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Effect of mycorrhizal inoculation and soluble phosphorus fertilizer on growth and phosphorus uptake of pearl millet

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Summary Six mycorrhizal fungi were tested as inoculants for pearl millet (*Pennisetum americanum* Leeke) grown in pots maintained in a greenhouse. VAM fungi varied in their ability to stimulate plant growth and phosphorus uptake. Inoculation with *Gigaspora margarita*, *G. calospora* and *Glomus fasciculatum* increased shoot drymatter 1.3 fold over uninoculated control. In another pot trial, inoculation with *Gigaspora calospora* and *Glomus fasciculatum* resulted in dry matter and phosphorus uptake equivalent to that produced by adding phosphorus at 8 kg/ha.

The influence of inoculating *Gigaspora calospora* on pearl millet at different levels of phosphorus fertilizer (0 to 60 kg P/ha) as triple superphosphate in sterile and unsterile alfisol soil was also studied. In sterile soil, mycorrhizal inoculation increased dry matter and phosphorus uptake at levels less than 20 kg/ha. At higher P levels the mycorrhizal effect was decreased. These studies performed in sterilized soil suggest that inoculation of pearl millet with efficient VAM fungi could be extremely useful in P deficient soils. However, its practical utility depends on screening and isolation of fungal strains which perform efficiently in natural (unsterilized) field conditions.

Introduction

Vesicular arbuscular mycorrhizal (VAM) associations are formed with wheat[1], maize[5], barley[18] and finger millet[13], and VAM inoculation can have large effects on the growth and nutrient uptake of the plants[8]. For instance barley grown in the greenhouse in irradiated soil showed a 56% increase in shoot dry matter due to mycorrhizal inoculation[12]. Growth and/or yield increases of cereal crops due to VAM inoculation have also been recorded in the field. Mosse and her colleagues have shown that inoculation of barley in a field containing 10 ppm soil P ($NaHCO_3$ extractable) resulted in 30 per cent increase in shoot growth[18]. In the same field, when the soil P level was 40 PPM, another trial gave a 2 fold increase in the barley ear growth after VAM inoculation[3]. Beginning from the studies made by Hattingh[7] and Rhodes and Gerdemann[22,23] depicting the role of hyphal translocation of

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phosphorus and other nutrients and that of Pearson and Tinker[19] concerning the increased phosphorus inflow into the mycorrhizal root, there have been consistent research efforts made to understand the fundamental aspects of phosphorus uptake due to mycorrhiza. The applied value of the mycorrhiza in terms of substitution of phosphorus has been estimated by many workers but seems to vary. Under certain conditions it is estimated that mycorrhiza can substitute up to 500 lb phosphorus/acre[14].

Most of the work on cereal crops has been done on soils from the temperate regions. VAM occur on several semi-arid tropics (SAT) plant species[21,25], and because the SAT soils usually have a low fertility, such associations may have a large effect on plant growth. Ther are no reports on the mycorrhiza of pearl millet (*Pennisetum americanum* Leeke) and its benefits, except for a passing reference to the occurrence of *Glomus leptotichum* on a crop grown in Georgia, USA[24]. We report here our studies on the effect of inoculation with different VAM fungal species and levels of P fertilizer application on the growth and phosphorus uptake of pearl millet.

Materials and methods

The VAM fungi used as inocula were maintained on *Cenchrus ciliaris*, a perennial host, grown in sterile sand:Alfisol soil mixture (1:1 v/v) for a minimum period of 90 days. For VAM inoculation, extramatrical chlamydospores of the particular fungus were collected by wet sieving and ca. 600 of them were layered 2–3 cm below the planting hole in each pot. Mycorrhizal spores were collected from the sieves, washed with sterile distilled water and the spore washings were added to the control pots.

