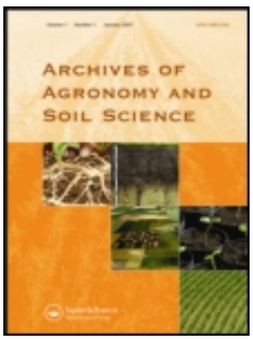

Archives of Agronomy and Soil Science

ISSN: 0365-0340 (Print) 1476-3567 (Online) Journal homepage: https://www.tandfonline.com/loi/gags20

Improvement of wheat and maize crops by inoculating *Aspergillus* spp. in alkaline soil fertilized with rock phosphate

Himani Singh & Sudhakara M. Reddy

To cite this article: Himani Singh & Sudhakara M. Reddy (2012) Improvement of wheat and maize crops by inoculating *Aspergillus* spp. in alkaline soil fertilized with rock phosphate, Archives of Agronomy and Soil Science, 58:5, 535-546, DOI: 10.1080/03650340.2010.532125

To link to this article: https://doi.org/10.1080/03650340.2010.532125

Published online: 18 Jul 2011.

Archives of Agronomy and Soil Science
Vol. 58, No. 5, May 2012, 535–546

Taylor & Francis
Taylor & Francis Group

Improvement of wheat and maize crops by inoculating *Aspergillus* spp. in alkaline soil fertilized with rock phosphate

Himani Singh and Sudhakara M. Reddy*

Department of Biotechnology, Thapar University, Patiala, India

(*Received 20 August 2010; final version received 7 October 2010*)

Aspergillus tubingensis and *A. niger* were isolated from the landfills of rock phosphate mines and tested for their efficacy to solubilize rock phosphate (RP), and improve plant growth and phosphate (P) uptake by plants grown in soil amended with RP. The results showed that they effectively solubilized RP in Pikovskaya's (PKV) liquid medium and released significantly higher amounts of P into the medium. *A. tubingensis* solubilized and released 380.8 μg P mL^{-1}, *A. niger* showed better efficiency and produced 403.8 μg P mL^{-1}. Field experiments with two consecutive crops in alkaline agricultural soil showed that inoculation of these fungi along with RP fertilization significantly increased yield and nutrient uptake of wheat and maize plants compared with control soil. P uptake by wheat and maize plants and the available P increased significantly in the RP-amended soil inoculated with fungi compared with control. These results suggest that the fertilizer value of RP can be increased, especially in alkaline soils, by inoculating P-solubilizing fungi.

Keywords: *Aspergillus tubingensis*; *A. niger*; fertilizer value; inorganic P; nutrient uptake

Introduction

Phosphorus (P) is one of the major essential nutrients for plant growth and development. Despite its wide distribution in nature, P is deficient in most soils and its content is $\sim$0.05%, of which only 0.1% is available for the plant (Vassilev and Vassileva 2003). Phosphorus is added in the form of phosphatic fertilizers, part of which is utilized and the remainder converted into insoluble fixed forms (Narsian and Patel 2000). Phosphate fertilizers are expensive and there is a need for alternative sources. Natural phosphate rocks have been recognized as a valuable alternative to P fertilizers. In India, it is estimated that there are almost 260 million tons of phosphatic rock deposits and this material should provide a cheap source of phosphate fertilizer for crop production (FAI 2002). Unfortunately, rock phosphate (RP) is not plant available in soils with a pH $>$ 5.5–6.0. However, the availability of phosphorus in the soluble state is of high agronomic value (Relwani et al. 2008). Many soil fungi and bacteria can solubilize inorganic phosphate into soluble forms through processes of acidification, chelation, exchange reactions and production of organic acids, which not only compensates for the higher cost of manufacturing

*Corresponding author. Email: msreddy@thapar.edu

ISSN 0365-0340 print/ISSN 1476-3567 online

http://dx.doi.org/10.1080/03650340.2010.532125
http://www.tandfonline.com

fertilizers in industry, but also mobilizes fertilizers added to soil (Rodriguez et al. 2004; Chung et al. 2005).

