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Arbuscular Mycorrhizal Inoculation and Phosphorus Mobility in Phosphorus-Fixing Sweetpotato Soils

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ABSTRACT

Phosphorus (P) mobility in three P-fixing (laterite, red and sandy) sweetpotato soils in relation to inoculation with arbuscular mycorrhizal (AM) fungi *Glomus microcarpum* has been studied through a pot culture trial for two seasons. In all the inoculated soils, irrespective of season, the sweetpotato plants had a comparatively high rate of colonisation. However, increased P level in the soil tended to decrease colonisation. Percentage root colonisation increased with days after planting (DAP) while, the spore density in the root-zone soil decreased with DAP. Soil P availability varied between inoculated and uninoculated treatments and different soil types. In general, inoculated treatments showed a low soil P availability but the rate of removal of P from soil to plant tissue was more in mycorrhiza inoculated treatments in both the seasons and at different DAP. The rate of removal of P by mycorrhizal plants was maximum in laterite and sandy soils at all DAP. Mycorrhizal inoculation did not give any added benefit on soil P release and fixation in the soil types studied.

Keywords: AM fungi, *G. microcarpum*, P-fixing soil, P mobility, sweetpotato

INTRODUCTION

Phosphorus (P) is one of the most important nutrients for plant growth, development and reproduction. In many regions of the world, soil P is often a major factor limiting crop production. To improve P nutrition of plants, the conventional approach is to apply large amounts of P fertilisers to soils. It has been estimated that nearly 36.78 million tonnes of P-based fertilisers (in terms of P_2O_5) are applied world wide every year (International Fertilizer Industry Association 2006) However, use efficiency of applied P is generally very low ranging from 10 to 30% in the year applied (McLaughlin *et al.* 1991).

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Many soils in the tropics are fragile and prone to degradation. Some characteristics of tropical soils put severe constraints on plant production. This includes soil moisture stress, low nutrient capital, erosion risks, low pH with aluminium toxicity, high P fixation, low levels of soil organic matter and loss of soil biodiversity (Sanchez *et al*. 2003). Improving plant uptake of P from soils is an obvious alternative to the management of such soils and the enhancement of use efficiency of P fertilisers.

The association of arbuscular mycorrhizal (AM) fungi with plant roots alters plant-soil interactions and enhances plant growth and nutrition under stressful edaphic conditions (Smith and Read 1997). Increased growth of mycorrhizal plants compared with non-mycorrhizal plants commonly observed in low P soils in glass house studies and also in the field, has largely been related to increased P uptake and more effective P nutrition. Exploration of large soil volume (Tinker 1978), faster movement of P into AM hyphae (Cress *et al*. 1979; Bolan *et al*. 1987) and solubilisation of relatively immobile sources of P (Hetrick 1989) are reported as the mechanism for increased P uptake by AM plants. AM fungal hyphae contribute to absorption and translocation of P from sites in soil that are not accessible to plant roots (Sanders and Tinker 1971) and depletion zone around non-mycorrhizal plants of a few millimeters (Li *et al*. 1991; George *et al*. 1992).

Sweetpotato (*Ipomoea batatas* L.) is a foremost tuber crop in respect of calorific value and is grown in almost all soil types (Harikumar 1997). Several reports document the incidence (Potty 1978; O'Keefe and Sylvia 1993), genotype dependent variation in AM colonisation (Harikumar and Potty 2002a) and response of the crop to P fertilisation (O'Keefe and Sylvia 1992). Despite these advances, we still know little about the role of AM association on P mobility in P-fixing soils where the crop is usually grown. We report in the present study the role of AM inoculation on changes in P mobility in three sweetpotato growing soils.

MATERIALS AND METHODS

Soils

Three different soils (Table 1) were collected one month after harvesting of the standing crop from fields where sweetpotato cultivation was practised for several years. They were cleared off weeds and debris prior to filling the pots.

Biological Materials

Sweetpotato (cv. Kanjangad) was selected for use in the study. A local isolate (CTAM 69) of AM fungi *Glomus microcarpum* Tul. & Tul. served as the mycorrhizal symbiont.

