



Development of a Regression Equation to Measure the Relative Efficiency of P-Mobilization by Phosphatase, Phytase and Organic Acids

Amita Gharu Dhariwal¹ and J.C. Tarafdar^{2*}

¹Assistant Professor, Department of Chemistry,
Jai Narain Vyas University, Jodhpur (Rajasthan), India.

²Former Principal Scientist and National Fellow,
ICAR - Central Arid Zone Research Institute, Jodhpur (Rajasthan), India.

(Corresponding author: J. C. Tarafdar*)

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ABSTRACT: It is important to know the relative contribution of phosphatases, phytase and organic acids which enable us to distinguish their individual importance in P mobilization. Therefore, we have developed a regression equation to understand the relative contribution of acid phosphatase, alkaline phosphatase, phytase and organic acids towards the P mobilization/solubilization from different organic matter soil. Our result suggested that acid phosphatase was most effective P mobilizers followed by phytase except under low soil organic matter condition where phytase was dominant P mobilizers. Among the organic acids, formic acid was most effective in P solubilization followed by citric acid while malic acid was found to be poor contributor under P solubilization. The effects of organic acids are found to be more under low organic P soil.

Keywords: Organic acids, Phosphatases, Phytase, P mobilization, P solubilization, Regression equation.

INTRODUCTION

Almost 97% phosphorus in soil exists as insoluble inorganic phosphates and organic phosphates, therefore, higher plants have developed several strategies for adaptation to low phosphorus (P) condition. These include the secretion of acid phosphatase and organic acids, induction of the inorganic phosphate (Pi) transporter and the substitution of some enzyme activity as alternative pathways to increase P utilization efficiency (Wasaki *et al.*, 2003). The mobilization of soil P by soil biota mainly involved the solubilization of insoluble inorganic P by the excretion of proton and organic acids, and hydrolysis of organic P by phosphatases (acid and alkaline) and phytase (Zhang *et al.*, 1998). The process of P mobilization was regulated by the equilibrium between soil P supply and plant P demand. The simultaneous exudation of organic acids and phosphatases (phosphoric mono ester hydrolases) could increase both P solubility by releasing bound organic P (Po) and Po-mineralization by increasing the rate of hydrolytic cleavage (George *et al.*, 2002). In general, under P deficient condition organic acid and acid phosphatase activity significantly increased (Shen *et al.*, 2001). Frago *et al.* (2005) found acid phosphatase activity increased 10 – fold in the absence of phosphate while this increase was only 4 or 5 – fold in the other phosphate deficiency treatments (phytic acid 500 μ M or AlPO_4 50 μ M). Phosphate amount in plant was high under phytic acid and AlPO_4 treatments, indicating that the plants produce phytase and organic

acids may help in phosphate mobilization. Enhanced secretion of acid phosphatase and phytase (Li *et al.*, 1997) by plant roots and also by rhizosphere microorganisms (Tarafdar and Marschner 1994) under P-deficient conditions may contribute to Pi acquisition by hydrolysis of organic P esters (Janes-Bassett *et al.*, 2022) in the rhizosphere. In a P-deficient sandy soil, more Pi was liberated by simultaneous application of acid phosphatase and organic acids identified in rhizosphere soil solution of *Hakea undulata* than by separate application of organic acids or acid phosphatase, respectively (Beissner, 1997). Ibrahim *et al.* (2022) examines how plants fulfill P requirements from tissue stored P during P starvation. The importance of acid phosphatase, alkaline phosphatase, phytase and organic acids are clear from the literature for native P mobilization for plant nutrition but no attempt has been made to find out their relative contribution in P mobilization. Therefore, it is very important to find out an equation to measure the relative efficiency of phosphatases, phytase and organic acids towards the contribution of P mobilization for plant nutrition under different soil organic matter conditions.