Plants were grown in Alfisol soil alone or mixed with sand (1:1 v/v), in 20 cm pots filled with 5 kg root medium. For P additions finely ground weighed amounts were added to the soil and mixed thoroughly in a plastic bag before addition to each pot. The VAM inoculum was added to each of 3 centrally placed planting holes before seeds of the pearl millet hybrid BJ 104 were sown. Two weeks later the seedlings were thinned to one per pot. Plants were watered to 60% moisture holding capacity by weight. Half-strength Hoagland's nutrient solution without phosphate source added twice during the growth period (15 and 30 days after planting). In Experiment 3, the nutrient solution was not added but nitrogen as urea was incorporated into the soil prior to sowing at the rate of 60 kg N/ha (275 mg N/pot). The experiments were conducted in a glasshouse with temperatures ranging from 26 to 35°C. Pots were arranged on tables as randomized blocks. Replicates were reallocated every week among the benches in the glasshouse (on a round robin basis) to reduce positional effects within the glasshouse.

At harvest, plant heights were measured, and shoot and root dry weights recorded after drying in a hot air oven at 60°C for 72 h. To determine the percentage of the length of root colonized by the VAM fungi, roots from each plant were carefully washed free of adhering soil and initially cut into 3 cm segments mixed thoroughly. Four subsamples containing approximately 3–5 g roots were removed, pooled and cut into smaller segments of 1 cm length. They were then transferred into bottles, 10% KOH added and the tissue cleared by steaming in a steam sterilizer at 100°C for 5 min followed by staining with 0.05% trypan blue[20]. The percentage root colonization was calculated as follows:

$$\frac{\text{Number of VAM positive segments}}{\text{Total number of segments scored}} \times 100 = \% \text{ VAM colonization}$$

Phosphorus in the plant tissue was estimated by the vanadomolybdate method[11] after digestion with tri-acid mixture ($HNO_3 : HCLO_9 : H_2SO_4 : 10 : 3 : 1$).

Mycorrhizal dependency values were calucated as the ratio of the dry weight of mycorhizzal plants to that of non-mycorrhizal plants, and expressed as a percentage[16].

Experiment 1

A pot trial was conducted to see if pearl millet derived benefits from mycorrhizal inoculation using 6 different VAM fungi: *Glomus fasciculatum, Glomus fasciculatum* (= E3?), *Glomus mosseae, Gigaspora calospora, Gigasopra margarita* and *Acaulospora* sp. Plants of pearl millet hybrid BJ 104 were grown in steam sterilized Alfisol soil mixed with sterilized sand (1:1 v/v), pH 8.2, 10.4 mg/kg soil P extracted with $NaHCO_3$) and harvested 54 days after planting. Each treatment was replicated five times.

Experiment 2

The two VAM fungi giving the greatest response in Experiment 1 were used as inoculants for BJ 104 grown in sterile Alfisol soil:sand (1:1 v/v, pH 7.2, 7.0 mg P/kg soil extracted with $NaHCO_3$). Growth of inoculated plants without added P was compared with plants grown in soil with added P (230 mg P per pot as triple superphosphate), equivalent to 8 kg P per ha when calculated on a weight basis). Plants were harvested 63 days after planting. Each treatment was replicated eight times.

Experiment 3

This experiment examined the effects of different P fertilizer additions and inoculation with the VAM fungus *Gigaspora calospora* on pearl millet grown in sterile and unsterile Alfisol soil. The soil contained VAM fungi of the genera *Glomus, Gigaspora* and *Sclerocystis* (at a total population of 192 spores per 25 ml soil); had a pH 8.3 and 8 mg P kg soil extracted with $NaHCO_3$. Triple superphosphate was added at rates equivalent to 0, 5, 10, 20, 40, and 60 kg per ha on a weight basis. Three replicates of each treatment was harvested at 39 and 47 days after planting.

Results

Three of the six cultures tested as VAM inoculants, *Glomus fasciculatum, Gigaspora calospora* and *G margarita* produced more than 30% increase in shoot dry matter over the uninoculated control (Table 1). *Glomus mosseae* and *Acaulospora* sp. did not significantly increase the yield inoculation with some species more than doubled the concentration of P in the plant tissue, and the highest concentrations were obtained with treatments giving the highest yields.

In Experiment 2, the addition of spore washings to the control treatments not receiving mycorrhizal inoculum gave VAM-free plants (Table 2) and VAM inoculation gave more than 50% root colonization. The shoot dry matter measurements of inoculated plants and that of P fertilized plants were similar. *Gigaspora calospora* significantly increased shoot dry matter production when compared with the uninoculated control. Inoculation also increased the percentage concentration and total P uptake two fold.

