The Use of Rock Phosphate and Phosphate Solubilising Fungi (*Aspergillus niger*) to Improve the Growth and the Yield of Upland Rice on Typic Kandiudalf

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Abstract

Field experiment was conducted to study the effect of rock phosphate (RP) and phosphate solubilizing fungi application on upland rice yield intercropped with pigeon pea from 2009 to 2011 at the Agricultural Research Centre, Kade, University of Ghana. In the first year, the main plot treatment consisted of five levels of phosphate fertilizer *viz.* 0 kg/ha P_2O_5, 40 kg/ha P_2O_5 RP, 80 kg/ha P_2O_5 RP, 120 kg/ha P_2O_5 RP and 45 kg/ha P_2O_5-triple super phosphate (TSP), while the planting dates of pigeon pea was set up as subplot (40 DAS –pigeon pea planted 40 days after sowing rice seed and 80 DAS- pigeon pea planted 80 days after sowing rice). In the second year, phosphate fertilizer was the main plot treatment, consisting of (0 kg/ha P_2O_5, 45 kg/ha P_2O_5 RP, 90 kg/ha P_2O_5 RP and 45 kg/ha P_2O_5 TSP), and the subplot treatment was inoculated and uninoculated with phosphate solubilising fungi *Aspergillus niger*. The highest grain yield of 1.051 t/ha was obtained with the 120 kg P_2O_5/ha RP followed by the 40 kg P_2O_5 /ha RP. Phosphate levels did not significantly influence the grain yield in the first year ($p = 0.08$) but planting pigeon pea as an intercrop at the different planting dates had a significant positive effect on both the grain and straw yields of rice ($p = 0.016, .07$). There was no significant interaction between the use of different phosphorus levels and the different planting dates of pigeon pea ($p = 0.348$). In the second year, phosphate levels had significant influence on grain yield ($p = 0.014$) and the highest grain and straw yields occurred where TSP and *Aspergillus niger* were used to plant rice. Inoculating rice with or without *Aspergillus niger* had no significant influence on grain yields ($p = 0.447$). No significant interaction existed between phosphate levels and level of inoculation ($p = 0.206$). The use of TSP and *Aspergillus niger* to grow rice improved rice yield followed by 45 kg P_2O_5/ha RP + Inoculation.

Introduction

Soils in the humid and sub-humid zones of sub Sahara Africa are generally deficient in available P and this constraint limits food production (Chien & Mennon, 1995; Sanchez *et al.,* 1997). Some of the reasons for the low available P in the soils are the low inherent *P* status of the parent material, intensive weathering of the soil minerals (Sanchez *et al.*, 1997; Ssali *et al.*, 1986) and also the reversion of soluble P into insoluble forms through reactions with iron and aluminum oxides. Therefore, large amounts of inorganic P fertilizer need to be applied to tropical soils to attain appreciable crop yields and that can be expensive to the farmer. If P supply is not adequate in such soils, crop response to other major nutrients including N would also be limited, hence, the overall fertility and productivity of the soils would be affected (Sahrawat *et al.*, 2001).

An alternative and cheaper source of P is rock phosphate (RP), which abounds in West Africa. The application of rock phosphate can be effective in increasing crop production. The rock phosphate enhances the

