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Factors Affecting Solubilization of Rock Phosphates in Soils

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Authors' contributions

This work was carried out in collaboration between both authors equally. Author AME read and approved the final manuscript.

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ABSTRACT

The aim of this work was to conduct laboratory and pot experiments to study the ability of plant roots on solubilizing various sources of rock phosphate and factors that may facilitate or inhibit their activity. The effect of adding organic matter or sulphur in solubilizing rock phosphate added to alkaline and calcareous soil was studied. A split medium – split root technique experiment was conducted to study also the effect of N form, soil type, source and nature of rock phosphate on the pH, P solubility in the medium and P uptake by bean plants (*Vicia faba* var. balady). Results show that the highest $NaHCO_3$ extractable P of incubation experiment were found for both soils treated with superphosphate. The behavior of rock phosphates in the two soils indicated slight solubilization in the alluvial soil and no remarkable change of extractable P in the calcareous soil as compared to the control. Regarding the effect of N form and P on growth and root exudates of bean plants, results indicated that the highest recorded total dry weight was found when both N forms were applied in the ratio of 1:4 NO_3^- : NH_4^+ or NO_3^- alone. Results of root exudate analyses for amino acid content indicated the presence of several amino acids and variations in the amount were only with lysine. The highest content (115 μδ/100 ml) was in the root exudate of the complete nutrient solution

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(Hoagland solution) and the lowest value (24 μδ/100 ml) in the exudates of bean plants fed with NO_3^- as the only N form. The rock phosphate from Abou zaabal gave the highest P content in acid soil in the presence of plant. The highest drop in pH value in the collected solutions was when NH_4^+ alone was the N source.

Keywords: Organic matter; sulphur; alkaline soil; calcareous soil; split medium technique; bean plants.

1. INTRODUCTION

In Egypt, phosphorus is the second major fertilizer after nitrogen and it is added to the soil mainly as superphosphate. P is an insoluble element in alkaline soil particularly in soils containing high calcium carbonate and it precipitate rapidly to insoluble phosphate forms. Under acid soil condition rock phosphates proved to be a useful source of P depending on the reactivity of the material [1].

It is evident that the usefulness of P to crops can be influenced by intrinsic soil and plant factors [1,2,3]. Concerning the soil, there may be factors controlling rock phosphate solubility, as the use of acidifying materials, presence of humus and the use of extensive amount of water as in rice fields. Moreover, precipitation of P can occurs under conditions of high pH, $CaCO_3$ content, clay content and soluble Ca, Al, Fe [4,5,6].

With respect to plant factors, P-solubilization by root exudates has been put forward as a mechanism leading to enhance P-availability to the plants. Acidification of root medium and excretion of organic acids, amino acids and other chelating substances were suggested as a possible mechanism of increasing P-uptake particularly under P-stressed conditions and with the use of rock phosphate as a source of P [7,8,9,10]. Accordingly, getting benefit of factors that help in solubilizing hardly insoluble sources of P may encourage the use of rock phosphate even under alkaline condition.

So the aim of this work was to conduct laboratory and pot experiments to study the ability of plant roots on solubilizing various sources of rock phosphate and factors that may facilitate or inhibit their activity. The effect of adding organic matter or sulphur in solubilizing rock phosphate added to alkaline and calcareous soil was studied. A split medium – split root technique experiment was conducted to study also the effect of N form, soil type, source and nature of rock phosphate on the pH, P solubility in the medium and P uptake by bean plants (*Vicia faba* var. balady).

2. MATERIALS AND METHODS

2.1 Soil and Rock Phosphates Samples

Three soil samples of which 2-alkaline Egyptian soils and one-German acid soil were used in this study. The alkaline soils represent an alluvial clay soil, *Typic Torrents*, collected from Shalakan Experimental Station and a calcareous sandy loam soil, *Calcids*, collected from Burg Elarab area, while the acid soil was collected from Bayern, Germany, *Weihenstephan soil*. Some chemical characteristics of the studied soils are presented in Table 1.