Table 1. Experiment 1. Effect of VAM inoculation on growth and P uptake of pearl millet hybrid BJ 104 grown in sterile alfisol soil for 54 days

Mycorrhizal cultures	Shoot dry weight (g/plant)	Phosphorus uptake	
		Concentration (% dry wt)	Total (mg/plant)
Gigaspora calospora	21.1	0.11	23.2
Glomus fasciculatum	19.5	0.13	15.2
Gigaspora margarita	19.1	0.11	21.4
Glomus mosseae	17.2	0.09	15.9
Acaulospora laevis	16.5	0.09	15.2
G. fasciculatum (E3)	7.5	0.08	5.4
Control	14.5	0.05	8.0
SE	1.19	0.006	1.42
CV (%)	12	15	20

Table 2. Experiment 2. Comparison of mycorrhizal inoculation and phosphorus fertilization on pearl millet cv BJ 104 at 43 days

Treatment	Plant colonization	Plant height (cm)	Shoot dry-matter (g/plant)	Phosphorus uptake	
				conc. (% dry wt)	Total (mg/plant)
Glomus fasciculatum	60 A	72	7.2	0.14	9.8
Gigaspora calospora	57 A	76	9.1	0.14	13.2
Phosphorus-uninoculated[b]	0 B	96	8.0	0.09	7.2
Control-uninoculated	0 B	56	5.8	0.08	4.2
SE	–	4.0	0.64	0.009	0.81
CV (%)	13	15	24	21	26

[a] Data for percentage root colonization by mycorrhiza were analysed after (x + 0.5) square root transformations and values with different postscripts are significantly different at $P < 0.05$; CV (%) calculated on transformed data.
[b] 230 mg P/pot applied as triple superphosphate.

Experiment 3 examined the response of pearl millet to VAM oculation at different levels of P fertilizer addition in sterile and unsterile soil. In sterile soil, the control given spore washings was free of fungal colonization. Increasing P fertilizer tended to reduce the length of root colonized, by the fungus, both in sterile and unsterile soil (Fig. 1). VAM inoculated plants grown in sterile soil were generally larger and produced more foliage than the uninoculated plants (Plate 1). Plant growth increased with P fertilizer addition for uninoculated controls in sterile soil, but inoculation with VAM masked the response. In sterile soil the total dry matter produced by VAM inoculated plants was more than controls between 0 and 20 kg P added per ha. As more P fertilizer was added mycorrhizal and non-mycorrhizal treatments recorded similar dry weights (Fig. 2). In