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replenishment of N through biological fixation, and also in the maintenance or improving soil chemical properties and soil fertility (Sahrawat *et al.*, 2001). The dissolution of RP can be increased through microbial mediation. *Penicillium bilaii* produces citric and oxalic acids to solubilize calcium phosphate (Cuuingham & Kuiack, 1992), *Penicillium variable* P16, also produces gluconic acid (Vassilev *et al.*, 1996). Similarly, *Aspergillus niger* produces gluconic, citric and oxalic acids (Manguson & Lasure, 2004), as well as acid and alkaline phosphatases (Phukan *et al.*, 2011). The dissolution of different types of rock phosphate by *Aspergillus niger* has been well demonstrated (Bojinova *et al.*, 1997; Goenadi *et al.*, 2000, Reddy *et al.*, 2002, Seshadri *et al.*, 2004). Also, ferric phosphate was best solubilized by *Aspergillus niger* (Naik *et al.*, 2013). *Aspergillus terreus* solubilizes both organic and inorganic phosphates (Oliveira *et al.*, 2009). Phosphate solubilization by microbes is mediated by several different mechanisms including organic acid production and proton extrusion (Dutton & Evans, 1996; Nahas, 1996). It is generally recognized that organic acids solubilize RP through protonation and/or chelation reactions (Sagoe *et al.*, 1998). Besides the acid strength, the type and position of the ligand determine the effectiveness of the organic acid in the solubilization process (Kpomblekou & Tabatabai, 1994).

Illmer *et al.* (1995) indicated the level of organic acids resulting in significant P dissolution in the order of 3–30 uM/ml, distinctly below the efficiency of biotic leaching. Plants grown as green manures that are capable of extracting P from rock phosphate have the potential to supply P to a subsequent crop through organic P mineralization (Stewart & Tiessen, 1997). McLenaghen *et al.* (2004) found that maize yield and P uptake increased with the combined application of rock phosphate and planting of lupin whereas maize yield was unaffected when either lupin or rock phosphate was used alone. Similarly, Cuero (2006) observed that by combining the use of the legume fallow crop *Cajanus cajan* with rock phosphate, high yield of the upland rice was maintained after 3 years of cultivation.

The objectives of the study were: (1) to determine the growth and yield of Nerica rice and the use of a leguminous crop as an intercrop, (2) assess the effectiveness of phosphate solubilizing microorganism (PSM) in solubilizing rock phosphate and making phosphate available for plant use, and (3) examine the possible interactions between factors investigated, e.g., levels of phosphates, levels of PSM used and time for planting leguminous crops.

Material and methods

A two-year field experiment was conducted on the Kokofu series (Typic Kandiuldalf) in a semi-deciduous agroecological zone of Ghana. The study site was at the Agricultural Research Centre, University of Ghana, Okumaning, Kade in the Eastern Region, Ghana (6° 05' N; 0° 05' W). The site is in the moist semi-decidous forest zone, approximately 175 km from Accra. The climate of the area is humid tropical. Average annual temperature is 28 °C, with the maximum temperature in March and minimum temperature in August. The rainfall pattern is bimodal and the average annual rainfall is 1179 mm with about 80% falling from March to mid-July and from September to November.

Profile pits were dug and soils sampled according to their genetic horizons. This was to determine the physical and chemical characteristics of the soil at the experimental area. In addition to the profile pits, grid soil samples (0–20 cm) were taken at intervals of 10 m for fertility evaluation and spatial variability of physical and chemical properties of the soils within the sites.

Soil analyses

Analyses were performed on air-dried soil fractions (< 2 mm). The soil *p*H was measured potentiometrically in 1:2 (w/v) suspension of water and in 0.01*M* $CaCl_2$. Organic carbon was determined by the Walkley & Black (1934) method. This method involved the reduction of Cr ion by the organic matter and the unreduced $Cr_2O_7^{2-}$ measured by titration with ammonium ferrous sulphate. The quantity of organic matter oxidised is calculated from the amount of $Cr_2O_7^{2-}$ reduced.

The total soil nitrogen was determined by the Kjeldahl method. The nitrogen in the sample was converted to ammonia by digestion with concentrated H_2SO_4 using a nitrogen catalyst (selenium powder). The ammonium formed was determined by distillation of the digest with an alkali and titrated with a standard acid (hydrochloric acid).

Available phosphorus was determined using the method of Bray & Kurtz (1945). Five grams of soil was weighed into a centrifuge tube and 50 ml of 0.03 *M* NH_4F in 0.025 *M* HCl was added. The suspension was shaken for 3 min on a mechanical shake and filtered. Five ml aliquot of the filtrate was taken into a 50 ml volumetric flask. A yellow colour was developed when a drop of para-nitrophenol indicator was added together with a drop of ammonia solution into the filtrate. Available P was determined by using the method of Watanabe & Olsen (1965).

