EXPLORING PLANT FACTORS FOR INCREASING PHOSPHORUS UTILIZATION FROM ROCK PHOSPHATES AND NATIVE SOIL PHOSPHATES IN ACIDIC SOILS

GUANG-LIN FENG, LI-MING XIONG
Chinese Academy of Sciences,
Nanjing, China

Abstract. Six plant species with contrasting capacity in utilizing rock phosphates were compared with regard to their responses to phosphorus starvation in hydroponic cultures. Radish, buckwheat and oil rapeseed are known to have strong ability to use rock phosphates while ryegrass, wheat and sesbania are less efficient. Whereas other plants acidified their culture solution under P starvation (-P), radish plants make alkaline the solution. When neutralizing the pH of the solutions cultured with plants under either -P or + P conditions, solutions with P starved buckwheat, rapeseed, and radish had a higher ability to solubilize Al and Fe phosphates than did those cultured with sesbania, ryegrass and wheat. Characterization of organic ligands in the solutions identified that citrate and malate were the major organic anions exuded by rapeseed and radish. Besides citrate and malate, buckwheat exuded a large amount of tartrate under P starvation. In contrast, ryegrass, wheat and sesbania secreted only a limited amount of oxalic acid, regardless of P status. Changes in activities of phosphoenolpyruvate carboxylase, acid phosphatase, and nitrate reductase in these plants were also compared under P- sufficient or -deficient conditions. The results indicated that plant ability to use rock phosphates or soil phosphates is closely related to their responses toward P starvation. The diversity of P starvation responses was discussed in the context of co-evolution between plants and their environment. Approaches to use plant factors to enhance the effectiveness of rock phosphates were also discussed.

1. INTRODUCTION

The effectiveness of rock phosphates for direct application is determined by an array of factors. These include the characteristics of the rock materials, soil and environmental conditions and last but not the least, plant factors. It has long been recognized that some plants are more efficient in utilizing soil phosphates or rock phosphates than other plants. For example, plants such as buckwheat, radish and oil rapeseed were identified as highly efficient in utilizing Chinese rock phosphates in field and greenhouse experiments [1]. The underlying mechanisms accounted for the high P efficiency in these plants have been speculated to include high Ca uptake, the exudation of protons and secretion of organic acids. However, comprehensive analysis of responses to P deficiency with these high P-efficient crop plants is still not available. It is also not clear why different plants utilize different mechanisms in their adaptation to P deficiency. In the frame of the FAO/IAEA coordinated research project on evaluation of the effectiveness of rock phosphate-based fertilizers, we attempted to explore those plant factors that are expected to contribute significantly to an enhanced utilization of P from rock phosphates or soil native phosphates. The understanding of the adaptation of these high P-efficient plants to P starvation may eventually help us to engineer crop plants with improved ability to use rock phosphates or soil P accumulated from long-time application of P-fertilizers.

2. MATERIALS AND METHODS

2.1. Plants

Six plant species were used in the present study: buckwheat (*Fagopyrum esculentum* Moench), radish (*Raphanus sativus* L.), oil rapeseed (*Brassica napus* L.), sesbania (*Sesbania cannabina* Pers), ryegrass (*Lollium multiflorum* Lam), and wheat (*Triticum aestivum* L.). Except for wheat, these plant species used are commonly cultivated in acidic soils of southern China and they have economic or ecological significance.

2.2. Hydroponic culture

The nutrient solution consisted of the following: 1 mM $NH_4H_2PO_4$, 0.34 mM NH_4NO_3, 4 mM Ca $(NO_3)_2$, 1.31 mM $CaCl_2$, 2mM $MgSO_4.7H_2O$, 5.98 mM KNO_3, 14.3 μM Fe EDTA, 23 μM H_3BO_3, 0.0235 μM $CuCl_2.2H_2O$, 4.55 μM $MnCl_2.4H_2O$, 88 μM $ZnCl_2$, and 0.0123 μM $H_2Mo_4H_2O$. Treatments with phosphorus (+P) did not include NH_4NO_3 and those without phosphorus (-P) did not have $NH_4H_2PO_4$.

2.3. Quantification of H^+ secreted in cultured solutions

The pH of the cultured solution was measured with a Beckman pH meter. The amount of protons excreted by the plants under indicated conditions was quantified by titrating aliquots of the cultured solutions with standard alkaline or acid solution.

2.4. Dissolution of rock phosphate, Fe and Al phosphates by root exudates or organic acids

Each of the following phosphates was extracted at 25°C for 2 hr with 20 ml solution individually cultured with the plant species mentioned above: 0.5 g North Carolina Rock Phosphate (100 mesh), 0.1 g $FePO_4.nH_2O$ (Fe-P) [2] or 0.1 g $AlPO_4.nH_2O$ (Al-P) [3]. A control treatment with distilled water was also run simultaneously. Phosphorus in the solution was determined by molybdate blue method.