Table 1. Some chemical characteristics of the studied soils

Character	Alluvial clay soil	Calcareous sandy loam soil	German acid soil
pH	8.00	8.50	6.10
Clay content, %	40.0	14.0	16.0
OM, %	1.30	0.80	2.22
$CaCO_3$, %	3.80	28.0	1.20
Total N, %	0.93	0.38	0.16
Total P, %	0.22	0.11	0.07

Three-rock phosphate (RP) samples were used, 2-samples represents the main rock phosphate mines in Egypt (Red Sea mine), namely Abou Zaabal and Abou Tartour. The third sample was collected from Kodjari mine in South Africa. The rock phosphate samples are different in their total P citric acid-soluble P content and the calculated reactivity values, as shown in Table 2.

$$\text{Reactivity, \%} = \frac{(\%\,citric\ acid\ soluble\ \ P)}{\%\,Total\ P} \times 100 \ [11]$$

2.2 Incubation of Alkaline Soils Enriched with Rock Phosphate

Dry sieved alluvial and calcareous soil samples (400 g) were placed in a plastic container. To a group of pots ground alfalfa residues containing about (8 mg P/pot) were mixed with the soil at a rate of 2% and to another group sulphur powder

Table 2. Total P and its reactivity values in different sources of rock phosphates

Rock phosphate source	Total P (%)	Citric acid soluble –P (%)	Reactivity (%)
Egypt, Red Sea - Abou Zaabal	9.4	4.3	46
Egypt, Red Sea - Abou Tartour	8.2	3.7	46
South Africa, Kodjari	13.6	2.2	16

was mixed with the soil at the rate of 0.2% S. The organic matter and S enriched pots were first incubated for 2-weeks at 30℃ after adjusting the water holding capacity to 100% and then adjusting the moisture content daily. The P treatment was added at the rate of 150 mg P/kg as superphosphate or rock phosphate to all pots except the control (without P source). The pots were incubated for one-month at 30℃ and adjusting the moisture content as mentioned above. Soil samples were taken at 0, 15 and 30 days for evaluating $NaHCO_3$ extractable P as described by [12].

2.3 Split Medium Technique

The combined sand and solution culture technique previously designed and used by [5] was used in this investigation, as shown in Fig. 1.

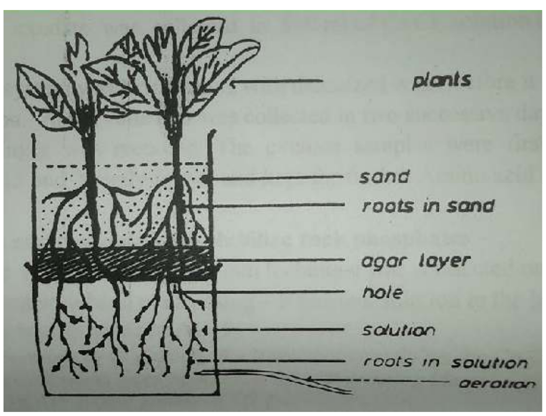

Fig. 1. Split medium technique

The technique was used to study the role of plant roots in mobilizing P from rock phosphates. In order to understand and maximize this role, root exudate was collected and analyzed with respect to changes in pH value of the medium and the effect of N form (NO_3^- and NH_4^+) and ratio on the amino acid contents in the exudate. Also, the ability of root exudate to mobilize different sources of rock phosphates added separately or with different soil samples on plant growth and P content under different treatments was studied in the following experiments.

2.4 Effect of N Form and Ratio on the pH and Amino Acid Composition of Root Exudates

In this work broad bean plants (*Vicia faba* var. balady) was used as test plants. Seeds were first germinated between filter papers. After one week, five seedlings were transferred to each of the upper Neubauer pots to which 400 g of acid washed sand were placed. The seedlings were irrigated with various composition of a nutrient solution [5] as follow: (A) nutrient solution with P, (B) complete nutrient solution NO_3^- : NH_4^+ is 1:4, (C) solution B with N in the NH_4^+ form, (D) solution B with N in the NO_3^- form.

The pH of the nutrient solution was adjusted to pH 5.5. The pots were placed in a growth chamber (26℃ with 60% humidity, 10-hrs day of 20.000-foot candle and 14-hrs night.) After 15-days from seedling, each pot was placed over another pot (Fig. 1) and the solution in the lower pot was the same as the irrigation solution of the upper pot. The roots penetrated the agar layer and the root systems grew normally in the lower pots. The changes in pH of the lower solution was recorded, also the root exudate was collected in 500 ml of $CaCl_2$ solution 0.5×10^{-4} M pH 6.85.

The root system was first washed with de-ionized water before it was immersed in the solution. About one liter was collected in two successive days for each pot and pH changes were recorded. The exudate samples were first filtered with Whitman No 15 and directly frozen and kept for further amino acid analysis.

2.5 Root Exudates and the Ability to Solubilize Rock Phosphates

The above mentioned split medium technique was conducted on another group of pots planted with bean plants using –P nutrient solution in the lower and upper compartments beside the control (+P).