At present, RP is chiefly employed to sustain soil P levels in an available form for plants. In this context, P-solubilizing microbes have been reported to solubilize the RP via the production of organic acids, ion chelation and exchange reactions in the growth environment (Yadav and Dadarwal 1997). As a result of this activity, P-solubilizing microorganisms play an important role in supplementing P to plants and allowing the sustainable use of phosphatic fertilizers. It has been reported that inoculation of P-solubilizing micro-organisms in soil amended with RP is a promising technique because it can increase P availability (Reyes et al. 2002) and improves the physio-chemical, biochemical and biological properties of RP-amended soil (Caravaca et al. 2004). Several authors have reported increasing yields of wheat and mungbean (Whitelaw et al. 1997; Omar 1998; Saber et al. 2009) through simple inoculation of P-solubilizing fungi, but reports indicating the utilization of RP as a fertilizer along with P-solubilizing microbes are scarce, especially in field conditions.

In this study, P-solubilizing fungi *Aspergillus tubingensis* and *Aspergillus niger* were isolated from the mine landfills of RP, which have more adaptability to RP solubilization. These fungi were inoculated to RP-amended alkaline soils and the growth and yield of wheat and maize sown in two consecutive seasons were studied.

Materials and methods

Isolation and screening of P-solubilizing fungi

Rhizospheric soils of plants *Jatropha curcas* growing in mine landfills of rock phosphate of Rajasthan State Mines and Minerals Limited (RSMML), Udaipur, India were collected. The fungi were isolated from these samples on Pikovskaya (PKV) medium (Pikovskaya 1948) supplemented with RP (5 g RP L^{-1}). The RP used was high-grade and obtained from RSMML, Udaipur; it consists of 31.5% P_2O_5, 45.4% CaO, 3.4% MgO, 8.4% Al_2O_3, 3.1% fluoride, 0.044% organic carbon and 0.003% available P. Mineralogically, the material is predominantly fluorapatite, with some carbonate fluorapatite and chlorapatite. Minor minerals are oligoclase, sillimanite, quartz, etc. The grain-size of RP material is 90–99% <74 μm.

Distinct colonies on the medium showing halo zones were selected, purified by repeated culturing and maintained on PKV agar slants at 4°C. Two efficient strains of *Aspergillus* were selected, based on their efficiency in solubilizing RP and were identified at the species level based on the ITS sequence database. rDNA fragments were amplified using fungal universal primers, ITS1 and ITS4 (White et al. 1990). Nucleotide sequence data were compared with GenBank data (http://www.ncbi.nlm.nih.gov/) using a BlastN search (Altschul et al. 1997). The isolates were identified as *Aspergillus tubingensis* (*At*) and *A. niger* (*An*). The sequences were submitted to the National Center for Biotechnology Information (NCBI) under accession numbers HM801881 (*At*) and HM801882 (*An*).

Solubilization of rock phosphate

Solubilization activity was carried out in 50 mL PKV broth amended with RP equivalent to 1% P_2O_5. The fungi were inoculated and incubated on a rotary shaker at 30°C for 15 days. Cultures were harvested after different growth periods in order to record the change in pH and concentration of P released in the medium. Soluble

phosphorus in the culture filtrate was estimated using the molybdenum blue method (Bray and Kurtz 1945) and expressed in terms of $\mu g\ mL^{-1}$ phosphorus released.

To determine the organic acid released and enzyme activity of P solubilization by the fungi, the experiment was repeated. In Pikovskaya broth, RP was added in amounts equivalent to 500 mg P_2O_5 in 50 mL in a 250 mL conical flask, then inoculated with *A. tubingensis* and *A. niger* and incubated at 30°C for 7 days on rotary shaker. The organic acids produced by these fungi during P solubilization were determined using the HPLC method. After 7 days of growth, culture filtrates were passed through a 0.22 μm filter and subjected to HPLC with a polypore H column (Perkin–Elmer, USA). The mobile phase consisted of 0.008 N H_2SO_4 at a flow rate of 0.3 mL min^{-1}. Detection was performed by a UV/Vis detector at 210 nm (Relwani et al. 2008). HPLC profiles of the culture filtrates were analyzed by comparison with the elution profiles of pure organic acids (Bio-Rad standard containing oxalic acid, succinic acid, acetic acid, citric acid, malic acid and formic acid, whereas gluconic acid from Sigma-Aldrich was injected separately). Acid phosphatase activity was estimated using the method described by Dorn and Rivera (1966) and phytase activity using the method of Kim and Lei (2005) in culture filtrate.

