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Response of different crops to arbuscular mycorrhizal inoculation in phosphorus deficient soil

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Abstract

Arbuscular mycorrhizal fungi (AMF) have the capability to improve crop yields by increasing plant nutrient supply. A pot experiment was conducted under natural conditions to determine the response of AMF inoculation on the growth of maize (Zea mays L.), sorghum (Sorghum bicolor L.), millet (Pennisetum glaucum L.), mash bean (Vigna mungo L.) and mung bean (Vigna radiata. L.) crops during the year 2008. The experiment was conducted as completely randomized design in three replications using P deficient soil. Three plants were grown in 10 kg soil up to the stage of maximum growth for 70 days. Spores of AMF were isolated from rhizosphere of freshly growing wheat and berseem crops and mixed with sterilized soil with fine particles. Crops were inoculated in the presence of indigenous mycorrhiza with the inoculum containing 20 g sterilized soil mixed with 40-50 AMF spores. Inoculation with AMF improved yield and nutrient uptake by different crops significantly over uninoculated crops. Inoculated millet crop showed higher percentage of increase in shoot and root dry matters when compared with other inoculated crops. Higher percentage of increases in plant N and Fe were observed in millet, P in mash beans, Zn in maize and Cu and Mn in sorghum crop. Maximum root infection intensity by AMF and their soil spores density were observed in millet crop followed by mash beans. Results suggest that inoculation of AMF may play role in improving crops production and varied response of different crops to fungi signifies the importance of evaluating the compatibility of the fungi and plant host species.

Keywords: AMF inoculation, yield, nutrient uptake, AMF root, infection intensity and spores density

Introduction

Mycorrhizal symbiotic association with roots and fungi found in most of the plant species was found to play an important role in plant nutrient supply (Beringer *et al.*, 1987). Symbiosis of arbuscular mycorrhizal fungi (AMF) is recognized for its multiple positive effects on plant growth and for its contribution towards the maintenance of soil quality (George *et al.*, 1995). The realization of the full potential of this symbiosis has not been fully recognized. The understanding of interactions existing among crops, fungal partners and environmental conditions must be improved for the efficient management of crops and mycorrhizal symbiosis. Large number of AM fungi in soil have been used for inoculum production as alternative to chemical fertilizers (Duponnois *et al.*, 2006). Root colonization and spore population of AM fungi may vary greatly in different plant species grown in different types of soils (Jakobsen and Nielsen, 1983).

The beneficial effect of indigenous AMF is most important in stressed environments and circumstances termed as "ecological crunches" (Allen and Allen, 1986). However, few studies have been attempted to explain how plant species and their mycorrhizal status are related to varying environmental factors in natural ecosystem. It is now widely accepted that climatic and edaphic factors can substantially influence AM association and their population. Rapid changes in soil nutrients may affect AM association and spore numbers (Sharif *et al*., 2005). Tropical regions are dominated by soils, low in available nutrients and moisture (Osonubi *et al.*, 1991) The AM association increases uptake of immobile nutrients, especially P, Zn and Cu (Douds and Miller, 1999). The AM association contributes to the success of plant establishment and survival, increased uptake of water and osmotic adjustment under drought stress and also improves soil plant and water relationships (Jastrow *et al.*, 1998).

Legumes, in general, have a root system typically restricted in their morphology, which along with low soil nutrients makes them mycorrhizae-dependent and mycorrhiza enhances the competitive ability of these plants in obtaining the nutrients (Munn and Mosse 1980). Nodulating legumes require an optimum level of phosphorus in their tissue for nodulation and nitrogen fixation by the bacterial symbiont, because these processes are P-dependent (Hayman, 1986). Arbuscular Mycorrhizal fungi help in these processes with the bacterial symbiosis

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by satisfying the host nutritional needs (Barea and Azcon-Aquilar, 1983). More than 80% of flowering plants establish symbiotic associations with AM fungi, during which fungal hyphae expand the functional root-soil interface and enhance access to inorganic phosphate and other mineral nutrients (Smith and Read, 1997; Brundrett, 2002).