After one-week from placing, the lower pot containing the $CaCl_2$ solution, the following treatments were applied on the –P growing plants:

1. 1 g RP-Abou Zaabal
2. 1 g RP-Abou Tartour
3. 1 g RP-Kodjari
4. As No 1 + 10 g Alluvial soil
5. As No 1 + 10 g Calcareous soil
6. As No 1 + 10 g Weiheenstephan soil
7. Control (+P treatment)

The same above treatments were applied on another group of the lower Neubauer pots which were fitted with aeration but without plants.

After one week from applying the treatments, plants were harvested and separated to shoots and roots, washed with de-ionized water, dried at 70℃, dry weight was recorded then kept for P analyses. The solution in the lower pots of the two groups was filtered (Schleicher and Schull No 15); completed to 500 ml solution, the pH was measured and then analyzed for soluble-P.

2.6 Analytical Methods

pH was measured with the glass electrode. P was determined by the method of [13]. Amino acids in the residues of the exudate samples were dissolved in 5 ml LiCl buffer and the amino acids were evaluated by the use of amino acid analyzer [11].

The experiment was designed in a completely randomized design and each treatment was replicated three times. Data were statistically analyzed using the analysis of variance adopting a SAS software package at $P \leq 0.05$ [14].

3. RESULTS AND DISCUSSION

3.1 Solubilization of Rock Phosphates in Soils

After 30 days results in Table 3 showed, in general, that the highest $NaHCO_3$ extractable P of incubation were found for both the alkaline alluvial and calcareous soils treated with superphosphate. The behavior of rock phosphates in the two soils indicated slight solubilization in the alluvial soil and no remarkable change of extractable P in the calcareous soil as compared to the control. The presence of relatively high soluble Ca^{2+} and low native organic matter beside the presence of relatively high pH value could be the reason for the limited solubility of the rock phosphate recorded after 15 days from incubation. The decreases in solubilization effected by time could be related to decomposition of humic substances.

However, the extractable P from the rock phosphate + organic matter treatments increased in both soils compared to the control and the percentage increase represented about ½ to ⅔ from the percentage increase of exactable P from the superphosphate. The researchers [11,15] found that composting rock phosphate materials with straw or other organic waste materials resulted in phosphate mobilization. They further indicated that the driving force for rock phosphate solubilization is the pH decrease and the chelating effect of simple organic acids as citric and malic, and complex acids as humic and fulvic acids on Ca^{2+} ions from insoluble phosphate forms. The researchers [16] reported that humic substances in interaction with P in the soil could decrease the P fixation and increase the P uptake of plants. Humic acid served as a buffer at a broad pH interval and several macro and microelements could be taken by the plants since the soil was neutralized.

The sulphur treatment caused change of less than 10% in the amount of extractable P in the calcareous and alluvial soils due to the rapid disappearance of locally formed sulphuric acid produced from the oxidation of sulphur by soil microorganisms. The researchers [17] found that sulphur facilitated the solubility of the P, Fe and Zn by soil microorganism in the calcareous soil.

3.2 Effect of N Form and P on Growth and Root Exudates

Results in Table 4 indicated a decrease in the pH value of about 2-units in the solution when NH_4^+ was used as a source of N in the nutrient solution, while there was an increase of about one-unit of the pH when NO_3^- was the only N-form added. Such phenomenon was reported by several investigators for leguminous and non-leguminous plants [7,18,19,20]. It was further postulated that with NH_4^+ as N source more cations than anions were taken up to maintain an electrical charge balance and consequently a net H^+ ion extrusion from the root occurred [8].

The results found for bean plants showed also a drop in pH of about 2.2 units for P- deficient plants. It was reported that under certain stress conditions, roots, in order to overcome such phenomena exudates organic molecules as citric and malic acids [7,8,21], amino acids and reducing or chelating compounds [5,10,15,22].

The dry weight of shoots shows relatively higher values when NH_4^+ the only form was supplied. However, the highest recorded total dry weight found when both N forms were applied in the ratio of 1:4 NO_3^- : NH_4^+ or NO_3^- alone. It is interesting to note that the root/shoot ratio was the highest for P-deficient plants, [8,23] indicated that root growth and number of root hairs and length increased, also the amount of H^+ ion increased as plants became more P-deficient.

Results of root exudate analyses for amino acid content under various treatments indicated the presence of several amino acids and variation in the amount was only with lysine. The highest content (115 μδ/100 ml) was found in the root exudate of the complete nutrient solution and the lowest value (24 μδ/100 ml) in the exudates of bean plants fed with NO_3^- as the only N form. For P-deficient plants the value did not greatly changed as with variation in the N form. The researchers [22] mentioned that exudation of amino acids was induced by P-stressed Sudan grass plants.