Field experiment

Fungal inoculum for field inoculation was prepared by growing the fungi on PKV plates. Spores of *A. tubingensis* (At) and *A. niger* (An) were scraped and mixed separately with sterile vermiculite (2–4 mm). The inoculum size for fungi was 4.8–5.4 $\times$ 10^5 and 3.3–3.6 $\times$ 10^5 spores g^{-1} vermiculite for wheat and maize, respectively. The field experiment was carried out from December 2008 until September 2009. The temperature range was: December–February 4–25°C, March–June 35–44°C, July–September 25–37°C (rainy season). An agricultural field was used for the experiment having soil with: sand, 66%; silt, 15.45%; clay, 18.75% (sandy loam); 1.3 g cm^{-3} bulk density; pH 8.1; available P, 1.6 mg kg^{-1}; EC, 0.3 mS cm^{-1}; CEC, 12.7 meq 100 g^{-1}; total N, 0.10% and organic carbon, 0.09%. Eighteen field plots measuring 2 $\times$ 2 m^2 each were prepared. A complete randomized block design with three replicates per treatment was used. Three plots were amended with 35 g each of RP (20 mg P_2O_5 kg^{-1} soil) before seeding, whereas the other three served as non-fertilized controls (having 3.6 mg total P_2O_5 kg^{-1}) for each microbial treatment. The treatments consisted of: soil; soil + RP; soil + RP + *A. tubingensis* (At); soil + RP + *A. niger* (An); soil + *A. tubingensis* (At) and soil + *A. niger* (An). Irrigation was performed once before wheat was sown to have adequate soil water storage for seedling establishment. The seeds of wheat (variety HD2733) were sown (120 kg ha^{-1}) in December 2008/2009. At the time of seeding, 100 g of vermiculite containing the inoculum (4.8–5.4 $\times$ 10^5 spores g^{-1} vermiculite with a moisture content of 30%) was added to each plot. Plants were irrigated regularly with tap water and no fertilizers were added. The crop was harvested after five months (late April 2009) and studied for various growth parameters such as yield, biomass and P content in grain, shoots and roots. Phosphorus (P) content was measured using the molybdovanadate method described by Reuter and Robinson (1997). From each plot, 10 randomly selected plants were uprooted and shoot height, shoot and root dry mass were measured. The soil was analyzed for its organic carbon (Walkley and Black 1934), available P (Olsen et al. 1954) and pH (Richards 1954).

Two months after wheat harvest (late June 2009), maize was sown (30 kg ha^{-1}) in the same field. The field was tilled and irrigated without disturbing the experimental design. Maize cultivar Kanchan 25 was sown and at the same time 50 g of inoculum (3.3–3.6 $\times$ 10^5 spores g^{-1} vermiculite) was added to each treatment. Urea was added twice at a rate of 25 g N $plot^{-1}$ at the knee-high stage and at flag leaf emergence. After 90 days of growth (late September 2009), the crop was harvested and various growth parameters and yield were measured. From each plot, 10 randomly selected plants were uprooted and shoot height, shoot and root biomass were measured. Soil analysis was performed for organic carbon and available P and pH.

Analysis of wheat for aflatoxins

Monitoring grain for the presence of aflatoxins is important to ensuring consumer safety. Wheat samples from plots inoculated with P-solubilizing fungi were analyzed for B1, B2, G1 and G2 aflatoxins. The extraction method was based on AOAC-990.33 (Association of Official Analytical Chemists International 2005). Fifty grams of dry wheat sample was mixed with 200 mL methanol and 50 mL 0.1 N HCl and homogenized. After homogenization, 125 mL of the sample was filtered through filter paper in a separatory funnel; 50 mL of 10% NaCl and 50 mL hexane was added to the filtrate and shaken for 1 min. After layer separation, the lower layer was collected in another separatory funnel (upper layer was discarded); 50 mL dichloromethane (DCM) was added and shaken for 1 min. After separation, the lower layer of DCM was collected in a beaker. The extraction was repeated two more times with 25 mL DCM and all the extracts were collected. The extracts were filtered through sodium sulfate to remove water. The filtered extract was evaporated to 2 mL on rotavapour.

HPLC was used to separate these potentially carcinogenic aflatoxins using Zorbax-ODS (Agilent). The mobile phase consisted of a solvent mixture of deionized water, methanol and acetonitrile at a flow rate of 0.8 mL min^{-1}. The concentration of aflatoxins B1, B2, G1 and G2 was calculated by comparing with the peaks obtained for reference standards.

