Phosphorus nutrition of crops through arbuscular mycorrhizal fungi

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Phosphorus (P) is one of the diffusion limited major nutrients, which is essential for plant growth. In soil, phosphorus occurs in three forms namely, soluble inorganic P, insoluble inorganic P and organic P. Uptake of P from soil solution is mediated by arbuscular mycorrhizal fungi (AMF) in addition to plant roots. AMF are ubiquitous occurring in most of the soils. They are commonly found in association with agricultural crops. It is now proved beyond doubt that AMF greatly enhance plant growth. The improved growth is mainly attributed to uptake of diffusion limited nutrients such as P, Zn, Cu, etc. from soil. The other beneficial effects are their role in the biological control of root pathogens, hormone production, greater ability to withstand water stress and synergistic interaction with beneficial microorganisms. It is believed that mycorrhizal plants absorb P only from the soluble P pools in the soil. Synergistic interactions between AMF and P-solubilizing microorganisms (PSM) are present which in turn helps plant growth. This is because PSM solubilize and release H_2PO_4 ions from unavailable forms of P and AMF help in the uptake of H_2PO_4 ions from soil. Field studies have shown that inoculation with efficient AMF not only increases growth and yield of crop plants but also reduces the application of phosphatic fertilizer by nearly 50%, especially in marginal soils deficient in nutrients. Though the rock phosphates available in India are of low grade and not fit for the manufacture of phosphatic fertilizer, they can be used with PSM plus AMF as a potential source of P for crop plants, thus bringing down the import of P fertilizers/rock phosphate in our country. Advantages of AMF have been attained through application of suitable AM fungal inoculum and augmenting native AM fungal activities in soil through manipulating agricultural practices in favour of these fungi.

Keywords: Agricultural practices, AM fungi, P nutrition, rock phosphate.

Introduction

NITROGEN, phosphorus (P) and potassium are the three major plant nutrients of which P is non-renewable. Rock phosphate is a major ingredient for the manufacture of phosphatic fertilizers. Even though India has an estimated amount of 250 mt of rock phosphate, much of it is of low grade having less than 25–30% P_2O_5 and not suitable for manufacture of P fertilizer. Thus, India is mostly dependent on imports to an extent of 90% for meeting its domestic requirement of P in the form of rock phosphate, phosphoric acid and P fertilizer. Plants require adequate P from the very early stages of growth for optimum crop production[1]. Plants draw nutrients from the soil. Ion uptake by plant roots from soil is governed by two major factors: transfer of ions through soil and the absorbing power of the root. The transfer of ions through the soil occurs either by mass flow or by diffusion. Some of the ions such as NO_3, SO_3, Ca, etc. move through mass flow which is faster and easy. These are referred to as mobile elements. The uptake of these ions would be limited by the absorbing capacity of the root. In contrast, some ions (such as H_2PO_4, NH_4, Zn, Cu) are poorly mobile and move by diffusion. These are referred to as diffusion limited or immobile elements. The uptake of such ions depend on the movement of these ions to the root surface and then the absorbing capacity of the root. So, root interception to the adsorbed P on soil particles is required for acquisition which is enhanced by arbuscular mycorrhizal (AM) fungal external hyphal network which extends beyond root zone increasing effective root surface area.

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P in soil and plant P uptake

Phosphorus is one of the diffusion limited major nutrient, which is essential for plant growth. It is an integral part of the cellular activities of living organisms. It has a defined role in plant metabolism such as cell division, development, photosynthesis, breakdown of sugar, nutrient transport within the plant, transfer of genetic characteristics from one generation to another and regulation of metabolic pathways[2]. Phosphorus constitutes about 0.2% of plant dry weight[3]. Plants obtain their P requirements from soil. In soil, phosphorus occurs in three forms, namely soluble inorganic P, insoluble inorganic P and organic P. The soluble inorganic P occurs in soil solution. The soluble inorganic P occurring as primary orthophosphate ($H_2PO_4^-$) and secondary orthophosphate (HPO_4^{-2}) are taken by plants. Of these two forms, plants prefer to take P in the monovalent H_2PO_4 form[4]. The average orthophosphate concentration in soil solution is around 10^{-6} M, which is near the limit at which plants can absorb adequate phosphate. Insoluble inorganic P is found in crystal lattices. The inorganic unavailable P forms are varisite, strengite, fluorapatite, hydroxyapatite and tricalcium phosphate. These forms occur mainly due to the weathering of rocks such as apatite or from the fixation of available inorganic P to unavailable forms. The mineral forms of iron and aluminium phosphates are predominant in acidic soils, while calcium phosphate predominate in neutral to alkaline soils. These unavailable forms are converted to the available orthophosphate by some microorganisms. Such microbes are said to have mineral phosphate solubilizing ability[5]. There are several reviews on these phosphate-solubilizing microorganisms (PSMs) and their usefulness in increasing crop growth and yield, when applied with a cheaper source of rock phosphate[6,7].