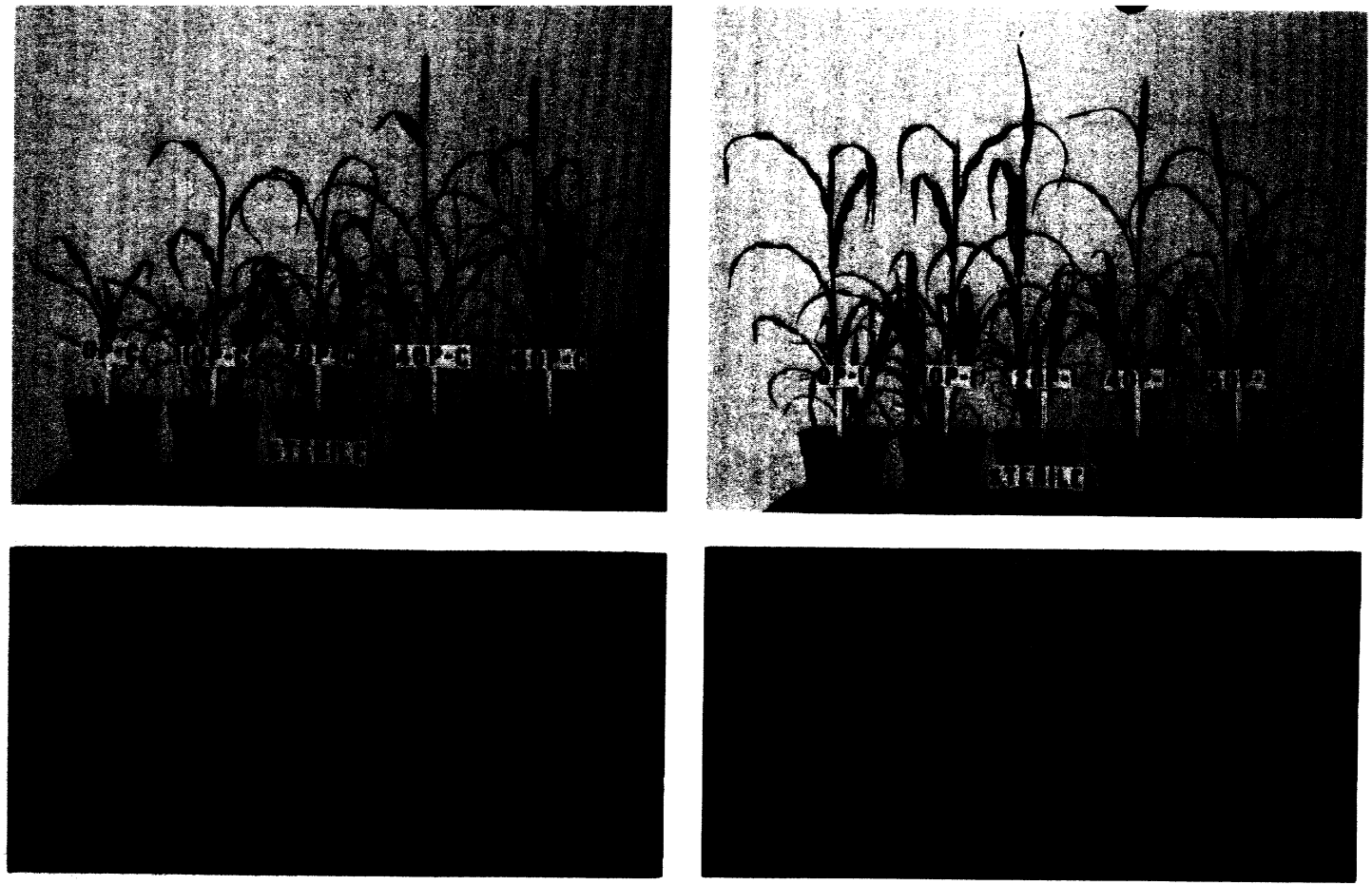

Plate 1. Influence of soluble phosphate fertilizer and vesicular arbuscular mycorrhiza (VAM) on shoot and root growth of Pearl millet hybrid BJ 104 (C = Control; I = Inoculated; Sterile = Sterilized soil; P = kg P/ha).