Statistics

The data were analyzed by analysis of variance (ANOVA) and means were compared with Tukey's test at $p < 0.05$. All the analysis was performed by using GraphPad Prism 4.03 software.

Results

Solubilization of RP by the fungi

The two fungal isolates *A. tubingensis* and *A. niger* were tested for their ability to solubilize RP. Maximum P solubilization was recorded at the sixth day in both fungi (Figure1a). *A. tubingensis* solubilized 380.8 μg P mL^{-1}, whereas *A. niger* showed better efficiency of RP solubilization and produced 403.8 μg P mL^{-1}. P solubilization was accompanied by a decrease in the pH of the culture filtrate. The pH of the medium was reduced to 3.2 in both the cases (Figure 1b). P solubilization by the fungi was accompanied by the production of organic acids in the culture media. The main organic acids excreted by these isolates were oxalic acid, gluconic acid, malic acid, succinic acid and acetic acid. The production of organic acids varied

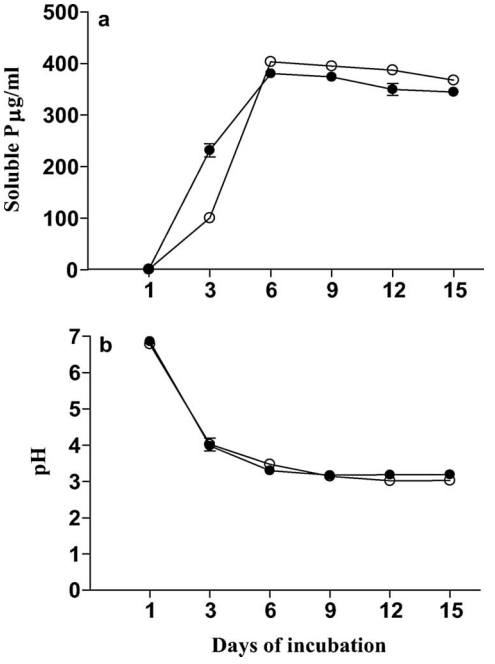

Figure 1. (a) Solubilization of rock phosphate and (b) change in the pH of the media for the tested P-solubilizing fungi *Aspergillus niger* (clear circles) and *Aspergillus tubingensis* (black circles) during 15 days of incubation. (Values are the mean of three replicates).

Table 1. Production of organic acids, acid phosphatase and phytase enzymes by *Aspergillus niger* and *Aspergillus tubingensis* grown in the presence of rock phosphate.

	A. niger	*A. tubingensis*
Organic acids (nmol mL^{-1})		
Oxalate	331 ± 9	343 ± 34
Gluconate	8958 ± 295	8421 ± 1004
Malate	5397 ± 587*	1849 ± 251
Succinate	249 ± 6	362 ± 27
Acetate	57 ± 2	127 ± 24*
Acid phosphatase (μmol mL^{-1} h^{-1})	1361 ± 62	3114 ± 57*
Phytase (μmol mL^{-1} h^{-1})	17703 ± 328	18386 ± 109

*Significant at $p < 0.05$.

significantly between the fungi (Table 1). Among the five organic acids, gluconic acid was predominant in both fungi, followed by malic acid. *A. niger* secreted higher amounts of gluconic acid and malic acid compared with *A. tubingensis*. Acid phosphatase as well as phytase activity was significantly higher in *A. tubingensis* than *A. niger* (Table 1).

Wheat experiment

Inoculation of *A. tubingensis* and *A. niger* significantly increased the growth of wheat plants compared with noninoculated ones. The shoot height increased more in