MATERIAL AND METHODS

To develop a regression equation indicating the relative contribution of acid phosphatase, alkaline phosphatase, phytase and organic acids towards hydrolysis of native soil P, experiments were conducted by using four different types of soil collected from Tonk, Jalawar and

Bundi (Rajasthan) are presented in Table 1. Random surface soil samples (0-15 cm) from 15 different sites of each selected field were collected and properly mixed. The soil was air-dried, passed through a 2 mm sieve, and stored in plastic barrels prior to use. Selected chemical and physical properties were determined by standard methods (Jackson, 1967).

Four organic acids (citric, formic, lactic, and malic) were selected for the study as both plants and micro-organisms may secrete an appreciable amount of all the four organic acids (Jones, 1998). The organic acid solutions were prepared for extraction from extra pure grade material (E-merk) and deionized water was used for preparation of different solution concentrations (0.2, 0.4, 0.6, 0.8 and 1 mM). Two experiments were conducted. In the first experiment the release of available P due to the action of different concentration of four organic acids in four different types of soil were studied. Triplicate samples of 5g air dried soil were placed in 250 mL plastic bottles and 50 mL of extracting solution of known concentration (0.2, 0.4, 0.6, 0.8, 1.0 mM) were added to the soil. The bottles were capped and shaken (100 rpm) at $35 \pm 2^\circ\text{C}$ temperature. Samples were removed after four hours and filtered through Whatman No. 1 filter paper. The filtrate was analyzed for P release by using the standard method of Jackson (1967). For blank instead of acid extracting solution only deionized water was used for extraction.

In second experiment, phosphatases (acid and alkaline) and phytase activity of left over soil under different treatments after complete removal of water were estimated. Acid and alkaline phosphatases were assayed by adopting the standard procedure of Tabatabai and Bremner (1969) using acetate buffer (pH 5.4) and sodium tetraborate- NaOH buffer (pH 9.4), respectively and 4-nitrophenyl phosphate as enzyme substrate. Phytase activity was estimated by using a standard method of Ames (1966), with acetate buffer (pH 4.5) and sodium phytate (1 mM) act as enzyme substrate.

RESULTS AND DISCUSSION

A regression equation was developed to understand the relative contribution of acid phosphatase, alkaline phosphatase, phytase and organic acids towards the total P mobilization. Individual contribution was also recorded under different soil types (low, medium and higher soil organic matter condition as well as low available soil P conditions). The soil chosen under the study for different parameters was presented as Table 1. In general, significant contribution towards P mobilization was observed in the order: acid phosphatase > phytase > organic acids > alkaline phosphatase. The β -weight of each parameter towards mobilization of unavailable P was presented as Table 2. The higher β -weight indicate, more contribution towards the mobilization of unavailable phosphorus. The results clearly showed that contribution from acid phosphatase was more as compared to the other agents, if we look at the overall situation. But under low organic matter condition phytase was more efficient (β -

weight; 0.5219) followed by organic acids (β -weight; 0.4902). Under medium organic matter soil condition more efficiency was observed from phytase (β -weight; 0.5772) followed by acid phosphatase (β -weight; 0.3981). The P mobilization from high organic matter soil was mostly contributed by organic acids (β -weight; 0.4319) followed by alkaline phosphatase. If the soil had low available phosphorus, then the P mobilization from native sources were mainly contributed by organic acid (β -weight; 0.2676) closely followed by alkaline phosphatase (β -weight; 0.1805).

The results clearly indicated (Table 2) that acid phosphatases are most active and efficient in mobilizing phosphorus followed by phytase and organic acids. Behera *et al.* (2017) also found that acid phosphatase is the most important enzyme for P mobilization. Enhancing phytase activity in soils and phytate P acquisition by plants was also described by Liu *et al.* (2022). The results also showed that phytase was most efficient under high organic matter and low available P soils. Under Pi- deprivation, plant species and cultivars evolved classical array of morphological, physiological, biochemical and molecular adaptations that enable them to scavenge P from such conditions. These adaptation include, beside enzyme and organic acid secretion, modifications in root architecture e.g. increased functional plasticity viz. increased root : shoot ratio and structural plasticity via occurrence of proteoid roots, increased root hair density and elongation of lateral roots (Vance *et al.*, 2003), increased organic acid exudation (Jones, 1998), rhizosphere acidification (Rengel, 2002), increased amount of phosphatases and RNAses (Radersma and Grierson 2004), enhanced phosphate uptake rate (Gilroy and Jones 2000) and increased synthesis of Pi transporter (Hammond *et al.*, 2004).