2.5. Solubilization of rock phosphate by organic acid

Twenty-five ml of 0.1 M organic acids (adjusted to pH 6.0 or unadjusted) was mixed with 0.5 g of NCPR or 0.1 g $FePO_4.nH_2O$ or $AlPO_4.nH_2O$ and extracted at 25°C for 2 hr. Extracts were filtered and analyzed for P.

2.6. Analysis of organic acids

Hydroponic solutions with plants growing for specific periods of time under -P or +P conditions were collected and passed through cation exchange resin column and 0.25 μM filter. The resulting clear solutions were then condensed on a vacuum rotary evaporator until 1 to 2 ml remained. The solutions analyzed for organic acids by HPLC. Separated organic acids by Bondapak C-18 column were eluted with dilute H_3PO_4 (pH 2.65) and detected at 214 nm against organic acid standards. Organic acids in plant samples were assayed by enzymatic methods following procedures provided by the supplier of the enzyme kits (Boehringer-Mannhein, Germany) [4].

2.7. Assay of enzyme activities

The activity of phosphoenolpyruvate carboxylase (PEPC) was assayed as described in [5]. Acid phosphatase activity was determined using *p*-nitrophenyl phosphate (*p*NPP) as substrate. Nitrate reductase activity (NRA) was determined according to [6].

3. RESULTS

3.1. Excretion of protons in response to phosphorus deficiency

Three plant species were cultured in full strength nutrient solution with P (+P) for 10 days before they were transferred to solution deprived of P (-P). Changes in pH of the -P solution were evident one day following the transfer (Table I). For oil rapeseed and buckwheat, there was a decrease in pH initially, but a gradual restoration to the original value eventually occurred. On the contrary, pH values of solution with radish increased after transferring to P deficient solution (Table I). Clearly, under P deficient condition, acidification of the rhizosphere was not a strategic response adopted by radish.

To quantify the amount of protons excreted or consumed during P starvation, aliquots of solutions from cultures 1 or 5 days after deprivation of P were titrated with standard alkaline solution. Except for radish, all other plant species showed a significant increase in excretion of protons to the medium under -P condition (Table II). Sesbania, wheat and ryegrass all exhibited a high excretion of protons under normal P supply (+P). They also showed an enhanced excretion under P starvation. The background proton excretion with P supplied (+P) may be related to a preferential uptake of NH_4^+ relative to NO_3^- in these plants or a general higher cation uptake than anion uptake. On the other hand, buckwheat, oil rapeseed, and radish excreted very little protons under P-sufficient condition, yet under P starvation, buckwheat and oil rapeseed increased the excretion of protons while there was little increase in proton excretion with radish.

3.2. Solubilization of various forms of phosphates by nutrient solutions cultured with plants

The above results indicated that there were changes in pH in solutions cultured with different plants under -P relative to +P conditions. Thus, it was interesting to study the capacity of these cultured solutions to solubilize different forms of phosphates. Results are presented in Table III.

The data showed that solutions from these plant species had various capacities to solubilize different forms of phosphates. In most cases, solutions from -P treatments had higher capacity to solubilize phosphates than did those cultured with + P plants. Solutions cultured with oil rapeseed, buckwheat, and wheat had high ability to dissolve Fe and Al phosphates regardless of the supply of phosphates. The solutions cultured with rapeseed and buckwheat also showed a higher solubilizing ability to RP with -P treatment compared with +P treatment. On the contrary, radish, sesbania, and ryegrass had enhanced solubilizing ability to these phosphates only under -P condition. Among them, the increase was most significant for radish whose +P-cultured solution had little effect on these phosphates.

3.3. Solubilization of Al-P was enhanced by more alkaline solution

The above extraction of P from various phosphate compounds with cultured solutions was done with the solution without any adjustment in pH. The effect was therefore, a result of combined proton effect (Tables I and II) and other root exudates. Thus the effect of pH adjustment on the solubilization of Al phosphates was investigated. The results indicated that when rising solution pH from 4.5 to 6.0, all cultured solutions increased significantly in the solubilization of P from Al phosphates (Table IV). On the other hand, there was no change in the dissolution of either Fe phosphate and RP (Table V and data not shown).