RP-amended soil than in normal soil inoculated with these fungi. The shoot biomass increased significantly in RP-amended soil inoculated with fungi compared with nonamended inoculated soil. The root biomass was significantly higher in RP-amended soils inoculated with *A. tubingensis* compared with other treatments (Table 2). An increase in the yield of wheat was also recorded with the application of P-solubilizing fungi compared with noninoculated RP-amended or nonamended control soil. Inoculation with *A. tubingensis* and *A. niger* considerably increased P uptake and enhanced plant growth, even in the absence of RP, but inoculation by these fungi in plots having RP fertilization increased the total P content of wheat plants by a greater amount than reported for plants receiving inoculum without fertilization. Total P levels were higher in both RP-amended and nonamended soils inoculated with *A. tubingensis* compared with *A. niger*. N content increased in the RP-treated inoculated soil compared with control soils (Table 2). There was not much change in the pH of the soil compared with initial values. The available P was increased significantly in RP-amended soils inoculated with the fungi compared with nonamended soil. In general, inoculation of these fungi increased the available P compared with the respective control treatments. Inoculation of *A. niger* increased the organic carbon in both RP-amended and nonamended soils compared with *A. tubingensis* and control treatments (Table 2).

Maize experiment

The maize seeds were sown two months after wheat harvest and booster doses of *A. tubingensis* and *A. niger* were inoculated. The shoot height and the biomass of shoot and root were significantly increased in soils inoculated with these fungi compared with controls. The growth parameters were higher in RP-amended soil inoculated with the fungi than in nonamended soils (Table 3). The yield of maize significantly increased in RP-amended soils inoculated with fungi compared with other treatments. *A. tubingensis* and *A. niger* when inoculated in RP-amended soil showed $>77\%$ increase in yield compared with noninoculated and nonamended control (Table 3). The P uptake in grain was significantly higher in RP-amended soil inoculated with fungi compared with nonamended soils. In general, P levels were increased both in RP-amended and nonamended soils inoculated with these fungi. The N content increased significantly in all treatments. In the treatment having RP and inoculum, the N content was highest compared with control soils. The pH of the soil decreased compared with the initial pH of the soil. The addition of P-solubilizing fungi increased the available P of the soil many folds compared with control soil. The greatest increase was observed where RP fertilization was involved. A general increase in organic carbon occurred in soil after harvesting the maize compared with control soil.

Aflatoxins in the wheat grain samples were tested by HPLC analysis and these results revealed that aflatoxins such as B1, B2, G1 and G2 were absent in the grains.

Discussion

When the efficiency of two fungal strains of *Aspergillus* to solubilize RP was tested, a significant decrease in the pH of culture filtrates of both the fungi was observed. This was attributed to the varying diffusion rates of different organic acids secreted by the tested organisms. The significant decreases in pH and high P solubilization have been

Table 2. Growth parameters of wheat plants and soil characteristics after harvest as affected by rock phosphate (RP), *Aspergillus niger* (An) and *Aspergillus tubingen*sis (At).

Treatments	Shoot height (cm)	Shoot dry mass (g)	Root dry mass (g)	Yield (kg plot^{-1})	Total plant P (mg kg^{-1})	Total plant N (%)	Soil characteristics		
							pH	Available P (mg kg^{-1})	OC (%)
Soil	43 ± 5b	1.07 ± 0.04c	0.60 ± 0.02e	0.76 ± 0.02c	141 ± 11f	0.13 ± 0.0d	8.07 ± 0.01a	1.6 ± 0.1c	0.10 ± 0.0ab
Soil+An	50 ± 3ab	1.24 ± 0.09bc	0.87 ± 0.02bc	1.10 ± 0.03a	364 ± 17d	0.17 ± 0.0c	7.88 ± 0.01b	2.8 ± 0.2b	0.11 ± 0.0ab
Soil+At	51 ± 3ab	1.30 ± 0.03bc	0.76 ± 0.02cd	0.97 ± 0.02b	419 ± 23c	0.18 ± 0.1c	7.80 ± 0.01c	2.8 ± 0.1b	0.09 ± 0.01b
Soil+RP	50 ± 3ab	1.48 ± 0.11b	0.73 ± 0.09d	0.78 ± 0.02c	309 ± 22e	0.18 ± 0.0c	7.86 ± 0.01b	3.8 ± 0.3ab	0.09 ± 0.00b
Soil+RP+An	59 ± 2a	1.82 ± 0.03a	0.90 ± 0.04b	1.10 ± 0.02a	483 ± 19b	0.22 ± 0.1b	7.49 ± 0.01e	4.9 ± 0.5a	0.11 ± 0.01a
Soil+RP+At	60 ± 3a	1.89 ± 0.02a	1.14 ± 0.07a	1.10 ± 0.02a	553 ± 32a	0.25 ± 0.2a	7.68 ± 0.03d	4.9 ± 0.3a	0.09 ± 0.0ab

Note: Values sharing a common letter within the columns are not significant at $p < 0.05$.

