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Response of Arid Legumes to VAM Fungal Inoculation

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Abstract

A field study was conducted to determine the effect of vesicular arbuscular mycorrhizal (VAM) fungi on growth and nutrient uptake of the drought-hardy legumes, clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.), mung bean (*Vigna radiata* (L.) Wilczek) and moth bean (*Vigna aconitifolia* (Jacq.) Marechal). Nodulation, nitrogenase activity, percent root infection by VAM fungi, and the number of VAM spores in the soil were increased significantly upon inoculation. Phosphatase activity was enhanced significantly due to VAM inoculation. An improvement in dry matter production (20 to 38%) and grain yield (15 to 22%) upon inoculation was obtained. Concentrations of N, P, Cu and Zn in the shoot were found to be significantly higher in inoculated plants. However, in general, concentrations of K, Ca, Mg, Na, Fe and Mn remained unaffected. From the results, a positive interaction between *Rhizobium* and VAM fungi is evident under arid field conditions. All the legumes showed similar effects upon inoculation with *Glomus mosseae* and *Glomus fasciculatum*.

Keywords: Drought-resistant legumes, *Glomus*, nutrient uptake, arid environment, VAM

1. Introduction

Arid legumes are cultivated mostly in sandy loam soils of the drought-prone areas of Western Rajasthan where most of the soils are deficient in P. Use of chemical fertilizers is limited in this region because of the risk of crop failure

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due to drought. The crops are grown sometimes by inoculating with rhizobial strains, but inoculation often fails because of P-deficiency and of frequent droughts. VAM fungi enhance drought tolerance of plants (Hardie and Leyton, 1981; Allen and Boosalis, 1983) and help in the uptake of various nutrients especially P (Tarafdar and Marschner, 1994), Zn (Thompson, 1990; Kothari et al., 1991) and Cu (Li et al., 1991), by tapping a large volume of soil, mobilization of scarce nutrient sources, and modification of the root environment (Hayman, 1983). In general, VAM fungal associations with plants are wide-spread both taxonomically and geographically. Most of the plants growing in Indian deserts carry VAM infections on their roots (Kiran Bala et al., 1989).

Mycorrhizae are beneficial to plants only under some environmental conditions especially in soils of low fertility (Fitter, 1989). Thus, to estimate VAM contributions to plant growth under field conditions, plants differing in their VAM status should be compared in the field. The aim of the present experiment was to investigate and compare the possible benefits of VAM fungal inoculation on growth, nodulation, nutrient uptake and yield of different legumes under arid environment.

2. Materials and Methods

The experiment was conducted under field conditions at Central Research Farm, Central Arid Zone Research Institute, Jodhpur, which is located at 26°18' N and 73°01' E. The soil is loamy sand (hypothermic typic haplocambids) and last year only clusterbean was grown with the inoculation of *Rhizobium* DRG-3 but without addition of any fertiliser. The organic C content 0.23%, total N content 0.031%, Olsen P content 7.0 mg/kg, organic P content 50 mg/kg, total P content 270 mg/kg, pH 8.1, EC 0.18 dS/m, and 70 VAM spores/100g soil. The legumes were grown during the 1993 wet season (mid-July to end of October). The VAM fungal cultures isolated from maize, and maintained on *Cenchrus ciliaris* (L.) grass were obtained from ICRISAT. The grass was grown for 90 days, and spores and infected roots present in the medium served as inoculum. A thin layer of inoculum containing about 8 to 10 spores/g was placed below the soil surface in the row before planting the seeds. Each row (4 m length) received 800–1000 surface sterilized (with 0.2% chloramin T and 0.02% streptomycin sulphate) infective VAM propagules (spores) of 90–250 μm in size. After germination, the inoculated field soil was tested for infective propagules of VAM fungi and compared with the inoculum in an MPN trial (Daniels and Skipper, 1982); the inoculated plots contained a 14–15 fold

concentration of infective propagules per cm^3 volume compared with control plots.