To initiate AM symbiosis, fungal hyphae first differentiate on the surface of the root to form an appressorium, which in turn gives rise to a penetration peg that facilitates entry into the plant. Once inside the root, fungal hyphae continue to grow until they reach and penetrate the cell wall of an inner cortical cell, where further differentiation yields highly ramified fungal hyphae, termed arbuscules (Harrison, 1997). In parallel, AM fungi also develop extensive hyphae outside the plant root. The intra radical and extra radical hyphae constitute a filamentous network that bridges rhizosphere and plant roots and consequently facilitates bi-directional nutrient transfer where soil nutrients move to the plant and plant photosynthesis flow to the fungus (Jakobsen, 1995; Harrison, 1997; Smith *et al.,* 2001). Most of the economically important crops are infected by AM fungi (Hayman, 1982). The positive impact of inoculation with AM fungi has been shown by Guehl and Garbaye (1990). Chen *et al.* (2005) reported the beneficial effect of mycorrhiza on phosphate nutrition of crop plants in soil with low phosphorous concentration.

Keeping in view the important role of AMF in crop production, this experiment was designed to study the effect of AM fungal inoculation on the growth and nutrient uptake of maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), millet (P*ennisetum glaucum* L.), mash bean (*Vigna mungo* L.) and mung bean (*Vigna radiata*. L.) in P deficient soil under natural conditions.

Materials and Methods

Pot experiment was conducted under natural conditions to evaluate the response of maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), millet (P*ennisetum glaucum* L.), mash bean (*Vigna mungo* L.) and mung bean (*Vigna radiata*. L.) plants to AMF inoculation and to identify the best host for AMF multiplication under the prevailing soil conditions. Treatments of the experiment were arranged as completely randomized design (CRD) with three replications. Pots were filled with 10 kg sandy loam soil collected from river side of Jamrud Road Peshawar containing low phosphorous concentration. Crops were inoculated in the presence of indigenous mycorrhiza with the inoculum containing 20 g sterilized soil mixed with 40-50 AMF spores. For this purpose, Spores of AMF were isolated from rhizosphere of freshly growing wheat and berseem crops of the research farm of Agricultural University, Peshawar and mixed with sterilized soil with fine particles. Soil containing AMF spores were mixed well with the top soil of the pot followed by irrigation. Pots were sown with eight seeds each of maize, sorghum, millets, mash bean, and mung bean crops at field capacity and were thinned to three plants accordingly to have equal plants stand in the pots. Crops were harvested at maximum growth stage after 70 days. All cultural practices were strictly followed to have optimum growth after sowing and throughout the plant growth.

After harvest, the plant and root samples were washed with distilled water and dried in oven at 80-90 ^{0}C for 48 h till constant weight and shoot and dry matter yields were recorded. Soil samples were also collected from each treatment after harvesting the crops. Some of the fresh soil from each sample was stored at 4 ^{o}C for AM fungal spores determination, while the remaining soil was dried at room temperature, ground and sieved through ≤ 2 mm sieve and packed in labeled plastic bags for laboratory analysis.

Soil and Plant Analysis

Representative soil sample from each treatment was analyzed for various physical and chemical properties following standard analytical procedures. Soil texture was determined by hydrometer method (Koehler *et al*., 1984). The pH of soil and water suspension (1:5) was determined by the method as described by McClean (1982), soil organic matter with the method of Nelson and Sommers (1982), total N by Kjeldahl digestion method (Bhargna and Raghupathi, 1993) and AB-DTPA extractable P, Cu, Zn, Fe and Mn were determined by the method of Soltanpour and Schawab (1977). Plant samples were analyzed by wet digestion method (Walsh and Beaton, 1977). To separate the effect of dilution or concentrations caused by variation in crops shoot yield, tissue nutrient concentrations were multiplied with total dry matter yield and converted to total amount of nutrients accumulated by plants ha^{-1} on the basis of mass of soil per pot (Jarrell and Beverly, 1981). Data regarding crops shoot and root dry matter yields, plants nutrient concentration and their accumulation by plants were analyzed statistically according to the procedures given by Steel and Torrie (1980) using MSTATC package.

Isolation and Identification of AM Fungal Spores

Mycorrhizal spores were isolated from soil by wet-sieving and decanting techniques (Brundrett, 1996). The isolated spores were identified according to their morphological characteristics including shape, size, colour, distinct wall layer, attached hyphae and surface orientation

of spores as described by Schenck and Parez (1990).

Estimation of AM Fungal Infection

Infection rates by AM fungi in the crop roots were determined by staining the mycorrhizal chitin with lactic-trypan blue according to the procedure explained by Philips and Hyman (1970) and Koske and Gemma (1989). The presence of vesicles, arbuscules or hyphae were measured by the techniques described by Giovannetti and Mosse (1980).

Results and Discussion

Data on physical and chemical characteristics of the soil under investigation showed textural class of sandy loam with organic matter content of 1.0 %, pH value was 8.7 and N content was recorded as 0.071%. AB-DTPA extractable P was 2.58; Zn, 0.129; Mn, 0.960; Fe, 1.10 and Cu was noted as 0.750 mg kg^{-1}.