3.3 Solubilization of Different Rock Phosphate Sources and Interaction with Soil Components

Results in Table 5 showed that in the absence of plant roots the solubility of the different RP sources was governed by their solubility product in the solution, also the pH value of the suspension showed slight variation according to the type of rock phosphate. It is generally known that rock phosphates consist of different forms of apatite (hardly soluble fluorine apatite or slightly soluble hydroxyl and carbonate apatite). The dissolution rate depends on the reaction products Ca^{2+} and $H_2PO_4^-$ ions in the solution as well as the pH.

Table 3. $NaHCO_3$- soluble P in soils treated with rock phosphates as a function of time

Treatment	NaHCO$_3$ – Extract, ppm P					
	Incubation period					
	Zero time	% increase	15-days	% increase	30-days	% increase
	Alluvial soil					
Control	0	158	0	129	0	127
Superphosphate	28.3	202	78.3	230	33.7	170
RP (Abou-Zaabal)	4.00	164	24.0	160	9.70	140
RP+OM	17.7	186	40.1	180	43.5	183
RP+Sulphur	8.50	171	32.1	173	12.8	144
	Calcareous soil					
Control	0	88.4	0	94.4	0	88.4
Superphosphate	80.1	159	83.4	173	35.1	130
RP (Abou-Zaabal)	0	84.3	0	88.8	0	87.6
RP+OM	42.4	125	47.0	138	21.7	107
RP+Sulphur	9.30	96.7	0	84.4	0	75.2

Table 4. Effect of P and N form on the pH and amino acids (Lys) content of root exudate and P concentration in bean plants

Treatments	pH			Dry weight, g			% P		Amino acid lys in root exudate, μδ/100 ml
	Before treat.	After treat.	Root exudate	Shoots	Roots	Total	Shoots	Roots	
-P (1:4 NO_3^-: NH_4^+)	5.35	3.12	3.19	3.10	1.31	4.41	0.54	0.53	90.0
+P (1:4 NO_3^-: NH_4^+)	5.30	3.20	3.14	3.35	1.35	4.70	0.54	0.46	115
+P (NH_4-N)	5.20	3.05	3.22	3.00	1.10	4.10	0.54	0.57	62.0
+P (NO_3-N)	5.20	6.32	4.62	3.65	1.10	4.75	0.57	0.48	24.0
$LSD_{0.05}$	**0.05**	**1.06**	**0.86**	**0.23**	**0.03**	**0.22**	**0.03**	**0.02**	**8.24**

Table 5. Effect of root exudate and soil type on the solubility of different rock phosphates

Treatments	Plant	pH of solution	P in solution, ppm
0- -P	Without Plant	5.35	0.00
	With Plant	3.15	0.00
1- RP (Abou zaabal)	Without	6.14	0.44
	With	4.03	2.72
2- RP (About tartour)	Without	5.97	0.54
	With	4.00	2.60
3- RP (Kodjari)	Without	5.30	0.44
	With	3.41	8.94
4- RP (1) + Alluvial soil	Without	6.44	0.50
	With	6.15	0.38
5- RP (1) + Calcareous soil	Without	6.42	0.44
	With	6.45	0.44
6- RP (1) + Weihenstephan soil	Without	6.41	0.06
	With	4.80	3.50
7- +P	Without	5.50	15.50
	With	3.00	9.90
$LSD_{0.05}$		**0.14**	**0.13**

As mentioned above the Abou zaabal and Abou tartour sources resulted in a slight increase in the pH and the pH value further increased when 10 g of the tested soils was added. However, such slight increase in pH did not result in variation among treatments in the solubility of rock phosphate sources in the absence of plants.

On the other hand, results of pH value in the presence of plants show a pronounced decrease as a result of root activity in releasing protons which reached to a decrease of about 2 to 2.5 units in all P treatments (having N in the ratio of 1:4 NO_3^- : NH_4^+), [18,20]. When the rock phosphate materials were added to the P medium the drop in pH were about 2 units with RP of Kodjari and only a drop of 1.3 units for Abou zaabal and Abou tartour sources. It is interesting to mention that more drops in pH was recorded with the low reactive source of RP (16% compared to 45%) which indicates that with hardly soluble RP; plant roots respond vigorously by releasing more protons or dissolving substance. However, P concentration increased in the solution about 5 and 6 times for Abou zaabal and Abou tartour and to about 20 times more for RP of Kodjari characterized by low reactivity. These results agreed with those obtained by [20] who reported that soil pH at the root surface of ryegrass plants decreased by up to 1.6 units and these changes extended to a distance between 1 and 4 mm from the root surface depending on the soil type. However, [8] indicated that the major part of local rhizosphere acidification by P-stressed plants had to be attributed to exudation of organic acid malate and to a lower extent citrate. The authors [24] divided root exudates into two classes of compounds. Low-molecular weight compounds such as amino acids, organic acids, sugars, phenolics, and other secondary metabolites account for much of the diversity of root exudates, whereas high molecular weight exudates, such as mucilage (polysaccharides) and proteins, were less diverse but often composed a larger proportion of the root exudates by mass.

