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Inorganic phosphorus fractions and recovery efficiency in aerobic rice (*Oryza sativa*) in relation to sources and levels of phosphorus

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Rice (*Oryza sativa* L.) is the staple food for the millions of people in the world and it plays a vital role in food security and economy of many countries in the world. Aerobic rice (AR) system can save 50 to 60% water as compared to transplanted rice (Prasad 2011). In aerobic rice system, drying a submerged soil decreases the availability of both native soil P and applied P (Sah *et al.* 1989). However, a large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly and becomes unavailable to plants. At present, water-soluble P source like di-ammonium phosphate (DAP) is being widely used but this fertilizer is expensive creating financial burden on marginal farmer who prefers to opt not to use P fertilization to crops. The situation demands the need to make use of economical and indigenously available P fertilizer sources which can save money. Rock phosphate (RP) is one such indigenous source that can supply P at a unit cost much lower than superphosphate and DAP. Earlier studies have shown that incorporation of finely ground rock phosphate directly into the soil (pH 7.0 or above) in conjunction with PSB has the potential to improve plant available P (Sharma *et al.* 2010). Hence, there is growing interest in biological manipulation of RP to enhance its agronomic effectiveness. With this aim the present study was undertaken to evaluate the effect of rock phosphate and microbial inoculation on soil inorganic P fractions and enzymatic activities of soil.

The field experiment was conducted during rainy season (June-October) of 2013 at the experimental farm of IARI, New Delhi situated at a latitude of 28°40'N and longitude of 77°12' E, altitude of 228.6 meters above the mean sea level. The soils of experimental field had a pH- 8.3 (1: 2.5 soil and water ratio), available N of 142.3 kg/ha, available P of 13.46 kg/ha and K of 252.2 kg/ha. The field was disc-ploughed twice and leveled after puddling. The experiment consisted of fifteen treatments including; T_1: Control (Po), T_2: Phosphate-solubilizing bacteria (PSB), T_3: Arbuscular mycorrhizal fungi (AMF), T_4: PSB+AMF, T_5: rock phosphate (RP) @15 kg P/ha, T_6: RP_{30}, T_7: diammonium phosphate (DAP) @15 kg/ha, T_8: DAP $_{30}$, T_9: RP_{15}+ PSB, T_{10}: RP_{15}+ AMF, T_{11}: RP_{15} + PSB+ AMF, T_{12}: RP_{30} + PSB, T_{13}: RP $_{30}$ + AMF, T_{14}: RP $_{30}$ + PSB+ AMF and T_{15}: P on soil test basis. Chemical N at 120 kg/ha was applied as urea in all the treatments. Potassium (K) was applied at 40 kg K/ha in all the treatments through muriate of potash. Inoculation with PSB was carried out using liquid bio-fertilizer and applied on seed at 250 ml/ha. Soil based AMF culture was applied @ 12.5 kg/ha in the furrows at the time of sowing. The soil samples were manually drawn from a depth of 0-15 cm of respective treatment plots after 7 days of sowing (DAS) and at crop harvest. Soil P was sequentially fractionated following the method of Hedley *et al.* (1982).

For determination of alkaline phosphatase activity one gram of air dried soil sample was incubated with 4 ml of modified universal buffer and 1 ml *p*-nitro phenyl phosphate disodium used as substrate at 37 °C for 1 hour (Tabatabai and Bremner 1969). After incubation, reaction was stopped by adding 1 ml 0.5 M $CaCl_2$ and 4 ml 0.5 M NaOH. The contents were centrifuged at 4000 g for 5 min. The *p*-nitrophenol in the sample was determined spectrophotometrically at 400 nm using Thermofischer UV-vis spectrophotometer. The alkaline phosphatase activity was expressed as µg p-NP/g soil/hr. All the data under randomized block design were statistically analyzed using the *F*-test as per the standard procedure. LSD values at $P = 0.05$ were used to determine the significance of difference between treatment means.