3.4. Different ability of various organic acids in solubilizing phosphates

Organic acids are the major components in root exudates that enhance the solubilization of phosphates in rhizosphere soils. Several organic acids have been identified in root exudates from plants experiencing P starvation (for review, see Jones, 1998[7]). Nevertheless, data regarding the relative ability of these acids in attacking phosphates are incomplete. Although information from the reaction equilibrium parameters of these organic acids with various phosphates can be obtained, it is more straightforward to compare their ability in a single experiment. Table V presents the results of the assay of P dissolved by organic acids from various forms of phosphates with or without adjustment of solution pH. Tha data indicated that different acids have quite different ability in solubilizing these phosphates. With regard to forms of phosphates, it can be seen that the solubilization of RP was largely dependent on the proton effects of these acids. When adjusting solution pH to 6.0, the amount of phosphate solubilized by these organic acids decreased dramatically, while its effect on Fe phosphate was not very significant, suggesting protons were not the major factor controlling P release from Fe phosphate. Adjustment of the pH to 6.0 resulted in increased solubilization of Al phosphates, as also demonstrated in Table IV. Among all these organic acids, oxalic acid was the strongest in solubilizing Fe phosphate, while oxalic acid, citric acid, and tartaric acid had stronger ability in solubilizing Al phosphate. Oxalate was particularly efficient in solubilizing Al phosphate after adjusting the pH to 6.0 (Table V).

TABLE I. CHANGES IN pH IN -P SOLUTION AT DIFFERENT TIMES AFTER TRANSFERING PLANTS FROM +P TO -P SOLUTION (INITIAL pH=6.2)

Days after Changing Solution	Radish	Buckwheat	Rapeseeds
1	6.16	5.73	5.86
3	6.45	5.78	5.89
5	6.80	5.93	6.07
7	6.81	6.07	6.13
9	7.64	6.15	6.45
13	7.32	6.40	6.70

TABLE II. PROTON EXUDATION 1 OR 5 DAYS AFTER TRANSFERRING PLANTS TO P SOLUTIONS (meq/g FRESH WEIGHT)

Plant Species[1]	Treatment	1 day	5 days
Buckwheat	-P	0.26	0.31
	+P	0.04	0.03
Rapeseed	-P	0.47	0.38
	+P	0.01	0.01
Sesbania	-P	2.14	2.76
	+P	1.26	1.38
Wheat	-P	3.15	3.29
	+P	2.76	2.87
Ryegrass	-P	3.37	3.78
	+P	2.68	2.74

[1] Solution cultured with radish had a higher pH than background control and proton excretion was not determined.

TABLE III. SOLUBILIZATION OF ROCK PHOSPHATE (NCPR) AND SYNTHESIZED PHOSPHATES BY CULTURED SOLUTIONS (μg P/ml)

Plant Species	Fe-P		Al-P		RP	
	-P	+P	-P	+P	-P	+P
Rapeseed	4.70	4.03	4.92	4.06	0.36	0.12
Buckwheat	4.72	4.56	4.89	4.27	0.26	0.11
Radish	5.67	0.77	5.86	1.24	0.45	0.23
Sesbania	3.79	1.02	4.16	1.15	0.21	0.18
Wheat	4.73	4.13	4.82	4.01	0.17	0.16
Ryegrass	3.80	1.41	4.07	1.26	0.23	0.18

TABLE IV. SOLUBILIZATION OF Al PHOSPHATE BY CULTURED SOLUTIONS ADJUSTED TO DIFFERENT pH (μg P/ml)

Plant Species	pH of Solution	P Concentration in Solution (μg/ml)
Rapeseeds	4.5	6.54
	6.0	16.1
Buckwheat	4.5	9.59
	6.0	17.4
Radish	4.5	4.92
	6.0	12.0
Sesbania	4.5	1.85
	6.0	5.92
Wheat	4.5	4.42
	6.0	7.88
Ryegrass	4.5	2.69
	6.0	6.46

TABLE V. PHOSPHORUS SOLUBILIZED BY VARIOUS ORGANIC ACIDS (0.1 M) AT THEIR NATURAL pH OR AT pH 6.0 (0.1 g PHOSPHATES) (μg P in 25 ml)

Organic Acids	pH adjustment	Fe-P	Al-P	RP
Citric	No	31.3	176	1218
	6.0	31.2	870	39.3
Malic	No	30.8	111	2785
	6.0	68.8	514	123
Oxalic	No	529	186	2655
	6.0	141	1233	138
Acetic	No	52.0	25.0	730
	6.0	38.8	196	n.d.
Lactic	No	14.5	179	1757
	6.0	59.5	181	n.d.
Tartaric	No	37.5	257	2940
	Yes	88.3	391	69.5
Succinic	No	n.d	56.2	1500
	Yes	191	n.d.	200

n.d., not determined.