The experiment was laid out in a randomized block design. The size of each plot was 5 × 4 m. Each plot contained 3 treatments: two VAM-fungi and one native VAM control, with four replications. Seeds of clusterbean (cultivar: Maru Guar), moth bean (cultivar: Maru Moth-1) and mung bean (cultivar: S8) were hand-sown in rows 50 cm apart and with a 10 cm spacing within the row. One meter distance was maintained between plots from all the sides to avoid contamination. The control soil was not inoculated. No fertilisers were applied.

After 35 d of growth, four plants from each plot were uprooted carefully at random. The root systems with intact nodules were recovered and rhizosphere soil samples (soils adhering to the root surface) were collected. Nitrogenase activity of the roots was assayed immediately by placing the root system of the plants in 200-ml assay bottles closed with an air-tight serum cap. Ten per cent of the air inside the bottle was replaced with C_2H_2, and the C_2H_4 produced after incubation for 1 h at 30°C was estimated by an AIMIL-NUCON gas chromatograph fitted with Poropak-N-Column (2 × 0.03m) using N_2 as carrier gas at a flow rate of 25 ml/min. The assay was performed with 4 replicates for each treatment. Nitrogenase activity was expressed as n mol C_2H_4 produced per plant per hour (Rao and Venkateswarlu, 1987). Following the assay, the percentage of VAM colonization of the roots was estimated by the root slide technique (Read et al., 1976) after clearing root segments of 1 cm length with KOH and staining with trypan blue. Viable VAM-fungal spores were isolated by wet sieving and decanting from 100 g wet soil (Gerdemann and Nicolson, 1963). The spore sizes between 90–250 μm were counted under a dissecting stereomicroscope. Activities of acid and alkaline phosphatases were assayed by the method of Tabatabai and Bremner (1969) with an acetate buffer (pH 5.4) and borate-NaOH buffer (pH 9.4), respectively, using 4-nitrophenyl phosphate as the substrate at 35°C, for 1h. The enzyme activity was expressed in nano-Katals as the amount of enzyme required for hydrolysing 1.0 nmol of 4-nitrophenyl phosphate per second at 35°C at a specific pH.

At maturity, grain yield and dry matter production were determined. Shoots were dried at 60°C to a constant weight, ground to a fine powder and subjected to a mixed acid (di or triacid) digestion. Estimates of N, P, K, Ca, Mg and Na were made by following the standard methods outlined by Jackson (1967) after wet digestion, while Cu, Zn, Fe and Mn contents were determined by using atomic absorption spectrophotometry (Varian AA 1475).

Standard errors of means were calculated and, when appropriate, analysis of variance carried out and means of mycorrhizal and control (native-mycorrhizal) treatments separated by the Scheffe test for planned comparisons (Sokal and Rohlf, 1981).

3. Results and Discussion

Because of the presence of an efficient strain of *Rhizobium* in the test soil, plants were healthy and had adequate N supplied through N_2 fixation. Inoculation with different VAM fungi improved nodulation and nitrogenase activity (Table 1) in all the legumes tested. This observation confirms that of Manjunath and Bagyaraj (1984), who reported enhanced nodulation in different legumes with VAM infection. Enhanced nodulation might be due to better plant nutrition and higher levels of growth regulating compounds in the host plants (Dodd et al., 1983; Ek et al., 1983). The enhancement in percent root infection and build up of VAM spores in the rhizosphere soil due to inoculation varied slightly among the legumes as well as from one fungus to the other. In general, under control soil conditions the maximum infection was observed with mung bean (47%) followed by moth bean (42%) and clusterbean (35%). But after inoculation, moth bean was most infected (83 to 85%) with a production of 872–936 spores per 100 g soil. However, the differences among the legumes were slight.

Table 1. Effect of VAM fungi on nodulation, N_2-ase activity, spore build-up and percent root infection in arid legumes

Inoculant	Number of nodules per plant	Nitrogenase activity (n mol C_2H_4 per plant per h)	Root infection (%)	VAM spores per 100 g soil
A. Clusterbean				
Control	16.5	385	35	352
Glomus mosseae	21.0***	510***	83***	916***
Glomus fasciculatum	20.8**	490***	78***	725***
B. Moth bean				
Control	13.0	272	42	390
Glomus mosseae	18.5*	483***	85***	936***
Glomus fasciculatum	15.6	471***	83***	872***
C. Mung bean				
Control	25.0	265	47	283
Glomus mosseae	29.5*	428***	82***	875***
Glomus fasciculatum	35.0***	440***	79***	862***

Statistical significance calculated for comparison between control and inoculated treatment. *p<5%; **p<1%; ***p<0.1%.