Arbuscular mycorrhizal fungi and P nutrition

Uptake of P from soil solution is mediated by mycorrhizal fungi in addition to plant roots. Though there are different kinds of mycorrhizal fungi, only arbuscular mycorrhizal fungi (AMF) are discussed here. AMF are ubiquitous, occurring in most of the soils. They belong to the phylum Glomeromycota, which has three classes (Glomeromycetes, Archaeosporomycetes and Paraglomeromycetes) with five orders (Glomerales, Diversisporales, Gigasporales, Paraglomerales and Archaeosporales), 14 families and 26 genera[8]. The commonly occurring genera of AMF are *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora* and *Entrophospora.* These fungi are obligate symbionts and have not been cultured on nutrient media. AMF are not host specific although evidence is growing that certain endophytes may form preferential association with certain host plants[9,10]. They are commonly found in association with agricultural crops, most shrubs, most tropical tree species and some temperate tree species. The fungi being obligate biotrophs do not grow on synthetic media. It is now proved beyond doubt that AMF greatly enhance plant growth. The improved growth is mainly attributed to uptake of diffusion limited nutrients such as P, Zn, Cu, etc. from soil. The other beneficial effects are their role in the biological control of root pathogens, hormone production, greater ability to withstand water stress and synergistic interaction with nitrogen fixers, P solubilizers and plant growth promoting rhizomicroorganisms (PGPRs)[10]. The role played by these fungi in improving plant growth is much more significant in tropical soils compared to temperate soils. This is mainly because most of the soils of the tropics are of low inherent fertility. They are deficient in phosphorus. In addition to being deficient in phosphorus, they are P-fixing, i.e. 75–80% of the phosphatic fertilizers added get fixed in the soil and is not readily available over the crop period necessitating fresh additions. In acidic soils, they are fixed as iron and aluminum phosphates, while in neutral soils they are fixed as calcium phosphates. Continuous application of P fertilizers will result in increased concentration of total phosphorus in the soil over times, resulting in large reserves of fixed P. According to Ozanne[11], less than 10% of soil P enters the plant–animal cycle.

Experiments with P^{32}-labelled phosphorus conclusively proved that AMF cannot solubilize unavailable inorganic phosphorus sources, but draw extra phosphate only from the labile pool in soil solution[12]. The rate in which plant roots absorb phosphorus from the soil solution is much faster than the rate in which phosphorus moves in soil solution by diffusion. This results in a phosphorus depletion zone around the root. It is here AMF play the most significant role. The external hyphae of AMF travel much beyond the P depletion zone and scavenge a large volume of soil and supply P to the plants. Early experiments showed that hyphae can travel 8 cm away from the root system.

The improved P nutrition in plants has been explained mainly by the extension of AM fungal hyphae beyond the root system which allows for the exploration of spatially unavailable nutrients[13]. In exchange, the AMF receive carbohydrates from its host plant[14]. Mycorrhizal hyphae of *Glomus* sp. and *Acaulospora* sp. can transport P from distances of up to several centimetres from host plant roots[15,16]. The efficiency of P uptake by AMF has been related to both the spatial distribution of the AMF extraradical hyphae in the soil and to the capacity of P uptake by unit length of the hyphae[14]. Native P-pool efficiency can also be enhanced through cropping sequences. Recently, Maiti *et al.*[17] demonstrated enhancing indigenous AMF-mediated P acquisition efficiency in upland rice in plots grown with maize–horse gram/rice rotation (2 years rotation of maize relay cropped by horse gram in first year and rice in second year) than farmers' rotations. This practice also encouraged native AM fungal population in soil, root colonization, P uptake and rice grain yield. This AM-supportive rice-based crop rotation reduced phosphatic fertilizer dose by 33.3% in rice to obtain comparable grain yield[18]. Further functional differences within AMF species exists with respect to plant growth response, mycelial growth pattern, and spore production per unit of hyphal length; while the P uptake per unit of hyphal length seems to be more conserved on AMF species level[19]. Jansa *et al.*[20] demonstrated the variation in P acquisition in different AM species. They observed *Glomus mosseae* and *Glomus intraradices* to be more efficient in P uptake than *Glomus claroideum* and *Glomus mosseae*.