3.5. Characterization of organic acids secreted by plants under P deficient condition

The above studies indicated that in addition to protons, P-components were released in the cultured solution because after adjusting the pH of the solution to neutral, there was still much P-releasing capacity from solution cultured with plants under P deficient conditions. Additional results presented in Table V also demonstrated the ability of various organic acids in solubilizing insoluble phosphates at near-neutral pH. Further experiments were conducted to characterize these components.

Results in Table VI indicated that under P deficient conditions, all plants exhibited enhanced excretion of organic acids, yet the extent and kinds of the acids varied with plant species. Four organic acids were identified, i.e., citric, malic, oxalic and tartaric. Radish and oil rapeseed secreted a large amount of citric acid, while buckwheat showed significant increase in tartaric acid and malic acid secretion. Although wheat excreted malic acid under phosphate deficient condition, only oxalic acid was detected under P sufficient condition. Wheat, sesbania, and ryegrass showed a limited response in oxalic acid excretion under P starvation. This pattern of organic acid excretion matched closely with the ability of these plants in utilizing different forms of soil phosphates and rock phosphates.

3.6. Acid phosphatase activity in plants under phosphate starvation

It is well known that acid phosphatase activity in plants will dramatically increase when plants suffer from P deficiency. However, information on comparison of acid phosphatase activity among plant species under P deficient conditions and how it relates to plant P efficiency is still not available. In this study, we determined acid phosphatase activity in roots and plant leaves at various positions under P starvation (Table VII).

Results indicated that acid phosphatase activity was higher in new leaves than in older leaves, the lowest levels were found in roots (Table VII). It is also noted that radish, oil rapeseed, and buckwheat had higher acid phosphatase activities in leaves than those of wheat, sesbania, and ryegrass.

TABLE VI. SECRETION OF ORGANIC ACIDS (μg)
UNDER P SUFFICIENT AND DEFICIENT CONDITIONS

Plant Species	Treat-ments	Citric Acid	Malate Acid	Oxalic Acid	Tartaric Acid
Radish	-P	547	168	80.2	bd
	+P	87.2	34.7	47.6	bd
Rapeseed	-P	490	147	92.7	bd
	+P	189	99.6	62.4	bd
Buckwheat	-P	128	253	57.4	428
	+P	72.6	46.3	52.6	20.2
Wheat	-P	bd	177	67.6	bd
	+P	bd	bd	62.3	bd
Sesbania	-P	bd	bd	57.8	bd
	+P	bd	bd	55.4	bd
Ryegrass	-P	bd	bd	87.6	bd
	+P	bd	bd	79.5	bd

bd, below determination limit.

TABLE VII. ACID PHOSPHATASE ACTIVITY IN DIFFERENT PARTS OF PLANTS (μmol NPP/g f.w.)

Plant Species	New leaf	2nd Leaf	4th or 5th Leaf	Roots
Radish	13.8	6.32	3.26	0.62
Rapeseeds	12.5	5.27	3.42	0.57
Buckwheat	9.96	4.27	2.78	0.48
Wheat	7.84	2.76	1.56	0.42
Sesbania	2.36	1.07	0.86	0.32
Ryegrass	2.84	1.27	0.91	0.39

To further compare the acid phosphatase activities, as induced by phosphorus deficiency, we choose the first expanded leaf to assay the activity under either +P or -P conditions. Results were presented in Figure 1. Obviously, there are two patterns of responses in acid phosphatase under phosphate deficient condition. Activities of acid phosphatase in buckwheat, oil rapeseed, and radish increased 3.2, 3.9 and 3.8 times under -P than under +P conditions, respectively. On the contrary, there was little change in the activities for ryegrass, sesbania, and wheat (Fig. 1).

The activities of acid phosphatase in root exudates presented a slightly different picture. Wheat, radish, buckwheat and rapeseed showed an obvious increase in secreted acid phosphatase activities in their root exudates under -P condition while the increase in ryegrass and sesbania was to a lesser extent (Fig. 2).

3.7. Secreted acid phosphatase activities, as affected by sparingly soluble phosphates

Acid phosphatase activity and the secretion of the enzyme are mainly regulated by interior inorganic P status in plants. It is of interest to know if any sparingly soluble inorganic P sources in the root environment could regulate the enzyme activities. We tested the effect of RP, synthesized iron phosphate and Al phosphate on acid phosphatase activity in the exudates under -P or +P conditions. The enzyme activity in the exudates was normalized with regard to plant fresh weight. Results in Fig. 2 indicated that except for sesbania and oil rapeseed the addition of RP to culture solution resulted in an elevated acid phosphatase activity compared with -P alone. Iron and Al phosphates also had similar effect albeit the effect was not as strong as with RP.