Experimental Set-up and Design

The experiment was carried out over two seasons in earthen pots of 13 cm diameter having a holding of 5 kg soil. The pots were filled with soils sterilised with

TABLE 1
Characteristics of soils used in the experiment

Characteristics	Soils		
	Laterite	Red	Sandy
Soil Group	Oxisol	Alfisol	Entisol
pH	5.5	4.6	5.6
Organic Carbon (%)	0.92	0.46	0.87
Available P (ppm)	151.12	154.57	118.54
Al (ppm)	0.68	12.10	88.16
Fe (ppm)	0.29	1.12	12.07
Zn (ppm)	0.22	0.21	0.29
Sesquioxide (ppm)	0.02	0.24	0.26

formalin 15 days prior to planting. Before the onset of the experiment, each pot was fertilised per kg soil with 2.8 ppm nitrogen (N) as urea, 4.2 ppm potassium (K) as muriate of potash and 140 ppm phosphorus (P) as Mussorie rock phosphate. Half of the pots received mycorrhization by way of planting rooted *G. microcarpum* infected vine cuttings (Harikumar and Potty 2002b), one per pot. All pots received native microflora (except AM fungi) as sieving. The experiment consisted of three soil types (laterite, red and sandy) and two levels of endomycorrhization (mycorrhizal (M1) and non-mycorrhizal M0) with a complete 3 × 2 factorial design with 10 replications per treatment. The set-up was maintained in a glass house for observing the changes under different treatments. Two pots were set aside from each treatment at 15-day interval for monitoring various parameters. Final sampling of pots from each treatment was done at the termination of the experiment at 45 days after planting (DAP).

Assessment of AM Colonisation

For determination of AM colonisation, fresh root samples (approximately 0.2 g) were thoroughly washed in running tap water and cut into 1 cm long segments. The root segments were cleared in 10% (w/v) KOH (30 min, 90^0C), acidified with lactic acid (10 min) and stained with 0.5% Trypan blue (Phillips and Hayman 1970). Fifty root fragments (approximately 1 cm long were mounted on slides in a polyvinyl alcohol-lactic acid-glycerol solution (Koske and Tessier 1983) and examined at 100× magnification under Nikon Eclipse E400 microscope to obtain the percentage of root length colonised by AM fungi. The percentage of root length colonised by AM fungi was determined using the magnified line-intersect method of McGonigle *et al*. (1990).

Recovery and Counting of AM Fungal Spores

Spores of AM fungi were extracted from 50 ml of air-dried sub-samples of each soil sample by wet sieving followed by floatation centrifugation in 50 % sucrose (Dalpé 1993). The finest sieve used was 45 µm. The spores were collected on a grid patterned (4x4) filter paper, washed three times with distilled water to spread

them evenly over the entire grid and counted using stereo microscope (Zeiss Stemi- DV4) at 30 × magnification.

Measurement of Soil P Mobility

Soil available P was estimated by Bray's No.1 extract method (Jackson 1973). Soil P mobility was calculated at different DAP using the following formulae:

$$\text{P released (\%)} = \frac{\text{(Soil available P + P absorbed by plants)}}{\text{Initial soil P}} \times 100$$

$$\text{P removed (\%)} = \frac{\text{P absorbed by plants}}{\text{Initial soil P}} \times 100$$

P fixed (%) = Initial soil P- (Soil available P + P absorbed by plants)

Statistical Analysis

Data were statistically analysed by analysis of variance with IRRISTAT PROGRAM (IRRI Philippines). Treatment means were separated by Duncan's Multiple Range Test (DMRT) (Little and Hills 1978).

RESULTS

Mycorrhizal Colonisation

In all the inoculated soils, irrespective of seasons, the sweetpotato plants had a comparatively higher rate of colonisation. However, increased P levels in the soil led to decreased colonisation in all soil types. Among the inoculated soils, during Season I, maximum percentage root colonisation was observed in plants grown in sandy soil while the lowest was in laterite soil. In Season II, the trend was, however, reversed with a relatively higher colonisation in plants raised in laterite and red soils. In inoculated treatments percentage root colonisation increased with DAP. The same trend was observed in season II as well. Uninoculated plants remained non-mycorrhizal during both the seasons.