There was significant enhancement of nitrogenase activity in these legumes upon VAM fungal colonization with the maximum increase in moth bean. Higher nitrogenase activity in inoculated plants compared to control plants probably were due to better P-nutrition of the VAM-inoculated plants. Increases of available soil P through P fertilization are known to result in better nodulation and enhanced N_2 fixation in different legumes (Israel, 1987), resulting in improved grain yield and dry matter production (Bremer et al., 1989). Mosse et al. (1976) observed that P supplied through VAM fungi led to enhanced N_2 fixation. The improved P nutrition of these plants could be due to the tapping of a larger volume of soil beyond the phosphate depletion zone by the ramifications of VAM-fungal hyphae, as the mobility of phosphate in soil is limited (Hayman, 1978), or to the increased availability of P in the rhizosphere soil resulting from the breakdown of organic phosphates by the phosphatases secreted by VAM fungi or VAM infected plants.

In the rhizosphere of all studied legumes the activity of acid and alkaline phosphatases was increased by inoculation with VAM fungi (Table 2). The increase in activity was more pronounced with the inoculation of *Glomus mosseae*. In the VAM inoculated plants, the acid phosphatase activity was increased up to 27% and alkaline phosphatase activity was up to 23% compared with the uninoculated soils. Production of extracellular acid phosphatase (Tarafdar, 1995) which catalyses the release of P from organic sources (Tarafdar and Marschner, 1994) is well documented for VAM fungi. The present results provide evidence of production of both the phosphatases by extraradical mycelium under field conditions. Increased VA-mycorrhizal and root activity in the rhizosphere may generally account for higher phosphatase activities. Plants secrete only acid phosphatase (Tarafdar, 1989), the increase in alkaline phosphatase in soil is solely microbial in origin (Tarafdar and Claassen, 1988). Kumari et al. (1990) demonstrated elevated alkaline phosphatase activity on root surface in mycorrhizal French bean plants. In view of the above, it has been suggested that the increased alkaline phosphatase activity in the mycorrhizosphere might be due to release of this enzyme by VAM fungi, or by other microorganisms whose activities are enhanced by the mycorrhizosphere effect (Bethlenfalvay and Franson, 1988). Dodd et al. (1987), found that both plant roots and VAM contributed to the higher activities of phosphatase in the rhizosphere of plants infected with *Glomus mosseae*. Histochemical studies (Gianinazzi et al., 1979) to localize phosphatase activity within VAM roots at an ultra-structural level demonstrated acid phosphatase activity in the cytoplasm of the plant root cells as well as at the growing tips of VAM hyphae. Among the legumes tested maximum phosphatase activity was observed with moth bean.

Table 2. Effect of VAM fungi on phosphatase activity in arid legumes

Inoculation	Clusterbean	Moth bean	Mung bean
A. Acid phosphatase (n Kat per 100 g soil)			
Control	8.0	9.8	8.2
Glomus mosseae	9.8***	12.1***	10.4***
Glomus fasciculatum	9.5***	11.0*	9.7***
B. Alkaline phosphatase (n Kat per 100 g soil)			
Control	13.0	17.5	11.0
Glomus mosseae	15.5***	20.0**	13.5*
Glomus fasciculatum	14.8**	19.8**	13.6*

Statistical significance calculated between control and inoculated treatment. *p<5%; **p<1%; ***p<0.1%.

Table 3. Dry matter and grain yields of arid legumes affected by different VAM fungi

Inoculation	Clusterbean	Moth bean	Mung bean
A. Grain yield (Q per ha)			
Control	5.4	4.2	4.8
Glomus mosseae	7.0***	5.1***	6.0***
Glomus fasciculatum	6.2**	4.9***	5.8***
B. Shoot dry mass (Q per ha)			
Control	23.0	18.2	21.5
Glomus mosseae	31.7***	23.5***	27.9***
Glomus fasciculatum	29.9***	21.8*	25.8**

Q-Quintal; Statistical significance calculated for comparison between control and inoculated treatment. *p<5%; **p<1%; ***p<0.1%.