3.8. Changes in activities of other related enzymes

Increased excretion of organic acids under P starvation may suggest a concurrent increase in the synthesis of organic acids within the plants. One key enzyme in organic acid metabolism is phosphoenolpyruvate carboxylase (PEPC). We assayed the enzyme activity in -P and + P treated roots and shoots. Table VIII showed that whereas there was no obvious increase in PEPC activities in roots of rapeseed and radish, obvious increase in its activity was observed in the shoots. On the other hand, with buckwheat, there was an increase in PEPC activity in both roots and shoots.

TABLE VIII. ACTIVITY OF PEPC IN SHOOTS AND ROOTS
UNDER +P OR -P CONDITIONS (nmol NAD/min/g f.w.)

	Rapeseed		Radish		Buckwheat	
	+P	-P	+P	-P	+P	-P
Shoot	264	548	284	463	148	206
Roots	166	169	181	192	97	123

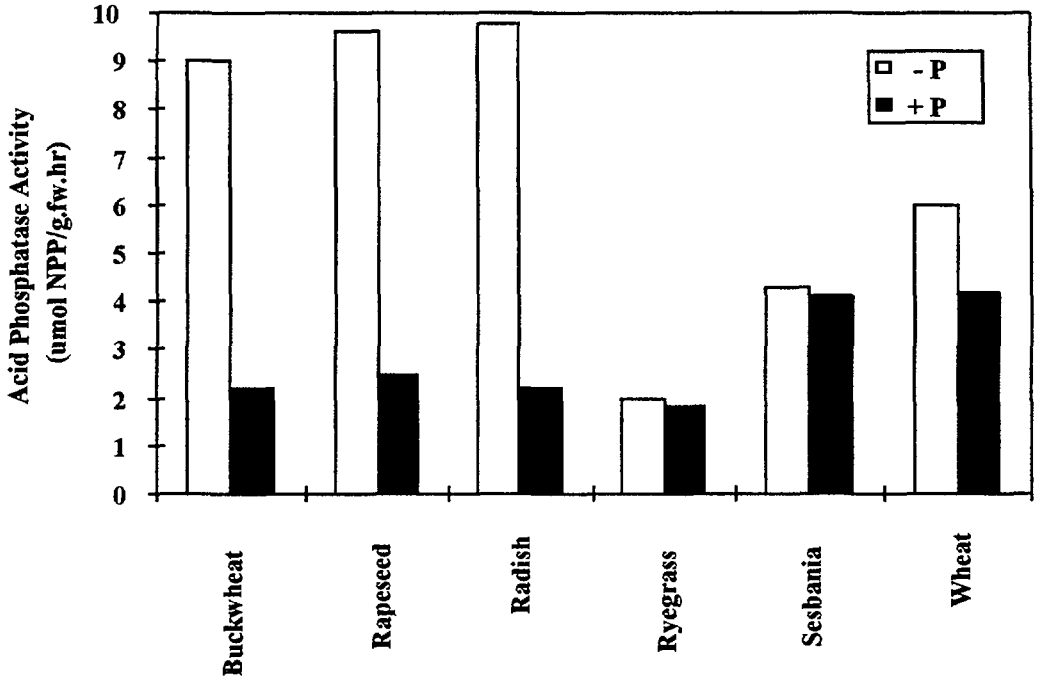

Fig. 1. Acid phosphatase activity (μmol NPP/g fresh weight.hr) in newly expanded leaf under P starvation (-P) *or with phosphorus supply* (+P) *(μmol NPP/g fresh weight.hr). Data are the averages of duplicate determinations.*