Table 3. Growth parameters of maize plants and soil characteristics after harvest as affected by rock phosphate (RP), *Aspergillus niger* (An) and *Aspergillus tubingensis* (At).

Treatments	Shoot height (cm)	Shoot dry mass (g)	Root dry mass (g)	Yield (kg plot^{-1})	Total plant P (mg kg^{-1})	Total plant N (%)	Soil characteristics		
							pH	Available P (mg kg^{-1})	OC (%)
Soil	145±3b	31±3d	3.1±0.3b	1.7±0.01f	232±7d	0.18±0.0d	8.03±0.07a	1.6±0.1d	0.65±0.03a
Soil+An	147±4b	51±3c	4.0±0.4b	2.8±0.01d	388±13b	0.21±0.1c	6.98±0.02b	3.6±0.2c	0.72±0.03a
Soil+At	162±2a	48±5c	7.5±0.8a	2.9±0.01c	422±22b	0.20±0.0c	6.95±0.04b	3.0±0.1c	0.72±0.04a
Soil+RP	149±4b	49±2c	6.3±0.2a	2.4±0.01e	357±19c	0.21±0.1c	6.98±0.03b	4.7±0.1b	0.71±0.02a
Soil+RP+An	167±3a	81±2a	7.8±0.5a	3.0±0.02b	428±29b	0.25±0.2b	6.76±0.04b	6.4±0.5a	0.73±0.01a
Soil+RP+At	163±2a	61±3b	7.9±0.4a	3.2±0.01a	524±38a	0.31±0.2a	6.85±0.06b	6.0±0.1a	0.75±0.04a

Note: Values sharing a common letter within the column are not significant at $p < 0.05$.

reported for filamentous fungi (Pradhan and Sukla 2006; Reddy et al. 2002). Maximum P solubilization was observed after six days of incubation in both the fungal isolates. The decrease in P concentration at the beginning stages of the experiment is consistent with the findings of Seshadri et al. (2000) who stated that the existing P is utilized for growth and development of the organism during this period. The increase in P concentration in the later stages might be due to the action of the fungi on the substrate for demands of nutrients, thus releasing more P from insoluble sources. Cell lysis and P solubilization brought about by organic metabolites may also increase the released P during the later stages (Illmer and Schinner 1992).

Increase in soil weathering and the enhancement of nutrient availability in soil are frequently associated with the production of organic acids. The production and release of organic acids are attributed to ion chelation and solubilization of inorganic P sources (Illmer and Schinner 1995). Our results showed that high amounts of organic acids were produced during solubilization of RP by both isolates. Phosphate solubilization and organic acid secretion are positively related, *A. niger* inducing more P solubilization showed higher organic acid exudation. This finding is similar to those of Illmer and Schinner (1995) and Rashid et al. (2004), indicating exudation of organic acids by all phosphate-solubilizing microbes. Cunningham and Kuiack (1992), Gadagi et al. (2007) and Reyes et al. (2006) also reported that the production and release of organic acids (mainly citric acid, oxalic acid, malic acid and gluconic acid) with ion chelation solubilizes inorganic P sources.

Phosphatases (phytase, acid phosphatase, etc.) produced by soil microorganisms play a major role in the mineralization of organic forms of soil P to release phosphate (Raghothama 1999). Acid phosphatase and phytase enzyme activity were significantly higher in both *A. niger* and *A. tubingensis*, which shows that apart from other mechanisms, both acid phosphatase and phytase played a major role in P solubilization also in these experiments.

In this study, the results showed that the addition of RP along with fungal cultures greatly enhanced plant growth and nutrient uptake in wheat and maize plants. It is generally thought that P-solubilizing microbes, in addition to solubilizing inorganic P, also release growth-promoting substances (Kucey et al. 1989), which improve the germination and growth of plants and stimulate microbial activity in the rhizosphere. A highly significant interaction was recorded between RP amendment and inoculation with fungi. Significant increase in the yield of wheat as well as maize was also recorded with the application of RP with P-solubilizing fungi in the soil. Inoculation with the fungal strains in RP-fertilized soils improved the growth of wheat and maize plants. Vassilev et al. (2006) and Omar (1998) reported that there is a positive effect of RP fertilization and phosphate-solubilizing fungi on the growth of plants.