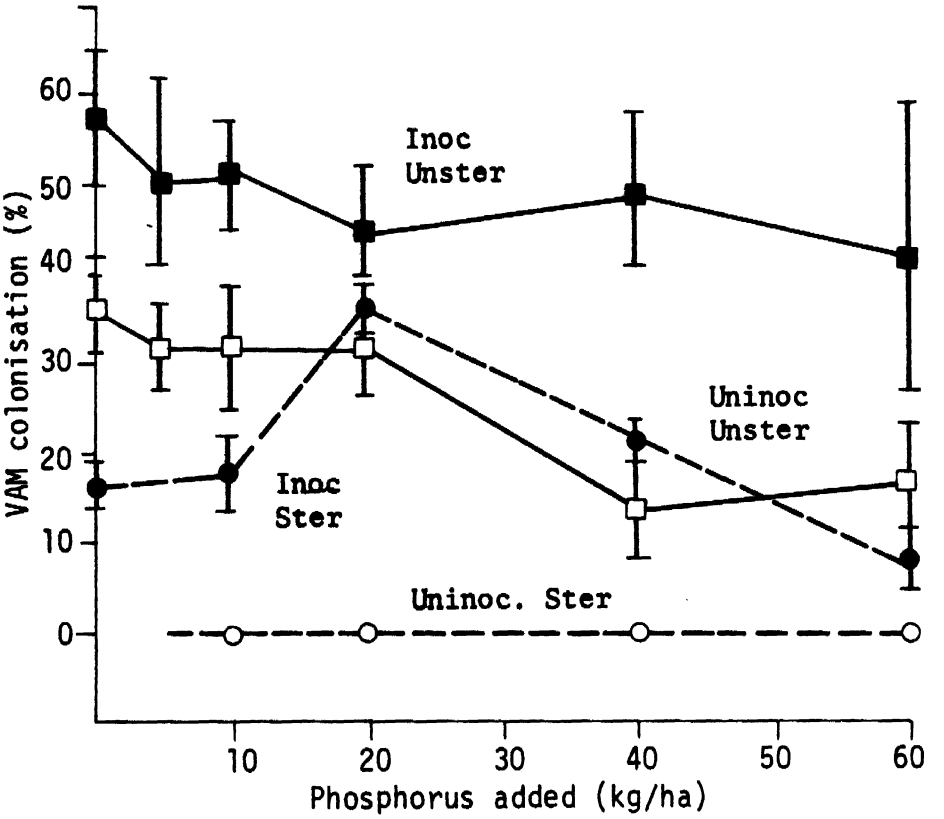

Fig. 1. Percentage VAM colonization in *inoculated* and *uninoculated* Pearl millet BJ 104 grown in pots containing *sterile* and *unsterile* alfisol soil. ■ = Inoculated, unsterile soil; □ = Uninoculated, unsterile soil; ● = Inoculated, sterile soil; ○ = Uninoculated sterile soil.

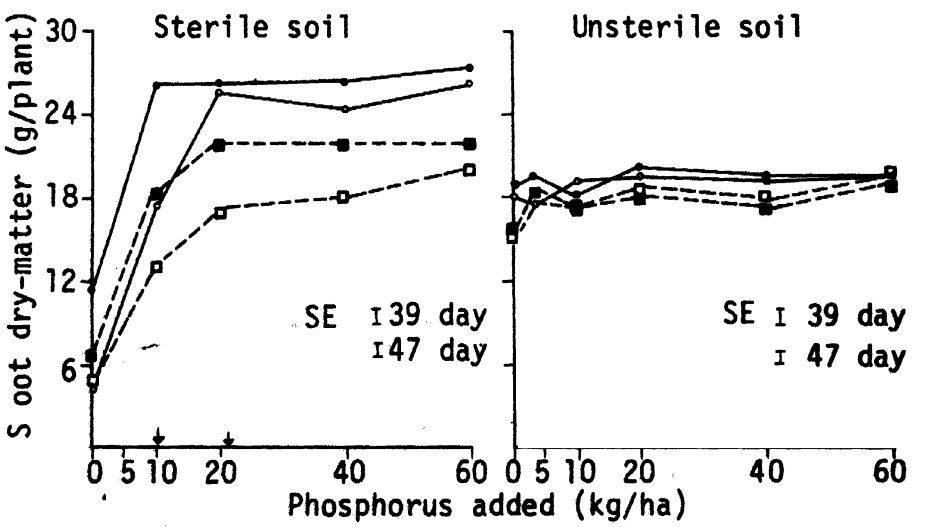

Fig. 2. Dry weights of VAM *inoculated* and *uninoculated* pearl millet CV BJ 104 grown in *sterile* and *unsterile* alfisol soil. (■ VAM inoculated, □ Uninoculated on 39th day; ● VAM inoculated, ○ Uninoculated on 47th day).