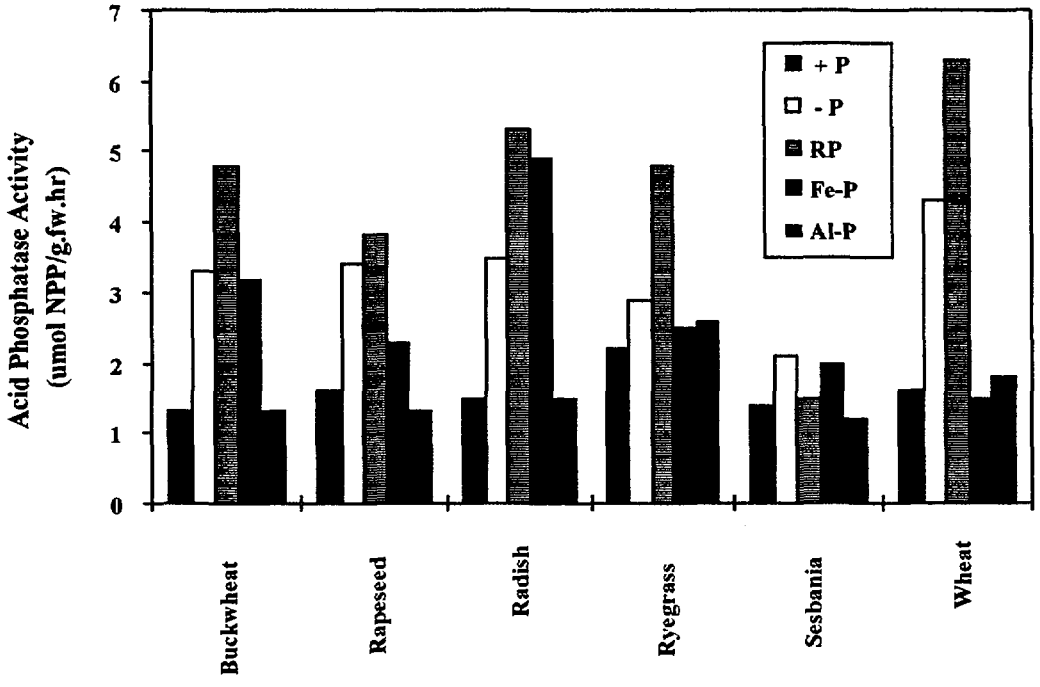

Fig. 2. Acid phosphatase activity (μmol NPP/g fresh weight.hr) in root exudates under P starvation condition as affected by the addition of NCPR (PR, bars with horizontal strips), iron phosphate (Fe-P, bars with down diagonal strips) and aluminum phosphate (Al-P, bars with up diagonal strips). Also shown are -P alone (open bars) and +P treatment (black bars). Data are the averages of duplicate determinations.

Another enzyme that was also affected by P starvation is nitrate reductase (NR) Table IX presented results with NRA in both leaves and roots. Results indicated that while there was a general trend that under phosphorus deficiency condition, NRA decreased, the trend was particularly clear in roots. A higher NRA may associate with higher rhizosphere pH [8]. Reduced NRA may shift the preference from the uptake of NO_3^- to NH_4^+ and decrease rhizosphere pH [9].

TABLE IX. NITRATE REDUCTASE ACTIVITY IN ROOTS AND SHOOTS UNDER -P OR +P CONDITIONS (μg NO_2^--N/g fw/hr)

Plant	*Leaves*		*Roots*	
species	-P	+P	-P	+P
Rapeseed	19.2	40.2	4.6	21.7
Radish	28.7	16.8	3.6	38.4
Buckwheat	32.6	17.5	4.9	41.2
Sesbania	12.4	20.3	2.8	2.5
Ryegrass	8.7	9.6	1.2	1.8
Wheat	20.4	26.3	3.1	10.6

4. DISCUSSION

4.1. Diversity of plant responses to phosphorus deficiency

Under condition of P limitation, plants exhibit a large diversity of responses, which are presumably used to increase P acquisition from the surrounding environment or mobilize internal P resources for use in key processes that are vital for survival. At the molecular level, increased transcription of some genes was observed. These included, for example, genes that encode root-specific phosphate transporters (e.g., [10]). Changes at cellular or morphological levels that are well-documented include alteration of root architectures, excretion of protons, organic ligands, and acid phosphatases. Although it is still not clear whether these responses are adaptation strategies or simply consequences of damage caused by P starvation, these processes are probably of great significance for plants to survive in P-insufficient habitats. Plants may select one or several responses and the choice of these strategies may be related to the natural habitats, which they have long been co-evolved with.

Our study indicates that under P deficiency, wheat and ryegrass had a significant secretion of protons, which acidified the solution. However, cultured solutions with these plants were less efficient in dissolving either Fe or Al phosphates (Table V). Presumably, these plants originated from temperate regions, where the predominant form of phosphorus in soils is calcium phosphates. Fe or Al phosphates are little component fractions of the total P in these soils [11, 12]. A small pH decrease in the rhizosphere would increase significantly the available phosphorus for plants in such a Ca phosphate abundant soil. In natural conditions, these plants also establish symbioses with mycorrhizal fungi and the resulting mycorrhizae are very important for acquisition of phosphorus from bulk soils that extend far away from the otherwise limited rhizosphere.