Chemical analysis of aerobic rice soil showed that application of rock phosphate at 30 kg P/ha in conjunction with PSB and arbuscular mycorrhizal fungi (AMF) resulted in the highest content of available P (62.94 µg/g soil), a two fold increase over diammonium phosphate @ 30 kg P/ha

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that recorded a value of 31.93 µg/g soil. Moreover, the less labile sodium hydroxide (NaOH) extractable P content (29.86 µg/g soil) was also doubled with combined application of PSB and AMF, compared with DAP_{30}. The efficiency of PSB to release Ca bound P into soil solution was evaluated by its sequential extraction into different P fractions of highly labile and less labile and most stable forms and compared with diammonium phosphate fertilizer soil. Higher values for stable HCl-P fraction indicated the high P fixation capacity of the test soil. The sodium bicarbonate extractable P (Ca-P) was extracted higher from the soil samples collected at 7 days after sowing compared to that from sample of harvest stage (Table 1). In most of the treatments, availability of P declined as the crop matured. The DAP_{15} and DAP_{30} showed higher available P values compared with un-inoculated rock phosphate at both levels of applied P. However, seed inoculation with *Pseudomonas striata* in conjunction with both levels of rock phosphate, viz. RP_{15} and RP_{30} resulted in higher P values compared with RP_{15}+ AMF and RP_{30} + AMF treatment. The dual inoculation of PSB and AMF along with RP_{30} enhanced the available P content of soil by 61.67% in RP_{30}+ PSB and 89.63% in RP_{30}+AMF treatment. The level of available P content of soil with this treatment was significant higher compared to other treatments. At harvest, the treatment receiving sole inoculation of AMF performed better compared with other treatments as indicated by the available P content of soil (70.09 µg/g soil). It was followed by un-inoculated RP_{30} treatment. In all other treatments, available P content at harvest stage varied between 18.53 to 35.92 µg/g soils. Inoculation certainly improved the available P content of soil over their respective controls. However, between both the inoculants tested, effect of PSB for improving availability of P was more pronounced compared to AMF. Sodium hydroxide extractable P fraction showed a reverse trend compared with sodium bicarbonate extractable P fraction. The values were lower at 7d sampling and increased at harvest stage. At initial sampling RP_{30} + PSB+ AMF treatment recorded the highest value of 29.86 µg/g soil. The peak value for this fraction at harvest was recorded in DAP_{15} followed by RP_{30} + PSB. The values for NaOH-P fraction in other treatments ranged from 4.63-11.37 µg/g soil. HCl-P fraction is the most stable P fraction that is generally not available to the plants. The higher values for this fraction were recorded at initial stage followed by a decline at harvest stage. Application of 30 kg P/ha through RP + PSB +AMF showed higher reduction of P content in this fraction. Hedley method of sequential P fractionation is used to chemically fractionate the continuum of soil P. It is assumed that P availability to plants decreases with increasing strength of the chemicals used in the fractionation procedure. Bicarbonate-extractable-P fraction contributes most to plant available-P, while hydroxide-P and acid-extractable P fractions are the forms of moderate or low availability to plants.

Table 1 Effect of rate and sources of phosphorus on different fraction of phosphorus of rice soils

Treatment	$NaHCO_3$-P (µg/g soil)		NaOH-P (µg/g soil)		HCl-P (µg/g soil)	
	At 7 DAS	At harvest	At 7 DAS	At harvest	At 7 DAS	At harvest
P_0	20.21	18.53	11.37	45.60	188.72	82.55
PSB	32.00	27.69	11.12	34.81	146.97	48.34
AMF	27.00	70.09	9.90	29.78	147.90	107.34
PSB + AMF	26.21	35.92	10.14	13.62	160.85	92.75
RP_{15}	24.71	25.07	9.42	33.41	66.92	58.25
RP_{30}	24.83	47.28	6.34	16.00	108.76	29.85
DAP_{15}	27.69	25.31	5.65	44.70	118.04	48.43
DAP_{30}	31.93	29.78	15.60	23.85	142.80	38.04
RP_{15}+ PSB	36.31	28.51	10.36	15.85	195.27	18.14
RP_{15} + AMF	26.72	21.34	6.95	19.87	161.15	97.80
RP_{15} + PSB + AMF	28.06	27.97	11.79	28.69	159.04	75.10
RP_{30} + PSB	38.93	25.51	7.81	41.77	127.25	44.96
RP_{30} + AMF	33.19	27.63	4.63	33.83	154.17	47.13
RP_{30}+ PSB + AMF	62.94	26.17	29.86	31.58	145.74	71.86
P on soil test basis	35.37	28.21	32.94	20.77	45.76	35.01
SEm±	0.24	0.31	0.07	0.24	0.25	0.05
CD (P=0.05)	0.71	0.91	0.20	0.70	0.72	0.14