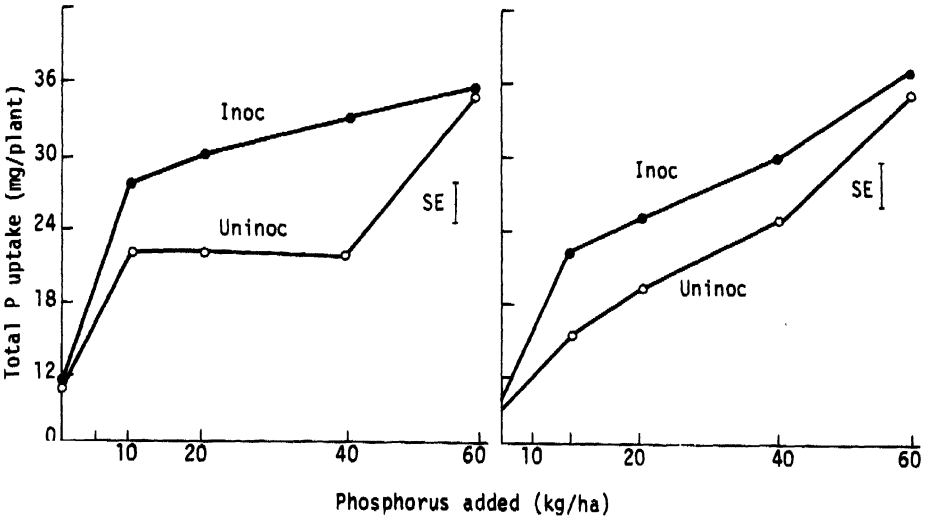

Fig. 3. Total phosphorus uptake of pearl millet cv BJ 104 with and without mycorrhizal inoculation at different levels of added phosphorus and grown in sterilized alfisol soil.

unsterile soil, response to VAM inoculation at 0 and 5 kg P added per ha was not significant and no response was observed at higher levels of P fertilizer addition. Total phosphorus uptake was superior with mycorrhizal inoculation in sterile soil (Fig. 3). Table 3 shows that there was a considerable response to mycorrhizal inoculation with little or no added P in sterile soil. The dependency decreases as the level of applied P increases. There was no effect of inoculation on relative growth in unsterile soil.

Discussion

There are differences between the three major genera of VAM ıngi in their ability to stimulate plant growth and P uptake of pearl _ıillet (Table 1). Similar variations among VAM fungi isolates have been shown for soybean[2]. There may be specific interactions between the plant species and the mycorrhizal strain. Hence, selection of an efficient VAM strain needs to be made for each crop but little work has been done in this direction. Selection based on the final dry matter production is a time- and space-consuming process when a large number of isolates is to be tested; thus there is a need for a technique which measures the efficiencies of VAM fungi at an early stage in the plant-VAM symbiosis which correlates well with the enhancement of final yield and P uptake.

The decrease in the intensity of VAM colonization in sterile soils when large amounts of P are added as fertilizer confirms earlier

Table 3. Mycorrhizal dependency of pearl millet grown in sterilized and unsterile soil at different phosphorus levels

P level kg/ha	Sterilized soil	Unsterilized soil
0	184 ± 66	106 ± 14
10	135 ± 17	98 ± 12
20	113 ± 17	96 ± 9
40	112 ± 14	104 ± 29
60	107 ± 12	101 ± 8

[a] Ratio of the dry weight of mycorrhizal plant to that of non-mycorrhizal plant, expressed as percentage.

studies[5,7]. The physiological basis for the inhibition of VAM colonization is not well understood. Menge *et al.*[15] found that it was the concentration of phosphorus in the plant tissue responsible for inhibition of mycorrhizal colonization and sporulation.

Very little is known about the P requirement or physiological effects of P deficiency on pearl millet. Earlier experiments in the field, at ICRISAT Center, India, have shown the critical P level to be 5 Kg P/ha[10]. Field-grown pearl millet appears to be usually colonized with VAM. Our results show a lower critical P level (10 kg P/ha) for VAM inoculated plants than for non mycorrhizal (20 Kg P/ha) in sterile soil. *Stylosanthes* sp. grow at normal rates in soils containing as little as 3 ppm available P when they are mycorrhizal[8]. In the case of citrus, Menge *et al.*[14] found mycorrhizal fungi to substitute for up to 56 ppm phosphorus (100 lb/acre) in greenhouse culture of Troyber citrange and 278 ppm (500 lbs/acre) for Brazilian sour organge. Cassava[9], Stylosanthes and Citrus demonstrate an almost obligate dependence on VAM, especially at lower levels of P availability[8]. In addition to the fact that mycorrhizal efficiency is largely under the influence of soil characteristics, different sources of phosphorus[17], mycorrhizal benefits in terms of phosphorus substitution is perhaps also dependent on plant species and the cultivar[14] used.

Pearl millet obviously benefits from the presence of mycorrhiza at low levels of available P in sterilized soil and there are differences between fungal species in stimulating millet growth and P uptake. The utility of mycorrhiza depends on selection of efficient plant-VAM fungal isolate combinations which give response in natural field situations.

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