The consensus in the literature is those acid and alkaline phosphatases are differentiated by their source of production. Plant-produced phosphatase is reported to be exclusively acid phosphatase (Tarafdar and Marschner 1994). In addition, acid phosphatase is produced by bacteria, fungi, yeasts and protozoa, so that enhanced acid phosphatase activity in the rhizosphere may be produced directly by plant roots or indirectly via stimulation of the microbial biomass. Alkaline phosphatase does not have a plant origin and is produced by bacteria, fungi and earth worms (Hebrien and Neal, 1990). P depletion with concurrent enhanced enzyme activity has often been demonstrated in nutrient solution or sand culture and in soil with powerful techniques such as NMR (Adams and Pate 1992). However, it is rarely demonstrated in soil with standard chemical extractants. For example, Adams and Pate (1992) demonstrated the IHP and KH_2PO_4 were equally effective as P sources in sand (attributing this to phytase activity) while IHP was a much poorer source in soil. The release of organic acids from roots or microbes can operate by multiple mechanisms in response to a number of well-defined environmental stresses. The present results indicate that the sorption of organic acids to the mineral phase and mineralization

by the soils microbial biomass are critical to determine the effectiveness of organic acids in most rhizosphere process. The results clearly showed that the highest P mobilization was observed due to formic, lactic and citric acid at a concentration of 0.8 mM. Citric acid was found to be most efficient in P mobilization in low available P soil.

The regression equation with different parameters under different soil conditions was presented as Table 3. The results clearly showed that in low organic matter soil phytase and organic acids are the main contributors towards the P mobilization, while phosphatases are insignificant contributors. In medium organic matter soil main contribution was noticed from phytase and acid phosphatase, but alkaline phosphatase and organic acids seems to be insignificant contributors. In high organic matter soil, although the combined effect of all the parameters was significant but not a single agent was found to be significantly contributed towards P mobilization while organic acids was found to be most dominant component (β weight; 0.4319) followed by alkaline phosphatase. A similar observation was noticed under the soils of low available phosphorus where not a single parameter was significantly contributed but their overall contribution was significant and organic acids as well as alkaline phosphatase seems to be more contributors.

The pattern of P solubilization by different organic acids was also analyzed after considering four important P solubilizing organic acids (formic, lactic, citric, malic) with variation of their concentration under different soil condition. The P solubilization from low organic matter soil after variation in different organic acid concentrations was presented as Fig. 1. The results clearly showed that moderate P solubilization was noticed with formic acid concentration from 0.2 to 0.8 mM. But with increase in concentration above 0.8 mM, a significantly higher ($p < 0.01$) P solubilization was noticed due to formic acid, which increased from 9.7 mg kg⁻¹ to 17.1 mg kg⁻¹. There was no significant increase in P solubilization with increase in lactic acid concentration from 0.2 to 1 mM, where the solubilization between 9.4 to 10.2 mg kg⁻¹. A significant ($p < 0.01$) increase in P solubilization was observed with increase in citric acid concentration till 0.8 mM, with further increase in concentration there was declining in P solubilization. The least effect on P solubilization was observed with malic acid where the P solubilization varies between 22 to 39% more due to increase of malic acid concentration up from 0.2 to 1 mM.