RP_{15}- 15 kg P/ha through rock phosphate, RP_{30} - 30 kg P/ha through rock phosphate, DAP_{15} -15 kg P/ha through diammonium phosphate, DAP_{30} -30 kg P/ha through diammonium phosphate

The better performance of RP_{30}+ PSB+AMF showed that phosphate solubilising bacteria could solubilize P from rock phosphate and AMF being phosphate mobilizers brought more P into soil solution. AMF can explore more area of soil due to the presence of extra radical mycelium. Sharma *et al*. (2009) reported that increasing rate of P application significantly increased available P content in soil and recorded an increase of 12.99% (in available P content) over the control when level of DAP was increased from 17.5 kg P/ha to 35 kg P/ha. The role of PSB in increasing the soil P availability through solubilisation of insoluble P into plant available forms was in agreement with the reports of Gaind (2013) who documented that using PSB and AMF as biofertilizer in conjunction with rock phosphate input of costly chemical P fertilizers could partially be substituted. *P. striata* a phosphate mineralizer also improved the available P content of soil by mineralizing the organic P present in soil. The low level of phosphatase activity under DAP treatment may be attributable to the fact that enzyme synthesis is inhibited under conditions of P availability and is induced under P-deficiency.

Phosphatase enzyme activity in soil under rice was significantly higher in RP_{30} + PSB+ AMF than DAP at both the levels of P applied. Phosphatase enzyme activity was enhanced when RP, PSB and AMF were applied in combination. However, the activity declined when DAP was used as a source of P compared to RP (Fig 1) The

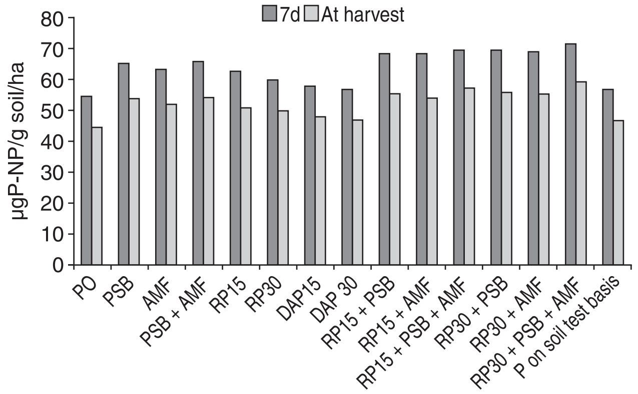

Fig 1 Effect of rate and sources of phosphorus on phosphatase activity of soil under aerobic rice

Table 2 Effect of rate and sources of phosphorus on grain yield, agronomic, crop recovery and physiological efficiency in aerobic rice

Treatment	Grain yield (tonnes/ ha)	Agronomic- efficiency (kg grain increased/ kg P applied)	Crop recovery efficiency (%)	Physiological efficiency (kg grain/ kg P uptake)	Total P uptake (kg/ha)
P_0	3.83				11.02
PSB	3.95				13.19
AMF	3.89				13.09
PSB + AMF	3.97				13.80
RP_{15}	4.44	40.67	18.53	219.48	13.97
RP_{30}	4.93	36.67	9.83	373.04	15.60
DAP_{15}	4.56	48.67	30.53	159.42	14.57
DAP $_{30}$	5.24	47.00	11.83	397.30	15.89
RP_{15}+ PSB	4.53	46.67	32.47	143.73	14.25
RP_{15} + AMF	4.65	54.67	21.53	253.92	15.04
RP_{15} + PSB + AMF	4.75	61.33	26.80	228.84	15.36
RP_{30} + PSB	5.57	58.00	14.47	400.83	17.45
RP $_{30}$ + AMF	5.45	54.00	21.43	251.98	16.91
RP_{30}+ PSB + AMF	5.62	59.67	19.63	219.48	18.36
P on soil test basis	5.45				16.79
S Em±	0.03				0.50
CD (P=0.05)	0.09				1.44

increased activity of phosphates enzyme in soil at the time of harvest might be due to increased solubilization and mobilization of soil through the activity of phosphatase enzyme which was increased when PSB and AMF were used in combination with rock phosphatase. Nath *et al.* (2011) found similar findings that application of rock phosphate in combination with PSB increased the phosphatase and dehydrogenase activity in rice.