Now, it is believed that mycorrhizal plants absorb P only from the soluble P pools in the soil and they are unable to utilize sources of P which are unavailable to uninfected roots[21,22]. Good evidence that AM hyphae produce substances to solubilize P is not yet available. Some workers have observed improved plant growth and P-uptake in soils amended with tricalcium phosphate or rock phosphate with mycorrhizal inoculation. Critical analysis of the study revealed that PSMs in soil in those experiments solubilized the insoluble P sources releasing the orthophosphate ions which in turn were taken by the AM hyphae thus enhancing the P uptake and plant growth[23,24].

The concentration of P in root tissue or mycorrhizal hyphae is 1000 times greater than to the concentration of P in soil solution, necessitating an active uptake mechanism requiring energy. Once Pi enters the hyphae they get linked and form poly-P granules (Pi-Pi-Pi-Pi-) and the enzyme which mediates is polyphosphate kinase. Poly-P granules move along with the hyphae through cytoplasmic streaming as evidenced by the cytoplasmic streaming inhibitor, cytochalasin-B[25]. At the arbuscular tip, the poly-P granules are broken down to individual Pi units by the enzyme polyphosphatase. Pi travels through the arbuscule and gets deposited in the interfacial matrix. From the interfacial matrix, Pi enters the host through an active process involving ATPase and alkaline phosphatase. Calculations have shown that hyphal inflow of P (uptake per unit length of hyphae per unit time) is around 18×10^{-14} mol cm^{-1} s^{-1} or about six times more compared to non-mycorrhizal roots[26].

In mycorrhizal plants, the movement of P from root to the leaves is also faster. This is attributed to the steepness of P gradient from the root to the leaves. Conversion of inorganic P takes place at a faster rate in the shoot system of mycorrhizal plants resulting in less inorganic P in the shoot, thus increasing the steepness of P gradient from root to the leaves.

The major organic P source in the soil is phytate. AMF produce acid phosphatase which acts on phytate and releases the H_2PO_4 ions[27].

P fertilizers, PSMs and AMF

It is well-established that heavy doses of phosphatic fertilizers inhibit AM colonization. Attempts have been made to establish whether AM colonization is more affected by the soil or plant P status. Kurle and Pfleger[28] concluded that plant P status modulates colonization, whereas Miranda and Harris[29] demonstrated that soil P might have a direct effect on AM external hyphal growth. AM colonization of plants and mycorrhizal soil infectivity are decreased by both mineral and organic P fertilizers[30]. Plant mycorrhizal colonization and mycorrhizal dependency are negatively correlated with phosphorus concentration in the soil solution[31]. Synergistic interactions between AMF and PSMs are present which in turn help plant growth. This is because P solubilizers solubilize and release H_2PO_4 ions from unavailable forms of P and AMF help in the uptake of H_2PO_4 ions from soil[12,24]. Some workers found that PSMs inoculated onto seeds or seedlings maintained high populations, longer in the rhizospheres of mycorrhizal than non-mycorrhizal roots.

Some PGPRs and endophytes, which live in the host tissue, have also been reported to solubilize unavailable form of P to a soluble form, in addition to their ability to produce plant growth promoting hormones and to suppress phytopathogenic organisms via production of antibiotics, siderophores, chitinases, etc. The ability of PGPRs such as *Paenibacillus* spp.[32], *Exiguobacterium* spp.[33] and *Pantoea stewartii*[34] to solubilize unavailable phosphates has been reported. Similarly endophytic bacteria *Ewingella* spp. and *Rahnella* spp. from banana[35] and fungi such as *Trichoderma* spp. and *Papulaspora* sp.[36] isolated from bush mint have also been reported to solubilize unavailable forms of P. Synergistic interactions between AMF and P solubilizing PGPRs/endophytes with consequential benefit on plant growth has been demonstrated not only in crop plants but also in forest trees[22,37]. Plants treated with AMF and PSM also record increased plant dry matter and P uptake in soils amended with rock phosphate[38]. Some researchers have reported that a combined inoculation of PSM with AMF along with rock phosphate could improve crop yield in nutrient deficient soils[7]. Some studies also revealed that the total cost of cultivation and the gross income, net profit per hectare and the cost benefit ratio are also high when AMF and PSM are inoculated together with rock phosphate under field conditions[39].

Significant increases in plant growth and yield of several plants important in agriculture, horticulture and forestry because of AM inoculation in unsterile soils containing less or insufficient indigenous endophytes have been reported by several workers. These studies also brought out that application of phosphatic fertilizer in crops can be reduced by nearly 50% (refs 9, 40) (Table 1). In medicinal and aromatic plants, AM fungal inoculation not only increased the crop yield but also that of the active ingredient[10,22].