P solubilization by different organic acids from medium organic matter soils was presented (Fig. 2). The result indicate significantly higher ($p < 0.01$) P solubilization above 0.4 mM concentration of formic acid. But with increase the concentration of more than 0.8 mM the solubilization decreases. An interesting result was obtained with lactic acid where the solubilization significantly increased (140%) upto 0.2 mM but

decreased thereafter till 0.8 mM and after that enhanced with increased in concentration till 1 mM. A typical pattern on P solubilization by citric acid from medium organic matter soil was observed where 0.2, 0.4 and 1 mM concentration showed similar solubilization of plant unavailable phosphorus. Surprisingly, least P solubilization was noticed at 0.6 mM concentration. Citric acid seems to be better acid for more P solubilization (up to 364%, from control) from medium organic matter soil. The P solubilization by malic acid was also observed more at 0.2 mM concentration. Thereafter, the solubilization was in decreasing trend till 0.6 mM and further marginal improvement in solubilization was noticed up to 1 mM concentration.

The pattern of P solubilization by different organic acids from high organic matter soils (Fig. 3) showed more solubilization of P by citric acid (up to 188%). Formic acid showed greatest solubilization at a concentration of 0.8 mM, although the increase of solubilization was up to 79%. The effect of lactic acid also was observed maximum at a concentration of 0.8 mM, where 43% improvement in P solubilization was visualized. A similar trend in solubilization was also observed with citric acid, where 0.8 mM concentration found to be the best for solubilization. However, the P solubilization by malic acid was found only up to 34% with the highest at 1 mM concentration.

The effect of different organic acids on low available P content soil was presented (Fig. 4), and showed an individualistic pattern for different organic acids. The highest P solubilization was observed at 0.8 mM (formic acid), 0.6 mM (lactic acid), 0.4 mM (citric acid) and 0.6 mM for malic acid. In general, 5 to 7 times improvement in P solubilization was observed with formic acid, 0.7 to 6 times by lactic acid, 11 to 16 times by citric acid and 0.9 to 4 times by malic acid. The present result reflected that the effect of organic acids was much more in low P soil than any other soil types.

The reaction of organic acids can be expected to vary both spatially and temporally in the rhizosphere with the operation of many fluxes simultaneously. While microbes can consume root exudates, they are also responsible for the production of a wide range of organic acids especially in situations where nutrients may be limiting. Due to the negative charge associated with their carboxyl groups, organic acids can become rapidly and readily sorbed to the soils solid phase, a fact which is often ignored. Sorption appears to be largely dependent on soil type as for P (Jones and Brassington 1998). It is also probable that all these anions share similar sorption sites, as all four organic acids tested are capable of inducing P desorption or preventing the sorption of newly added P (Bolan *et al.*, 1994, Jones and Darrah 1994). Future work should concentrate on close identification of the constituents of the organic P pool and monitor their dynamics in the rhizosphere of plants releasing non-rate limiting activities of enzymes and organic acids.

Table 1: Soil chosen for regression analysis for different P parameters.

Parameter	Type of soil studied
Low organic matter soil	Organic matter content < 0.5%
Medium organic matter soil	Organic matter content > 0.5% but < 1.0%
High organic matter soil	Organic matter content > 1.0% but < 2.0%
Low available P soil	Available P content < 5 mg kg ⁻¹

Table 2: β -weight of different parameters towards the mobilization of native soil phosphorus under different soil condition.

Soil characters	Acid phosphatase (X ₁)	Alkaline phosphatase (X ₂)	Phytase (X ₃)	Organic acid (X ₄)
Low organic matter	0.2763	0.0272	0.5219	0.4902
Medium organic matter	0.3981	0.1941	0.5772	0.0921
High organic matter	0.0891	0.1314	0.1056	0.4319
Low available P	0.0318	0.1805	0.0486	0.2676
Overall	0.4247	0.0610	0.3615	0.3098

Table 3: Regression equation under different soil parameters towards the contribution of native P mobilization by recognizing agents.

