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Improvement of Maize Plant Growth by Phosphate Solubilizing Fungi in Rock Phosphate Amended Soils

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Abstract: *Aspergillus tubingensis* and *A. niger* were tested for their efficacy to solubilize rock phosphate (RP) and also to improve the growth of maize (*Zea mays*) in rock phosphate amended soils. Both the species were able to grow and solubilize rock phosphate and soluble P levels were significantly increased in the culture medium as the concentration of RP increased. The results of nursery experiment showed that the growth of maize plants and shoot P levels were significantly increased by these fungi compared to control soil. Soil analysis results showed that the available P, organic carbon levels were significantly increased when compared to initial soil. The soil pH was also lowered compared to initial pH of the soil. These results suggested that *A. tubingensis* and *A. niger* serves as excellent phosphate solubilizers in alkaline soils amended with RP.

Key words: Rock phosphate · phosphate solubilizers · *Aspergillus tubingensis* · *Aspergillus niger* · better plant growth

INTRODUCTION

Phosphorus (P) is an essential nutrient for plant growth and development. Despite its wide distribution in nature, P is deficient in most soils and its content is about 0.05% of which only 0.1% is available for plant [1]. Phosphorus is added in the form of phosphatic fertilizers, part of which is utilized by plants and the remainder converted into insoluble fixed forms [2]. Phosphatic fertilizers are expensive and there is a need for alternative sources. For this reason, the possibility of the practical use of rock phosphate (RP), which has been recognized as a valuable alternative source for P fertilizer has received significant interest in recent years. Consequently, there is a growing interest in ways of manipulating such rock to obtain a more valuable product. Rock phosphate as such is not available for plant in soils with a pH greater than 5.5 - 6.0 and even when conditions are optimal, yields are lower than those obtained with soluble phosphate [3]. Because of this, extension services are reluctant to be recommended and farmers hesitant to utilize RP directly.

As most of the traditional techniques to increase the solubility of P from RP such as thermal alteration or partial acidulation require substantial capital investments, there is a need for alternative approaches to increase the availability of P from RP [4]. One approach for rock phosphate solubilization is the application of microorganisms able to excrete organic acids. It has been repeatedly shown that low molecular weight organic acids can strongly increase phosphorus solution concentration by mechanisms involving chelation and exchange reactions [5].

Filamentous fungi are widely used as producers of organic acids [6] and, particularly some *Aspergillus* and *Penicillium* species have been tested by inoculating directly into the soil in order to solubilize rock phosphate [7, 8]. Inoculation of phosphate solubilizing fungi and mycorrhizal fungi improves the physico-chemical, biochemical and biological properties of RP amended soil [9]. It has also been reported that the available P and aggregate stability levels, higher soil C levels, enzyme activities and lower soil pH were also reported due to inoculation of these fungi [9].

In India, it is estimated that about 260 million tons of phosphate rock deposits are available and this material should provide a cheap source of phosphate fertilizer for crop production [10]. The objective of this study was to quantify the ability of *A. tubingensis* and *A. niger* to use P from phosphate rock, to assess the impact of RP addition along with these fungi to improve the growth of maize seedlings and to quantify changes in the soil characteristics.

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MATERIALS AND METHODS

Aspergillus tubingensis and *A. niger* were isolated from the rhizospheric soil of the *Eucalyptus* plantations from Punjab, India. The P solubilization ability of these fungi was tested on rock phosphate. The rock phosphate used was Rajasthan Rock Phosphate which consists of 34.1% P_2O_5; 52.6% calcium as CaO; 3.85% fluoride as F and 0.95% acid insoluble. In Pikovskaya broth [11], tricalcium phosphate was replaced with RP in the amount equivalent to 50, 100 and 150 mg P_2O_5 in 50 ml in 250 ml conical flasks. The fungi were inoculated to these flasks and were incubated at 30°C for six days under shaking conditions. Water soluble P in the culture filtrate was estimated by the chlorostanous reduced molybdophosphoric acid blue method described by Jackson [12].

