31 ± 0.12 [7]
South–West Finnish	-1.14 ± 0.17 [6]	-0.86 ± 0.14 [7]	1.89 ± 0.31 [8]	-0.93 ± 0.18 [7]	-0.21 ± 0.20 [7]
V84–77	-1.02 ± 0.22 [7]	0.13 ± 0.43 [7]	1.25 ± 0.23 [7]	-1.14 ± 0.16 [7]	-0.20 ± 0.28 [7]
93–893	-0.30 ± 0.45 [7]	-0.68 ± 0.31 [6]	1.86 ± 0.36 [7]	0.44 ± 0.53 [9]	0.55 ± 0.70 [8]
H	13.484	1.312	7.586	19.838	5.372
Significance	$0.02 > p > 0.01$	$p < 0.001$	ns	$0.01 > p > 0.001$	ns

by chloramphenicol treatment (SUBBARAO et al. 1997). Our unpublished results show that barley in axenic conditions is a species unable of using FeP as a P source, but evidently has genotypes which are temporarily capable of mobilizing P from FeP.

The six pea genotypes did not differ in their ability to solubilise phosphate from CaP significantly (Table 3, 4) even though AHOKAS et al. (2007) found differences among the barley genotypes. Iron phosphate is one of the major deposited forms of P in peat soils in Finland (VALMARI 1970; NIEMINEN and JARVA 1996) and in field soils that have been fertilized (HARTIKAINEN 1989) with a surplus of P since the 1940s (SAARELA et al. 2003).

The P immobilisation by roots from FeP by most pea genotypes (Table 2, 3, 4) and by barley (AHOKAS et al. 2007) agrees with the accumulation in soils where roots also contribute to the precipitation of FePO₄. This highlights the soil binding of P (KAILA 1965) being either a characteristic of the soil or possibly a long-lasting effect of the vegetation in the soil. The circumstance that the plants (pea, barley and some others) immobilise phosphates from several P substances making P more water-insoluble also under conditions of P starvation shows that the root activities responsible for the phenomenon have functions entirely unrelated to P acquisition. Our results obtained in simplified experimental conditions support the notion of complex organic acid behaviour in soils (JONES et al. 2003).

Root-released organic acids have been seen to be related to phosphate uptake. In general they are found to benefit the P supply (HOCKING 2001; VANCE et al. 2003). The significant and positive correlation observed in dissolving phosphate from CaP or inorganic P from MBM and acidification of the medium suggest that this is true with Linie von Nord with these phosphorous substances, but the relationship is the reverse with apatite, AIP and FeP as the source of P, since the acidity tended to make P unavailable (Table 1). The curve of phosphate solubility in the presence of an equivalent of iron in the solution was shown to be going down to pH 2.2 (GAARDER 1934). P solubility from FePO₄ was low over a wide pH range in a mixture of sand and vermiculite (AE et al. 1990, 1993).

In the case of apatite, including Siilinjärvi Apatite studied by us, the field trials with barley in organic farming (SEURI et al. 2001) corroborate the unavailability or poor availability of apatite P to crops. Likewise INDIATI and NERI (2004) found that an Algerian rock phosphate (with 12.6% total P) contributed little or no soluble phosphate in two different soil types. The effectiveness of apatite, AIP or FeP as seasonal fertilizers to most pea genotypes is questionable.

The peas tested by us represent old, partly wild (*Pisum elatius*) genotypes. In pea landrace populations a high level of genetic variation was probably maintained also in relation to the ability of the plants to acquire P from soil. The alternating or rhythmic activities of the modes of P

acquisition observed on the primary roots in Linie von Nord might benefit a plant in an unpredictable environment and in the neighbourhood of other genotypes of the population where the plant happened to germinate. On the other hand, genotypes efficient at certain means of phosphorus acquisition may constitute a genetic load in relation to some other effects of the environment. A temporal expression, a 72-h rhythm observed with Linie von Nord might mitigate any adverse effect of the property. Many crops are grown as mixtures of genotypes. Mixed genotypes that are able to acquire P from different sources might prove to be valuable in conditions where soil P sources are limiting and multiple P substances are found in the soil.

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