Cation exchange capacity (CEC) and exchangeable basic cations Ca^{2+}, Mg^{2+}, K^+ and Na^+ (1 *M* NH_4OAc at *p*H 7) were determined. Ten grams of soil was weighed into a 200-ml extraction bottle and 100 ml of 1 *M* neutral ammonium acetate (NH_4OAc, *p*H 7.0) solution added. The bottle and its content were placed on the mechanical shaker and shaken for 1 h, centrifuged at 3000 r.p.m. for 10 min. The supernatant was then filtered through No. 42 Whatman filter paper. Aliquots of the extract were used for the determination of exchangeable cations (i.e. Ca, Mg, Na and K).

To determine the exchangeable calcium, 10 ml aliquot of the sample solution, 10 ml of 10% KOH and 1 ml triethanolamine (TEA) were added. Three drops of 1 *M* KCN solution and a few crystals of cal-red indicator were then added, after which the mixture was titrated with 0.02 *M* EDTA solution from red to blue end point. The titre value was used in the calculation of calcium.

Exchangeable magnesium was determined by pipetting 10 ml of the sample solution, 5 ml of ammonium chloride-ammonium hydroxide buffer solution was added followed by 1 ml of triethanolamine. Three drops of 1 *M* KCN solution and a few drops of Eriochrome black T solutions were added after which the mixture was titrated with 0.02 *M* EDTA solution from red to blue end point. The end point titre value determined the amount of calcium and magnesium in the solution. The titre value of magnesium was then determined by subtracting the value obtained for calcium above and the new titre value obtained. The titre value of magnesium was then used for the calculation of concentration of magnesium.

The flame photometer was standardized in a way that 10 mg/kg of Na gave 100 full scale deflections. After the standardization of the photometer, the concentration of sodium in 10 ml aliquot was determined. The result was then used in the calculation of the amount of sodium (Na) present in the soil. The flame photometer was standardized such that 10 mg/kg of K gave 100 full scale deflections. The flame photometer after standardization was used to determine the concentration of potassium in the aliquot. The result was used in the calculation of the amount of potassium percent in the soil.

For the exchangeable acidity, 10 g of soil were transferred into a dry filter paper in a funnel placed in a 100-ml volumetric flask. The soil was successively leached with 10 ml batches of 1 *M* KCl to a total volume of 100 ml. A 25-ml aliquot was taken and four drops of phenolphthalein added. The solution was titrated with 0.02 *M* NaOH to first permanent pink end point. A correction for the blank of NaOH titre on 100 ml of KCl solution was made and the KCl extracted acidity (extractable acidity Al and H) was calculated.

The soil at the experimental site was strongly acid (*p*H 4.5–5.7 in water and 4.4–5.2 in 0.01 *M* $CaCl_2$, Table 1). The soils were free from gravels and had low to medium bulk densities (1.3–1.5 Mg m^{-3}). Total nitrogen content was highest in the surface soils and decreased with increasing profile depth from 0.04 to 0.08%. Organic carbon (OC) content decreased with depth of the profile and contained low to very low organic carbon (Table 1). Available P (Bray 1) also decreased with depth of profile and ranged between 7.02 mg kg^{-1} in the surface soil to 0.64 mg kg^{-1} in the 150–190 cm depth. The ECEC values at the 0–19 cm depth were above 4 cmol(+) kg^{-1} soil, suggesting that the soil was rich enough in exchangeable cations to support rice production.

Year one field experiment

The first year field experiment involved the clearing of the field using farmers' practice of cutlass and hoes and stumping off trees. Nerica 2 rice seeds were sown 20 cm by 20 cm apart with 5 seeds per hole. The seedlings were thinned to three per hole and basal fertilizer was applied to the different experimental plots. The basal fertilizer consisted of 45 kg N/ha (ammonium sulphate) + 40 kg K_2O ha in the form of muriate of potash. Top dressing of N with ammonium sulphate (45 kg N/ha) was done 45 DAS.