Rock phosphate fertilization, as well as fungal inoculum, had a significant effect on total plant P in this study, in contrast to Omar (1998) who reported that RP fertilization had no significant effect on total plant P, but was significantly increased by plant inoculation. Inoculation with fungi considerably increased P uptake and enhanced plant growth compared with uninoculated soil, even in the absence of RP. These results could be attributed to the ability of these microorganisms to solubilize organic and inorganic phosphorus already present in soil. RP-fertilized soil inoculated with isolates of *Aspergillus* showed higher organic carbon than noninoculated soils (Table 3), which may be due to the production of microbial organic colloidal materials and solubilization of P, as suggested by Mba (1994). The

significant increase in N content of the soil after final harvesting may be due to an increase in organic matter. Kucey et al. (1989) reported that, besides providing P, phosphate-solubilizing microbes produce considerable amounts of N and plant growth-promoting substances in the rhizosphere.

The improved crop yield in this investigation may also have been due to the significant increase in available P in all the treatments compared with noninoculated and nonfertilized soil. The application of P-solubilizing fungi is recommended as a sustainable way to increase crop yield, under all field conditions. Many reports support our study and have shown an improvement in plant growth using P-solubilizing fungi (Whitelaw et al. 1997; Richa et al. 2007).

Mycotoxins are toxic compounds produced by certain fungi under specific conditions. Among various mycotoxins, aflatoxins have assumed significance due to their deleterious effects on human beings, poultry and livestock. Grain is susceptible to aflatoxins produced either while the crop is growing by fungal species or during storage. Among 18 different types of aflatoxins identified, major members are aflatoxin B1, B2, G1 and G2. For this reason, the wheat samples were analyzed for these aflatoxins. The results showed that the fungi used in experiment do not produce aflatoxins, therefore, no test was needed with maize. This suggests that the phosphate-solubilizing fungi *A. tubingensis* and *A. niger* can both be used as a biofertilizer to enhance plant growth and nutrition.

Conclusion

In conclusion, this study revealed that RP-solubilizing fungi enhanced the fertilizer value of RP, and RP can be used as a crude phosphatic fertilizer by direct application to alkaline soils. The application of RP and rock-phosphate-solubilizing fungi enhanced the growth and yield of wheat and maize. We report for the first time the sustainable release of P from RP-amended soil for two consecutive crops spread over a year. This report suggests that inoculation of *A. tubingensis* and *A. niger* significantly enhance the fertilizer value of RP especially in alkaline soils where solubilization of RP as such is not possible. This may assist in solving problems encountered in the crop production economy, and might contribute to reversing the trend in soil degradation and actually encourage soil conservation during cultivation of the land.

Acknowledgements

The authors are thankful to University Grant Commission for financial assistance and TIFAC-CORE for facilities.

References

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389–3402.

Association of Official Analytical Chemists International [AOAC]. 2005. In: Horwitz W, editor. Methods of analysis. 18th ed. Gaithersburg, MD: AOAC.

Bray RH, Kurtz LT. 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59:39–45.

Caravaca F, Alguacil MM, Azcon R, Diaz G, Roldan A. 2004. Comparing the effectiveness of mycorrhizal inoculation and amendment with sugar beet, rock-phosphate and *A. niger* to enhance field performance of the leguminous shrub *Dorycnium pentaphyllum* L. Appl Soil Ecol. 25:169–180.

Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H, Sa T. 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol Biochem. 37:1970–1974.

Cunningham JE, Kuiack C. 1992. Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaii*. Appl Environ Microbiol. 58:1451–1458.

Dorn G, Rivera W. 1966. Kinetics of fungal growth and phosphatase formation in *Aspergillus nidulans*. J Bacteriol. 92:1918–1922.

Fertilizer Association of India [FAI]. 2002. Fertilizer statistics. 2001–2002 I: 39–47. New Delhi (India): FAI.

Gadagi RS, Shin WS, Sa TM. 2007. Malic acid mediated aluminum phosphate solubilization by *Penicillium oxalicum* CBPS-3F-Tsa isolated from Korean paddy rhizosphere soil. Dev Plant Soil Sci. 102:285–290.

Illmer P, Schinner F. 1992. Solubilization of inorganic phosphate by microorganisms isolated from forest soils. Soil Biol Biochem. 24:389–395.