Soil characters	Regression equation
Low organic matter	$Y = 7.8607 - 0.2723 X_1^{NS} - 0.0814 X_2^{NS} + 0.0034 X_3^{***} + 8.4347 X_4^{***}$ (R ² = 48.07%)
Medium organic matter	$Y = 20.4247 - 0.2815 X_1^{**} - 0.4421 X_2^{NS} + 0.0023 X_3^{***} + 1.2307 X_4^{NS}$ (R ² = 64.11%)
High organic matter	$Y = 18.1529 - 0.0617 X_1^{NS} - 0.2534 X_2^{NS} - 0.0015 X_3^{NS} + 4.3605 X_4^{NS}$ (R ² = 20.51%)
Low available P	$Y = 4.9329 - 0.0078 X_1^{NS} - 0.1423 X_2^{NS} + 0.0001 X_3^{NS} + 1.11793 X_4^{NS}$ (R ² = 7.35%)
Overall	$Y = 8.0346 - 0.3711 X_1^{***} - 0.1555 X_2^{NS} + 0.0018 X_3^{***} + 4.5849 X_4^{***}$ (R ² = 36.46%)

*** p < 0.001; ** p < 0.01; * p < 0.05; NS: Non-significant; X₁: Acid phosphatase; X₂: Alkaline phosphatase; X₃: Phytase; X₄: Organic acid

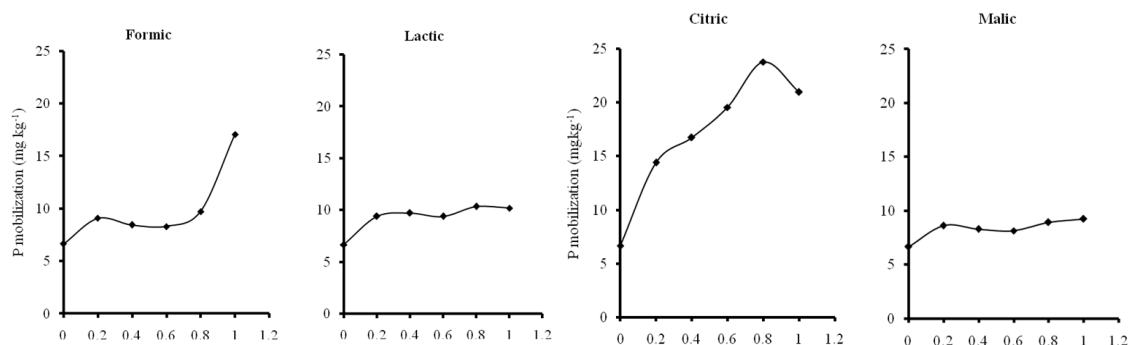


Fig. 1. Pattern of P mobilization from low organic matter soil by the action of different organic acids.

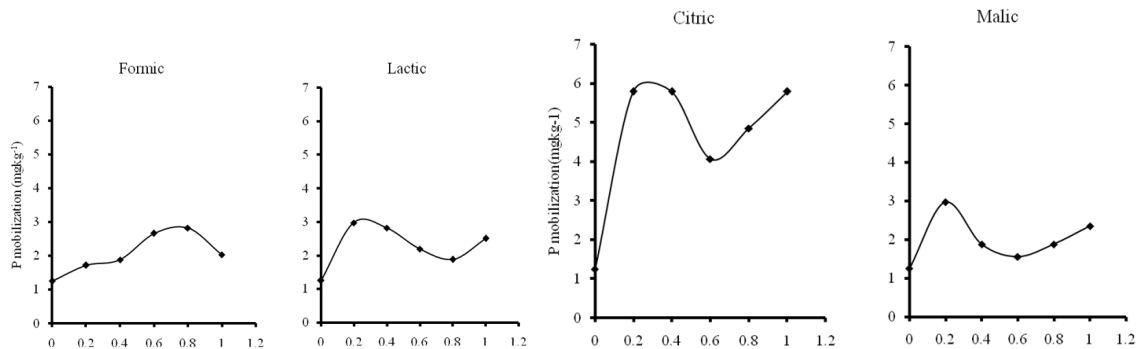


Fig. 2. Pattern of P mobilization from medium organic matter soil by the action of different organic acid.