The Nerica rice used for the field experiment was strictly rain fed (i.e. minor season rains). The experimental design was split plot with the main plot treatment being different rates of application of phosphate fertilizer *viz.* 0 kg P_2O_5/ha (control), 40 kg P_2O_5/ha rock phosphate (RP), 80 kg P_2O_5/ha RP, 120 kg P_2O_5/ha RP and 45 kg P_2O_5/ha TSP. TSP was included in the treatment to be a check so that it would be known what happens to treatment where P is readily available as compared to RP treatments where P is not readily available. The subplot treatments had pigeon pea planted at different dates at 0 DAS, 40 DAS and 80 DAS.

The rock phosphate used for the experiments was from Togo and it had a total P of 10.89%, water soluble P of 15. 8 mg P/kg and citric acid soluble P of 16.9 mg P/kg. The reactivity of the Togo rock phosphate was 16 (Abekoe, 1996). The experiment was replicated three times. Pigeon pea seeds were planted as relay crop in between the rice rows so that the plants continued to grow after harvesting the rice and the pigeon pea residue was cleared from field before the second year experiment. Harvesting the rice was done at 100 DAS.

Year two field experiment

The second year's experiment was conducted in the same field as in Year one. The field was cleared as described earlier on without the crop residues being incorporated into the soil. The surface soil from 0–10 cm deep was thoroughly tilled with hoes to level the experimental field, and the different plots were re-laid taking into consideration the gradient of the field. At 21 days after seeding, basal fertilizer of 45 kg N/ha (ammonium sulphate) + 40 kg K_2O/ha, in the form of muriate of potash were applied to the different experimental plots. Top dressing of N using ammonium sulphate (45 kg N/ha) was done 45 DAS.

The main plot treatment was phosphate levels of 0 kg P_2O_5/ha, 45 kg P_2O_5/ha RP, 90 kg P_2O_5/ha RP and 45 kg P_2O_5 /ha TSP, whilst the subplot treatment was inoculation with or without (I or UN) phosphate solubilizing microorganism (PSM) *Aspergillus niger* (obtained from Soil Science Department, University of Pretoria, South Africa). The number of replication was three. Two days after applying the basal fertilizers, i.e. 23 DAS the liquid inoculant was applied to the rhizosphere of the rice plants. A second application of the inoculant was done 37 DAS. Harvesting of the rice was done 100 DAS. Plant height and tiller numbers were recorded weekly until the time of harvesting.

TABLE 1

Physico-chemical characteristics of the Typic Kandiudalf (Kokofu series)

Depth (cm)		*BD (Mg m^{-3})*	*pH_w	*Ca*	*OC (%)*	*Total N (%)*	*Available pH_{ca} (mg kg^{-1})*	*Exch. bases (cmol$_c$ kg^{-1})*				*Exch. Acidity cmol$_c$kg^{-1}*	*ECEC cmol(+) /kg*
								Ca	*Mg*	*K*	*Na*		
0-7	L**	1.3	5.7	5.5	0.79	0.08	7.02	2.4	2.0	0.27	0.45	0.64	5.76
7-19	L	1.3	5.2	5.1	0.53	0.06	5.63	2.0	1.4	0.16	0.42	0.74	4.72
19-41	CL***	1.4	5.1	4.6	0.24	0.06	4.01	1.4	1.0	0.14	0.35	0.84	3.73
41-90	CL	1.5	4.7	4.5	0.20	0.05	2.41	1.0	1.0	0.10	0.27	0.94	3.31
90-150	CL	1.5	4.6	4.4	0.16	0.04	1.16	0.8	0.6	0.10	0.31	1.00	2.81
150-190	CL	1.4	4.5	4.4	0.10	0.04	0.64	0.6	0.4	0.08	0.32	1.02	2.42

*Soil pH was determined in distilled water (W) and in 0.01M $CaCl_2$ (Ca); ** Loam; *** CL - Clay loam
Exch. Bases stands for exchangeable bases.