Results also indicated that adding 10 g of either alluvial, calcareous or the acidic soil resulted in fixation or precipitation of soluble phosphate reaching to almost the amount in the absence of plants for the alkaline soil but with the acid soil the fixation was limited. The researchers [2,3] indicated that the usefulness of rock phosphates to crop can be influenced by intrinsic soil and plant factors and concerning the former, rock phosphates dissolve more readily in acid than neutral and alkaline soils. The authors [4] reported that from the factors affecting P-fixation in soils were pH, clay minerals, $CaCO_3$ content as well as soluble Ca in alkaline and soluble Al and Fe in acid soils.

3.4 Growth and P Content in Bean Plants

Data of plant dry mass in Table 6 confirm the previous finding of the role of root exudates in mobilizing insoluble rock phosphate sources and also the action of soil components as the dry weight of both shoots and roots remarkably increased with the lowest effect with Kodjari when different rock phosphate sources were applied alone. The dry weight reached about the

Table 6. Effect of root exudate and soil type on the solubility and uptake of P by bean plants from different sources of rock phosphates

Treatments	Shoots			Roots			Total	%
	Dry weight g	P ppm	P uptake µg	Dry weight g	P ppm	P uptake µg	P uptake µg	increase
0- -P	3.10	0.39	1.21	1.30	0.37	0.48	1.69	0
1-RP (Abou zaabal)	3.24	0.49	1.59	1.61	0.37	0.60	2.19	30
2-RP (About tartour)	3.26	0.44	1.43	1.53	0.41	0.63	2.06	22
3-RP (Kodjari)	3.10	0.45	1.40	1.33	0.42	0.56	1.96	16
4-RP (1)+Alluvial soil	3.15	0.38	1.21	1.32	0.35	0.46	1.62	0
5-RP (1)+ Calcareous soil	3.21	0.34	0.10	2.05	0.29	0.59	0.69	0
6-RP (1)+Weihenstephan soil	3.63	0.33	1.20	1.61	0.63	0.58	1.78	5
7- +P	3.73	0.64	2.39	1.58	0.48	0.76	3.09	82
$LSD_{0.05}$	**0.06**	**0.04**	**0.11**	**0.03**	**0.03**	**0.03**	**0.12**	

value of P treatment when 10 g of different soil samples were added with Abou zaabal source with the highest fixing action by alkaline soils compared to the acid one; the highest dry weight was recorded for the complete nutrient solution treatment.

Similar trend of P concentration and total uptake were recorded. P concentration increased in the shoots and roots of bean plants immersed in solution to which rock phosphate sources were added separately but such effect was eliminated when the 10 g of soil samples were added. The percentage increase in total uptake reached about 30%, 22% and 16% for Abou-zaabal, Abou-tartour and Kodjari sources, respectively, compared to 82% increase for the complete nutrient solution. The total uptake did not change when the soil was added with Abou zaabal.

Thus, it is clear that roots of bean plants were able to utilize insoluble sources of rock phosphate placed in solution but soil components interacted with solubilized P and hinder it unavailable to plants. So the mechanism of solubilizing rock phosphate by exudating protons or organic, amino and other organic compounds is possible at the root surface even in alkaline soil as long as the rock phosphate material was added near the root and organic matter was added to limit the fixing power of the inorganic components as clay, $CaCO_3$ and excess soluble Ca. Also, the availability of P from rock phosphate sources depends on its reactivity value.

4. CONCLUSION

Rock phosphate can be used as a source of P in alluvial and calcareous soils as long as organic or sulphur is mixed close to the root system. The behavior of rock phosphates in the studied soils indicated slight solubilization in the alluvial soil and no remarkable change of extractable P in the calcareous soil as compared to the control. The effect of N form and P on growth and root exudates of bean plants indicated that the highest recorded total dry weight was found when both N forms were applied in the ratio of 1:4 NO_3^- : NH_4^+ or NO_3^- alone. The RP from Abou zaabal gave the highest P content in acid soil in the presence of plant. The highest drop in pH value in the collected solutions was obtained when NH_4^+ alone was the N source.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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