The spore density in the rhizosphere of inoculated plants also showed significant ($p<0.05$) differences in most cases. In general, more spore density was recorded in laterite and sandy soils during both the seasons. In both the seasons spore density decreased with DAP (Table 2).

Soil P Availability and Removal

There was significant ($P<0.05$) difference in the soil P availability between inoculated and uninoculated treatments and between different soil types. The differences were observed at all DAP. In general, the soil available P was low in inoculated treatments in all soil types during Season I. However, in Season II a noticeable reduction was observed only at late stages of plant growth, that is, at 45 DAP. Soil P availability did not show any definite trend with an increase in DAP in Season I. In Season II, the soil availability, however, decreased with an increase in DAP.

TABLE 2
Effect of *G. microcarpum* inoculation on percentage colonisation and rhizosphere spore density in sweetpotato grown in different soil types

AMF	Soil type	Season I						Season II					
		% Colonisation			Spore density/50ml soil			% Colonisation			Spore density/50ml soil		
		15 DAP	30 DAP	45 DAP	15 DAP	30 DAP	45 DAP	15 DAP	30 DAP	45 DAP	15 DAP	30 DAP	45 DAP
M0	Laterite	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	0.00^{c}	0.00^{b}	0.00^{d}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{c}
	Red	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	0.00^{c}	0.00^{b}	0.00^{d}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{c}
	Sandy	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	0.00^{c}	0.00^{b}	0.00^{d}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{c}
M1	Laterite	31.50^{b}	32.50^{b}	36.45^{c}	33.00^{b}	23.00^{a}	21.00^{a}	14.15^{b}	16.33^{a}	33.17^{a}	47.00^{a}	16.00^{a}	24.00^{a}
	Red	55.00^{a}	77.50^{a}	53.50^{b}	19.00^{c}	21.00^{a}	18.00^{a}	19.50^{a}	8.16^{b}	32.67^{a}	23.00^{c}	19.00^{a}	15.00^{b}
	Sandy	52.00^{a}	75.00^{a}	72.50^{a}	41.00^{a}	15.00^{b}	17.00^{a}	7.42^{c}	16.33^{a}	15.63^{b}	41.00^{b}	16.00^{a}	15.00^{b}

Means in column with the same superscripts do not differ significantly at P=0.05 level by DMRT
Initial soil P in Season I: Laterite 149 ppm; Red 152.75 ppm; Sandy 116.5 ppm
Soil P in Season II: Laterite M0-253.04 ppm M1-261.45 ppm
Red M0-274.31 ppm M1-274.80 ppm
Sandy M0-236.81 ppm M1-237.35 ppm

TABLE 3
Effect of *G. microcarpum* inoculation on soil P availability and removal in sweetpotato soils

AMF	Soil type	Season I						Season II					
		Soil P (ppm)			P removal (%)			Soil P (ppm)			P removal (%)		
		15 DAP	30 DAP	45 DAP	15 DAP	30 DAP	45 DAP	15 DAP	30 DAP	45 DAP	15 DAP	30 DAP	45 DAP
M0	Laterite	9.53^{c}	17.25^{b}	13.25^{c}	0.69^{b}	0.26^{c}	0.73^{a}	76.25^{c}	49.50^{c}	58.96^{c}	1.03^{cd}	0.86^{d}	1.23^{b}
	Red	3.92^{d}	5.42^{e}	10.00^{d}	0.40^{c}	0.38^{bc}	0.40^{b}	12.50^{f}	12.25^{f}	4.00^{e}	0.54^{e}	0.50^{e}	0.63^{c}
	Sandy	23.17^{a}	20.96^{a}	20.98^{b}	0.70^{b}	0.52^{ab}	0.36^{b}	104.25^{a}	45.25^{d}	90.75^{a}	1.26^{b}	1.12^{c}	1.35^{b}
M1	Laterite	4.08^{d}	13.17^{c}	13.17^{c}	0.94^{a}	0.71^{a}	0.72^{a}	79.25^{b}	71.25^{b}	32.25^{d}	1.10^{bc}	1.40^{b}	1.57^{a}
	Red	2.92^{d}	8.16^{d}	4.15^{e}	0.50^{bc}	0.43^{bc}	0.46^{b}	25.25^{e}	18.75^{e}	3.96^{e}	0.88^{d}	0.59^{e}	0.69^{c}
	Sandy	15.42^{b}	18.17^{b}	25.83^{a}	1.07^{a}	0.54^{ab}	0.46^{b}	60.38^{d}	91.25^{a}	71.25^{b}	1.82^{a}	1.82^{a}	1.58^{a}