Preparation of inoculum

Aspergillus niger was cultured in Pikovskaya medium (1948) with the following composition: glucose - 10 g; $Ca(PO_4)_2$ - 5g; $(NH_4)_2SO_4$ - 0.5 g; NaCl - 0.2 g; $MgSO_4.7H_2O$ - 0.1g; KCl - 0.2 g; yeast extract - 0.6 g; $MnSO_4$ - 0.04 g; $FeSO_4.3H_2O$ - 0.04 g, all were dissolved in 1 liter distilled water, the *p*H was adjusted to 6.5 and the culture medium was sterilized. Culturing was done in an orbital shaker at a temperature of 28 °C until the solution was turbid. The solution was diluted into one half the initial concentration and used as a liquid inoculant.

Statistical analysis

Data from the various treatments were analyzed by using Genstats (9th edition) statistical software. Analysis of variance (ANOVA) was run on the parameters to ascertain significant differences among treatments. The means were separated using the least significant difference.

Results

Grain and straw yields as influenced by phosphate and pigeon pea in the first year

The highest grain yield (1.051 t/ha) was obtained with the 120 kg P_2O_5 RP/ha treatment, and it was followed by the 40 kg P_2O_5 RP/ha, but there were no significant differences in yield between the two treatments (Table 2). Rice grain yields were generally low, and increased with the different planting dates of pigeon pea, thus, the highest grain yield was obtained with planting pigeon pea 80 DAS rice, and the next high yield was when planting was done 40 DAS rice, and the least grain yield occurred when rice was monocropped. For the monocropped rice, at 0 kg P_2O_5/ha, the yield of rice for plots where intercropping was done was higher than for the monocropped rice (Table 2). A similar observation was made for treatments where 40 kg P_2O_5/ha RP, 80 kg P_2O_5/ha RP and 45 kg P_2O_5 TSP/ha were applied (Table 2).

Increasing the phosphate levels generally increased the yield of rice (Table 2). Phosphate levels, however, did not significantly influence the rice grain yield ($p = 0.08$). There was no significant interaction between the phosphate levels and the days to planting pigeon pea ($p = 0.348$). There was a significant effect on the grain yield with the different planting dates of pigeon pea ($p = 0.016$) as an intercrop. The highest straw yield was observed for the 40 kg P_2O_5 RP/ha treatment but that was not significantly different from straw yields at 120 kg P_2O_5 RP/ ha treatments (Table 3). Phosphate levels did not significantly influence the straw yields ($p = 0.115$). There was no significant interaction between phosphate levels used and the dates of planting pigeon pea as an intercrop in the rice plant ($p = 0.177$).

The different pigeon pea planting dates had a significant effect on straw yields ($p = 0.007$). Plots where no pigeon pea was planted and rice was monocropped, the straw yields were lower (Table 3) compared to plots with pigeon pea planted. Monocropped plot where 0 kg P_2O_5/ha was applied, lower yield was obtained than the same treatment intercropped with pigeon pea suggesting some extra benefits the pigeon pea plots were obtaining. Similar observations were made with 40 kg P_2O_5 /ha RP and 45 kg P_2O_5/ha TSP plots (Table 3). Straw yields when 45 kg P_2O_5 TSP/ha was applied was lower than straw yields when 40 kg P_2O_5/ha RP had been applied.

TABLE 2

Effect of phosphorus levels on grain yield (t/ha) of rice monocropped or intercropped with pigeon pea at different dates

Phosphate level (kg P_2O_5 ha^{-1})	*0 DAS**	*Pigeon pea planting* *40 DAS***	*80 DAS****	*Mean*
No P	0.294	0.449	0.666	0.470
40 as RP	0.806	0.872	1.098	0.925
80 as RP	0.638	0.720	0.701	0.686
120 as RP	0.963	1.210	0.980	1.051
45 as TSP	0.463	0.515	0.849	0.609
Mean	0.633	0.753	0.859	

* No pigeon pea was planted, i.e. rice was monocropped; ** Pigeon pea was planted 40 days after sowing rice; *** Pigeon pea was planted 80 days after sowing rice; LSD ($p \leq 0.05$): Phosphate levels = 0.436; DAS = 0.146; Phosphate levels × DAS = 0.485.