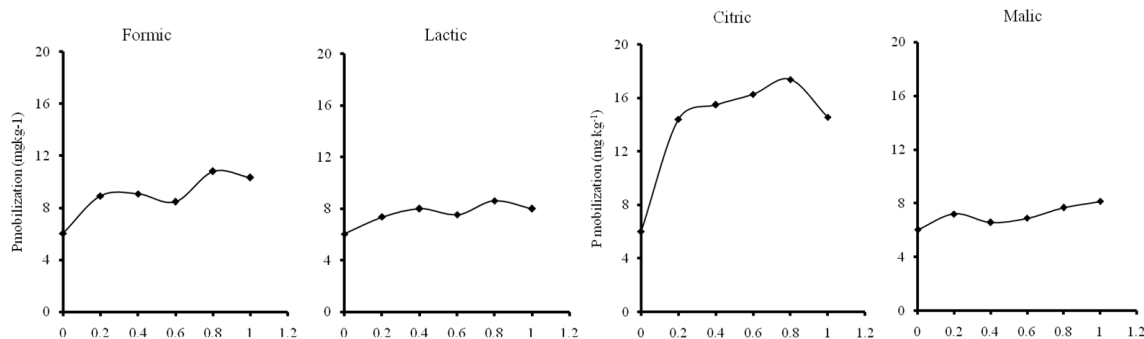


Fig. 3. Pattern of P mobilization from high organic matter soil by the action of different organic acids.

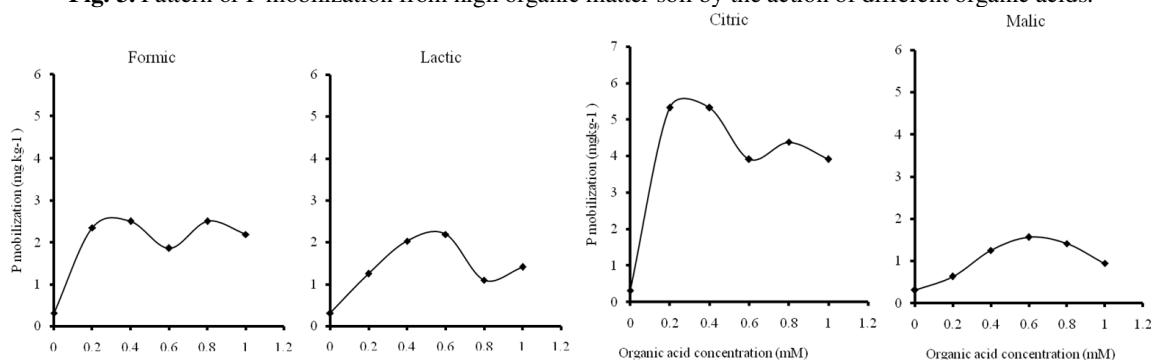


Fig. 4. Pattern of P mobilization from low available P soil by the action of different organic acids.

CONCLUSIONS

The present study evaluates a regression equation regarding the relative contribution of all four important P mobilizing/solubilizing agents (acid phosphatase, alkaline phosphatase, phytase and organic acids) present in soil after originating from both plant and microbial sources. The data indicates the relative contributions of all the agents under different soil organic matter and low P conditions. The results have the indication that although some of the agents are more effective under certain soil conditions but all four of them are equally important for soil P mobilization in one way or other for plant nutrition. The present findings also indicate that under agricultural settings phosphatases group of enzymes and organic acids plays an important role in P acquisition especially from native organic sources.

FUTURE SCOPE

Further research is needed to quantify the role of each P mobilizing/solubilizing agents to enhance higher P mobilization from native sources for more plant nutrition and to enhance resilience of food production.

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Conflict of Interest. None.

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