Means in column with the same superscripts do not differ significantly at P=0.05 level by DMRT

TABLE 4
Effect of *G. microcarpum* inoculation on P release and fixation in sweetpotato soils

AMF	Soil type	Season I						Season II					
		P release (%)			P fixation (%)			P release (%)			P fixation (%)		
		5 DAP	30 DAP	45 DAP	15 DAP	30 DAP	45 DAP	15 DAP	30 DAP	45 DAP	15 DAP	30 DAP	45 DAP
M0	Laterite	6.31^{c}	11.29^{ab}	6.62^{c}	93.71^{bc}	88.21^{b}	94.54^{ab}	43.35^{c}	27.50^{c}	45.35^{b}	56.15^{d}	82.43^{c}	66.32^{c}
	Red	2.53^{d}	3.64^{d}	6.46^{c}	97.46^{ab}	94.88^{a}	95.15^{ab}	8.22^{b}	8.11^{e}	2.55^{e}	91.38^{a}	93.60^{a}	97.44^{a}
	Sandy	13.42^{a}	12.42^{a}	12.44^{b}	91.11^{c}	87.57^{b}	90.23^{c}	64.05^{a}	27.37^{c}	59.31^{a}	35.45^{f}	72.13^{d}	40.68^{d}
M1	Laterite	2.63^{d}	8.41^{c}	6.33^{c}	94.53^{abc}	94.76^{a}	92.56^{ab}	51.50^{b}	46.08^{b}	6.13^{d}	48.50^{e}	53.43^{e}	92.57^{b}
	Red	1.89^{d}	5.27^{d}	2.68^{d}	98.11^{a}	94.72^{a}	97.32^{a}	16.39^{e}	13.21^{d}	3.28^{e}	83.11^{b}	87.41^{b}	96.73^{a}
	Sandy	8.60^{b}	10.44^{b}	15.32^{a}	91.44^{c}	85.44^{b}	89.75^{c}	37.09^{d}	55.50^{a}	37.16^{c}	62.41^{c}	44.25^{f}	62.83^{c}

Means in column with the same superscripts do not differ significantly at P=0.05 level by DMRT

When the rate of P removal by plant was compared, it was more in inoculated treatments in both the seasons at all DAP. In general, higher values for the percentage of removal were noted in laterite and sandy soil. The rate of removal showed a general decline as DAP advanced (Table 3).

P Release and Fixation in Soil

The P release and fixation in inoculated and uninoculated treatments varied significantly ($p<0.05$) in all soil types. However, mycorrhizal inoculation did not give any added benefit to soil P release and fixation irrespective of season. Among the inoculated treatments, the rate of P release and fixation continued to be good in laterite and sandy soils (Table 4).

DISCUSSION

A series of edaphic constraints of the tropics limit crop production. Infertile soils are either acidic or alkaline and represent deficiencies of P and high P-fixing capacity. In acid soils, when phosphatic fertilisers are incorporated, the major share of P is fixed thereby making it unavailable to plants. Most of the phosphate fertiliser in P-fixing soils ends up in fixed pools, having a recovery of only approximately 10 - 20% (Janssen 2006).