TABLE 3

Straw yield (t/ha) of rice as influenced by phosphate levels and days after seeding pigeon pea

Phosphate level (kg P_2O_5 ha^{-1})	*Pigeon pea planting (DAS)*			*Mean*
	0	*40*	*80*	
No P	1.133	1.367	1.600	1.367
40 as RP	1.733	2.600	2.733	2.356
80 as RP	1.500	1.733	1.467	1.567
120 as RP	2.067	1.633	2.367	2.022
45 as TSP	1.200	1.867	2.333	1.800
Mean	1.527	1.840	2.100	

LSD ($p \leq 0.05$): Phosphate levels = 0.778; DAS = 0.333; Phosphate levels × DAS = 0.931

Grain and straw yields as influenced by phosphate application and inoculation with A. niger *in the second year*

The highest grain yield was observed for the treatment where 45 kg P_2O_5/ha TSP was applied and inoculation was done (Table 4). Applying 90 kg P_2O_5 /ha RP the grain yield was lower than when 45 kg P_2O_5/ha RP was applied even though difference in yield was not significant. Inoculation depressed the grain yield at 0 kg P_2O_5/ha and 45 kg P_2O_5/ha RP as compared to the uninoculated treatments (Table 4). Phosphate levels applied had a significant effect on the grain yield ($p = 0.014$). The first year application of phosphates might have contributed to phosphates significantly influencing the yield of rice in the second year. Generally, increasing the phosphate levels also increased the yield of rice grain. Inoculation did not significantly influence rice grain yield ($p = 0.477$). The mean for the inoculated grain yield was, thus, slightly higher than for the uninoculated rice plants (Table 4). There was no significant interaction between phosphate levels and inoculation ($p = 0.206$).

Straw yields were generally higher than the grain yield. The highest straw yield was observed with 45 kg P_2O_5/ha TSP combined with inoculation (Table 5). Straw yields of 90 kg P_2O_5/ha RP, was lower than straw yields of 45 kg P_2O_5/ha RP, even though there was no significant difference in yield. Straw yields at 45 kg P_2O_5/ha TSP was higher than straw yields at 45 kg P_2O_5/ha RP whether inoculated or not inoculated. The straw yield was influenced by the phosphate levels applied ($p = 0.009$). Inoculation or no inoculation did not significantly influence the yield of straw ($p = 0.237$). There was no significant interaction between the phosphate level and the level of inoculation ($p = 0.627$). Straw yields were generally higher for the inoculated treatments than for the uninoculated treatments (Table 5).

Growth characteristics of rice plant

For all the treatments, plant height increased with time (Table 6). There was no lodging and this is characteristics of Nerica rice that has been bred to be hardy, resistant to drought, lodging, common rice diseases and pests. Nerica rice varieties grow well in acid soils and because they grow profusely they are able to control weeds (WARDA, 2001). From 45 DAS onwards, the treatment with the highest height was where 45 kg P_2O_5/ha TSP + Inoculation was applied. Depression of plant height was observed for treatments 0 kg P_2O_5/ha and 45 kg P_2O_5/ha RP, with inoculation than the same treatments that were not inoculated (Table 6) upto 50 DAS.