A nursery experiment was undertaken to evaluate the effectiveness of these fungi in improving the growth and P levels in maize seedlings in rock phosphate amended soil. The soil was amended with 3.5% (w/w) of RP. The soil used was the top 0-20 cms of agricultural field with a pH of 8.3; 12.2mg/kg of P and 0.9% of organic carbon. The experiment consisted of four treatments: soil, soil + RP; soil + RP + *A. tubingensis* and soil + RP + *A. niger*. The soil with above treatments was filled in polythene bags (500g capacity) and the spore inoculum of *A. tubingensis* and *A. niger* (2 - 2.5 x 10^9 cfu/bag) was inoculated. The seeds of maize (*Zea mays*) were planted and grown for 6 weeks. Various growth parameters such as height, dry biomass and P levels in shoots were studied. Shoot P content was determined by molybdovando method described by Kitson and Mellon [13]. The soil was analyzed for its organic carbon [14] and available P [15]. The data were subjected to analysis of variance (ANOVA) and the means were compared by Tukey's test.

RESULTS AND DISCUSSION

Both *Aspergillus* species were able to grow and solubilize the rock phosphate in RP amended medium. The soluble P levels were significantly increased in the culture medium as the concentration of RP increased from 50 to 150 mg P_2O_5/50 ml. The maximum soluble P was recorded at 150 mg of P_2O_5 for both species (Fig. 1). A reduction in the pH of the medium was observed in both the species of *Aspergillus* when grown in different concentrations of RP. The pH of the spent medium was reduced to below 3.6 in all the concentrations of RP.

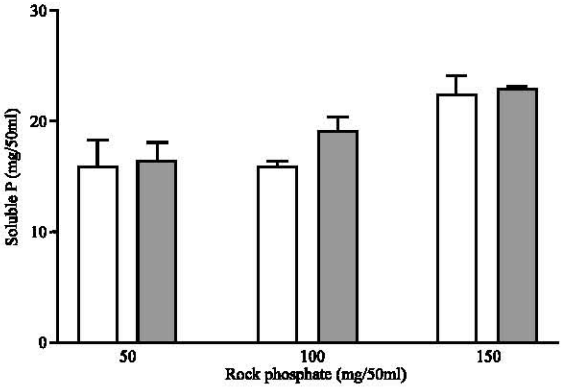

Fig. 1: Effect of increasing concentration of rock phosphate on soluble P levels of the spent medium of *A. niger* (empty bars) and *A. tubingensis* (filled bars)

Inoculation of *A. tubingensis* and *A. niger* increased the growth of maize plants in RP amended soil. The shoot height was significantly higher in presence of RP amended soil inoculated with these fungi compared to control soils. The shoot biomass was significantly increased in RP amended soil inoculated with these fungi than control soils. The root biomass was significantly higher in soils inoculated with *A. tubingensis* compared to control soils. The shoot P content was significantly increased by the addition of *A. tubingensis* followed by *A. niger* when compared to control soils (Table 1). To evaluate the effectiveness of these fungi on the changes of soil characteristics, the soil was analyzed for available P, organic carbon and the pH. Inoculation of *A. tubingensis* and *A. niger* improved the soil characteristics in RP amended soil. The pH of the soil was slightly decreased (about 0.2 units) due to inoculation. The available P levels were significantly increased in all the treatments compared to the initial levels of the soil. Inoculated soil had a higher organic carbon levels compared to control soils. In general, the organic carbon levels were increased in all the treatments compared to the initial levels (Table 2).

It is generally accepted that the mechanisms of phosphate solubilization in soils is closely related to the complex forming properties of low molecular weight organic acids [16]. Soil microorganisms are deeply involved in this process and their role in solubilization of phosphate bearing materials has been the subject of an increasing number of studies in soil plant systems. The attractive approach of microbial mediated solubilization of rock phosphate has successfully proved using many filamentous fungi [17, 18, 19]. In this study, *A. tubingensis*

Table 1: Growth parameters and P content in shoots of maize plants as affected by rock phosphate (RP), *A. tubingensis* (At) and *A. niger* (An)