The principle function of AM is to enhance P acquisition of the host plants from, primarily, liable sources in soil (Smith and Read 1997) although non-labile sources (e.g. Al-and Fe-bound P, Ca-phytate) can also be used by some plants (Shibata and Yano 2003). Sweetpotato is widely grown in P-fixing acidic soils of Kerala. The situation, however, favoured the growth and establishment of AM fungi *G. microcarpum* in the crop grown in all soil types as evident in the present study.

Most studies testing the relationship between P availability and mycorrhizal colonisation suggest that increased P availability in soil (Abbot and Robson 1984; Liu *et al*. 2000) as well as plant tissue (Smith and Read 1997) leads to decreased colonisation. This hypothesis might have worked for the present study also where increased P availability in soils and removal to the plant tissue led to a decrease in percentage colonisation and spore density in the rhizosphere of plants grown in all soils types during the second season. However mycorrhizal inoculation significantly improved spore density in the rhizosphere of sweetpotato, which was particularly apparent in laterite and sandy soils. A similar increase in rhizosphere spore density as a consequence to mycorrhizal inoculation has been reported in several crops such as rice (Secilia and Bagyaraj 1994) and cow pea (Muthukumar and Udaiyan 2002).

Another important feature observed in our study was the rhizosphere spore densities decreased with increasing percentage root colonisation and DAP in all the soils types. This could be due to the reason that more spore germination might have taken place to offer infection to the newly emerged roots as the days advanced. This is in accordance with the findings of Gaur and Adholeya (2002)

who also observed a significant correlation between infective propagules, spore density and percentage colonisation.

In P-fixing soils, the P deficiency is mainly caused by strong adsorption of $H_2PO_4$to aluminium (Al) and iron (Fe) (hydro) oxides, which turns large proportions of total P into forms that are unavailable to plants. The role of AM symbiont in enhancing P availability in such soil is by blocking Fe and Al from coming into contact with H_2PO_4. This is possibly by retaining the Fe and Al in the hyphae of the fungus within the root cells or the fungus provides the plant additional binding sites for metal adsorption. The finding of Fabig (1982) and Schüpp *et al*. (1987) that fungal cell walls have a large number of complexing sites for metallic cations strengthens the above postulation. Therefore, the roots highly infected by AM fungi provide the plant an increased surface area for metal adsorption. Perhaps this may be the mechanism at work in P-fixing acid soils in enhancing P availability to mycorrhizal plants. However, in the present study, we noticed a comparatively low level of P availability in mycorrhizal treatments. This is likely to have resulted from fast removal of P by an increased development of external hyphae around the root system of mycorrhizal plants. The general increment in P removal to the tissues of mycorrhizal plants coincided with the decrease in soil P availability, further supporting this theory. Previous works (Jacobsen *et al*. 1992; Johansen *et al*. 1993; Liu *et al*. 2003) also show that increased external hyphal growth in soil enhanced depletion of P in soil. In this context, it is worthwhile to mention the recent finding of Smith *et al*. (2003; 2004) that P uptake at the root surface can be reduced in AM plants and that much of the P enters via the AM pathway.

We could not disentangle the relationship between mycorrhizal inoculation P release and fixation in the soil types studied. It seems that mycorrhizal inoculation did not give any added benefit to P release and fixation. If this is the case in P-fixing sweetpotato soils, the introduction of suitable phosphate solubilising microorganisms (PSM) in conjunction with AM fungi may yield promising results. The P-solubilising organisms dissolve unavailable forms of P by exerting organic acids and chelating substances (Kucey *et al*. 1989; Kapoor 1995) which can be readily absorbed by the AM fungus. The potential of dual inoculation with PSM along with rock phosphate has been reported by many workers (Azcon *et al*. 1976; Barea *et al*. 1983; Singh and Singh 1993). However, extensive evaluation particularly in field conditions in the presence of native microbial competitors is required before arriving at a definite conclusion.

CONCLUSION

In summary, increased removal of P from P-fixing soils to the plant tissue as a result to the inoculation of *G. microcarpum* can be considered as an improvement in plant nutrition under the conditions of our experiment.

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