Discussion

Grain yield as influenced by phosphate in the first year

The rice yield of 1.051 t/ha obtained with 120 kg P_2O_5 RP/ha in the first year was low. Applying 120 kg P_2O_5 RP/ha was uneconomical for growing the Nerica rice. Rock phosphate applied to the soil with time will solubilize under strongly acidic soil condition and the phosphate becomes available for plant use. Some of the phosphates released will be fixed by iron and aluminum oxides, thus, becoming unavailable for plants to use. However, if the soil environment is dry due to little or no rainfall, as occurred during the period of the experiment, it would not favour the production of protons and solubilization of rock phosphate. Other studies on upland rice reported by Saharawat *et al.* (1994) showed that grain yields increased with increasing application of triple superphosphate (TSP).

Grain yield of rice with 45 kg P_2O_5/ha TSP was lower than when 40 kg P_2O_5/ha RP was used to plant the rice. One would have expected that phosphate was readily available in TSP as compared to RP, thus, more vigorous growth of rice with TSP than RP would have occurred. The low rainfall could have caused some of the available phosphates from TSP to be in the soil solution for plants to benefit at the right time. The rock phosphate, on the other hand, is sparingly soluble, therefore, the rest of the insoluble RP could be solubilized by later rainfalls that could have benefitted the crop.

The use of pigeon pea as a fallow legume crop

Pigeon pea enhanced grain and straw yields, and the effect was great when pigeon pea was sowed 80 DAS rice than 40 DAS

TABLE 4

Grain yield (t/ha) of rice as influenced by phosphate levels and inoculation with A. niger

Phosphate levels (kg P_2O_5 ha^{-1})	*Inoculation*		*Mean*
	Inoculated	*Un-inoculated*	
0	1.135	1.388	1.262
45 as RP	1.749	1.948	1.849
45 as TSP	2.688	1.937	2.312
90 as RP	2.031	1.792	1.912
Mean	1.901	1.766	

LSD ($p \leq 0.05$); Phosphate levels = 0.513; Inoculation = 0.388; Phosphate levels × Inoculation = 0.681

TABLE 5

Straw yield (t/ha) of rice as influenced by phosphate levels and inoculation with Aspergillus niger

Phosphate levels(kg P_2O_5 ha^{-1})	*Inoculation*		*Mean*
	Inoculated	*Un-inoculated*	
0	3.50	2.34	2.92
45 as RP	4.13	4.38	4.25
45 as TSP	6.12	5.11	5.62
90 as RP	3.80	3.45	3.63
Mean	4.39	3.82	

LSD ($p \leq 0.05$); Phosphate levels = 1.25; Inoculation = 1.01; Phosphate levels × Inoculation = 1.73

TABLE 6

Plant height (cm) of rice at different sampling dates in the second year of field experiment

Phosphate levels (kg P_2O_5 ha^{-1})	*Inoculated*				*Un-inoculated*				*Mean*
	45 DAS*	*50 DAS*	*56 DAS*	*70 DAS*	*45 DAS*	*50 DAS*	*56 DAS*	*70 DAS*	
0	66.56	82.15	94.13	107.13	69.6	95.3	97.56	113.5	90.7
45 as RP	66.26	96.72	94.28	110.62	71.78	91.97	96.8	116.0	93.1
45 as TSP	72.16	92.95	101.05	125.02	71.6	87.35	99.62	112.3	95.3
90 as RP	66.48	89.92	96.22	117.03	69.6	84.74	91.01	113.9	91.1
Mean	67.8	69.7	96.4	114.9	70.6	89.8	96.2	113.9	

*DAS stands for days after seeding of rice

rice. Harvesting of rice in all plots was done 100 DAS rice. Pigeon peas planted 40 days after seeding rice (i.e. pigeon peas in 40 DAS plots), the rice plants would have established and about to boot before the pigeon pea was planted, therefore, pigeon pea would not strongly compete with such established plants. If pigeon pea and rice had been seeded together at the time of planting, then competition for nutrients, water, sunlight, etc. would have been stronger. Pigeon pea planted 80 days after seeding rice (80 DAS) by the time of harvesting the rice plant, the seedlings would have been about 20 days old. Competition between pigeon pea plants and rice plants that have already tillered and over 70 days old would almost be minimal. Therefore, strong competition between the pigeon pea and Nerica rice in all plots that pigeon pea was planted was not envisaged since Nerica rice was well established before pigeon pea was planted.