Treatments	Height (cms)	Shoot dry wt. (g)	Root dry wt. (g)	P content in shoots (mg g^{-1})
Soil	18.8±0.44b	1.23±0.1b	0.83 ±0.07b	35.4±0.73c
Soil+RP	17.0±1.10b	1.26±0.18b	0.80±0.06b	32.8±1.28c
Soil+RP+At	21.4±0.47ab	1.83±0.13a	1.05±0.11a	57.1±1.56a
Soil+RP+An	22.8±0.75a	1.57±0.14a	1.0±0.05ab	51.8±0.73b

Mean ± SEM sharing a common letter within the column are not significant at $p<0.05$

Table 2: Change of soil characteristics after inoculation of *A. tubingensis* (At) and *A. niger* (An) amended with rock phosphate (RP)

	pH		Available P(mg kg^{-1})		Organic carbon (%)	
Treatments	Initial	Final	Initial	Final	Initial	Final
Soil	8.3	8.2	12.2	20.2*	0.9	1.03
Soil+RP	8.2	8.1	14.3	20..8*	1.10	1.12
Soil+RP+At	8.2	8.0	14.3	21.8*	1.10	1.36
Soil+RP+An	8.2	8.0	14.3	22.1*	1.10	1.35

*Significant compared to initial values within treatment

and *A. niger* was tested for their efficacy to solubilize rock phosphate and also to improve the growth of maize in RP amended soils. Both the species of *Aspergillus* were able to increase the soluble P as the concentration of RP increased in the culture medium. Narsian and Patel [2] also reported increased levels of soluble P with increase in RP concentration in the medium by *A. aculeatus*. Contrary to these, Vassileva *et al.* [20] reported the decrease of soluble P with increasing amount of RP in the medium. Although the levels of soluble P are higher in the spent medium, it is difficult to estimate the complete solubilized P concentration in the medium. The soluble P liberated during the growth processes is influenced by the overall behavior of the microbial cultures. A great part of the soluble P consumed by the abundant free mycelium and the negative effect of soluble P on the microbial acid productivity [21] causes changes in the final soluble P concentration.

A nursery experiment was undertaken to evaluate the effectiveness of *A. tubingensis* and *A. niger* in RP amended soils with respect to improvement of maize plant growth and physico-chemical properties of the soil. Both the fungi were significantly improved the growth of maize plants. Many reports have shown the plant growth improvement by using P solubilizing fungi [7, 8, 22]. Gerke [23] reported that the addition of citric acid increased phosphate concentrations in solutions of alkaline soil and this effect was detectable even after 140 days. The soil used in this study was alkaline in nature and both the species are capable of producing organic acids. This assumption could be considered while assessing the positive effects of RP amendment along with the fungi on plant growth. Despite the possibility of rapid degradation of acid by the soil components, citrate ions can be adsorbed at the same sites as phosphate and, consequently, may desorbs phosphate ions directly [24]. It was also found that organic acids added to soils increased the plant uptake of P from a water soluble P [25]. In addition, organic acids can be considered as a source of available carbon or serve as plant growth stimulators [16]. Hence, the results presented in this study could be interaction of these factors that were taking place in improving the plant growth. The shoot P content was increased by the inoculation of these fungi in RP amended soil. The increased P uptake by plants was also reported due to inoculation of *A. niger* by Medina *et al.* [26]. The higher uptake of P might be a result of the solubilization of rock phosphate by these fungi in the soil system.

The soil characteristics were also improved after inoculation with these fungi. The available P levels were significantly improved in all the treatments compared to the initial P values. The improvement of physico-chemical, biochemical properties of RP amended soil with the inoculation of *A. niger* and mycorrhizal fungi was reported by Caravaka *et al.* [9]. They also reported higher available P, soil total carbohydrates, water soluble C and lower soil pH compared to control soils. In the present study also the available p, organic C levels were increased by the inoculation of *A. tubingensis* and *A. niger*. From the present study, it was clear that amendment of soils with rock phosphate along with the phosphate solubilizing fungi improves the plant growth and also the soil characteristics. These results concluded that *A. tubingensis* and *A. niger* serve as excellent phosphate solubilizers in RP amended soils.

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