Other plants in this study that also secreted protons under P starvation were sesbania, buckwheat, and oil rapeseed. When P supply was adequate, these plants did not exude much protons. In acidic soils where these plants are well adapted, increased proton exudation may lead to increased level of

toxic Al in their rhizosphere. In contrast to wheat or ryegrass, besides proton secretion, buckwheat and rapeseed also excreted quite large amount of organic ligands under P deficient conditions. On the other hand, radish plants did not acidify their rhizosphere, yet they also secreted a large quantity of organic acids. The organic acid excretion in radish, buckwheat and rapeseed endowed these plants the ability to exploit Al and Fe phosphates which are the major forms of phosphates in acidic soils. Interestingly, unlike cereal and legume plants, rape and radish do not establish any mycorrhizal symbiosis.

4.2. Role of organic ligands in plant acquisition of phosphorus

When experiencing P deficiency, many plants exude increased amounts of organic components to the surrounding environment. A great variety of organic acids have been detected in root exudates [7], however, some major acids seem preferentially to be secreted by plants. Among these are citric and malic acids (or citrate and malate depending on the pH of the environment involved). A striking example of organic acid excretion was found with white lupin. Under P deficient condition, white lupin secreted a large amount of both citric and malic acids through the segment 1 to 3 cm from the tips of their young proteoid roots [13]. The amount of citric acid secreted may account for more than 10 percent of total plant dry weight [14]. Another organic acid detected was piscidic acid, i.e., hydroxybenzyl tartaric acid, which was secreted by pigeon pea under P deficient conditions and contributed to solubilization of iron phosphates in Alfisols [15].

In the present study, citric and malic acids were the major organic acids secreted by oil rapeseed and radish. Ryegrass and sesbania secreted only small amount of oxalic acid under -P or +P conditions. In addition to oxalic acid, wheat also exudated some malate under P starvation, but the quantity was limited. A special case is with buckwheat. Besides some citric acid and malic acid, buckwheat secreted a significant amount of tartaric acid. The induction in tartaric acid excretion was particularly significant under -P condition (Table VI).

It is not known whether the citrate or malate secretion in oil rapeseed and radish is P starvation-specific. While the excretion of oxalic acid in buckwheat (the same cultivar as we used in this study) was reported to be specifically induced by Al [16], which is consistent with the observation that oxalate had the strongest ability to solubilize Al phosphate (Table V), oxalic acid was not induced by -P treatment. Whereas there was not as much increase in the exudation of citric and malic acids, 20 times increase in the exudation of tartaric acid under -P condition was found with buckwheat (Table VI). It was laso observed a more than 3 times increase in the content of tartaric acid in buckwheat shoots under -P condition. In comparison, the increase in citric and malic acids in oil rapeseeds and radish shoots was less than 2 times (data not shown). It is therefore, very likely that the exudation of tartaric acid in buckwheat is P starvation-specific. Detailed characterization of the specificity of organic acid excretion was first made by Delhaize et al. (1993) [17] in the case of Al toxicity. They demonstrated in a series of studies that the excretion of malate from an Al-tolerant ecotype of wheat was specifically induced by Al but not by other related stresses. While induction of the secretion of some organic ligand in our study is probably P starvation-specific, secretion of citrate and malate is likely stimulated by both P starvation and Al toxicity. Both stresses are common in acidic soils. It is, therefore, possible that an interaction between P deficiency and Al toxicity may lead to even higher secretion of organic ligands than that observed in this study, where only P starvation was applied.

Plants do not normally accumulate high amount of free organic acids. Increased excretion of organic acids under -P condition may imply there are profound changes in carbon metabolism within plants. An important enzyme in carbon metabolism, phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) plays an important role in organic acid synthesis. The enzyme catalyzes the carboxylation of PEP using HCO_3^- as a C source to form oxaloacetate and meanwhile releases the Pi from PEP. While it is not known to what extent this process increases cellular Pi supply, increased PEPC did occur under P deficiency condition, and the increased excretion of organic acids may attribute to this elevated activity of PEPC. Several reports have shown that P deficiency stimulated the activity of C_3 PEPC in leaves [18, 19] and nonphotosynthetic PEPC in roots [17,18]. Yet, in most cases, it is not clear where the increased amount of organic acid came from. Hoffland (1992) [18] suggested that shoot PEPC may contribute to increased citrate synthesis in P-deficient rape plants. Results with white lupin

demonstrated both roots and shoot contribution to the increased synthesis of organic acid [20]. In this experiment, we did not detect increased activities of PEPC in roots of rapeseed and radish, the increased activity of PEPC was found mainly in the shoots (Table VIII). This may suggest that citrate and malic acids excreted in these plants probably come from the synthesis in the shoots. On the other hand, a concomitant increase in PEPC was observed both in shoot and in roots of buckwheat, suggesting that non-photosynthesis carbon fixation may contribute to the synthesis and excretion of organic acids in buckwheat.