Pigeon pea is known to release piscidic acid (an organic acid) that enhances the dissolution of iron-phosphate (Fe-P) in soils (Ae *et al.,* 1991). The piscidic acid chelates the iron-phosphates and makes phosphates more available for the rice plant to use. This might have contributed to the yield of rice in plots where pigeon pea was planted. Pigeon pea's ability to fix atmospheric nitrogen even when intercropped and under droughty conditions (Valenzuela & Smith, 2002) also influenced grain and straw yields of rice. Pigeon pea is used by farmers in different parts of Ghana because of its advantage such as the ability to regenerate soil fertility (Adjei-Nsiah *et al.,* 2004).

Grain and straw yields of monocropped rice experienced no competition from intercropped plants and yet the yields were lower than that of intercropped rice. Apart from the P supplied to monocropped plots, such plots had no other sources of P which was critical for the growth of rice. Another probable reason for the monocropped rice experiencing lower yield than the intercropped rice was that the plot had none or little of the benefits that pigeon pea plots had such as piscidic acid solubilizing some of the fixed P (Fe-P) and making extra P available for rice plant to use and fixing of atmospheric N for the rice plant to use.

Grain and straw yields as influenced by phosphate in the second year

TSP significantly influenced the yield of rice and straw in the second year partly because of more available moisture, thus, plants were able to make use of TSP immediately it was applied. The rice plants established faster, growing more vigorously initially than the other plants not treated with TSP. The best treatment was where TSP + I were used to grow the rice. There was early development of the plant, thus, high plant heights, good grain yields and filled grain. There were some other benefits rice plants treated with TSP + I had more than plants in other treatments. Rahi *et al.* (2009) isolated phosphate solubilizing fungus that exhibited multiple plant growth promoting attributes of solubilization of inorganic phosphate substrates, production of phytase and siderophores and biosynthesis of indole acetic acid-like auxin.

Probably, the *A. niger* in the presence of available phosphate (TSP) exhibited such multiple plant growth promoting attributes. *A. niger* made use of some of the available TSP and the ammonium sulphate that had been applied earlier. Growth of *A. niger* was promoted and it secreted enzymes such as acid phosphatase that solublized organic phosphates making them available for rice plants to use. *A. niger* also secreted organic acids to solubilize rock phosphates, but because the soil was strongly acidic, some of the phosphates was converted to insoluble forms. *A. niger* could also solubilize ferric phosphate in the plots.

A similar observation has been made by Parvaze *et al.* (2007), who noted that the combined inoculation of N_2-fixing and phosphate solubilizing bacteria, considerably improved growth, nodulation, and nutrient uptake of chickpea. These combined inoculation effects were more than the sum of the individual inoculation effects, suggesting synergisms beyond simple additive effects (positive multiplicative interaction). In plots where no phosphates was supplied or little phosphate in the form of rock phosphate was applied, the *A. niger* depressed the yield of rice grain. Probably the fungi utilized the little available P and plants were initially deprived of available P. The present findings support the hypothesis that the use of inorganic phosphate fertilizer together with phosphate solubilizing fungi can improve plant growth considerably leading to significant increases in the grain yield of field-grown rice plants.

Conclusion

The use of phosphate solubilizing microorganisms is feasible under the tropical environmental conditions provided the organisms (PSM) are in the active growth stage. The soil environment that the rock phosphate and PSM are introduced should not be dry since soil solution is critical for the dissolution of rock phosphate. Rock phosphate rates above 80 kg P_2O_5/ha rather depressed growth of rice. The best experimental treatment was when TSP was inoculated with *A. niger* and used to grow rice. The next best treatment was rock phosphate at 45 kg P_2O_5/ha + I which also promoted high grain yield. The use of pigeon pea positively influenced rice yield and as a fallow crop it must be planted between 40-80 DAS rice.

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