Table 1. Effect of different arbuscular mycorrhizal fungi and added phosphorus on fruit yield of chilli

	Yield of chilli (kg/plot)		
	Addition of P fertilizer		
Inoculation	No addition	Half the recommended level	Recommended level (75 kg/ha)
Uninoculated	0.27	0.37	0.43
Glomus fasciculatum	0.40	0.52	–
G. albidum	0.38	0.42	–
G. macrocarpum	0.32	0.40	–
G. caledonicum	0.37	0.41	–

Agricultural practices and AMF

The intensive agriculture that developed from the mid-20th century was based on new cultivars and on increased use of fertilizers and biocides. As fertilizer and pesticide applications may decrease the development of AMF, their effects would be severely affected[30]. Plenchette *et al.*[41] while reviewing work on managing AMF through cropping systems mentioned that breeding programmes are generally conducted in experimental stations with high input conditions. Since increasing soil fertility diminishes mycorrhizal development, and therefore the benefits of mycorrhizal fungi, it was hypothesized that this could lead to the selection of varieties with high P requirements. In other words, breeders would be selecting against mycorrhizal dependency. Johnson and Pfleger[42] also suggested that there is no doubt that crop breeding programmes for selection of high yielding varieties under fertilized conditions may inadvertently select genotypes that are unresponsive to mycorrhizal fungi. Studies have shown that AM colonization is a heritable trait[43]. Breeding for better symbiosis could be an objective for a sustainable agriculture that would contribute to improved efficiency of P use[44]. Based on evidences, scientists have advocated greater use of AMF in agricultural practices[45], particularly to reduce the use of P fertilizers[46]. Hence, the formation and functioning of AM symbiosis is expected to be crucial in sustainable systems[47].

In agriculture, AM symbiosis is influenced by management practices such as the amount and type of the supplied fertilizer[48,49] and extent of tillage[50,51]. Soils from low-input farming systems have a greatly enhanced capacity to initiate the mycorrhizal symbiosis[52]. It is estimated that there are almost 40 million tonnes of phosphatic rock deposits in India[53], and this material should provide a cheap source of phosphate fertilizer for crop production[54]. Though the rock phosphates available in India are of low grade and not fit for the manufacture of phosphatic fertilizer, they can be used with PSM plus AMF as a potential source of P for crop plants, thus bringing down the import of P fertilizers/raw material for the manufacture of P fertilizer in our country.

Conclusion

Considering the fact that most of the Indian soils are not only deficient in phosphorus but also P fixing, more efficient method of utilizing phosphatic fertilizer has to be developed. Further, raw material for the manufacture of P fertilizer being imported necessitates the need for research in utilizing low-grade rock phosphate deposits in our country by judicial agricultural techniques involving PSM and AMF. Though some work has been concluded on PSM and AMF, systematic efforts to screen and select the most efficient PSM and AMF with better P acquisition and use efficiency and their role in improving the productivity of crop plants in different agro-climatic zones of the country has to be strengthened. Using the selected AMF, their ability to improve growth, nutrition and yield of different crop plants along with the possibility of saving P fertilizer usage needs investigation.

Plant breeding generally ignores the functional contributions of microorganisms in soil and rhizosphere such as AMF, PSM, etc. Conventional and modern tools of plant breeding can take advantage of this in breeding crop cultivars with enhanced response to AMF. Such cultivars shall be of greater advantage in low input sustainable agriculture. Further, AM-technology has also been shown to be efficient under moisture stress condition[55] emphasizing its importance in rainfed ecology, constituting a portion of Indian agriculture. Based on these conclusions, the following researchable and policy issues are to be addressed.

Researchable issues

- Considering the enormous diversity and heterogeneity of microorganisms in the different agro climatic regions of the country, research effort is needed to develop efficient strains of AMF with better P acquisition and use efficiency.
- Exploitation of AMF together with PSMs and other beneficial soil microbes in order to improve crop productivity and reduced use of fertilizers.
- Exploitation of appropriate species of AMF alone or together with PSMs to utilize low grade rock phosphates as a source of P fertilizer.
- It is suggested that during breeding programmes, breeders may consider breeding for better AM symbiosis that would contribute to improved P use efficiency.
- To develop laboratory media and techniques for the cultivation of AMF which will aid their easy mass multiplication.

Policy issues

- Mass awareness about the use of AMF, PSM, P fertilizers and rock phosphate.
- Provision for financial support to public and private sectors for production of quality AMF.
- Modification of the present quality control specifications prescribed for AMF by FCO as it lacks clarity and some of the methods suggested are not reproducible.
- Training the farmers on use of AMF, PSMs and rock phosphate.

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