Illmer P, Schinner F. 1995. Solubilization of inorganic calcium phosphates solubilization mechanisms. Soil Biol Biochem. 27:257–263.

Kim T, Lei XG. 2005. An improved method for a rapid determination of phytase activity in animal feed. J Anim Sci. 83:1062–1067.

Kucey RMN, Jenzen HH, Leggett ME. 1989. Microbially mediated increase in plant available phosphorus. Adv Agron. 42:199–228.

Mba CC. 1994. Field studies on two rock phosphate solubilizing *Actinomycete* isolates as biofertilizer sources. Environ Manage. 18:263–269.

Narsian V, Patel HH. 2000. *Aspergillus aculeatus*as rock phosphate solubilizers. Soil Biol Biochem. 32:559–565.

Olsen SR, Cole CV, Watanabe FS, Dean LA. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture Circular No. 939.

Omar SA. 1998. The role of rock-phosphate solubilizing fungi and vesicular-arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J Microbiol Biotechnol. 14:211–218.

Pikovskaya RI. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiology. 17:362–370.

Pradhan N, Sukla LB. 2006. Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afr J Biotechnol. 5:850–854.

Raghothama KG. 1999. Phosphate acquisition. Annu Rev Plant Physiol. 16:665–693.

Rashid M, Khalil S, Ayub N, Alam S, Latif F. 2004. Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (psm) under *in vitro* conditions. Pak J Biol Sci. 7:187–196.

Reddy MS, Kumar S, Khosla B. 2002. Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis* and *A. niger*. Bioresour Technol. 84:187–189.

Relwani L, Krishna P, Reddy MS. 2008. Effect of carbon and nitrogen sources on phosphate solubilization by a wild-type strain and UV-induced mutants of *Aspergillus tubingensis*. Curr Microbiol. 57:401–406.

Reuter DJ, Robinson JB. 1997. Plant analysis: an interpretation manual. 2nd ed. Clayton (Australia) CSIRO.

Reyes I, Bernier L, Antoun H. 2002. Rock phosphate solubilization and colonization of maize rhizosphere by wild and genetically modified strains of *Penicillium rugulosum*. Microb Ecol. 44:39–48.

Reyes I, Valery A, Valduz Z. 2006. Phosphate-solubilizing microorganisms isolated from rhizospheric and bulk soils of colonizer plants at an abandoned rock phosphate mine. Plant Soil. 287:69–75.

Richa G, Khosla B, Reddy MS. 2007. Improvement of maize plant growth by phosphate solubilizing fungi in rock phosphate amended soils. World J Agr Sci. 3:481–484.

Richards LA. 1954. Diagnosis and improvement of saline–alkali soil. Agriculture handbook No. 60. Washington (DC): USDA.

Rodriguez H, Gonzalez T, Goire I, Bashan Y. 2004. Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturewissenschaften. 91:552–555.

Saber WIA, Ghanem KM, El-Hersh MS. 2009. Rock phosphate solubilization by two isolates of *Aspergillius niger* and *Penicillium* sp. and their promotion to mung bean plants. Res J Microbiol. 4:235–250.

Seshadri S, Muthukumarasamy R, Lakshminarasimhan C. 2000. Solubilization of inorganic phosphates by *Azospirillium halopraeferans*. Curr Sci. 79:565–567.

Vassilev N, Vassileva M. 2003. Biotechnological solubilization of rock phosphate on media containing agroindustrial wastes. Appl Microbiol Biotechnol. 61:435–440.

Vassilev N, Medina A, Azcon R, Vassileva M. 2006. Microbial solubilization of rock phosphate on media containing agro-industrial wastes and effect of the resulting products on plant growth and P uptake. Plant Soil. 287:77–84.

Walkley AJ, Black IA. 1934. Estimation of organic carbon by chromic acid titration method. Soil Sci. 37:29–38.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego (CA): Academic Press. p. 315–322.

Whitelaw MA, Harden TJ, Bender GL. 1997. Plant growth promotion of wheat inoculated with *Penicillium radicum*sp. Aust J Soil Res. 35:291–300.

Yadav KS, Dadarwal KR. 1997. Biotechnological approaches in soil: microorganisms for sustainable crop production Jodhpur (India): Scientific Publishers. p. 293–308.