Shoot and root dry matter yield

Shoot and root dry matter yield of different crops as affected by AM fungal inoculation are presented in Table 1.

Table 1: Shoot and root dry matter yield of crops as affected by AM fungal inoculation

Crop	Treatment	Shoot dry weight	Root dry weight
		----- kg ha^{-1} -----	
Maize	Uninoculated	3423 b**	2061 b*
	Inoculated	3907 a (14)	2325 a (13)
Sorghum	Uninoculated	2457 b*	1447 b*
	Inoculated	2730 a (11)	1590 a (10)
Millet	Uninoculated	1582 b**	1090 b**
	Inoculated	1893 a (20)	1324 a (21)
Mash bean	Uninoculated	1607 b*	1169 b**
	Inoculated	1840 a (14)	1378 a (18)
Mung bean	Uninoculated	1578 b*	1105 b**
	Inoculated	1780 a (13)	1366 a (19)

*Means with different letter (s) in columns are significantly different at $P < 0.05$
**Means with different letter (s) in columns are significantly different at $P < 0.01$
Values in parentheses show the percentage increases with inoculation as compared to uninoculated treatments

Significantly ($P < 0.01$ and $P < 0.05$) increased shoot dry matter yield of 3907, 2730, 1893, 1840 and 1780 kg ha^{-1} over uninoculated treatments were observed for maize, sorghum, millet, mash bean and mung beans, respectively when inoculated with AM fungi. Data revealed that millet produced 20 % higher shoot dry matter yield followed by mash bean and maize crops with 14 % increase over uninoculated treatments. Root dry matter yield was recorded as 2325, 1590, 1324, 1378 and 1366 kg ha^{-1} for maize, sorghum, millet, mash bean and mung beans, respectively, in treatments inoculated with AM fungi. Root growth of crops showed similar trend of growth as in shoot dry matter yield. Millet produced 21 % increased root yield over uninoculated treatments which was higher than all other AM inoculated crops. The higher growth of millet was followed by mung bean and mash beans. Significantly ($P < 0.01$ and $P < 0.05$) increased yield of AM inoculated crops were observed as compared to uninoculated crops.

Bagayoko *et al.* (2000) reported that local genotypes of pearl millet (*Pennisetum glaucum* L.), sorghum (*Sorghum bicolor* L.) and cowpea (*Vigna unguiculata*) with the inoculation of AM fungi in a sterilized sandy soil from a farmers field in Niger showed greater growth enhancing effects. Inoculation with AM fungi led to 18 and 24 fold increases in pearl millet root and shoot dry matter yield, whereas the shoot and root dry matter yields of sorghum and cowpea also depend largely on AM fungi. Many other studies reported enhanced growth of cereals by inoculation of AM fungi (Mamatha *et al.,* 2002; Sharif and Jan, 2008). Ikram *et al.* (1992) noted 70 % increase in shoot dry matter yield of *Hevea brasilinsis* with AM fungal inoculation in the presence of indigenous AM fungi. According to Hayman (1987), millet, maize and sorghum form effective symbiotic association with indigenous Arbuscular Mycorrhizal (AM) fungi. Pot experiments with growing of these crops showed 3 to 101% increases in shoot dry matter yields with the inoculation of AM fungi. Tarafdar *et al.* (1992) reported that many fungi and *Aspergillus fumigatus* in particular, enhanced the growth of mung bean and mash bean.

Plant N and P uptake

Plant N and P uptake as influenced by the inoculation of AM fungi are shown in Table 2. Nitrogen uptakes by plants were recorded as 7.7, 5.2, 3.5, 4.5 and 4.2 kg ha^{-1} for maize, sorghum, millet, mash beans and mung beans, respectively. Data indicated that N uptake by AM inoculated millet was increased significantly by 67 % over

uninoculated crops followed by sorghum plants, which is higher than all other inoculated crops. Significantly higher plant N uptake were noted in AM inoculated crops as compared to uninoculated crops. Data in Table 2 showed that significant increase of plant P uptake by 166 % was noted by mash beans followed by sorghum with the inoculation of AM fungi. These increases were higher than all other inoculated crops. Plant P uptake was recorded as 1.3, 0.9, 0.7, 0.8 and 0.6 kg ha^{-1} for maize, sorghum, millet, mash beans and mung bean crops, respectively. Maximum plant P uptake was observed for maize as 1.3 kg ha^{-1} followed by sorghum, while minimum P uptake was found in mung bean crop.