4.3. How did rock phosphate enhance acid phosphatase activity

It has long been known that under nutrient-limited conditions, plants are able to sense and direct their roots toward the location where nutrients are relatively abundant. Once roots reach the nutrient source, a lot of new laterals are initiated to explore the resources. The molecular explanation for this phenomenon is emerging [21]. By analog, we hypothesized that when we supply a limited amount of available P in the form of rock phosphates the plants would show an increased responses in the excretion of organic acids or acid phosphatase.

Our preliminary experiments indicated that indeed, supply of rock phosphate stimulated the activity of acid phosphatase within the plants (Fig. 1) and increased the secreted enzyme activity (Fig. 2). However, it is not clear whether the effect is from a supply of trace amount of phosphate from the rock phosphate or is from the ions existed in rock phosphates which may be solubilized to some extent in the presence of root exudates. Several kinds of protein phosphatases (e.g., PP2B and 2C) depend on binding with Ca or calmodulin to function (e.g., [21, 23]) (for review, see [24]) yet the amount of Ca in plant cells or in the nutrient solution are more than sufficient for this purpose. Although acid phosphatases are not reported to need Ca for their function, evidence indicated that Ca did increase their activity [25]. On the other hand, detailed analysis of acid phosphatases indicated Fe and Zn are cofactors for their activity [26]. Again, the supply of these nutrients in nutrient solution should be sufficient.

Considering these factors, it is more likely that the small amount of phosphate in the solution caused the increase in acid phosphatase. Experiments with low concentration of soluble phosphate should prove this hypothesis. If this is true, one may wonder why orthophosphate ions should be able to stimulate the secretion of acid phosphatases which target organic phosphorus? In the soil environment, organic phosphate molecules would travel around much easier than inorganic phosphates and hence would be more likely to become a candidate signal to signify to the plants the existence of a P resource for consumption. The induction of phosphatase synthesis is in fact induced by Pi deficiency and the signal is internal. Trace amount of phosphate ions in the bulk soil environment as a result of decomposition of organic matter may therefore act together with internal P deficiency signals in plants to exaggerate the secretion of acid phosphatase and/or organic acids.

It should be pointed out that it is not clear whether the detected changes in acid phosphatase activity were due to changes in the amount of proteins or just due to alteration in activities. With *B. nigra* suspension cells or intact roots experiencing a transition from P sufficiency to deficiency, Duff et al. (1991) [27] quantified extracted acid phosphatase protein by immunoblots and found that the amount of total acid phosphatase protein was closely correlated with total enzyme activity. Additional experiments should be conducted to clarify the uncertainties encountered in the present experimental conditions.

4.4. Using plant factors to increase the efficiency of rock phosphate-based fertilizers

As demonstrated in the present experiments and in other studies, plants differ greatly in their responses to P deficiency. Likewise, some plants are more efficient in using soil phosphates or other sparingly soluble sources of P applied as fertilizers. Agronomic practices taking the advantage of this fact in crop rotations have been suggested. For example, it was recommended that legume crops which have stronger ability to utilize rock phosphate and soil phosphates be included in the rotation between double rice, and rock phosphates be applied to these legume crops and not directly to cereal crops [1].

In the Indian subcontinent, incorporation of pigeon pea was also practiced by farmers to increase the exploitation of soil phosphates [15]. Advances in molecular cloning techniques have made transferring of genes across genera border a routine practice. Recently, Herrera-Estrella and co-workers successfully transferred the citrate synthase gene from bacterium *Pseudomonas aeruginosa* to tobacco and papaya. The increased citrate content in plants and secreted citrate contributed to the detoxification of Al in these transgenic plants [28]. Given the great impact of organic acid on plant acquisition of phosphate, these transgenic lines should also be efficient in utilizing rock phosphates or soil phosphates. However, it is not known what other consequences of the heterogeneous genes will have on host plants. Also, the transferred genes may not function the way expected. This concern is particularly important considering that carbon metabolism as the primary metabolism of plants is not very flexible [29] and is recalcitrant to modification. Nevertheless, it is still very optimistic that similar studies should lead to the production of engineered plants with improved ability to use soil phosphates or rock phosphates. For example, modification of plants with enhanced organic acids synthesis and root exudation should increase plant utilization of inorganic phosphates. Over expression of genes encoded secreted acid phosphatases should also increase the possibility of using organic phosphates, although the role of acid phosphatase in plant phosphorus nutrition is still ambiguous [30]. As plant mutants defective in secreted acid phosphatases are being isolated [31], characterization of these mutants should provide insightful knowledge regarding the role of these enzymes in plant P nutrition under various soil conditions.

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