Crop recovery efficiency was higher at lower level of P and decreased with increased level of P (P_{30}). However, physiological efficiency showed a marginal increase with increase in P levels (Table 2). Fageria *et al.* (2013) reported a quadratic decrease in agronomic P-use efficiency, apparent P-recovery efficiency and P-utilization efficiency with increasing P rates, whereas physiological P-use efficiency increased quadratically and agro-physiological P-use efficiency decreased linearly with increasing P rates. The decrease in agronomic efficiencies with higher levels of P application may be due to the fact that absorbed P is not efficiently utilized for grain production at higher levels of P in agreement with the reports of Rao (2003). The agronomic efficiency (AE) of P decreased with the increase in P application levels from 15 to 30 kg P/ha. Highest AE was found with RP_{30}+ PSB+ AMF. Crop recovery efficiency also followed the trend similar to AE. It varied between 9.83% (RP_{30}) to 32.47% (RP_{15} + PSB). The highest crop recovery efficiency (32.47%) was observed with RP_{15}+ PSB. The physiological efficiency (kg grain/kg P uptake) showed a slight increase with increase in P levels from 15 to 30 kg P/ha and highest value was recorded with RP_{30}+ PSB and lowest with RP_{15}+ PSB. Afzal *et al.* (2005) also reported increased P use efficiency due to the inoculation of PSB.

The grain yield was significantly increased due to the inoculation of PSB and AMF with rock phosphate over 30 kg P through DAP (Table 2). Highest yield was obtained with the 30 kg P/ha through RP + PSB+ AMF.The increase in yield was due to the increased solubilization of phosphorus by inoculation of PSB. The increased solubility of P also enhanced uptake of phosphorus from the soil and its translocation inside the plant. Inoculation of AMF with rock phosphate increased the mobilization of phosphorus and thus increase in the absorbing surface of the roots resulted in vigorous growth of the plant. The results are in agreements with the findings of Sharma *et al.* (2009) who reported the highest grain and straw yield and harvest index when RP was combined with the inoculation of PSB. Increased amount of phosphorus resulted in improvement in leaf photosynthetic rate, biomass production and sink formation which increases the grain yield of the rice. These results are in agreement with the findings of Panhwar *et al.* (2010) who reported that application of PSB along with chemical P sources increased plant P uptake and resulted in higher plant biomass, grain and straw yield.

Results of this study indicated the highly beneficial effect of PSB inoculation on P release from rock phosphate and consequently increase in P uptake in aerobic rice plants compared to non-inoculated treatments. These results are found much similar to the study of Panhwar *et al.* (2011) who reported that inoculation of PSB with rock phosphate successfully increased the concentration of P in rice plants. Highest uptake of P in grain and straw was found due to the application of 30 kg P/ha through RP + PSB + AMF. Uptake of P in grain and straw of rice increased significantly due to the integrated application of 30 kg P/ha through RP over P control (Table 2). The application of 30 kg P/ha through RP + PSB + AMF increased the amount of available P in the soil because PSB strains releases some organic acids which make the phosphorus soluble in soil. Colonization of roots with AMF increases the absorbing surface of roots and improves the phosphorus mobility in soil and thus enhances the P availability to plants. Hajiboland *et al*. (2009) also reported that inoculation of microbial sources with insoluble P sources increased the P solubility in the soil.

SUMMARY

Integrated application of 30 kg/ha rock phosphate with inoculation of phosphate-solubilizing bacteria (PSB) and Arbuscular mycorrhizal fungi (AMF) not only improved the sodium bicarbonate extractable P fraction in rice soil but also enhanced the enzymatic activities of soil. This treatment also increased P use efficiency of rock phosphate in aerobic rice. Thus, application of PSB and AMF inoculants can provide the farmers, an option to use a cheaper phosphate fertilizer in the form of rock phosphate for cultivation of aerobic rice.

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