Table 2: Plant N and P uptake as affected by AM fungal inoculation

Crop	Treatment	N	P
		---------- (kg ha^{-1})----------	
Maize	Uninoculated	5.6 b**	0.8 b**
	Inoculated	7.7 a (38)	1.3 a (63)
Sorghum	Uninoculated	3.5 b**	0.5 b**
	Inoculated	5.2 a (49)	0.9 a (80)
Millet	Uninoculated	2.1 b**	0.4 b**
	Inoculated	3.5 a (67)	0.7 a (75)
Mash bean	Uninoculated	3.1 b**	0.3 b**
	Inoculated	4.5 a (45)	0.8 a (166)
Mung bean	Uninoculated	2.9 b**	0.4 b*
	Inoculated	4.2 a (45)	0.6 a (50)

* Means with different letter (s) in columns are significantly different at $P < 0.05$

** Means with different letter (s) in columns are significantly different at $P < 0.01$

Values in parentheses show the percentage increases with inoculation as compared to uninoculated treatments

These results were in accordance with the findings of Alvey *et al.* (2001) which provided strong evidence that plants inoculated with AMF can enhance P nutrient uptake. Tarafdar *et al.* (1992) reported that many soil fungi produce phosphatase as extra cellular enzyme, which lead to increase phosphate uptake in mung bean and mash bean. Ruiz (2006) reported that AM fungi improve uptake of nutrients by extra radical mycorrhizal hyphae. Similar effects of mycorrhiza were also reported by Mamta and Tilak (1987). They studied the effect of different *Rhizobium* species and mycorrhizal fungus (*Glomus versiforme*) on nutrient uptake of mung bean. Single inoculation with *G. versiforme* had non-significant effect over the corresponding controls, while dual inoculation significantly improved nutrient status of mung bean. George *et al.* (1995) and Chen *et al.* (2005) found that colonization of plant roots by AM fungi greatly increased the plant uptake of phosphorus and nitrogen. The most prominent contribution of these fungi was increased uptake of nutrient by extra-radical mycorrhizal hyphae. Many tested fungal isolates increased phosphorus and nitrogen uptake of the plants by absorbing phosphate, ammonium and nitrate from soil. The contribution of AM fungi to plant phosphorus uptake however, was in general much greater than contribution of plant nitrogen uptake.

Plant micronutrients uptake

Effects of AM fungal inoculation on plant micronutrient uptake (plant concentration × shoot dry matter yield) are given in Table 3.

Data given in Table 3 show that AM fungal inoculation improved the plant uptake of micronutrients significantly ($P < 0.01$ and $P < 0.05$) as compared to uninoculated treatments. Maximum Zn, Fe, Cu and Mn were observed in inoculated treatments of different crops as compared to uninoculated treatments with values of 0.332, 0.751, 0.384 and 1.41 g ha^{-1}, respectively. Data indicated that percent increase in plant uptake of Zn with the inoculation of AM fungi was higher in maize, Fe in millet and Cu and Mn were higher in sorghum crops.

Aliasgharzad *et al.* (2009). found that inoculation of sorghum plants with AMF help to absorb enough micronutrients through chelate formation with siderphores, Bagayoko *et al.* (2000) reported 2.5 - 6 folds increases in total uptake of P, Zn, Fe and Cu in local genotypes of pearl millet (*Pennisetum glaucum* L.), Sorghum (*Sorghum bicolor* L.) and cowpea (*Vigna unguiculata*) with the inoculation of AM fungi.

Root infection intensity and soil spores density

Data in Table 4 indicate root infection intensity by AM fungi and their soil spores density in different host plants in soil under investigations. Data revealed significant increase in AM fungal root infection intensity and their soil spores density in inoculated treatments when compared with uninoculated treatments in different crops. Maximum root infection intensity of 35 % with spores' density of 22 per 20 g soil was recorded in millet crop followed by mash bean. Figure 1 showed that AM spores density of soil increased with higher AM fungal root infection intensity and there was positive correlation between percent increase in shoot and root dry matter yield and that of the AM root infection intensity of crops (Figure 2).