Dry matter production and grain yield of legumes were significantly improved (Table 3) by inoculation. This was in conformity with results of Islam et al. (1980), who had observed increased dry matter production and grain yield of legumes in response to inoculation with VAM fungi in Nigerian soils. This could be attributed to a more extensive root system tapping a larger

volume of soil for water and nutrients. Linderman (1988) suggested that mycorrhizal plant responses involve the entire mycorrhizosphere, not just the fungus alone. Companion fungi or bacteria present in the mycorrhizosphere, may promote plant growth through a variety of mechanisms. The microbial community may stimulate the development of hyphae and rhizomorphs or decrease the growth of pathogens.

The increased N concentration in shoots of inoculated plants (Table 4) may be due to the improvement in symbiotic N_2 fixation. Azcon-Aguilar and Barea (1978) attributed higher N concentrations and contents in lucerne plants inoculated with phosphate-solubilizing bacteria and *Rhizobium*. If the P nutrition of plants is improved, either through fertilization or biological means, symbiotic N_2 fixation and plant N content are improved.

Table 4. Concentration of primary nutrients (mg/g) as influenced by inoculation with *Glomus mosseae*

Legumes	Nitrogen		Phosphorus		Potassium	
	Control	Inoculated	Control	Inoculated	Control	Inoculated
Clusterbean	28	33*	1.7	2.1***	28	36*
Moth bean	26	30*	1.5	1.9**	31	34
Mung bean	34	39*	1.4	1.9***	24	23

Statistical significance calculated for comparison between control and inoculated treatment. *p<5%; **p<1%; ***p<0.1%.

A significant improvement in the P concentration (Table 4) in the VAM plants was observed. Greater phosphatase activity has been directly implicated in the acquisition of P by plants (Dodd et al., 1987). Efficiency of VAM fungi in the production of phosphatases for the hydrolysis of organic P is clearly shown (Tarafdar and Marschner, 1994). Alternately, P mineralisation may be indirect if VAM hyphae acquire P only after it is mineralized by other soil microorganisms or those associated with the mycorrhizal fungi. The concentration of K was affected significantly only in clusterbean by VAM fungi.

No significant difference in the concentration of Ca, Mg and Na upon inoculation in any of the legumes was observed (data not shown). The role of mycorrhiza in the acquisition of Ca, Mg and Na is often small probably because

they are transported preferentially by mass flow of the soil solution to the root.

Copper and Zn concentrations in the shoot dry matter of legumes were consistently higher in the VA-mycorrhizal plants (Table 5). Usually VAM colonization is advantageous to plant growth by providing scarcely mobile mineral nutrients, such as P, Zn and Cu. It is generally recognized that Zn and Cu can be absorbed and then translocated through VAM hyphae, and then released to the host (Li et al., 1991; Kothari et al., 1991). In contrast, the concentrations of Mn and Fe in the shoots are not affected due to inoculation (results not shown). The trends are similar in all the legumes tested. Mn apparently did not decrease in inoculated vs. control plants, because the controls were already with AM, i.e. there were no additional effects as with N, P, Cu and Zn. It is concluded that under arid conditions introduced VAM fungi can compete with the native VAM fungi and proliferate better in the rhizosphere of drought hardy legumes resulting in the improvement of biological activity in the rhizosphere. These fungi further helped in improving nutrient uptake, grain yields and dry matter production under field conditions. In the light of the above there is a possibility of employing exotic AM fungi as inoculants for enhancing biomass production.

Table 5. Concentration of micronutrients (μg/g) as influenced by inoculation with *Glomus mosseae*.

Legumes	Copper		Zinc	
	Control	Inoculated	Control	Inoculated
Clusterbean	14	19***	56	69***
Moth bean	14	18***	67	79***
Mung bean	14	18***	80	97***

Statistical significance calculated for comparison between control and inoculated treatment. ***$p<0.1\%$.

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