The higher root colonization percentages observed in millet may be due to the better compatibility between the AM fungi and the millet plant under given conditions. (Carrenho *et al.,* 2002). Babu *et al.* (2001) recorded highest i.e 460 spores in 200 g soil. Density of AM fungal spores and their roots infection intensity varied in different crops,

Table 3: Plant micronutrients uptake as affected by AM fungal inoculation

Crop	Treatment	Zn	Fe	Cu	Mn
		----------(g ha^{-1})----------			
Maize	Uninoculated	0.116 b**	0.384 b**	0.154 b**	1.49
	Inoculated	0.332 a (186)	0.751 a (95)	0.384 a (149)	2.02 (35)
Sorghum	Uninoculated	0.106 b*	0.303 b**	0.083 b**	0.95 b*
	Inoculated	0.228 a (115)	0.532 a (75)	0.256 a (208)	1.41 a (48)
Millet	Uninoculated	0.082 b**	0.180 b**	0.054 b**	0.58
	Inoculated	0.173 a (110)	0.359 a (99)	0.155 a (187)	0.83 (43)
Mash bean	Uninoculated	0.103 b**	0.270	0.061 b**	0.52
	Inoculated	0.173 a (67)	0.449 (66)	0.136 a (122)	0.77 (48)
Mung bean	Uninoculated	0.063 b**	0.240b**	0.081 b**	0.61 b*
	Inoculated	0.147 a (33)	0.401a (67)	0.167 a (106)	0.89 a (45)

* Means with different letter (s) in columns are significantly different at $P < 0.05$
** Means with different letter (s) in columns are significantly different at $P < 0.01$
Values in parentheses show the percentage increases with inoculation as compared to uninoculated treatments

Table 4: Root infection intensity of AM fungi and their soil spores density as affected by AM inoculation

Crop	Treatment	Root infection intensity (%)	Spores density (20 g soil)
Maize	Uninoculated	6 b*	9 b*
	Inoculated	25 a	15 a
Sorghum	Uninoculated	11 b*	7 b*
	Inoculated	28 a	12 a
Millet	Uninoculated	9 b*	8 b*
	Inoculated	35 a	22 a
Mash bean	Uninoculated	9 b*	7 b*
	Inoculated	32 a	20 a
Mung bean	Uninoculated	9 b*	6 b*
	Inoculated	30 a	21 a

* Means with different letter (s) in columns are significantly different at $P < 0.05$

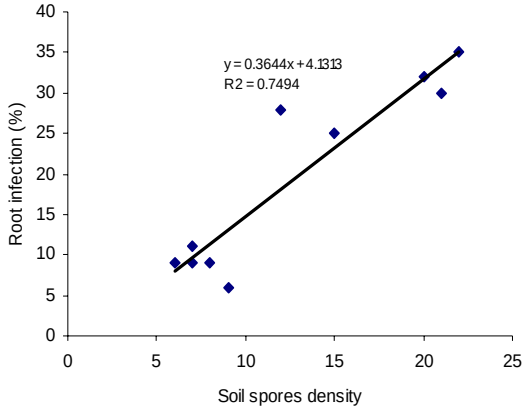

Figure 1: Relationship between root infection intensity and their soil spores density

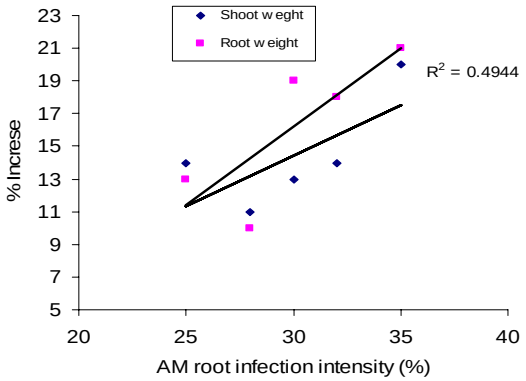

Figure 2: Relationship between AM root infection and % increase in shoot and root yields

may be attributed to the composition of root exudates of these crops, which stimulated the germination of mycorrhizal spores and their infection rates under the prevailing conditions.

Spores of *Glomus fasciculatum* were found in abundance in soil under investigations, where as spores of *G. intraradices, G. mosseae, G. Aggregatum*, *Acaulospora melleae* and *Sclerocystis* were also identified in lower densities. These identified spores still require further confirmation by crop inoculation, re-isolation and re-identification.

Conclusion

It could be concluded from the results of this experiment that shoots and roots dry matter yield of maize, sorghum, millet, mash bean and mung beans improved significantly by the inoculation of AM fungi under the given soil conditions with varied response in different crops. Inoculated millet crop showed higher percentage of

increases in shoot and root yields followed by mash beans. Significant increases in uptake of nutrients by different crops were recorded with the inoculation of AM fungi. Inoculation with AM fungi increased plants accumulation of nutrients and thus has potential to increase crops yield under favorable conditions.

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